Janet L. Leonard *Editor*

Transitions Between Sexual Systems

Understanding the Mechanisms of, and Pathways Between, Dioecy, Hermaphroditism and Other Sexual Systems

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To my husband, Bruce Karsh, with all my love

Preface

The stimulus for this book came from Verena Penning of Springer Verlag, who approached me about doing a book related to the topic of a symposium "Phenotypic plasticity and the evolution of gender" which I had organized for the 2013 meeting of the Society for Integrative and Comparative Biology and which was published that year in *Integrative and Comparative Biology*. With her encouragement, I began thinking about whether there were new and unexplored areas in the field, which could be the basis for a useful and groundbreaking book, and I decided there was. The focus of this book is on taxonomic groups that demonstrate substantial diversity of sexual systems with the goal of understanding what selective pressures are associated with changes in sexual system and what evolutionary pathways and sex-determining mechanisms are involved in changes in sexual systems when they change. George Williams (1975), in his seminal book, *Sex and Evolution*, (a) pointed out that sexual systems are often extremely conservative, in defiance of the theory of the era, and (b) suggested that understanding what factors were important in determining sexual system or evolutionary changes in sexual system would best come from analysis of taxa characterized by lability or diversity in sexual system. This book, then, is an effort to follow Williams' suggestion by offering an array of chapters, each dedicated to a taxon (or group of taxa) which is variable in sexual system, with a goal of analyzing the selective pressures, evolutionary pathways, and/or sex-determination mechanisms that explain this diversity.

When one begins to review a field, one always finds that it is riddled with rabbit holes, many of them full of fascinating facts, discoveries, and ideas that enrich one's understanding of evolution and biology (and sometimes also the workings of scientists' minds), even though this material often proves completely irrelevant to the theme of the review. Occasionally, there is real treasure down one of these holes. It is probably fair to say that there are two types of scientists, those who enjoy rabbit holes and those who do not. I happen to enjoy them, perhaps too much, and consequently planning, participating in, and particularly reading the chapters of this book has been a great pleasure. I hope all the authors who have contributed to this book have had the same experience. It is also my fervent hope that readers of this book will find both an authoritative source for the topics in question and a rich source of rabbit holes to explore.

My thanks to Verena Penning, the whole staff of Springer Verlag, and especially the authors of the book's chapters who gave their time to making this book possible and have taught me so much and the many reviewers who have made the book better.

Santa Cruz, CA Janet L. Leonard

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Chapter 1
The Evolution of Sexual Systems in Animals

Janet L. Leonard

Abstract A sexual system is the pattern of gender allocation that characterizes a species. In both plants and animals, simultaneous hermaphroditism and dioecy are the most common and stable sexual systems. Other sexual systems, sequential hermaphroditism, environmental sex determination, gynodioecy, androdioecy, and trioecy, are less stable and less widely distributed. The boundaries between these sexual systems are not always clear, largely because phenotypic plasticity is an important and prevalent component of sexual reproduction. One can view sexual systems in the Metazoa as lying on a gradient of phenotypic plasticity from simultaneous hermaphroditism at the high end through sequential hermaphroditism and environmental sex determination to genetically determined dioecy, which has a minimum of phenotypic plasticity in sex allocation. The distribution of sexual systems across the Metazoa gave rise to Williams' Paradox, which states that the pattern is best explained by phylogeny rather than sex allocation theory. Today, sex allocation theory seems to explain transitions in sexual system in those taxa with labile sexual systems adequately. However, the stability of either dioecy or simultaneous hermaphroditism in many major taxa, such as phyla and classes, remains inexplicable. While in angiosperms the evolutionary pathways between dioecy and simultaneous hermaphroditism are fairly well understood, a plausible evolutionary sequence for transitions between dioecy and simultaneous hermaphroditism in animals has been lacking. Here, the proposal is made that it is useful to view transitions from simultaneous hermaphroditism to dioecy as the result of selection for decreasing phenotypic plasticity and *vice versa.* A scenario for evolutionary transitions between simultaneous hermaphroditism and dioecy, in animals, through intermediate stages of sequential hermaphroditism and environmental sex determination is proposed.

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1.1 Introduction

A sexual system is the pattern of gender allocation that exists in a species (Box 1.1). Dioecy, in which there are two types of individuals, males (individuals reproducing through sperm) and females (individuals that reproduce through eggs), and simultaneous hermaphroditism, in which all individuals are capable of reproducing through both sperm and eggs in a single breeding season, are the most common, widespread, and evolutionarily stable sexual systems in both multicellular plants and animals (Ghiselin 1974; Williams 1975; Maynard Smith 1978; Leonard 2013; Chaps. 2 and 3, etc.). However, there are many other systems such as sequential hermaphroditism, in which an individual reproduces through eggs during one part of its life and sperm during another; gynodioecy, in which populations are composed of a mixture of females and simultaneous hermaphrodites; androdioecy in which populations consist of males and hermaphrodites; and even some examples of trioecy, with populations consisting of males, females, and hermaphrodites (Ghiselin 1974; Leonard 2010, 2013) (see Box 1.1). Understanding the evolution of sexual systems from the standpoint of natural and sexual selection acting on individuals has been a focus of evolutionary ecology for over 40 years (Williams 1975, etc.), but important questions remain.

Box 1.1

A. Dioecy = gonochorism

Dioecy is a sexual system in which individuals reproduce in one sexual role (male or female), only, during their lifetime. It is characteristic of many large taxa (phyla and classes) in both plants and animals (insects, birds, mammals, reptiles, amphibian, vast majority of nematodes, gymnosperms, etc.). The sex of an individual can be determined by either genetic or environmental factors or a combination of the two.

B. Simultaneous hermaphroditism (SH)

1. Selfing simultaneous hermaphroditism: individuals produce both eggs and sperm and reproduce through self-fertilization exclusively. This sexual system has evolved many times but is not characteristic of any major taxon and may be short-lived, evolutionarily.
2. Outcrossing simultaneous hermaphroditism: individuals are capable of reproducing in both sexual roles (male and female) during a single reproductive season. Outcrossing simultaneous hermaphroditism, in which individuals can mate with another individual in both the male and female sexual role, is characteristic of many large taxa (phyla and classes) in both plants and animals. Some outcrossing simultaneous hermaphrodites are also capable of self-fertilization.

(continued)

Box 1.1 (continued)

C. Sequential hermaphroditism

Individuals reproduce in different sexual roles during different reproductive periods of their life histories. This may involve a single change in sex with increasing size/age, or it may involve more than one change from male to female or the reverse depending on environmental variables. Sequential hermaphroditism is not characteristic of phyla or classes but is characteristic of many families of teleost fishes and some families of caenogastropods and has evolved several times in polychaetes, gastropods, and some groups of shrimps. It is also found in angiosperms.

D. Androdioecy

In androdioecy, breeding populations consist of a mixture of males and simultaneous hermaphrodites. Androdioecy is relatively rare in angiosperms but does occur and may offer an evolutionary path from dioecy to simultaneous hermaphroditism (Pannell 2002; Chap. 3; Delph 2009). In metazoan animals androdioecy has evolved many times but appears to be restricted taxonomically (Weeks 2012). The most common form of androdioecy in animals involves hermaphrodites that either self-fertilize or outcross with males but do not outcross with sperm (Weeks 2012). Another type of androdioecy, derived from hermaphroditic ancestors, does involve outcrossing by hermaphrodites. Some authors (Pannell 2002) restrict the term androdioecy to cases in which there is a genetic distinction between males and hermaphrodites as is usually the case in androdioecy derived from dioecious taxa (see Chaps. 3 and 4; Weeks et al. 2006).

E. Gynodioecy

In this sexual system, populations are made up of a mixture of females and simultaneous hermaphrodites. In angiosperms, it has evolved many times and it may represent part of a transition from simultaneous hermaphroditism to dioecy (see Charlesworth 1999; Delph 2009; Chap. 3). In animals it is extremely rare (review in Leonard 2010; Weeks 2012; see text). As with androdioecy, drawing a hard line between “true” gynodioecy with distinct, genetically determined morphs and cases of simultaneous hermaphroditism and a pure female state being developmental stages of the same individual is not always straightforward.

F. Trioecy

This sexual system involves populations consisting of a mixture of males, females, and simultaneous hermaphrodites. It is always very rare but has been found in both plants and animals, e.g., papaya and rhabditid nematodes. Trioecy may be either genetically or environmentally mediated (see text).

There are two major outstanding problems in understanding the evolution of sexual systems: (a) understanding the distribution of sexual systems among

Table 1.1 Sexual systems in the Plantae (Embryophyta)[a]

Phylum[a,b,c,d]			
	Class	Sexual system	Comments
			Spore plants
Anthocerophyta		Hermaphroditic[e]	Hornworts
Hepatophyta		Hermaphroditic[e]	Liverworts
Bryophyta		Hermaphroditic[e]	Mosses
Lycophyta			
	Lycopodiopsida	Hermaphroditic[e]	Clubmosses
	Isoetopsida	Hermaphroditic[e]	Spikemosses, quillworts
Monilophyta			
	Sphenopsida	Hermaphroditic[e]	Horsetails
	Filicopsida	Hermaphroditic[e]	Ferns
	Psilotopsida	Hermaphroditic[e]	Whisk ferns
			Seed plants
Gnetophyta		Dioecious	Vines, small trees, (Inc. *Ephedra*), and *Welwitschia*[d]
Coniferophyta		Dioecious or hermaphroditic	Conifers
Gingkophyta		Dioecious	One living species, *Ginkgo biloba*
Cycadophyta		Dioecious	
Magnoliophyta		Predominantly hermaphroditic, recurrent evolution of dioecy, gynodioecy, and occasionally androdioecy	Angiosperms

[a] Case and Jesson (Chap. 2)
[b] Lecointre and Le Guyader (2006)
[c] Margulis and Schwartz (1982)
[d] Wijesundara (2011)
[e] Sporophytes hermaphroditic as far as known; hence genets hermaphroditic, dioecious sporophytes theoretically possible but unknown (Chap. 2)

organisms and (b) identifying the evolutionary pathways from one sexual system to another. G.C. Williams (1975) pointed out that while the existing body of theory suggested that sexual systems should be sensitive to ecological factors (population density, encounter probability, length of reproductive lifetime, etc.), the distribution of sexual systems among organisms shows that this is often not the case. That is, for both plants and animals, the best predictor of sexual system in most taxa is not ecology but rather phylogeny, the phylum or class to which they belong (Williams' Paradox; see below and Leonard 1990, 2005, 2010, 2013). Table 1.1 shows the distribution of sexual systems across the Plantae, while Table 1.2 shows the distribution of sexual systems across the Metazoa. In both cases, whole phyla are characterized by dioecy, or hermaphroditism, while in both groups, a few taxa are

Table 1.2 Sexual systems in the Metazoa (Modified from Leonard 2013

Phylum		Mode(s) of sexuality	Comments
	Class		
Porifera		Usually hermaphrodites	No permanent gonads; not clear how often sperm and eggs ripen simultaneously versus sequentially; sessile
Placozoa		Yes	Very poorly known
Cnidaria		Either dioecious or hermaphroditic	Either pelagic or sessile
Ctenophora		Largely hermaphrodites	Pelagic
Platyhelminthes		Almost exclusively hermaphroditic	Free-living or parasitic; dioecy in parasitic family Schistosomatidae, sometimes in Didymozoidae[a]
Nemertea		Dioecious	Free-living
Rotifera		Dioecious	Free-living
Acanthocephala		Dioecious	Parasitic
Cycliophora		Dioecious; sessile female and dwarf male	Poorly known
Entoprocta		Protandric or simultaneous hermaphrodites	Sessile
Sipuncula		Dioecious except for one species	Tube-dwelling worms
Mollusca		Primitively dioecious	
	Solenogastres	Hermaphroditic	
	Caudofoveata	Dioecious	
	Polyplacophora	Mostly dioecious, one genus w/hermaphrodites[b]	Chitons
	Monoplacophora	Dioecious	
	Gastropoda[b]		
	Basal clades	Varied sexual systems	The basal clades include the Patellogastropoda, Vetigastropods, etc.
	Caenogastropoda	Largely dioecious; some sequential and simultaneous hermaphrodites[b]	
	Heterobranchia	Almost exclusively simultaneous hermaphrodites	
	Cephalopoda	Dioecious	
	Bivalvia	Largely dioecious; some hermaphrodites; various independent events	
	Scaphopoda	Dioecious	
Annelida			
	Polychaeta	Mostly dioecious	Both sedentary and errant families

(continued)

Table 1.2 (continued)

Phylum			
	Class	Mode(s) of sexuality	Comments
	Oligochaeta	Hermaphroditic	
	Hirudinea	Hermaphroditic	Predators and ectoparasites
	Echiura	Dioecious	Burrow-dwellers
	Pogonophora	Dioecious	Sessile tube-dwellers
Ectoprocta		Hermaphroditic	Sessile
Phoronida		Either dioecious or hermaphroditic	Sessile tube-dwellers
Brachiopoda		Dioecious, some hermaphrodites[c]	Sessile
Chaetognatha		Simultaneous hermaphrodites	Pelagic predators
Gastrotricha		Largely hermaphroditic	Interstitial
Priapulida		Dioecious	Sessile tube-dwellers or mobile predators
Loricifera		Dioecious	Interstitial
Kinorhyncha		Dioecious	Interstitial
Nematomorpha		Dioecious	Parasitic larvae; free-living adults
Nematoda		Dioecious or (rarely) androdioecious	Both parasites and free-living forms, dioecious
Onychophora		Dioecious	Terrestrial, tropical
Tardigrada		Dioecious	Aquatic
Euarthropoda			
	Chelicerformes	Dioecious	Pycnogonida, Merostomata, and Arachnida
	Remipedia	Hermaphroditic	Marine
	Cephalocarida	Hermaphroditic	Benthic, marine
	Maxillopoda	Dioecious or Hermaphroditic according to subclade	Copepods, ostracods, etc., dioecious; Cirripedia (barnacles; sessile, largely hermaphroditic)
	Branchiopoda	Largely dioecious, some hermaphroditic (notostracans) and androdioecious (chonchostracan) taxa	Largely freshwater; females often parthenogenetic
	Malacostraca	Mostly dioecious; some sequential and simultaneous hermaphrodites	Shrimps, crabs, and lobsters
	Hexapoda	Dioecious	Insects, 830,075 species
	Myriapoda	Dioecious	Centipedes, millipedes, etc.

(continued)

Table 1.2 (continued)

Phylum			
	Class	Mode(s) of sexuality	Comments
Mesozoa		Hermaphroditic and dioecious	Endoparasites; Rhombozoa are hermaphrodites which may self- or cross-fertilize; orthonectids dioecious
Echinodermata		Largely dioecious	Some hermaphrodites among the asteroids, holothuroids, and especially ophiuroids
Hemichordata		Dioecious	Sessile pterobranchs and free-living enteropneust worms
Chordata			
	Urochordata	Hermaphroditic	Sessile or pelagic in colonies
	Cephalochordata	Dioecious	"Amphioxus"
	Myxinoidea	Dioecious; some functional hermaphrodites	Hagfish; poorly known; highly female-biased sex ratios
	Petromyzontiformes	Dioecious	Lampreys; environmental sex determination
	Chondrichthyes	Dioecious	
	Actinopterygii	Largely dioecious; some sequential and simultaneous hermaphrodites among teleosts	Includes teleosts, sturgeons, gars, and bowfins
	Actiniata	Dioecious	Coelacanth; internal fertilization; live-bearing
	Dipnoi	Dioecious	Lungfishes
	Tetrapoda	Dioecious	Includes amphibians, reptiles, birds, and mammals

[a] Ghiselin (1974) and Anderson and Cribb (1994)
[b] Collin (2013)
[c] Kaulfuss et al. (2013)

more labile in their sexual system, such as the conifers among plants and the Cnidaria and annelids among animals. Assuming that taxa such as phyla and classes are, in general, evolutionarily older than orders and families, these patterns suggest that in many, if not most, cases, sexual systems are very old evolutionarily and therefore have been stable across hundreds of millions of years and a great variety of ecological conditions. For example, the class Hexapoda (insects) of the arthropods is believed to be about 400 million years old (Gaunt and Miles 2002) and is easily the most numerous class of animals in terms of numbers of species, but it is almost exclusively dioecious. In fact, if one includes the insects, the Metazoa are 95%

dioecious, whereas if one excludes the insects, the Metazoa are roughly 30% hermaphroditic (Jarne and Auld 2006). Similarly the phylum Platyhelminthes (13,780 species, Lecointre and Le Guyader 2006) is simultaneously hermaphroditic with very few exceptions (Ghiselin 1974). Sexual systems may be labile or conservative at any taxonomic level. Even in the cases in which the sexual system is labile within classes, sexual system may be quite conservative at the level of order or family [e.g., lysmatid shrimps (Chap. 10) or certain gastropods (Chap. 7)]. In other cases, species within a genus may vary in sexual system, as in the polychaete genus *Ophyrotrocha* (Chap. 5) or *Hydra* (Siebert and Juliano 2017) (see below).

Williams' Paradox (Williams 1975; Leonard 1990, 2013) states that theories of the advantages of hermaphroditism versus dioecy don't adequately explain the distribution of sexual system across the Metazoa. This observation gives rise to two questions: (a) What factors can explain this distribution? (b) Why are sexual systems so often very conservative? Comparative studies of groups that are relatively labile in sexual system may help in identifying the adaptive advantages of particular sexual systems as suggested by Williams (1975). This volume presents reviews of a variety of taxa that are labile in terms of sexual system, and, in general, the results show that sexual systems, in these labile groups, respond evolutionarily to the types of selective pressures that have been identified in theoretical and empirical studies over the last 50 years. The factors responsible for the extreme conservatism of sexual system in other taxa remain unclear at present.

The second major mystery of sexual system evolution concerns the evolutionary paths that lead from dioecy to simultaneous hermaphroditism or the reverse, in metazoan animals. Within angiosperms, there is a well-developed body of theoretical literature which predicts how dioecy evolves from simultaneous hermaphroditism. The usual path involves a situation in which a strong advantage to outcrossing for the producers of eggs favors the spread of genes for male sterility in a hermaphroditic population (Fig. 1.1). This results in a gynodioecious sexual system, and sexual selection favors hermaphrodites that emphasize pollen production at the expense of ovules, leading eventually to dioecy. In dioecious species, females may experience an advantage to some pollen production (perhaps for reproductive assurance) which can put males at a relative disadvantage. Then, selection for more male sex allocation in hermaphrodites in an androdioecious population will lead to stochastic loss of males and ultimately to a hermaphroditic sexual system (Fig. 1.1; Delph and Wolf 2005; Delph 2009; see also Pannell 2002; Wilson and Harder 2003). However, there is also evidence that monoecy, in which an individual plant has separate male and female flowers, may be a pathway from SH to dioecy in many angiosperms (see Chap. 3). In principle, the same arguments should be applicable to metazoans, but as Weeks (2012) pointed out in a very comprehensive review, in reality, neither gynodioecy nor androdioecy appears to be an evolutionary path between dioecy and hermaphroditism in animals [with the possible exception of barnacles (see Chap. 8; Yusa et al. 2013)]. As I have argued previously (Leonard 2013), it may be more useful to think of the evolution of sexual systems in animals as involving a continuum from more to less plasticity in sex allocation as one moves from simultaneous hermaphroditism, where sexual role may

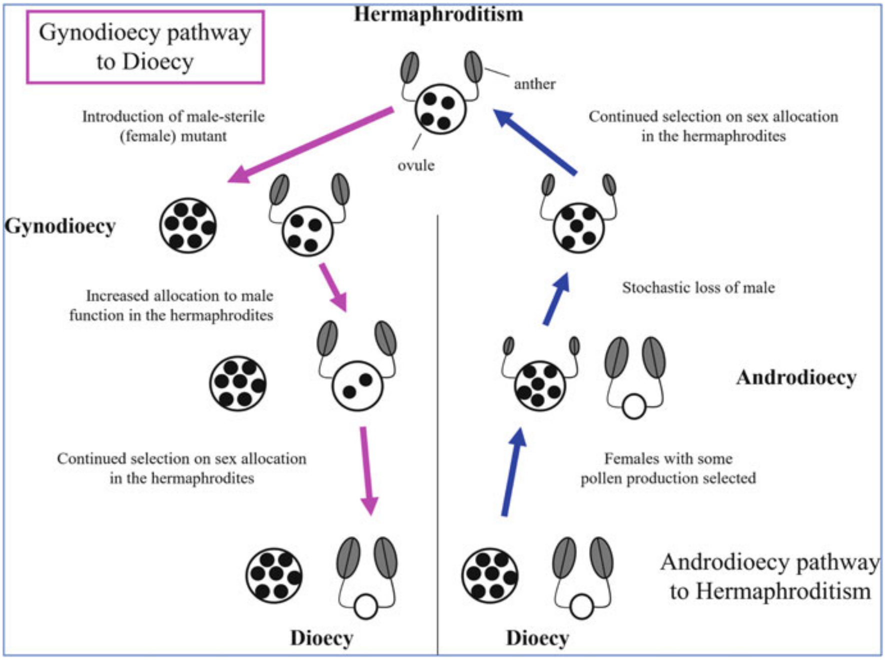

Fig. 1.1 A diagrammatic representation of the evolutionary pathways between dioecy and hermaphroditism in angiosperms (From Delph 2009). The hypothesized pathway from hermaphroditism through gynodioecy to dioecy is shown on the left in pink, and the pathway from dioecy to hermaphroditism through androdioecy is shown on the right in blue

be a purely behavioral choice, to dioecy with genetic sex determination, where sexual role is fixed at fertilization. Here I consider possible evolutionary paths between outcrossing simultaneous hermaphroditism (SH) and dioecy involving transitions through sequential hermaphroditism and/or environmental sex determination (Fig. 1.2). It remains to be seen to what extent these pathways actually are consistent with animal evolution.

1.2 Defining and Identifying Sexual Systems

Sexual systems can be defined in terms of the types of individuals that interact in a mating encounter. For example, Eppley and Jesson (2008) classified sequential hermaphrodites as dioecious for the purpose of their discussion of the evolution of sexual systems since mating interactions involve a functional male and a functional female. However, selection acts on the total reproductive success of individuals, which suggests that sexual systems should be defined in terms of the pattern of gender of genets (genotypic individuals), to use the botanical term. In both plants and animals, the two sexual systems that are strikingly stable evolutionarily, in that

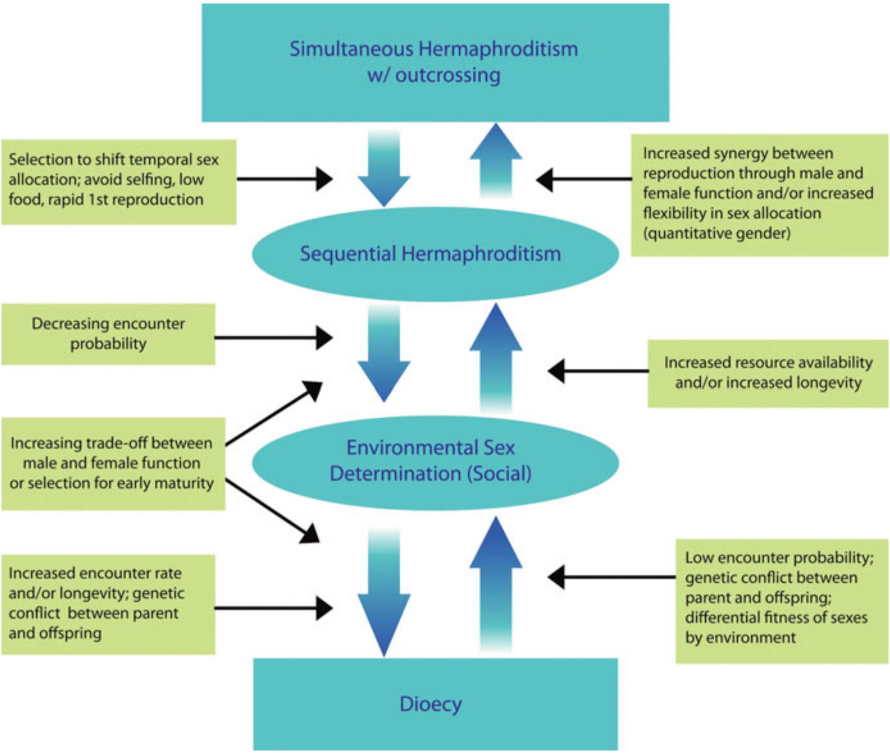

Fig. 1.2 A diagrammatic representation of hypothesized evolutionary pathways from simultaneous hermaphroditism with outcrossing to dioecy and back. The sexual systems are represented in blue with arrows indicating hypothesized directions of change in sexual system; the green boxes show suggested selective pressures that would promote the transition indicated by the black arrows. For the sake of clarity, dioecy, environmental sex determination, sequential hermaphroditism, and simultaneous hermaphroditism with outcrossing are depicted as discrete evolutionary states although in reality, there is a continuum between the sexual systems (see text)

they are conserved in whole phyla and classes, are dioecy (gonochorism) and simultaneous hermaphroditism with outcrossing in both roles. Other sexual systems tend to be both less common and less stable evolutionarily (see below).

1.2.1 Dioecy (Gonochorism)

The definition of dioecy is that it is a sexual system in which individuals reproduce in one sexual role (male or female), only, during their lifetime. It is characteristic of many phyla and classes in both plants and animals (e.g., insects, birds, mammals, reptiles, amphibians, the vast majority of nematodes, three of the four phyla of gymnosperms, etc.). Therefore, dioecy is, evolutionarily, a very stable sexual system, as is outcrossing simultaneous hermaphroditism (see below; Williams 1975). Theoretical studies show that in many cases, dioecy is maintained even though sequential hermaphroditism ought to be the more adaptive sexual system (Kazancioğlu and Alonzo 2009), indicating that there are factors that stabilize dioecy as a sexual system.

1.2.1.1 Genetic Sex-Determining Mechanisms in Dioecy

A wealth of sex-determining mechanisms have been identified in dioecious organisms. In classical genetic sex determination (GSD), the sex of an individual is determined at fertilization and does not change. Mechanisms of GSD include specialized sex chromosomes, genes not localized on sex chromosomes, haplodiploidy, or other phenomena (see below) [for a review of early literature, see White (1948); for more recent work, see Beukeboom and Perrin (2014)]. In addition to the familiar XY and ZW sex chromosome systems identified in many taxa, there are much more exotic types of sex determination by sex chromosomes. Spiders have great diversity among families in the number and arrangement of sex chromosomes (Araujo et al. 2012). While therian mammals use a XY sex determination in which the SRY gene on the Y chromosome is a dominant determinant of male sex, platypus and echidna lack evidence of an SRY gene and have multiple X and Y chromosomes, and there is evidence for homology with the DMRT1 gene found on the Z chromosome of birds (Rens et al. 2007; Veyrunes et al. 2008). In birds, males are ZZ and females are ZW. Infection with a variety of organisms, such as the bacterium *Wolbachia* (Werren et al. 1986; review in Beukeboom and Perrin 2014), can also influence genetic sex determination. There is a unique genetic sex determination system in bivalves, called doubly uniparental inheritance (DUI), in which females inherit mitochondria from their mothers, whereas males inherit mitochondria from their fathers, and this determines sex (Chap. 6; review in Zouros 2013). Interestingly, in freshwater mussels, DUI is prevalent in the dioecious species which predominate in the group but is not found in the few hermaphroditic species of freshwater mussel (Breton et al. 2011). Collin (Chap. 7) discusses various genetic sex-determining mechanisms in caenogastropods. The sex of an individual can be determined by either genetic or environmental factors or a combination of the two (Eggers and Sinclair 2012; Ono and Harley 2013; Bachtrog et al. 2014; Kuijper and Pen 2014; see also Chaps. 6, 11, and 12). Recent work has shown that sex-determining mechanisms have evolved relatively quickly in many groups (see Chaps. 4, 6, 11, and 12; Beukeboom and Perrin 2014 for review). It has been suggested that maternal-offspring conflict can lead to rapid evolution of genetic sex-determining mechanisms, including sex chromosomes (Werren et al. 2002). Within angiosperms, dioecy has evolved many times (Bawa 1980) and may involve either genetic sex determination (including sex chromosomes) or environmental sex determination. The interaction of the two may be complex (Diggle et al. 2011).

1.2.1.2 Environmental Sex Determination

Environmental sex determination (ESD) and genetic sex determination (GSD) can interact (Holleley et al. 2015) (see below). For example, in flounder, XY individuals are males, and XX individuals are female unless masculinized by extreme temperatures or other stressors (Mankiewicz et al. 2013). One may, in fact, think of GSD and ESD as ends of a continuum rather than discrete phenomena (see Kraak and Pen 2002 for discussion). In classic ESD, the genotype is capable of becoming either male or female until sexual differentiation is triggered, before first reproduction, by a factor such as social environment, or environmental temperature (Bull and Vogt 1979; Korpelainen 1990; Mankiewicz et al. 2013; see Chap. 12), i.e., sex is phenotypically plastic during early development. The Charnov-Bull (1977) model of the evolution of sex determination argues that it is adaptive when the environment during development is variable and one sex fares better in one type of environment and the other sex does better in a different environment. The molecular pathways involved in ESD have been extensively studied in turtles (see Chap. 12). In teleosts, estrogen signaling pathways have been implicated in sexual differentiation with ESD (Chap. 11). In addition to social cues and temperature, other factors such as photoperiod (e.g., a gammarid amphipod; Bulnheim 1967), settling substrate, food availability, and growth rate may also be involved (see below and discussion for bivalves in Chap. 6). An experimental study in sea lamprey showed an increased skew to males in a cohort exposed to a low-productivity environment, whereas there was an increased percentage of females in a group of individuals placed into a high-productivity environment (Johnson et al. 2017). It was hypothesized that the relevant variable was larval growth rate. A similar phenomenon was observed in the laboratory in Midas cichlids in which the relative size of juveniles in a group was shown to determine sex, independent of genetic factors (Francis and Barlow 1993). In that case it was hypothesized that social interactions rather than growth rate *per se* were the determining factor in sex determination. These authors emphasized the connection between ESD in this dioecious species and the size-advantage hypothesis (SAH) for sequential hermaphroditism (Ghiselin 1969; Munday et al. 2006a; Warner 1975). The term "conditional sex expression" has been used to emphasize the close association between ESD and sequential hermaphroditism (Frank and Swingland 1988; see below).

In dioecious barnacles, sex may be genetically determined with even the larvae being dimorphic, or sex may be determined by the substrate of settlement with larvae settling on an uninfected host becoming female and larvae settling on a host previously infected by a barnacle becoming (dwarf) males (Yamaguchi et al. 2014). The probable duration of the substrate may also have an effect on sex determination in barnacles (Ewers-Saucedo et al. 2015). Sex is also apparently environmentally determined in other taxa with dwarf males, such as at least some species of the siboglinid genus *Osedax* (Rouse et al. 2008; Vrijenhoek et al. 2008; Miyamoto et al. 2013) and echiuran annelids of the families Bonellidae and Ikedidae (Baltzer 1926; Jaccarini et al. 1983; Berec et al. 2005; Goto et al. 2013). Although there is some evidence of a role for genetic sex determination in both *Osedax* and *Bonellia* (see discussion in Rouse et al. 2015), Berec et al. (2005) argue that once ESD with dwarf males has evolved, it should be resistant to invasion by genetic sex determination. [Reversed sexual size dimorphism in spiders has also resulted in dwarf males in some taxa, although spiders seem to have chromosomal sex determination (see Araujo et al. 2012).] In spore plants, over 50% of bryophyte species may be dioecious at the gametophyte stage (McDaniel et al. 2012; Perley and Jesson 2015) although the genet is presumed to be hermaphroditic in all cases (see Chap. 2). Similarly, ESD determines sex in ferns that are dioecious in the gametophyte stage, with the relevant cue being a pheromone associated with population density (Atallah and Banks 2015; Goodnoe et al. 2016).

There are cases in which it is difficult to distinguish between dioecy and sequential hermaphroditism. In the polychaete *Grubea clavata*, Hauenschild (1953) demonstrated, in laboratory experiments, that initial sexual development resulted in either male or female worms and that male worms remained male throughout their lifetime, whereas after the initial egg-laying, a proportion of female worms regenerated the gonads as testes and then remained male throughout their lives. Other female individuals regenerated the gonads as ovaries after an initial egg-laying and laid eggs a second time. In the laboratory, the process repeated with a proportion of females changing to males after each egg-laying and remaining male, while other individuals regenerated ovaries and laid eggs again. Experimental interventions such as amputations, reduction in nutrition, and increased water temperature tended to increase the percentage of females becoming males, whereas only one (out of hundreds) of individuals, a very young male, was ever seen to change to female. Hauenschild concluded that young worms became female and remained female only under favorable conditions, whereas any loss of physical condition or deterioration of the environment led to a change to male. He reported similar phenomena in the related polychaete, *Exogone gemmifera*. Interestingly, in these polychaetes, maleness represents a type of “physiological sink” that individuals may fall into under unfavorable conditions or when the energy loss associated with egg-laying is sufficiently great (Hauenschild 1953; see also Franke 1986 for discussion of similar observations in other syllids). Breeding experiments indicated that while there was no obvious genetic sex determination (Hauenschild 1953), secondary males (those that started life as females) produce more daughters than do primary males (those who started reproduction as males) (Hauenschild 1959). Similar phenomena where sex depends on size and condition have been described in plants, but sex change can usually be in either direction (Heslop-Harrison 1957; Bierzychudek 1982, etc.; see below). In summary, there is neither a clear boundary between dioecy with genetic sex determination and dioecy with ESD nor a clear line between ESD and sequential hermaphroditism (see below).

1.2.2 Simultaneous Hermaphroditism

Simultaneous hermaphroditism (SH) is defined as a sexual system in which individuals reproduce through both sperm and eggs in the same breeding season, although not necessarily in the same sexual encounter. Simultaneous hermaphrodites may or may not be capable of self-fertilization. In fact, SH with, and without, outcrossing should, in principle, have rather different evolutionary consequences, and it is in taxa that show outcrossing that SH is stable across whole phyla and classes. It is important, therefore, to distinguish between species that have obligate selfing and species in which outcrossing occurs between simultaneous hermaphrodites. However, information as to the occurrence of selfing is often not available, and in some taxa such as the pulmonate gastropods, populations within a species may vary in the presence, absence, or frequency of selfing (Jarne and Charlesworth 1993; Jarne and Auld 2006; Baur 2010; Jarne et al. 2010). It is important to remember that the diversity found in nature will seldom be well captured by our terminology.

1.2.2.1 Obligately Selfing Simultaneous Hermaphroditism

In this sexual system, individuals produce both eggs and sperm and reproduce through self-fertilization exclusively. This sexual system has evolved many times in both plants and animals but is not characteristic of any major taxon and may be short-lived evolutionarily (see Darwin 1858; Weeks et al. 2006, 2009; Zierold et al. 2007; Denver et al. 2011; Weeks 2012; Chap. 4). Genetic analysis of progeny may be required to identify obligate selfing, which should lead to high levels of homozygosity. Obligately selfing individuals should show a reduced level of sperm production relative to outcrossing taxa since there will be no sperm competition and efficient mechanisms of fertilization. Reduced allocation to sperm has been demonstrated in populations of a freshwater mussel with high rates of selfing (Johnston et al. 1998), in a manner similar to that seen in angiosperms. Furthermore, in obligately selfing taxa, sperm and eggs should mature at approximately the same time, i.e., one would not expect a prolonged protandrous or protogynous period of development before maturation as in many simultaneous hermaphrodites. Also, the evidence suggests that obligately selfing hermaphroditic lineages are short-lived evolutionarily [for animals, see above (Zierold et al. 2007; Weeks et al. 2009; Chap. 4)]. Darwin (1858, p. 462) quoted a doctrine of Andrew Knight "that no plant self-fertilizes itself for a perpetuity of generations" and concluded "I am strongly inclined to believe that this is a law of nature throughout the vegetable and animal kingdoms." Ghiselin (1974) dubbed this the Knight-Darwin law, and it still seems valid, although the question of how many generations constitute "a perpetuity" remains open. Modern genetic work supports this conclusion: there is evidence of a 20–40% reduction in genome size in androdioecious *Caenorhabditis* species of nematodes, in which most reproduction is through selfing, suggesting that self-fertilization may lead to a rapid and substantial loss of genetic variance

(Fierst et al. 2015) as has been shown in angiosperms (Wright et al. 2008). It seems probable that this loss of genes may be associated with the short-lived quality of obligately selfing species.

1.2.2.2 Outcrossing Simultaneous Hermaphroditism

Again, individuals are capable of reproducing in both sexual roles (male and female) during a single reproductive season. However, in outcrossing simultaneous hermaphroditism, individuals are capable of mating with another individual in both the male and female sexual role. This mating system is characteristic of many large taxa (phyla and classes) in both plants and animals: most angiosperms, the genets of all spore plants, as far as is known (see Chap. 2; Table 1.1), heterobranch gastropods, barnacles, platyhelminthes, urochordates, clitellate annelids, etc. (Table 1.2). Some outcrossing simultaneous hermaphrodites are also capable of self-fertilization [e.g., some cestodes (Milinski 2006), many pulmonates (Baur 2010; Jarne et al. 2010), the ctenophore *Mnemiopsis leidyi* (Sasson and Ryan 2016), many angiosperms, etc.]. Features of outcrossing SH that are not found in obligately selfing SH include sex allocation that varies with the physical and biological (particularly social) environment. That is, hermaphrodites that are mating with other hermaphrodites may vary their investment in sperm versus eggs according to their size, food availability, availability of mates, social status, etc. What has been termed adolescent protandry or protogyny, in which individuals first reproduce in one sexual role and then mature into SH, is often observed in outcrossing SH (e.g., Dunn 1975a, b; Bauer 2006; di Bona et al. 2014; Chaps. 5 and 10).

Changes in sex allocation with size, age, social environment, and physical conditions occur frequently in outcrossing SH and have been termed "quantitative gender" (Lloyd 1982; Klinkhamer et al. 1997; Cadet et al. 2004). It has been suggested that in most cases of a size/age advantage of one sexual role over the other, shifting sex allocation with size/age can give the same adaptive advantage as an actual sex change. Klinkhamer and de Jong (2002) argued that there are two types of benefits of size: direct effects and budget effects. Budget effects are effects of size on reproductive success in one sexual role that are purely a product of the amount of resources available to invest. The fitness obtained for a given investment of resources will be the same for small and large individuals, but large individuals will have more resources to invest. In such cases a gradual change of sex allocation with size will be expected (Klinkhamer and deJong 2002). In contrast, direct effects are benefits to reproductive success in one sexual role that are associated with size *per se*; e.g., a taller wind-pollinated plant (or sessile broadcast-spawning invertebrate) will be able to disperse pollen/sperm over a wider area purely due to its height, thereby increasing its siring success. Therefore, in such species, large individuals should allocate resources preferentially to male function, becoming male; conversely since small plants will experience little siring success, they should become female. Similarly, in group-living fishes, if large individuals can dominate mating in a social group, large individuals should become male, making it profitable for small individuals to be female. If gain curves are linear, a sudden change of sex will be favored.

Also, in some species, individuals may act in only one sexual role although they have both testes and ovaries that are apparently functional (St. Mary 1993; Sadovy de Mitcheson and Liu 2008; di Bona et al. 2010; see also Chap. 5), indicating that histological and/or anatomical sex may not be a reliable guide to functional sex. In some cases, one of the types of gonads may not be fully functional [e.g., gobies (Cole and Hoese 2001) or spirochid trematodes (Anderson and Cribb 1994; Platt and Blair 1996)]. In bufonid toads, the testes of mature males are capped by Bidder's organ, which contains maturing oocytes (Farias et al. 2002), but there are apparently no reports of self-fertilization or functional hermaphroditism [personal communication, Marvalee H. Wake; but see Grafe and Linsenmair (1989)]. However, both sex change (see below) and hermaphroditism are easily induced experimentally (review in Wallace et al. 1999), so there would seem to be potential for selection to produce either sequential or simultaneous hermaphroditism. However, since amphibians are very susceptible to endocrine disrupters (e.g., Moresco et al. 2014), it is often hard to interpret reports of gonads with mixed gametes.

There are also cases intermediate between simultaneous and sequential hermaphroditism in which individuals change sex within a breeding season, sometimes repeatedly. For example, oysters of the genus *Ostrea*, which brood their zygotes, produce a clutch of eggs, then become male, and reproduce through sperm until the brood has hatched, when the gonad again becomes an ovary and produces eggs (Coe 1932; Chaparro and Thompson 1998; see also Chap. 6). Rapid alternation of sex within a breeding season has also been reported in a polychaete, *Ophryotrocha puerilis*, in which members of a pair change sexual role so that the larger individual is female, laying more eggs and growing more slowly. As the two individuals become more equal in size and changes become more frequent, the pair may ultimately both become simultaneous hermaphrodites (Berglund 1986). Such examples of alternating sex have traditionally been seen as sequential hermaphroditism, demonstrating the difficulty of fitting specific sexual systems into any overall scheme of definitions.

1.2.3 Sequential Hermaphroditism

In the classic definition of sequential hermaphroditism, individuals reproduce in distinct sexual roles during different breeding seasons of their life histories. This may involve a single change in sex, or it may involve more than one change from male to female or the reverse depending on environmental, physiological, or social variables (Leonard 2013). As stated above, this creates a gray area between simultaneous and sequential hermaphroditism (see also Klinkhamer et al. 1997; Cadet et al. 2004). Sequential hermaphroditism is not characteristic of phyla or classes but is characteristic of many families of teleost fishes (Erisman et al. 2013) and has evolved several times in polychaetes (Chap. 5), gastropods (see Collin 2013; Chap. 7), and some groups of shrimps (see Chap. 10). It is a relatively rare sexual system

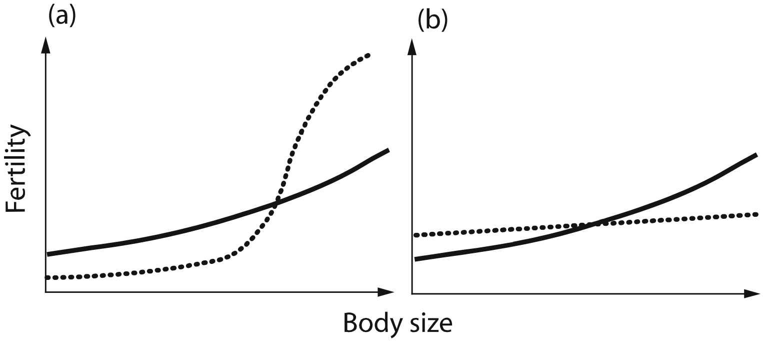

Fig. 1.3 The size-advantage model of sequential hermaphroditism (From Leonard 2013, based on Fig. 1 in Munday et al. 2006a). The offspring production expected for females (solid line) increases with body size if large females lay more eggs than small females. Expected male offspring production (dotted line) may or may not increase with body size, depending on whether large males have an advantage in securing mates. Sex change is favored when the size/age fertility curves of the two sexes cross. Protogyny (**a**) is predicted when the expected fertility of a male increases more rapidly with size/age than that of a female. Protandry (**b**) is predicted when the expected fertility of a female increases more rapidly with size than that of a male

(Kazancioğlu and Alonzo 2009; see also Ghiselin 1974; Policansky 1982, for review). Relatively little is known about the genetic correlates of sequential hermaphroditism. In the Pacific oyster *Crassostrea gigas*, in which populations show dioecy, protandric sequential hermaphroditism, and occasional simultaneous hermaphroditism, a genetic basis for sex change has been proposed with an interaction between a dominant male allele M and a protandric allele F, such that MF individuals are lifelong males and FF individuals are protandric sex changers (Guo et al. 1998; Zhang et al. 2014). Godwin and Roberts (Chap. 11) discuss the role of estrogen signaling pathways in sex change in teleosts. Although amphibians have chromosomal sex determination, as far as is known (Wallace et al. 1999), there is one report of protogynous sequential hermaphroditism in a laboratory population of a frog (Grafe and Linsenmair 1989). In angiosperms, sequential hermaphroditism is rarer, but it has evolved many times (Freeman et al. 1980; Charnov 1982) and involves very similar phenomena (Vega-Frutis et al. 2014). A large number of factors have been implicated as triggers for sex change in angiosperms, including light intensity, plant age, plant size, temperature, injury, disease, nutrients, etc. (Freeman et al. 1980).

The size-advantage hypothesis (SAH), first developed by Ghiselin (1969), states that sequential hermaphroditism will be adaptive when reproductive success (or more precisely, reproductive value; Warner 1988) increases more steeply with increasing size for one sex than the other (Fig. 1.3). In the well-known cases of protogyny in group-living fishes, individuals mature first as females, and then individuals that are able to grow large and socially dominant change sex to become male and defend spawning sites and/or harems (Fig. 1.3a; Warner 1984a, 1988; Munday et al. 2006a). This may occur even though individuals incur substantial costs in deferred current reproduction and growth, because of the substantial increase in reproductive value associated with being a dominant male (Warner 1984b). In labroids of the genus *Symphodus*, sex change is associated with the ability of large males to dominate mating, whereas in species with substantial paternal care, sex change does not occur (Warner and Lejeune 1985). In protandry, which is the common form of sequential hermaphroditism in many fish and invertebrates, individuals mature first as males, producing sperm and growing until they reach a point at which their reproductive value would be higher as a female, since they have attained a body size where they can produce more eggs than they could find to fertilize as males (Fig. 1.3b). Forty years of empirical and theoretical research have demonstrated that other factors can also explain sequential hermaphroditism (Munday et al. 2006a; Collin 2013). For example, both differential mortality and differential growth rate may be sufficient to provide an adaptive advantage for sex change (through an effect on reproductive value) even though size *per se* may not have a differential effect on reproductive success for the two sexual roles (Iwasa 1991). Levels of sperm competition (Muη̃oz and Warner 2004) and nutritional status (Yamaguchi et al. 2013) have also been shown to be important in determining whether an individual's reproductive value will be enhanced by sex change at a particular size in fishes. Thus, the decision of an individual to change sex may depend on a variety of proximate cues that reflect the environment of the individual and social group. An alternative hypothesis that size at sex change is usually invariant for a species (Charnov and Skúladóttir 2000; Allsop and West 2003) seems inconsistent with the evidence in most cases [(Buston et al. 2004; Cipriani and Collin 2005); see discussion in (Munday et al. 2006a)].

One well-studied system in angiosperms that is very consistent with the size-advantage hypothesis, which has been so important in animals [(Ghiselin 1969, 1974; Munday et al. 2006a); see below], is a perennial forest herb, the Jack-in-the Pulpit (*Arisaema triphyllum*). In this species, sex is associated with size: small individuals are immature, larger individuals are male, and the largest individuals are female (Policansky 1981; Bierzychudek 1982). Both genetic and environmental factors may influence the actual size at which individuals change sex in a given population (Viti et al. 2003). Therefore, *A. triphyllum* is a protandrous sex changer; i.e., it matures first as a male, producing pollen, and then, at a later time and larger size, becomes female, receiving pollen and producing seeds. Protogyny is the opposite pattern in which an individual first matures as a female and then becomes male. {N.B.: The terms protogyny and protandry are, unfortunately, used rather loosely in the literature. For example, many authors refer to protandry in heterobranch gastropods, by which they mean that in many species of this simultaneously hermaphroditic clade, sperm will mature before eggs in an individual, even though copulation may be reciprocal and the allosperm stored until the recipient matures eggs [e.g., the pteropod *Limacina* has been described as having copulation that is simultaneously reciprocal between mature males (Lalli and Wells 1978)]. In molluscs, this usage dates back at least to Pelseneer (1895) and is unlikely to be eradicated in the foreseeable future. Similar issues arise in other taxa.}

While sequential hermaphroditism has been dealt with, theoretically, as a discrete phenomenon [the SAH does not consider changing sex allocation in hermaphrodites (Henshaw 2018)], in the empirical data, the boundaries between sequential hermaphroditism and simultaneous hermaphroditism, on the one hand, and dioecy, on the other, are unclear. There are instances in which individuals may change sex more than once in their lives in both plants (Heslop-Harrison 1957; Freeman et al. 1980) and animals. In some plants, individuals may change sex each season depending on their current physical size, nutritional condition, or environment (Heslop-Harrison 1957). Some fish may change from female to male and back to female, or the reverse, if they lose a mate (Nakashima et al. 1996; Sadovy de Mitcheson and Liu 2008; Kuwamura et al. 2011; Sawada et al. 2017). Transcriptome analysis indicates that sex steroid pathways, including the aromatase gene, are involved in sex change in an anemone fish with bidirectional sex change (Casas et al. 2016). As discussed above, *Ostrea* oysters start reproduction as females, but the gonad converts to a testis when larvae are being brooded in the brood chamber, and once the larvae are released, the testis converts back to an ovary (Coe 1932; Chaparro and Thompson 1998), and there is a polychaete in which a pair of individuals alternate changing sex until the size difference and interval between sex changes become very short and the pair both become simultaneous hermaphrodites (Berglund 1986). In some mushroom stony corals, individuals may change sex between each spawning episode, correlated with consecutive full moons (Loya and Sakai 2008), although not all do. In one sabellid polychaete, *Bispira brunnea*, protandrous sequential hermaphroditism has been invoked to explain a pattern of populations consisting of males, females, and apparently functional simultaneous hermaphrodites, although we usually think of the transition from male to female in sequential hermaphrodites as being too rapid to involve a functional simultaneously hermaphroditic intermediate (Davila-Jimenez et al. 2017). Another possible explanation would be trioecy (see below). As is often the case, the species is not sufficiently well understood to allow us to distinguish between the hypotheses, although sequential hermaphroditism is well known in polychaetes (Chap. 5), whereas trioecy is not. More typically in sequential hermaphroditism, [e.g., the bivalve *Arca noae* (Bello et al. 2013)] during the intermediate phase with both types of gonads present, they are nonfunctional. In these cases, the line between simultaneous and sequential hermaphroditism is hard to draw.

The line between dioecy and sequential hermaphroditism is also rather fuzzy in many cases. In angiosperms, individuals of “dioecious” species may change sex in response to a variety of environmental variables (Heslop-Harrison 1957), indicating an overlap between dioecy with environmental sex determination (ESD) and sequential hermaphroditism. For example, shade is associated with maleness in a dioecious orchid, with females located in sunny spots, and if part of a female inflorescence is shaded, experimentally, it will become male (Zimmerman 1991). In animals, this has been less studied, but in cases such as the bluehead wrasse, which is the classic example of a protogynous sex changer, it has been found that whether individuals begin life as a female or develop directly into a male without a female stage depends on the social environment in which they are reared (Munday et al. 2006b). That is, the social environment determines whether an individual becomes a protogynous sex changer or a pure male, an example of ESD. In carid shrimp there are species in which populations consist of protandrous hermaphrodites along with either primary males or primary females, i.e., individuals that do not change sex during their lives (see Chap. 10), although whether phenotypic plasticity is involved is not clear. Also there is the case of polychaetes in which females, but not males, may change sex over the course of their reproductive lives [see above (Hauenschild 1953)]. There seems to be a continuum in terms of the phenotypic plasticity associated with ESD and the phenotypic plasticity associated with sequential hermaphroditism in some organisms [i.e., conditional sex expression (Frank and Swingland 1988); in fish, estrogenic signaling pathways seem involved in both (Chap. 11)].

It has been suggested that sequential hermaphroditism is rare relative to simultaneous hermaphroditism because many of the advantages that accrue to a particular sexual role as a result of size/age can be obtained by a gradual shift of sex allocation from one sexual role to the other over the course of the life of a simultaneous hermaphrodite (Cadet et al. 2004; Klinkhamer et al. 1997; Klinkhamer and deJong 2002). This concept of "quantitative gender" (see also Lloyd 1982) suggests that a complete sex change will only be advantageous when one sex has a "direct" advantage at a large size. A direct effect is one in which the advantage is due to large size *per se* and not just a greater availability of resources as a result of large size. Examples include advantages to male function of height in trees and advantages through social dominance and ability to sequester mates by large males in some fishes (see above).

1.2.4 Androdioecy

In androdioecy, breeding populations consist of a mixture of males and simultaneous hermaphrodites. Where two sexual morphs are determined genetically, their fitness must be equal for the sexual system to be evolutionarily stable, i.e., a stable polymorphism. This is the case for the two sexes in dioecy and for genetically determined androdioecy (or gynodioecy or trioecy; see below). To be stable, the single sex and hermaphroditic genotypes must also have equal fitness. On the other hand, where the sexual morph is determined facultatively, i.e., as a phenotypic response to environmental conditions, or as a developmental stage (e.g., protandrous or protogynous simultaneous hermaphroditism), the two morphs do not have to have equal fitness (see Chap. 9 for discussion). This distinction is important for understanding the evolution of the phenomenon, but it is often difficult to distinguish between the two types of sex determination in empirical data.

Androdioecy is relatively rare in angiosperms but does occur and may offer an evolutionary path from dioecy to simultaneous hermaphroditism (Pannell 2002, Chap. 3; Delph and Wolf 2005; Pannell et al. 2008; Delph 2009). This has been well studied in *Mercurialis annua*, in which populations may vary in sexual system (Pannell et al. 2008). Sex determination in hermaphroditic and dioecious *M. annua* has a genetic component, but the details remain unclear (Russell and Pannell 2015).

In metazoan animals, androdioecy has evolved many times but appears to be restricted taxonomically (Weeks 2012). The most common form of androdioecy in animals involves hermaphrodites that either self-fertilize or outcross through eggs, with males, but do not outcross through sperm (Weeks 2012). It typically occurs in strongly dioecious groups, where outcrossing through male function would require elaborate genitalia and/or behaviors (Weeks 2012). This type of androdioecy is found in a few nematodes, with the best-known example being *Caenorhabditis elegans* (Denver et al. 2011; see Chap. 4), one fish (Harrington 1971; Lomax et al. 2017), and two families of branchiopod crustaceans (Weeks et al. 2009; Zierold et al. 2007). It seems to be derived from dioecious ancestors and is often genetically mediated (Weeks et al. 2010; Mathers et al. 2015; Chap. 4). Some authors (Pannell 2002) restrict the term androdioecy to cases in which there is a genetic distinction between males and hermaphrodites as is usually the case in androdioecy derived from dioecious taxa (see Weeks 2012; Chaps. 3 and 4). Pannell (2002) argued that androdioecy generally evolves from dioecy as a means of reproductive assurance.

Another type of androdioecy does involve outcrossing by hermaphrodites. This is found in some angiosperms (see Chap. 3) where it serves as path from dioecy to SH and, with dwarf males, many barnacles (Yusa et al. 2013; Chap. 8). It has been assumed that in barnacles, hermaphrodites and dwarf males are genetically distinct. In fact, larval sizes are sexually dimorphic in some rhizocephalan barnacles (Yamaguchi et al. 2014) suggesting genetic sex determination, but recent evidence suggests that males may develop into hermaphrodites under the right conditions (Yusa et al. 2013; Chap. 9). In barnacles, androdioecy is often a path between simultaneous hermaphroditism and dioecy (Sawada et al. 2015). The development of males may be associated with small mating groups and high mortality on some host substrates (Ewers-Saucedo et al. 2015; see discussion in Chap. 9). Collin (2013) discussed the presence of dwarf males along with simultaneous hermaphrodites in molluscs, a phenomenon that is much less well understood.

As a third type of androdioecy, there are a variety of taxa in which a mixture of males and simultaneous hermaphrodites exists in the population as a matter of developmental or conditional sex allocation. For example, in some of the serranine fish (e.g., *Serranus psittacinus*, formerly *S. fasciatus* and *S. baldwini*), small individuals are simultaneous hermaphrodites, and under conditions of high density, large individuals become pure males and defend harems of smaller hermaphrodites. At lower densities, the population may consist of isolated pairs of simultaneous hermaphrodites (Hastings and Petersen 1986; Fischer and Petersen 1986; Petersen 1990; see reviews in Leonard 1993; Petersen 2006; Erisman et al. 2013). Similarly, so-called protandric simultaneous hermaphroditism, in which individuals mature first as males, and may mate as males, occurs in *Lysmata* shrimps (Bauer 2006; Chap. 10) and in some annelids (Chap. 5). A possible case of developmental androdioecy has been found in spirochid trematodes of the genus *Uterotrema* (Platt and Blair 1996). Where the line between androdioecy and simultaneous hermaphroditism lies is not always entirely clear. Androdioecy can represent a matter of extreme sex allocation in a basically simultaneously hermaphroditic species, such as in some serranids (see above). On the other hand, androdioecy can involve a basically dioecious taxon such as rhabditid nematodes, achieving reproductive assurance in that genetic females produce a few sperm for self-fertilization, as in *C. elegans* (see Chap. 4). The conditions that allow for stable androdioecy in barnacles are explored by Yamaguchi (Chap. 9) and include ESD, allowing androdioecy to exist as a contingent strategy.

1.2.5 Gynodioecy

In angiosperms, this sexual system, in which populations are made up of a mixture of females and simultaneous hermaphrodites, has evolved many times and seems to represent part of a transition from simultaneous hermaphroditism to dioecy (see Charlesworth 1999; Delph and Wolf 2005; Delph 2009, Chap. 3, for discussion). In angiosperms, a male-sterile mutation seems to be involved (Charlesworth 1999; Chap. 3). In the aquatic angiosperm *Sagittaria latifolia*, females were found to have more than twice the seed production of hermaphrodites, as predicted by the Delph model (Fig. 1.1), in low fertilizer conditions, but not in the presence of high levels of fertilizer (Dorken and Mitchard 2008). In animals, gynodioecy is extremely rare (review in Leonard 2010; Weeks 2012). Weeks (2012) reported gynodioecy in two sponges (Gatenby 1920; Fell 1975 cited in Weeks 2012), a few species of cnidarians (Dunn 1975a; Chornesky and Peters 1987), a sipunculid (Gibbs 1975 cited in Weeks 2012), a polychaete (Gregory 1905), a hagfish (Powell et al. 2005), and a teleost (Cole and Hoese 2001). In all of these cases, the difference between females and hermaphrodites seems to be a matter of developmental stage (i.e., protogynous simultaneous hermaphroditism), rather than genotype, with the exceptions of the coral *Porites* (Chornesky and Peters 1987), the sponge *Grantia* (Gatenby 1920), and possibly the hagfish *Myxine glutinosa* (Powell et al. 2005). A related phenomenon is "pseudo-gynodioecy" in the coral *Galaxea fascicularis* in which the population studied on the Great Barrier Reef consisted of females and hermaphrodites; however, the "eggs" produced by hermaphrodites were infertile, making the population effectively dioecious (Harrison 1988). This "pseudo-gynodioecy" implies that gynodioecy has evolved in the group and a gynodioecious population of the species has been found in Taiwan (Keshavmurthy et al. 2012). In both, females produce red eggs, whereas hermaphrodites produce white, buoyant "eggs" which were found to be infertile on the Great Barrier Reef and fertile in Taiwan. These observations imply that gynodioecy in this species may be part of a transition from simultaneous hermaphroditism to dioecy, as in angiosperms. *G. fascicularis* may therefore also represent a case in which gynodioecy is not a matter of a developmental transition, as it is in the best-studied case, the sea anemone *Epiactis prolifera*. In the latter, small individuals are female, and larger individuals become hermaphrodites (Dunn 1975a). This appears to be a case of protogynous simultaneous hermaphroditism even though a few individuals may remain female (Dunn 1975b). As with androdioecy, drawing a hard line between "true" gynodioecy with distinct, genetically determined morphs, and cases in which simultaneous hermaphroditism and a pure female state are developmental stages of the same individual, is not always straightforward. Most reports come from histological observations of field collected individuals where little is known about the whole life history.

1.2.6 Trioecy

This sexual system involves populations consisting of a mixture of males, females, and simultaneous hermaphrodites. It is always very rare but has been found in both plants and animals, e.g., papaya and a couple of other angiosperms (Aryal and Ming 2014; Renner 2014), a few nematodes (see Chaudhuri et al. 2015; Kanzaki et al. 2017; Chap. 4), two sea anemones (Jennison 1981; Armoza-Zvuloni et al. 2014), and a species of *Hydra* (Kaliszewicz 2011). In papaya, sex is chromosomally determined with females being XX and males and hermaphrodites carrying two different types of Y, Y and Y^H, respectively (Aryal and Ming 2014). In *Rhabditis* nematodes (Chaudhuri et al. 2015; Kanzaki et al. 2017), an interaction of genetic and environmental sex determination seems to be involved; sex is genetically determined with males being XY and females XX; if XX larvae are exposed to environmental conditions that produce a dauerlarva, they become simultaneous hermaphrodites as adults. Therefore, ESD and genetic sex determination interact in these examples of trioecy.

1.2.7 An Array of Sexual Systems

In reviewing the sexual systems of extant organisms, one immediately sees that they are both diverse and characterized by a gradient of phenotypic plasticity (Leonard 2013). At the one extreme are taxa with dioecy and genetic sex determination in which the sex of an individual is determined at fertilization and does not change; mammals, birds, and many insects fall into this category. At the other extreme are simultaneous hermaphrodites which may make a moment to moment decision whether to reproduce as a male or female and may be continuously adjusting their sex allocation, ranging from coral reef fishes, snails, barnacles, polychaetes, many corals, etc. In between, one finds dioecious species with ESD in which sex is determined by the environment but at a very young age and irreversibly (e.g., turtles and some other reptiles; see Chap. 12) and other species in which ESD may not be irreversible (many plants, some fish, bivalves, polychaetes, etc.; see above). Also there are species which alternate between asexual and sexual reproduction, in which sexual reproduction is a phenotypically plastic response to an environmental cue and both males and females are produced parthenogenetically from a single individual [i.e., aphids (Dixon 1973), some cladocerans (Walsh 2013), some nematodes (Denver et al. 2011)]. In these cases, it may be possible for a male and female offspring of the same individual to mate, creating a situation of self-fertilization. Androdioecy, gynodioecy, and trioecy also may involve phenotypic plasticity, with many examples involving developmental stages. For example, both *Ophyrotrocha* polychaetes (Chap. 5) and *Lysmata* shrimps (Bauer 2006; Chap. 10) have mating systems involving individuals that are purely male and others that are simultaneous hermaphrodites, but the systems are cases of protandric simultaneous hermaphroditism. Conversely, the best-studied case of gynodioecy, the sea anemone *Epiactis prolifera* (Dunn 1975a, b), also represents a shift in sex allocation with growth, i.e., protogynous simultaneous hermaphroditism. Angiosperms with either gynodioecy or androdioecy may also shift their sex allocation in response to the environment (see Chap. 3). The more we learn about sexual systems in diverse taxa, the more it becomes apparent that phenotypic plasticity plays a large role in the reproductive success of most organisms and that there are no hard and fast lines between sexual systems (see Leonard 2013 for review). However, it remains the case that dioecy and simultaneous hermaphroditism with outcrossing are both often extremely resistant to evolutionary change.

1.3 Williams' Paradox

A major stumbling block to understanding the evolution of sexual systems is understanding their current distribution among organisms. George C. Williams (1975), in his landmark book *Sex and Evolution*, pointed out that the prevailing theories of the benefits of hermaphroditism versus dioecy suggested that sexual system should be very sensitive to ecological parameters particularly the probability of encountering mates, whereas the empirical data demonstrate that the best predictors of sexual system, particularly in the Metazoa but also in some land plants, are phylum and class (Tables 1.1 and 1.2). That is, although theory predicts that simultaneous hermaphroditism should be associated with situations where encounter probability is low, such as parasitism, whereas dioecy should be associated with the ability to search for mates; nematodes, whether free-living or parasitic, are dioecious (with very few exceptions), whereas platyhelminths, whether free living or parasitic, are simultaneous hermaphrodites (with few exceptions, see below; Ghiselin 1974; see Williams 1975; Leonard 1990, 2013 for discussion). Sessility is another circumstance that is associated with low encounter probability; the only sessile subclass of Crustacea, the Cirripedia, are largely simultaneous hermaphrodites, but within this group, higher density is associated with simultaneous hermaphroditism, whereas low density and/or parasitism is associated with dioecy (Yusa et al. 2013; Chap. 8). While simultaneous hermaphroditism is the dominant sexual system in many sessile taxa, including angiosperms, spore plants (see Table 1.1, Chap. 2) ectoprocts, entoprocts, and urochordates, there are many other sessile taxa that are dioecious, such as most gymnosperms, pogonophorans, brachiopods, hemichordates, and the crinoids among the echinoderms (Table 1.2).

In Mollusca, Collin (2013; Table 1.2; Fig. 1.4) characterized the classes as consistent in sexual system with the exceptions of the Bivalvia (see also Chap. 6)

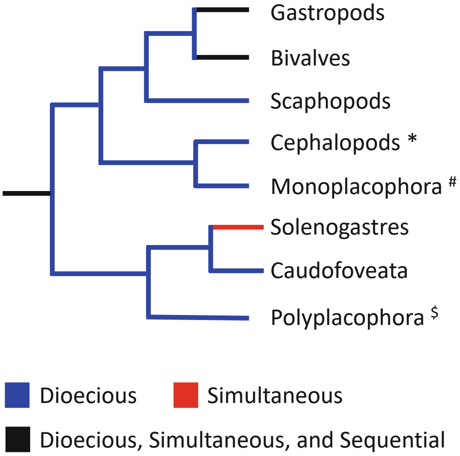

Fig. 1.4 The distribution of sexual systems in the classes of the Mollusca (reproduced with permission from Collin 2013). The red line indicates simultaneous hermaphroditism, the blue line indicates dioecy, and the black line indicates labile sexual systems with dioecy, sequential, and simultaneous hermaphroditism. *One known case of sequential hermaphroditism; #one genus with some simultaneous hermaphrodites; $one genus with some simultaneous hermaphrodites

and the Gastropoda. While these are much the largest classes of molluscs in terms of species [12,000 bivalves; 103,000 gastropods, as opposed to the next largest class, Polyplacophora, with 900 species (Lecointre and Le Guyader 2006)], within the Gastropoda, sexual system is quite conservative, with all 30,000 species (UCMP, Berkeley) in the Heterobranchia, being simultaneously hermaphroditic and many other groups being dioecious (see also Collin 2013; Chap. 7; for a discussion of the distribution of sexual system in gastropods). Similarly, the whole class of Hexapoda (insects) is dioecious despite a wide range of ecological conditions, with only minor exceptions. One family, the aphids, has a life cycle involving alternation of generations in which asexually produced individuals may begin to produce sexual individuals, both male and female, which may then mate with each other, or with sexual offspring of other clones, thereby making the parent individual (or genet to use a botanical term) effectively hermaphroditic (Dixon 1973). The only other example of "hermaphroditism" in insects involves three species of scale insect in which females have sperm-producing tissue that is apparently parasitic and derived from their fathers, exclusively (Gardner and Ross 2011). The empirical evidence therefore (Table 1.2) suggests that in animals, either dioecy or simultaneous hermaphroditism with outcrossing is often very stable evolutionarily. The phyletic distribution of sexual systems, and its relation to various ecological factors, has been reviewed several times (Ghiselin 1969, 1974, 1987; Williams 1975; Heath 1977, 1979; Clark 1978; Leonard 1990, 2010, 2013; Jarne and Auld 2006; Eppley and Jesson 2008; Iyer and Roughgarden 2008; Weeks 2012; for molluscs, see Collin 2013).

Interestingly, other sexual systems, such as sequential hermaphroditism, androdioecy, gynodioecy, or exclusively selfing hermaphroditism, are less stable, being confined to lower taxonomic categories. That is, although there are whole families of teleosts (Erisman et al. 2013), shrimp (Chap. 10), and gastropods (Chap. 7) characterized by sequential hermaphroditism, there are no phyla or classes with a predominance of sequential hermaphrodites (Weeks et al. 2006). Although androdioecy is found in groups that are evolutionarily ancient, such as notostracans and spinocaudatans, evidence suggests that androdioecy and selfing hermaphroditism have evolved recently (Zierold et al. 2007; Weeks et al. 2014). This is also the case for angiosperms. In angiosperms, gynodioecy has evolved frequently but is also evolutionarily short-lived (see Pannell 2002, Chap. 3; Delph 2009; Rivkin et al. 2016). Similarly, although simultaneous hermaphroditic taxa that reproduce exclusively by selfing have evolved many times in both plants and animals, they are evolutionarily unstable compared to taxa in which simultaneous hermaphrodites reproduce through outcrossing, at least occasionally (Darwin 1858; Ghiselin 1969; Williams 1975; Jarne and Auld 2006; see below).

It is clear, therefore, that in many metazoan taxa, either simultaneous hermaphroditism with outcrossing or dioecy are extremely stable evolutionarily (Table 1.2). This seems to be the case in plants also (Table 1.1). Why this should be the case is not yet clear. Williams (1975, p. 123) argued that the lack of flexibility in sexual system must indicate a lack of “preadaptations for changing to some other mode of reproduction.” In a sense this is a truism, since if a character hasn’t changed in hundreds of millions of years, change may not be possible. However, there should be efforts to find other explanations before we settle on “genetic inertia” to explain why some taxa have maintained a particular sexual system since (apparently) the Cambrian, whereas others, such as Cnidaria, teleost fishes, and polychaetes, are more flexible evolutionarily, such that sexual system varies by order, family, genus, or even species [e.g., *Ophyrotrocha* polychaetes (Chap. 5), *Hydra* (Kaliszewicz and Dobczynska 2017), didymozoid trematodes (Anderson and Cribb 1994), the jellyfish *Cassiopeia* (Hoffmann and Hadfield 2002), etc.].

On the other hand, there are few major taxa that do not show some instances of change of sexual system. For example, the phylum Platyhelminthes is almost exclusively hermaphroditic with a very few exceptions (see Ghiselin 1974 for review; also Charbagi-Barbirou and Tekaya 2009), notably the family Schistosomidae, which are exclusively dioecious parasites of vertebrate blood streams in which adult males hold the body of the smaller female (Basch 1990; Després and Maurice 1995; Platt and Brooks 1997). The parasitic digenean families Spirochidae and Didymozoidae also include species that are dioecious. In this group, a pair of worms are encysted together in the body of the host, and, depending on species, the pair may be simultaneously hermaphroditic, “incompletely hermaphroditic,” or dioecious (Anderson and Cribb 1994; Platt and Blair 1996). Platt and Blair (1996) also suggest protandrous hermaphroditism in one case. The class Hexapoda includes one family, the aphids with alternation of generations, whereby a single genet can produce both male and female offspring, i.e., a single genotype can produce both eggs and sperm. Also, in three species of scale insect, females inherit male spermatogenic tissue that is genetically identical to their father (Gardner and Ross 2011). If a change of sexual system was possible in these cases, why not in others?

If the change of sexual system was highly adaptive, wouldn't there have been a significant adaptive radiation in taxa that had changed sexual system? Has this been tested for? Comparative studies, elucidation of mechanisms of sex determination, and experimental studies testing responses to factors associated with changes in sexual system in some taxa may help in explaining this conservatism. In this volume, taxa with significant lability are reviewed, with a view to understanding the evolution and adaptive significance of their sexual systems. In general, the results of these reviews suggest that the changes of sexual system in these sexually labile taxa are consistent with the predictions of sex allocation theory, although new evidence suggests that food availability can influence sexual system in animals as well as plants in a way that was not accounted for in early models of sex allocation (see Chaps. 8 and 9).

There are obvious deficiencies in the classic theories explaining the adaptive advantages of hermaphroditism versus dioecy, referred to by Williams (1975). Firstly, the theories are simplistic, giving the two sexual systems as the only adaptive responses to environmental changes, whereas organisms can respond evolutionarily in a variety of ways that may solve the problem. For example, the classic low encounter probability model (Tomlinson 1966, etc.) predicts that declining encounter probabilities will result in the evolution of simultaneous hermaphroditism. However, there is a wide range of other evolutionary responses available to a dioecious organism faced with a reduced probability of encountering a mate (see Mosimann 1958). For example, an organism's probability of encountering a suitable mate would be increased by evolving (a) a longer life span and/or breeding season; (b) greater mobility or ability to detect mates; (c) living in groups, (d) sperm storage or (e) the formation of monogamous pairs when a mate is encountered. Warner (1978) discussed this deficiency in terms of terrestrial vertebrates and conservatism of sexual system. Another possibility is social control of sex determination such that individuals develop into the sex opposite to the first conspecific they encounter during larval dispersal (environmental sex determination, ESD; see above). Why these alternative adaptations would be easier to evolve than simultaneous hermaphroditism is not yet clear, perhaps because the questions have not been asked.

This gives rise to two questions: (a) What are the evolutionary advantages of a change in sexual system? (b) *Are* sexual systems less likely to change over evolutionary time than other characters?

1.4 Hypotheses of Advantages of Dioecy Versus Simultaneous Hermaphroditism

Since 1975 there has been substantial progress in (a) understanding the advantages of dioecy versus simultaneous hermaphroditism and (b) identifying factors that act to stabilize both dioecy and simultaneous hermaphroditism (review in Leonard 2013). In addition to the classic explanations of SH being advantageous in situations of low probability of encountering a mate and dioecy being advantageous in avoiding inbreeding and responding to sexual selection more easily, both theoretical and empirical work over the last 40 years has extended our understanding of the circumstances which may provide an evolutionary advantage to one or the other sexual system (see Leonard 2010, for review).

1.4.1 Advantages of Dioecy

Studies focused on the advantages of dioecy have come mostly from workers interested in angiosperms where dioecy has evolved frequently but is not characteristic of major taxa (see above). For angiosperms, reduction of inbreeding depression, and/or promotion of genetic variability of offspring, by prevention of self-fertilization (Mather 1940) remains the prevailing explanation for the evolution of dioecy (see Fig. 1.1; Bawa 1980; Lloyd 1982; Delph 2009; but see Chap. 3). However, a variety of mechanisms of self-incompatibility have evolved in both plants and animals (e.g., Jarne and Charlesworth 1993; Grosberg 2000; Charlesworth et al. 2005; Bishop and Pemberton 2006; Jarne and Auld 2006). Simultaneous hermaphrodites may avoid selfing by developmental, anatomical, or behavioral mechanisms (see Ghiselin 1974).

An alternative explanation for the evolution of dioecy in angiosperms has been resource limitation and/or predation. In harsh or nutrient-limited environments, or where predation levels are high, specialization in one sexual role may lead to more reproductive success (see Heslop-Harrison 1957; Givnish 1982; Charlesworth 1999; Ashman 2002; review in Thomson and Barrett 1981). A similar phenomenon may occur in barnacles (see Yamaguchi et al. 2014; Yusa et al. 2013; Chaps. 8 and 9). Nutrient limitation has also been invoked to explain the timing of sex change in some fish (Yamaguchi et al. 2013). The common factor in these studies is the idea that if reproductive success in one sexual role is "cheaper," energetically, then individuals in poor condition or facing a stressful environment may improve their reproductive success by specializing in the cheaper sexual role. In many cases, this will be the male role, since, in general, sperm are cheaper to produce than eggs (Bateman 1948). However, in cases in which mates become defensible resources within a social group and/or male–male competition for access to females is important, the male role may be more expensive because it requires larger body size and/or behavioral energy expenditure, and individuals in poor condition or facing a harsh environment should become females. This argument suggests that dioecy should be mediated by ESD.

For animals the classic explanation of the advantages of dioecy is that it represents an extension of anisogamy: i.e., divergent selection pressures for increased offspring survival on the one hand, and increased mate acquisition, on the other hand, lead to the evolution of two morphs, female and male, respectively (see discussion in Leonard 2010). In this view, males are specialized for access to females and/or their eggs (Puurtinen and Kaitala 2002; Iyer and Roughgarden 2008; see also Eppley and Jesson 2008). A related hypothesis is that sexual selection in general selects for dioecy since where individuals have only one sexual function, it is easier for sex-linked characters to evolve (Greeff and Michiels 1999). In turtles there is evidence that the evolution of sex chromosomes is associated with increased sexual dimorphism and sexual dimorphism at earlier ages (Chap. 12). There is evidence from some taxa (e.g., families of teleosts with variable sexual systems) that increased sperm competition selects for dioecy (Erisman et al. 2013; see also *Ophyrotrocha*, Chap. 5).

Another hypothesized advantage of having separate sexes that has been discussed in animals is “division of labor,” i.e., that the two sexes can each specialize in a manner that will increase individual reproductive success. The fact that in spirochid and didymozoid trematodes, in which pairs of parasites are encysted in the host in a manner that would apparently enforce monogamy, some species show either “imperfect hermaphroditism” or dioecy (Platt and Blair 1996; Anderson and Cribb 1994) would support this hypothesis. The most likely advantage to dioecy in perfectly monogamous pairs would be increased efficiency of gamete production. The most famous example of “division of labor” between the sexes also comes from the strongly hermaphroditic phylum, Platyhelminthes. In the schistosomids, a family of parasitic trematodes with dioecy and strong sexual size dimorphism, the muscular males hold the females in the blood vessel to feed, with the small, slender females leaving the male to move to thin-walled capillaries to deposit their eggs (Basch 1990; Deprés and Maurice 1995; Tchuem Tchuenté et al. 1996; Platt and Brooks 1997). Experimental data from *Ophyrotrocha* species (Prevedelli et al. 2006) shows that a dioecious species reproduced earlier than simultaneously or sequentially hermaphroditic species. This suggests that developing both types of gametes may require more energy/time than developing either eggs or sperm alone. Since earlier reproduction may increase fitness, this is a potential advantage to dioecy that might be looked for in other taxa. Theoretical considerations tell us that anytime there is a

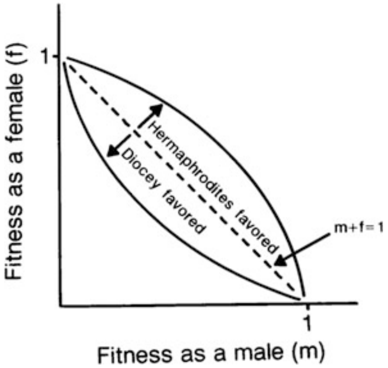

Fig. 1.5 Charnov’s (Charnov 1982) graph (reproduced from Leonard 2013) showing possible fitness sets for the trade-off between male and female function for hermaphroditism (convex) and dioecy (concave). The point of the graph is that where male fitness comes at the cost of female fitness, or *vice versa*, dioecy will be the evolutionarily stable strategy. In order for simultaneous hermaphroditism to be favored, there must be a correlation between fitness in the male role and fitness in the female role (see text)

trade-off between male and female reproductive success, there will be an advantage to dioecy (Charnov 1979, 1982; Fig. 1.5). The nature of such trade-offs is not always clear.

1.4.2 Advantages of Hermaphroditism

The earliest and simplest hypothesis for the advantage of hermaphroditism was that of reproductive assurance through self-fertilization. This hypothesis predates Darwin (see Ghiselin 1969 for discussion). Self-fertilization is widespread among simultaneous hermaphrodites (review in Jarne and Charlesworth 1993; Jarne and Auld 2006) and has evolved frequently; among stylommatophoran gastropods, the ability to self may vary among species in a genus and even populations within a species (Clark 1978; Baur 2010; Jarne and Auld 2006; Jarne et al. 2010). Evolution of the ability for females to make a few sperm for use in self-fertilization, thereby achieving reproductive assurance, clearly explains the evolution of simultaneous hermaphroditism and/or androdioecy in some groups, i.e., nematodes, clam shrimps, and tadpole shrimps (Weeks et al. 2006; see above). As in angiosperms, the genetic costs of inbreeding can be substantial (for review see Jarne and Auld 2006), making the magnitude of the advantage variable. The reproductive-assurance-through-selfing model does not work to explain much of the distribution of simultaneous hermaphroditism among organisms. There are many groups that do not self [e.g. barnacles, opisthobranchs, but see (Smolensky et al. 2009)] and, as in angiosperms, simultaneously hermaphroditic animals have evolved many mechanisms to prevent self-fertilization (Mather 1940; Ghiselin 1974). These range from genetic self-incompatibility (Grosberg 2000), anatomical separation of male and female gametes (Valdés et al. 2010), the timing of release of gametes, and requirements for activation of sperm to sequential hermaphroditism. Some simultaneously hermaphroditic corals release sperm and eggs together in packets of gametes, but do not self (Levitan et al. 2004).

In the mid-twentieth century, Tomlinson (1966) developed the low-density model which argues that, even without self-fertilization, simultaneous hermaphroditism will increase fitness when the probability of encountering a mate is low, since any conspecific is a potential mate, thus effectively doubling population size (Borgia and Blick 1981). This model explains the association of simultaneous hermaphroditism with sessility, parasitism, a planktonic lifestyle, and low dispersal potential (see Altenburg 1934; Ghiselin 1974 for discussion), but it does not explain why some phyla and classes that are sessile, or parasitic or planktonic, or have reduced dispersal ability remain dioecious (see above, discussion in Williams 1975). There are also cases in which simultaneous hermaphroditic taxa may be more mobile than related dioecious taxa; e.g., the heterobranch gastropods, in general, have light or reduced shells, whereas many of the dioecious gastropods have large, heavy shells and/or are sessile (see Chap. 7). Moreover, this does not explain why dioecy would be maintained in situations of low encounter probability, such as parasitism, or in taxa that undergo frequent founder events, such as is true for many insects and spiders.

Eric Charnov (1979, 1982; Charnov et al. 1976) demonstrated that where there is a trade-off between male and female reproductive success (i.e., egg and sperm production are derived from the same pool of resources), dioecy will be the stable evolutionary solution to the problem of sexual system (Fig. 1.5). Simultaneous hermaphroditism will only be evolutionarily stable where there is a positive correlation between male and female reproductive success, i.e., a convex fitness curve (Fig. 1.5). Charnov (1982) suggested that one way to produce a convex fitness function would be if the gain curve of one sexual function reaches a plateau (saturates) where increasing investment does not yield increasing fitness. In such a case, an individual may increase its fitness by putting further sexual investment into the other sexual function; examples include brooding (Heath 1979) in which the space for brooding eggs is finite, so that any excess resources available for reproduction will yield a bigger increase in fitness if invested in sperm. A well-known case is *Ostrea* (Coe 1932; Chaparro and Thompson 1998) oysters, which produce eggs and then convert the gonad to sperm production once the brood space is full. Once the brood has hatched, the gonad reverts to egg production. This is often referred to as alternating sequential hermaphroditism, but since an individual may act in both sexual roles in one breeding season, it may also be properly thought of as simultaneous hermaphroditism. Brooding is also associated with simultaneous hermaphroditism in other basically dioecious groups, e.g., the brachiopod *Argyrotheca* (Kaulfuss et al. 2013), some chitons, brittle stars, etc. Other examples of factors producing a convex fitness function include local mate competition (Charnov 1982) or local sperm competition (Schärer and Pen 2013) in which male function reaches a plateau when populations and/or local mating groups are small. Individuals which have resources remaining after being able to inseminate all of the neighbors may increase their fitness by devoting the remaining resources to egg production (Charnov 1982, 1987). This has become a major focus of interest in SH (e.g., West 2009).

Another way of achieving a convex fitness function is shared use of structures or behavior, or utilization of different resources, for reproductive success through both male and female function. For example, flowers may attract pollinators to both bring and remove pollen, creating a synergy between male and female reproductive success (Charnov 1979). Also in some cases, simultaneous hermaphrodites may produce their eggs and sperm at different times during the reproductive season, e.g., producing and exchanging sperm early in the season and yolking up eggs to be fertilized by stored sperm later in the season as in many euthyneuran gastropods, thus eliminating competition for resources between sperm and eggs (Charnov 1979). Similarly, Crowley et al. (1998) have argued that, in cases where one role is expensive, usually the female role, both partners benefit if they trade roles during production of a series of eggs. In this way simultaneous hermaphroditism is favored, not only through sexual selection for alternation of sexual roles (see Charnov 1979; Fischer 1980; Leonard 1990, 2006; below) but through natural selection for increased production of eggs by extending the period of egg production. This would explain the association of SH and small body size (see Clark 1978; Kaulfuss et al. 2013).

Another advantage of simultaneous hermaphroditism is that by reproducing in two sexual roles, individuals may be able to reduce their variance in reproductive success (Lloyd 1982; Leonard 1999; Wilson and Harder 2003), which, if it does not decrease average reproductive success, will increase fitness. This benefit comes from the reduced likelihood that both eggs and sperm will suffer the same hazards; i.e. the success of investment in sperm is less likely to be correlated with the success of investment in eggs, for an individual, than the success of investment in sperm (or eggs) is likely to be correlated with more investment in that same type of gamete. This is essentially the biological version of economic investment theory ("portfolio" theory; see discussion in Leonard 1999). The basic idea is that if, for example, two internally fertilizing hermaphrodites exchange sperm and then each goes off and lays its eggs, the success or failure of the two egg clutches is only weakly correlated with each other since they will be separated in space and/or time, so that an individual may lose its own clutch of eggs to predation or other factors and still have reproductive success as a sire from the other clutch. The idea that the fitness of simultaneous hermaphrodites may be enhanced by reduced covariance of fitness of offspring was first suggested for plants by Lloyd (1982). The major effect on fitness is that any reduction in variance or covariance will reduce the probability of reproductive failure, which is of critical importance in evolution. While theory often focuses on maximizing reproductive success, the first goal of any organism will be to avoid reproductive failure (see Leonard 1999).

A final, but perhaps very important, benefit of simultaneous hermaphroditism is the ability for individuals to adjust their allocation to each of the sex roles in response to either social or environmental conditions (Lloyd 1982; Klinkhamer et al. 1997; Klinkhamer and deJong 2002; Zhang 2002; Cadet et al. 2004). In such cases of "quantitative gender" (Klinkhamer et al. 1997), phenotypic plasticity in sex allocation need not involve significant cost (e.g., Lorenzi et al. 2006; Murren et al. 2015). In many species individuals can adjust their allocation to sperm versus eggs (Charnov 1982) or male behavior versus egg production (Lorenzi et al. 2006) in the face of changes in nutrient availability, mate availability, etc. in a way that should enhance individual fitness. While there has been substantial experimental work to demonstrate that various hermaphroditic taxa can and do change sex allocation in response to changes in the environment (Raimondi and Martin 1991; Klinkhamer and deJong 2002; Schärer 2009), experimental evidence to demonstrate that this actually increases fitness is still lacking. Some studies have shown that as access to mates increases, investment in male function increases, but investment in female function remains constant (Schärer and Ladurner 2002; Schärer 2009), which would mean that unless sperm were, in fact, free as in some of the early sexual selection models, some individuals are changing their sex allocation in a maladaptive manner, since they would probably enhance their fitness more by investing in a few more eggs as sperm become a saturating resource in the population. If it were true that increased access to mates led to the entire population increasing its allocation to sperm over eggs, it would be strong evidence for runaway sexual selection.

However, as demonstrated in *O. diadema* by Lorenzi et al. (2006), gamete production may not be the major component of allocation to a sexual role.

1.5 Stability and Transitions in Sexual Systems

While studies examining environmental correlates of sexual systems in taxa where significant variation occurs have found that sex allocation theory is consistent with most of the patterns found, there are two stubborn questions that remain. One is Williams' Paradox, that is, why is sexual system so conservative in so many taxa of both plants and animals? The second is what are the evolutionary pathways of changes of sexual system in animals?

1.5.1 Transitions in Sexual Systems

For angiosperms, there are well-developed models of how a species or population may evolve from simultaneous hermaphroditism to dioecy or the reverse, through either gynodioecy and androdioecy (see Fig. 1.1, Delph 2009, Chap. 3) or through monoecy (see Chap. 3). In Delph's model (Fig. 1.1), the transition from SH to gynodioecy begins when natural selection for inbreeding avoidance favors individuals that are male sterile, producing gynodioecy, populations consisting of pure females, and simultaneous hermaphrodites. Once gynodioecy has evolved, sexual selection will favor hermaphrodites that produce more pollen and fewer ova, leading to gradual transition to a dioecious population. In the reverse case, females in a dioecious population or species which are able to produce a small amount of pollen for self-fertilization are favored by natural selection when pollen is limiting. This produces an androdioecious population, consisting of males and simultaneous hermaphrodites. As fewer ova become available for males to fertilize, males experience reduced reproductive success and may be lost from the population, and sex allocation is driven by sexual selection to a condition of simultaneous hermaphroditism (Delph 2009). Phenotypic plasticity also seems to play an important role in these evolutionary changes of sexual systems (Delph and Wolf 2005). In monoecy, an individual hermaphroditic angiosperm produces separate male and female flowers, and response to selection pressures can drive the system to dioecy (see Chap. 3). It is interesting that in both the gynodioecious and androdioecious pathway, the initial move from the stable sexual system of either dioecy or simultaneous hermaphroditism is a result of natural selection, whereas subsequent movement toward the opposite sexual system is then driven by sexual selection.

Outside of the angiosperms, simultaneous hermaphroditism and dioecy are also the most common sexual systems, but the evolutionary paths between them are less clear (Chap. 2). There is a relationship between increased chromosome number and gametophyte hermaphroditism in spore plants, but the mechanisms are not clear. In mosses, liverworts, and hornworts, there is haploid chromosomal U/V sex determination, so with polyploidy, tetraploid UUVV sporophytes can produce UV spores that will become hermaphroditic gametophytes. However, there are dioecious allopolyploid mosses (see Chap. 2, for review). In other taxa, there may be a relationship between chromosome number and a change of sexual system, but it is not clear whether an increase in chromosome number produces the change in sexual system or creates genetic potential for a change in sexual system in response to selective pressures.

The logic and selective pressures in the Delph model (Fig. 1.1) would seem to apply to animals as well, but Weeks (2012) in a very comprehensive review, concluded that neither gynodioecy nor androdioecy were associated with transitions between SH and dioecy in animals, with the possible exception of androdioecy with dwarf males in barnacles (see Chap. 8). The evidence is that (a) gynodioecy is extremely rare in animals (see above) and (b) androdioecy, although it has evolved frequently, is usually a matter of females from dioecious taxa evolving the ability to produce a few sperm for self-fertilization and is evolutionarily short-lived, evolving either into populations/species that self exclusively or back to dioecy. Weeks (2012) attributes the failure of these androdioecious taxa to evolve toward outcrossing SH to the fact that they are, for the most part, internally fertilizing taxa, such as nematodes, clam shrimp, and tadpole shrimp, with strong sexual dimorphism, so that females would have to evolve, not only the ability to produce sperm but also complex intromittent organs and sexual behaviors, before being able to outcross through sperm. In barnacles, which have pseudocopulation, in which a penis fertilizes eggs which have been released into the mantle cavity, outside the body but inside the shell, and which do not appear to have self-fertilization, androdioecy involving dwarf males can be a sexual system that bridges dioecy and simultaneous hermaphroditism. The extent to which these changes in sexual system are genetic, as opposed to phenotypically plastic, responses is not entirely clear (Chap. 8). In any case, it is clear that those animal taxa that show gynodioecy or androdioecy are too rare to explain the existing transitions among sexual systems (see Weeks 2012).

One path from simultaneous hermaphroditism to dioecy that *is* seen in animals is that of incomplete hermaphroditism. As Picchi and Lorenzi describe in Chap. 5, in *Ophyrotrocha* polychaetes, high density can produce a tendency to dioecy in hermaphroditic species and in dioecious species, and individuals may produce some gametes of the opposite sex, even though those individuals act functionally only as male or female, not both. That is, individuals that have the secondary sexual characteristics of males may have a small number of eggs in the coelom, or apparent females may have a few sperm in the coelom. Similarly, in some of the trematodes that are dioecious (in a strongly hermaphroditic taxon), individuals are often characterized, anatomically, as "incomplete hermaphrodites" in that they have complete sexual organs of one sex and some portion of the sexual organs of the other (Anderson and Cribb 1994) suggesting that they can only function as one sex. These examples suggest that one path from SH to dioecy in animals may involve a shift in sex allocation to one sex or the other in individuals in a population, with a gradual loss of function of the sexual organs and/or gametes of the other sex. That is to say, in these species, individuals develop and function as either males or females, exclusively, while possessing nonfunctional vestiges, of a varying degree of development, of the sexual organs or gametes of the opposite sex.

Evolution of dioecy from SH in this manner can be easily explained if selection pressure tends to favor individuals that specialize in one sexual role, and decreased development of sexual organs associated with the other sexual role enhances the ability to function in the chosen sexual role. That is, once an individual has begun to specialize in one sexual role, decreased energy expenditure in the sexual organs and/or gametes of the other sexual role may free up energy to invest in the favored sexual role, enhancing fitness. By a gradual process, selection should eventually produce anatomically dioecious individuals, i.e., pure males and females. This would be a plausible scenario for the evolution of dioecy from SH in many animal taxa, although it could not account for the evolution of SH from dioecy. Furthermore, since in *Ophyrotrocha* the sexual role of an individual can be influenced by the social environment (Chap. 5) and in the trematodes, member of a pair encysted together show sexual specialization (Anderson and Cribb 1994), it seems likely that phenotypic plasticity plays a role in at least the early stages of the evolution of dioecy in these animals.

In caridean shrimps, Baeza (Chap. 10) has hypothesized that the protandrous simultaneous hermaphroditism characteristic of the lysmatids may have evolved from dioecy through stages involving (1) a system of sequential hermaphroditism with either primary males or primary females, (2) simple sequential hermaphroditism, and then (3) maintenance of the male sexual characters into the final adult phase with female sexual characters. Baeza proposes a sequence of genetic changes that could explain this. Collin (2013) also presented a hypothetical sequence of changes from simultaneous to sequential hermaphroditism in gastropods.

1.5.1.1 A Role for Phenotypic Plasticity?

Although much of theory on the evolution of sexual systems has started with the assumption that dioecy and simultaneous hermaphroditism are dichotomous evolutionary choices, it is clear that the sexual systems found in nature offer a continuum between these two extremes (Leonard 2013). That is to say, there is a gray area between dioecy with genetic sex determination on the one hand and ESD on the other, a very murky border between ESD and sequential hermaphroditism, and also a gray area between sequential hermaphroditism and simultaneous hermaphroditism. In angiosperms, dioecy often involves a degree of phenotypic plasticity, such that environmental influences may produce a degree of hermaphroditism (Heslop-Harrison 1957; Zimmerman 1991; Ashman 2002). In animals, we usually think of dioecy as involving genetic sex determination (GSD), but environmental sex determination (ESD) is also quite common (Beukeboom and Perrin 2014), and the two are not mutually exclusive (see above; Chaps. 8, 11, and 12). In nematodes (Pires-da Silva 2007; Denver et al. 2011; Chap. 4), teleost fishes (Mankiewicz et al. 2013; Chap. 11), and reptiles (Chap. 12), there are well-studied cases in which the sex of an individual is determined by the interaction of the genotype and the environment. Sex-determining mechanisms may evolve rapidly, and transitions between ESD and GSD may be frequent, and the two are by no means mutually exclusive (Kraak and Pen 2002; Beukeboom and Perrin 2014; Chaps. 6, 11 and 12; see above).

Frank and Swingland (1988) used the term "conditional sex expression" to refer to the similarities between dioecy with ESD and sequential hermaphroditism. The link between ESD and sequential hermaphroditism comes from the fact that both involve phenotypically induced gender development in response to a similar array of either physical or social cues. In dioecy with ESD, an organism develops as either male or female in response to a cue received early in development and then retains that sexual role for the rest of its life. In sequential hermaphrodites, on the other hand, an individual begins reproduction in one sexual role and then, as a function of size/age, social cues, or occasionally resource availability, changes sex and reproduces in that sex for the rest of its life. However, there are a variety of organisms that fall between these two patterns. For example, in polychaetes, there are cases in which young individuals develop as either males or females, but individuals that experienced adverse conditions were likely to change sex to male after spawning and then remain male (Hauenschild 1953; see above). In the perennial forest herb Jack-in-the-Pulpit, individuals are male in their first-growing season, but their sex in the subsequent season depends on the amount of stored resources; individuals with a large resource reserve emerge as females in the next season, whereas those with fewer resources emerge as males (Bierzychudek 1982; Viti et al. 2003).

Another sexual system that seems intermediate between dioecy and sequential hermaphroditism involves species that have both sequential hermaphrodites and individuals that are termed either primary females or primary males, depending on the sexual system. That is, in the bluehead wrasse (*Thalassoma bifasciatum*), which is a group-living protogynous sequential hermaphrodite, small individuals begin reproduction as females, spawning with large males that control mating sites. When one of these males disappears, a large female changes sex and eventually becomes the territorial male. However, some individuals in the population are male from a very small size and are termed "primary males." It was initially believed that these primary males were genetically determined, but it has become apparent that their sex is actually determined by social cues during early development (Munday et al. 2006b). A mixture of primary males and protandric sequential hermaphrodites, with a genetic basis, may be found in the Pacific oyster (Guo et al. 1998; Zhang et al. 2014). Environmental factors may also play an important role in sexual systems in bivalves (see Chap. 6). In carid shrimps, sexual systems may also involve mixture of "primary" males or females and protandrous sequential hermaphrodites (Chap. 10).

Similarly, although theory has usually dealt with sequential and simultaneous hermaphroditism separately, empirical studies demonstrate that it is often difficult to draw a clear line between simultaneously and sequentially hermaphroditic species. Hermaphrodites in general have significant phenotypic plasticity in sex allocation, which has long been taken for granted in angiosperms but has become more widely recognized in animals in recent years. Berglund (1986) demonstrated that in the polychaete *Ophryotrocha puerilis*, pairs of worms formed with the smaller individual acting as a male and having exclusively male gonads and the larger individual having ovaries and producing eggs, during spawning events, until the smaller individual had grown larger than the female, at which point the two individuals changed sex and spawned in reversed roles until again the male had grown larger than the female and sex reversal occurred again. This serial sex reversal is consistent with the size-advantage model (SAM), since the reproductive success of the pair in each spawning is dependent on the number of eggs produced, larger females produce more eggs and males grow more quickly than females because the expense of producing eggs is higher than the expense of fertilizing them. Over time, the size difference between members of the pair grows smaller, the interval between sex reversals grows shorter, and eventually both individuals become simultaneous hermaphrodites, alternating sexual roles in spawning bouts (that is, one hermaphrodite acts as female and the other as male in each spawning event).

In other examples, some of the serranine fishes are simultaneously hermaphroditic until, under densely packed conditions, some individuals change to become pure males and defend harems of simultaneous hermaphrodites which spawn as females with the males (Hastings and Petersen 1986; Fischer and Petersen 1986; reviews in Leonard 1993; Petersen 2006). The lines between simultaneous and sequential hermaphroditism are also blurred in taxa that change sex from one spawning bout to another such as *Ostrea* oysters (Coe 1932; Chaparro and Thompson 1998) and some mushroom corals (Loya and Sakai 2008). In other hermaphroditic taxa, both plants and animals, changes in sex allocation with size, age, or other environmental variables create a situation of "quantitative gender" (Cadet et al. 2004) where individuals within a species can vary their gender expression over short or long time periods. Therefore, it is clear there are no hard and fast boundaries between simultaneous and sequential hermaphroditism and that phenotypic plasticity often is responsible for the transitions between them. Pannell (Chap. 3) has suggested that this may explain the association between monoecy and evolution to dioecy in angiosperms.

1.5.1.2 A New Hypothesis

Seeing sexual systems as falling on a continuum rather than as necessarily discrete phenomena, and recognizing the role of phenotypic plasticity in creating gray areas between sexual systems, suggests that an important pathway from dioecy to simultaneous hermaphroditism in animals may run through ESD and sequential hermaphroditism with phenotypic plasticity acting as a bridge from one sexual system to another. This hypothetical pathway between dioecy and simultaneous hermaphroditism is shown in a schematic diagram in Fig. 1.2. In this scenario, in a population of simultaneous hermaphrodites that are capable of outcrossing with each other, selection pressures, such as (a) environmental conditions that favor temporal separation of male and female reproductive activity, e.g., a restricted period favorable for egg development, (b) reduced food availability, (c) selective pressure to reduce time to first reproduction, and/or (d) selective pressure to reduce possible self-fertilization, would favor individuals that alter their sex allocation to reduce overlap in male and female function. That is, individuals will begin to specialize in one sexual function at a smaller size and/or earlier age and the other at a larger size and/or later age, in accordance with the size-advantage hypothesis (Fig. 1.3). Eventually, selection associated with direct effects of size will favor individuals with complete separation of the two functions in time, producing sequential hermaphroditism with the cue for changing sex usually being an environmental variable, whether social structure, physical environment, or resource availability. A population of sequential hermaphrodites may evolve to ESD if there is increased selection for early sexual maturity, such as in conditions of high local mate availability, or decreased encounter probability, such that a larva settling near a conspecific may benefit from assuming the male sexual role, perhaps as a dwarf male, or if there is an increasing trade-off between male and female function. Furthermore, a stronger correlation between local environmental conditions and an individual's reproductive success in a particular sexual role may favor the evolution of ESD.

The selective forces responsible for the frequent evolutionary transitions and complex interactions between genetic and environmental sex determination are not entirely clear. While an offspring will benefit from assuming the sex that will have highest reproductive success under the environmental and/or social conditions (e.g., sperm competition, sexual selection) present in its environment (see Werren et al. 2002; Beukeboom and Perrin 2014), selection for a balanced sex ratio may drive a system to GSD (Bulmer and Bull 1982; van Doorn 2014). The effect of ESD versus GSD on sex ratio may vary with properties of the environment (see Bachtrog et al. 2014). Other selective pressures that might drive a population with ESD to pure dioecy would be increased encounter rate and consequently decreased benefit to dwarf males, high pressures of sexual selection, and greater longevity such that the benefits to an individual of assuming the sex that most benefits from the environmental conditions at hatching are reduced, although turtles, which are extremely long-lived, are characterized by ESD [(Bull and Vogt 1979); for discussion of the interaction of longevity and ESD vs. GSD, see (Chap. 12)]. Interestingly, in animals, there has been relatively little attention to the advantages of losing all plasticity in sex allocation to become dioecious with strict genetic sex determination as is found in birds, mammals, many insects, and spiders. However, Warner (1978) did argue that adaption to life on land resulted in fixed costs associated with internal fertilization and large offspring size that have made sex change prohibitively complicated for terrestrial taxa. The evidence is that dioecy is extremely stable as a sexual system over evolutionary time (see above).

Conversely, a strictly dioecious population with genetic sex determination may evolve to ESD when encounter probabilities become low, favoring (a) individuals that encounter a conspecific as larvae and become a dwarf male, as is seen in barnacles (Chap. 8), the echiuran, *Bonellia* (Jaccarini et al. 1983) and the pogonophoran, *Osedax* (Vrijenhoek et al. 2008), or (b) individuals that assume the minority sex in the social environment they encounter. Alternatively, if offspring "win" the parent-offspring conflict over sex determination, ESD may evolve (Werren et al.

2002; Beukeboom and Perrin 2014). This may occur if environmental conditions vary such that the fitness of one sex is strongly favored by one set of environmental conditions. A transition of conditional sex expression from ESD to sequential hermaphroditism may be favored by factors such as increased resource availability, increased longevity, or a tighter association between size/age and reproductive success in one sexual role over the other in accordance with the size-advantage hypothesis (see above).

An evolutionary transition from sequential hermaphroditism to simultaneous hermaphroditism, such as seen in carid shrimps (Chap. 10), may occur through any selective pressure that would increase synergy between male and female reproductive success, i.e., a situation in which male and female reproductive successes are both benefited by a trait (e.g., floral structures), monogamy, or reciprocal mating. This may be the case in some of the serranids in which low population density and lack of sperm competition are associated with simultaneous hermaphroditism (Petersen 2006; Erisman et al. 2013). Another factor would be a change in the social or physical environment such that the sex-specific benefits derived from "direct effects" of size, i.e., the role of size *per se* [e.g., the height of a tree's role in dispersing pollen or the ability to dominate a social group behaviorally (Klinkhamer and deJong 2002; Cadet et al. 2004)], become less important than the "budget effects" of size, i.e., the sheer quantity of resources available for allocation to a sexual role. Where subtle shifts in sex allocation through "quantitative gender" produce greater fitness than a complete sex change, simultaneous hermaphroditism will be favored. Reproductive assurance, through the ability to self, may also favor the evolution of simultaneous hermaphroditism from sequential hermaphroditism.

1.5.1.3 General Features of the Model

Interestingly, in the Delph scenario of evolutionary paths between dioecy and simultaneous hermaphroditism in angiosperms (Delph 2009; Fig. 1.1), the initial move away from either simultaneous hermaphroditism or dioecy depends on natural selection, i.e., inbreeding depression or reproductive assurance, respectively. Once the move to either androdioecy or gynodioecy has been made, subsequent shifts in sex allocation seem to be driven by sexual selection more than natural selection in the Delph model. In angiosperms, simultaneous hermaphroditism is the predominant sexual system, whereas dioecy, while it has evolved frequently, seems evolutionarily short-lived and less stable.

In animals both dioecy and simultaneous hermaphroditism are very stable sexual systems evolutionarily. The currently proposed scheme of transitions in sexual system in animals (Fig. 1.2) focuses on forms of phenotypic plasticity in sex allocation, suggesting a continuum of decreasing phenotypic plasticity from simultaneous hermaphroditism with outcrossing to stable dioecy, without ESD (Leonard 2013). Both forces of natural selection and sexual selection may contribute to evolutionary trajectories along this gradient. It will be important to examine the relative role of sexual selection and natural selection in transitions along the gradient. Given the general evolutionary stability of dioecy and SH, one might predict that initial movement away from these sexual systems might be more likely to come as the result of forces of natural selection than sexual selection. In Fig. 1.2 the shift from genetically determined dioecy to ESD is the critical point in increasing the degree of phenotypic plasticity in sex allocation. Here, either low encounter probability or an increase in the differential fitness of the two sexes in certain environmental conditions (see discussion in Chap. 12) would represent natural selection forces that could drive a population away from genetically determined dioecy to ESD and enhanced phenotypic plasticity in sex allocation. Valenzuela (Chap. 12) points out that the climate change associated with the Cretaceous/Paleogene transition resulted in fewer extinctions among families of reptiles with TSD than with GSD.

Similarly, in the scenario in Fig. 1.2, the transition from SH with outcrossing to sequential hermaphroditism might be a result of (a) natural selection for avoidance of inbreeding depression due to selfing; (b) natural selection for increased fitness through an earlier age of first reproduction, which has been shown to be later with SH in *Ophyrotrocha* polychaetes (Prevedelli et al. 2006); or (c) a response to low food or resource availability, through natural selection for maintaining some sexual reproduction at small sizes. Under resource limitation, it may pay to reproduce only in the cheap sexual role early in life, saving energy for somatic growth. In some groups, sexual selection may also help drive populations toward sequential hermaphroditism. For example, in some of the small serranines, SH is the norm, but where territories are densely packed, large individuals may lose ovarian tissue to become pure males and defend harems of simultaneous hermaphrodites (see above and reviews in Leonard 1993; Petersen 2006). One might predict that if the densely packed condition becomes the norm, populations might evolve toward protogynous sequential hermaphroditism as seen in many other coral reef fish. Looking at cases of transitions from SH or dioecy to either sequential hermaphroditism or ESD, respectively, may be useful in understanding how sexual systems shift from the stable patterns of dioecy or SH.

1.5.1.4 Testing the Model

The model in Fig. 1.2 can be tested by comparative methods, i.e., determining whether the scenario is consistent with the distribution of sexual systems across phyletic reconstructions in taxa in which both good reconstructions and sufficient information about sexual systems exist. In animals outside the vertebrates, this sort of detail is rare but is becoming available (see Chaps. 5, 7, 8, and 10). Detailed cladograms do exist, along with good information about sexual systems, for some of the families of teleosts found on coral reefs. Erisman et al. (2013) reviewed the relationship between phylogeny and sexual system in several families of fishes with very labile sexual systems and found that in general sexual system correlated well with social system and, in particular, the prevalence of sperm competition. High levels of sperm competition are generally associated with dioecy (gonochorism) in these families, and low levels of sperm competition such as seen with monogamy and low densities (often correlated) are associated with simultaneous

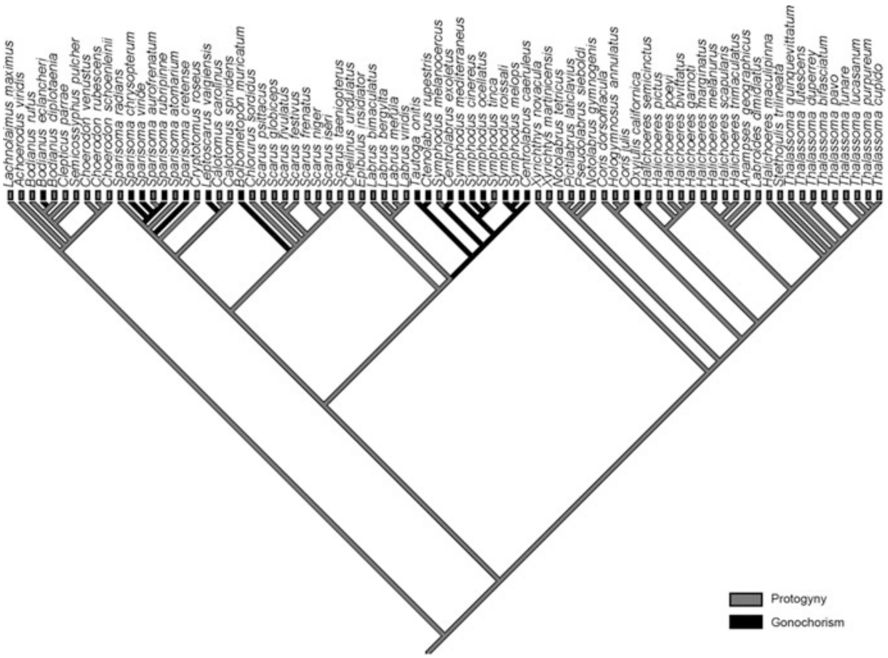

Fig. 1.6 A reconstruction of the transformations in character states for sexual system in labroid fishes (Reproduced with permission from Erisman et al. 2013). Two sexual systems, gonochorism (dioecy) and sequential hermaphroditism, with protogyny, occur in this group (see text). The tree shows dioecy as arising several times from protogynous sequential hermaphroditism (see text)

hermaphroditism. In many of these families, protogynous sequential hermaphroditism seems to represent the ancestral and most common sexual system (Erisman et al. 2013) (e.g., Figs. 1.6 and 1.7).

According to the model in Fig. 1.2, transitions in sexual system in these reconstructions (Figs. 1.6, 1.7, 1.8, and 1.9) should involve either transitions (a) from dioecy (gonochorism) to protogyny (protogynous sequential hermaphroditism); (b) protogyny to dioecy; (c) sequential hermaphroditism (protogyny) to simultaneous hermaphroditism; or (d) simultaneous hermaphroditism to protogyny. Transitions from dioecy to simultaneous hermaphroditism or the reverse would be inconsistent with the model. In the labroids (Fig. 1.6), only dioecy and protogyny are reported, and protogyny seems to be ancestral. The many transitions to dioecy (gonochorism) seen in the tree are all from a starting point of protogyny and are therefore consistent with the model, as are the reversions from dioecy to protogyny. Other groups of fishes are more complicated. In the serranids (Fig. 1.7), protogyny is seen to be the ancestral sexual system, and on the branch to the left in the diagram, there is one transition to dioecy (*Lepidoperca aurantia* and *Epinephelides armatus*) which is consistent with the model. The right branch of the tree includes a transition to dioecy in the *Paralabrax* branch and multiple branches of simultaneous hermaphrodites with two species marked as showing "androdioecy" which involves

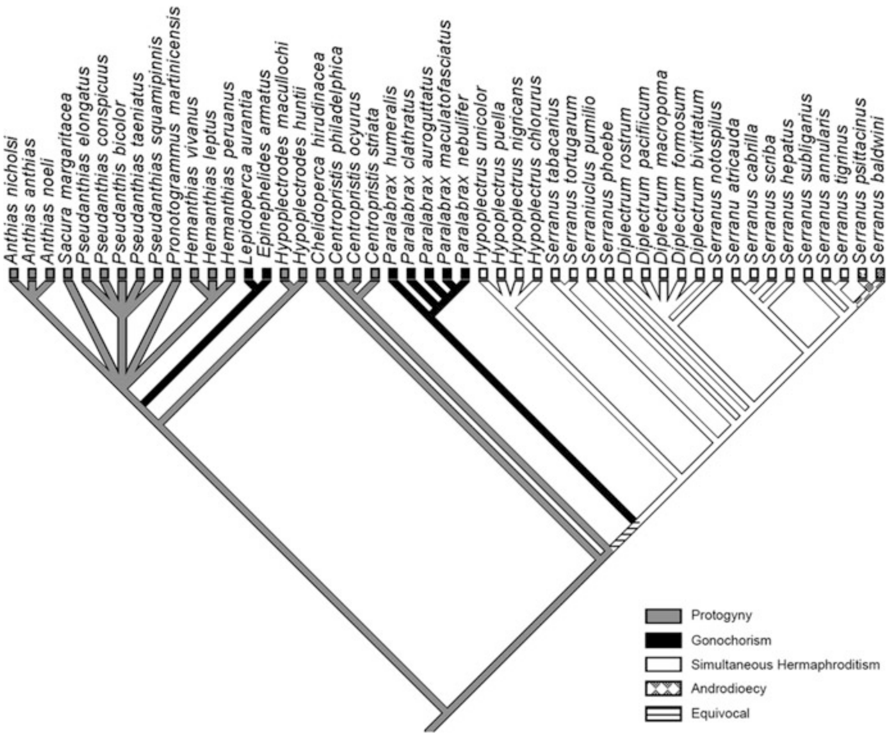

Fig. 1.7 A reconstruction of the transformations in character states for sexual system in serranid fishes (Reproduced with permission from Erisman et al. 2013). This sexually diverse group offers an excellent opportunity to test the hypothesis presented in Fig. 1.2 that sequential hermaphroditism (protogyny here) is the usual transition between dioecy (gonochorism) and simultaneous hermaphroditism and dioecy (see text). Androdioecy is used here to describe a sexual system in which, under certain conditions, large simultaneous hermaphrodites in a social group lose female function and become pure males (Hastings and Petersen 1986) (see text)

Serranus baldwini and *S. psittacinus*, which are the species of simultaneous hermaphrodites in which large individuals may become pure males, as discussed above. The model would regard this transition to androdioecy as incipient sequential hermaphroditism (protogyny) from SH, consistent with the model. The only point in the serranid tree which might be inconsistent with the model in Fig. 1.2 is the stretch of the right branch which is designated "equivocal." If the stretch of tree between the last protogynous branch, and the fork between the dioecious *Paralabrax* and the simultaneously hermaphroditic branches, were to be protogynous, the complete serranid tree would be consistent with the model in Fig. 1.2. If that "equivocal" stretch of tree represented either dioecy or SH, that transition would be inconsistent with the model. In the sparids (Fig. 1.8), the ancestral sexual system seems to be unknown, but all of the transitions in sexual system (except two) would be consistent with the model. The two problems for the model occur when dioecious *Sparodon durbanensis* and *Diplodus vulgaris* branch off from simultaneously hermaphroditic bases. Similarly, the aulopiforms (Fig. 1.9) are inconsistent with

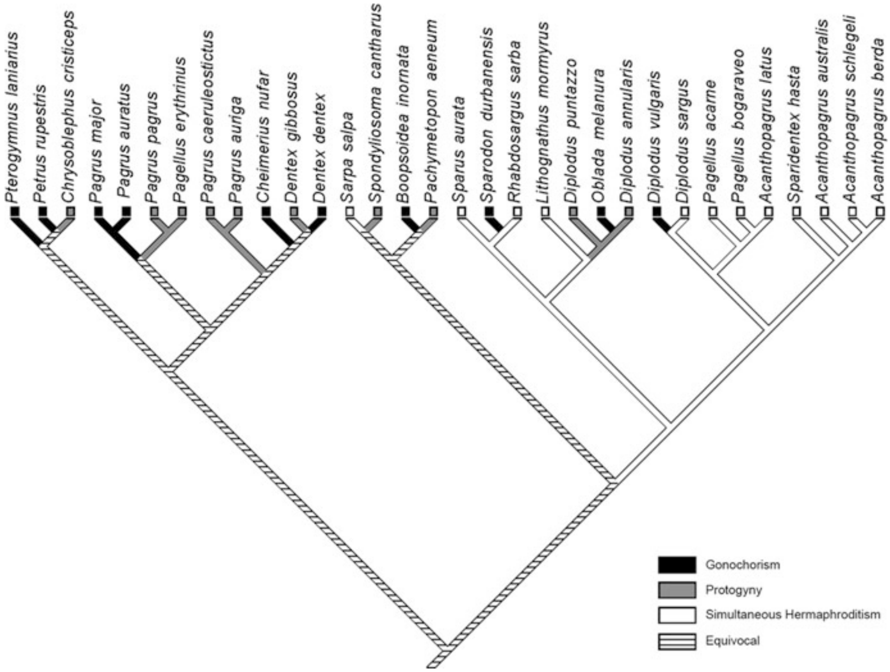

Fig. 1.8 A reconstruction of the transformations in character states for sexual system in sparid fishes (reproduced with permission from Erisman et al. 2013). This sexually diverse group shows several transitions from sequential hermaphroditism (protogyny here) to either dioecy (gonochorism) or simultaneous hermaphroditism (or the reverse) as predicted by the model in Fig. 1.2. However, it also shows two transitions directly from simultaneous hermaphroditism to dioecy (*Sparodon durbanensis* and *Diplodus vulgaris*) which would be inconsistent with the model. Further investigation of the sexual systems of the species located in those portions of the reconstruction would provide an opportunity to test the model in Fig. 1.2

the model since the tree shows simultaneous hermaphrodites branching off directly from a dioecious base. It may or may not be possible to improve resolution of these ancestral states to test the model, but the model is consistent with many of the transitions in sexual system in these families of reef fishes. This is not to say that the driving selective force in changes of sexual system in these groups is not sperm competition as suggested by Erisman et al. (2013). The model in Fig. 1.2 proposes the path of evolutionary change in sexual systems, not the driving selective forces. ESD is being ignored in the above discussion because it was not included in the reconstructions used.

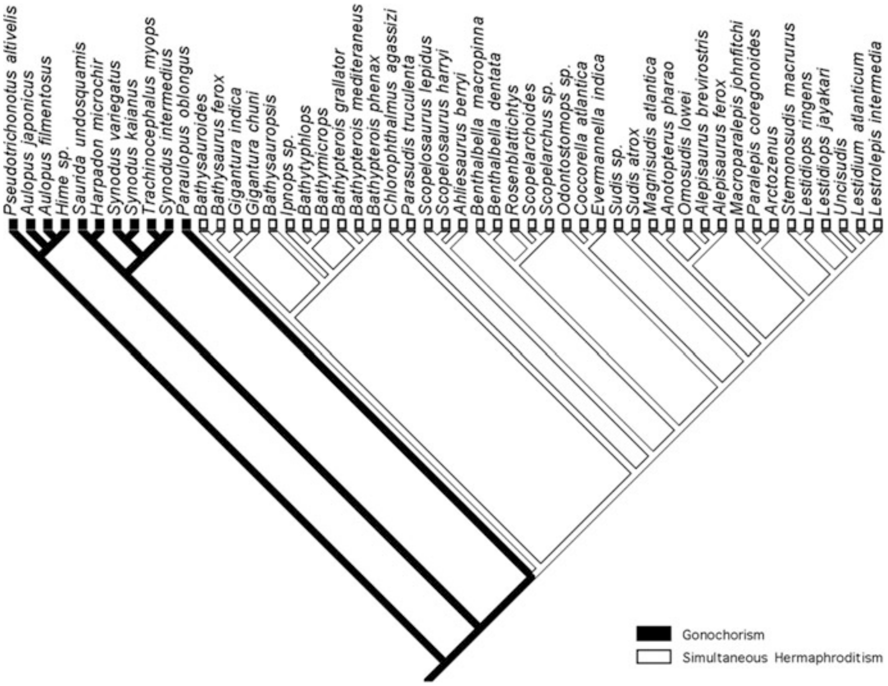

Fig. 1.9 A reconstruction of the transformations in character states for sexual system in aulopiform fishes (reproduced with permission from Erisman et al. 2013). This reconstruction features one transition from dioecy (gonochorism) directly to simultaneous hermaphroditism in violation of the model in Fig. 1.2 (see text)

1.5.2 *Stability in Sexual Systems*

The review of transitions between sexual systems given above, and in the other chapters of this volume, demonstrates that current sex allocation theory, with the important recent addition of considerations of resource availability (Yamaguchi et al. 2013; Johnson et al. 2017; Chap. 9), which has long been known to be important in angiosperms (Ashman 2002), can explain most of the transitions in sexual system seen in animals in those taxa that have substantial lability in sexual system. Also, a new hypothetical pathway from dioecy to SH or the reverse is proposed here (Fig. 1.2). However, it remains the case that in many animals, taxonomy remains the best predictor of sexual system. That is, as Williams' Paradox (see above) states, there are whole classes and phyla that remain either dioecious or simultaneously hermaphroditic in the face of many of the same selective pressures that are associated with changes in sexual system in other taxa (see also Collin 2013). The question of why this should be the case remains unanswered.

1.5.2.1 Is Williams' Paradox a Paradox?

Williams' Paradox stems from the obvious discrepancy between the observed distribution of sexual systems across the Metazoa and the body of theory from 40 years ago, as to the relative advantages of sexual systems (see discussion in (Williams 1975; Charnov et al. 1976; Leonard 1990, 2010, 2013). However, the hypothesis at the heart of Williams' Paradox (Williams 1975) has not yet been tested systematically. That is, the question remains as to whether sexual systems *are*, in fact, more stable, evolutionarily, than most traits, in many taxa. While either dioecy or SH are essentially fixed in some phyla and/or classes (Table 1.2; Fig. 1.4), is this unusual compared to other reproductive or life history traits? Studies that compare the rate of transitions among a range of reproductive traits, such as those identified in gastropods by Collin (Chap. 7) including sexual system, are rare. Kerr et al. (2011) compared stability of sexual system versus mode of spawning in scleractinian corals and found sexual system to be more stable. That is, corals are more likely to change from broadcast spawning to brooding or the reverse than they are likely to change between dioecy and simultaneous hermaphroditism. In fact, sexual system is quite conservative in many groups of corals (Baird et al. 2009; Kahng et al. 2011), even though corals lack permanent gonads so that fixed costs of sexual structures should be minimal. Further studies comparing the probability of transition in sexual system relative to other traits in major taxa will be helpful in determining whether sexual systems are, as they appear to be, more conservative than other traits across the Metazoa.

1.5.2.2 Stability in Dioecy and Outcrossing Simultaneous Hermaphroditism

If dioecy and SH with outcrossing are, in fact, particularly stable evolutionarily in many metazoan taxa, as they appear to be, the next questions are why are they so stable? One possible explanation for the stability of the sexual system in many taxa would be that sex-determining mechanisms, once evolved, are difficult to change. However, present evidence indicates that genetic sex-determining mechanisms evolve rapidly in many taxa (see Bachtrog et al. 2014; Beukeboom and Perrin 2014; Chaps. 4, 11 and 12). While the selective forces involved in the evolution of sex-determining mechanisms are not entirely clear (Bachtrog et al. 2014), maternal-offspring conflict may be one factor (Trivers 1974; Werren et al. 2002; Beukeboom and Perrin 2014). Taxa such as mammals and birds, in which sex chromosomes are fixed, seem to be the exception rather than the rule among metazoan animals. Godwin and Roberts (Chap. 11) also make the point that in mammals, sex steroids begin to function early in development and influence the development of all tissues in the body in a sex-specific manner, which is not the case in teleosts nor in many other taxa. In some taxa, therefore, such as birds, mammals, snakes (Chap. 12), and some insects, genetic sex-determining mechanisms may be resistant to selection pressures for phenotypic plasticity in sex allocation as Williams (1975) suggested; however, there is no evidence that it explains the paradox generally.

Most studies of evolutionary transitions to dioecy have focused on angiosperms, in which dioecy is relatively rare and may be evolutionarily short-lived (see Chap. 3). In angiosperms, avoidance of inbreeding is usually seen as the driving force in the evolution of dioecy (see Fig. 1.1; Delph 2009; Chaps. 2 and 3) although predation and resource limitation are also known to be important (Heslop-Harrison 1957; Ashman 2002). In animals the advantages and/or stability of dioecy have received little attention. Perhaps since both insects and tetrapod vertebrates are exclusively dioecious, dioecy has been taken largely for granted by zoologists. Since Darwin (1871), dioecy has been seen as representing an extension of the evolution of anisogamy, that is, disruptive selection acts to favor individuals that either devote their resources to the survival of offspring (females), a function of natural selection, or pour their resources into the production of large numbers of gametes and/or the acquisition of mates, a function of sexual selection (see discussion in Puurtinen and Kaitala 2002; Eppley and Jesson 2008; Iyer and Roughgarden 2008; Leonard 2010). Sexual selection has been invoked to explain the stability of dioecy in nematodes (Artieri et al. 2008), despite easy genetic changes to selfing hermaphroditism or androdioecy (see Chap. 4). Recent emphasis on the role of high encounter rates and sperm competition in favoring dioecy over simultaneous hermaphroditism (Erisman et al. 2013; see above) is consistent with this theory.

Another factor that may contribute to the stability of dioecy is a trade-off between male and female reproductive success (Fig. 1.2). That is, if there *is* a pool of resources dedicated to reproduction aside from that dedicated to somatic growth or other functions, as assumed in much of sex allocation theory (Charnov 1982), then it will usually be the case that an individual will achieve higher reproductive success by specializing in one sexual role over the other. For SH to be stable, it must be the case, obviously, that a simultaneous hermaphrodite will have higher fitness than either pure males or pure females (Fig. 1.2; Charnov 1979). However, as Charlesworth and Charlesworth (1981) demonstrated, self-fertilization can alter this relationship in favor of the stability of hermaphroditism under a wider range of conditions, although the precise relationship depends on factors such as inbreeding depression and/or fixed costs. The Charlesworths also make the important point that sex allocation models assume that the sexual system has existed for long enough that the population has reached an evolutionarily stable strategy. Given the evolutionary stability of dioecy in many groups of animals, this may often be the case.

What makes simultaneous hermaphroditism with outcrossing so stable in so many phyla and classes? While it was once thought that SH would be unstable relative to dioecy (Charnov et al. 1976), work over the last 40 years has demonstrated that there are a variety of factors that will act to stabilize SH, once evolved. Outcrossing is critical. The Knight-Darwin principle (Darwin 1858; Ghiselin 1974) that obligate selfing is evolutionarily short-lived seems correct for animals, in that it leads to reduced genome size (e.g., Fierst et al. 2015) and is an evolutionarily dead end (see above). Those phyla and classes that are simultaneously hermaphroditic are all characterized by simultaneous hermaphrodites able to outcross in both sexual roles. However, selfing has evolved frequently in taxa with SH, and as the Charlesworths pointed out (Charlesworth and Charlesworth 1981), the option of selfing may broaden the conditions under which SH is stable. Importantly, as discussed above, many simultaneously hermaphroditic taxa lack self-fertilization.

Eric Charnov (1979) was the first to hypothesize that sexual selection could stabilize simultaneous hermaphroditism by producing reciprocal mating systems through sexual conflict over a preferred sexual role (see also Leonard 1990, 1999, 2006). Where simultaneous hermaphrodites have reciprocal mating systems, it may be difficult for a single-sex mutant to compete for mates (Fischer 1980; Axelrod and Hamilton 1981; Leonard 1990, 2013; Henshaw et al. 2015). More recently, it has become apparent that hermaphrodites may increase fitness by reducing variance in reproductive success relative to dioecious individuals (Lloyd 1982; Leonard 1999, 2005, 2010; Wilson and Harder 2003; see also Chap. 3). This occurs because the reproductive success of offspring through sperm may be less tightly correlated to the reproductive success of offspring through eggs, for a single individual, than the success of offspring through a single type of gamete is correlated with other offspring through that same type of gamete. A reduction in variance in reproductive success would represent a strong force of natural selection for SH (Gillespie 1977; Leonard 1999). Moreover, the concept of quantitative gender (Lloyd 1982; Klinkhamer et al. 1997; Cadet et al. 2004) suggests that simultaneous hermaphrodites should be able to adjust sex allocation phenotypically to the current social or physical environment, which may be expected to increase fitness. Both the reduction in variance and the ability to adjust sex allocation phenotypically would tend to stabilize SH.

It is not clear, however, why factors such as selfing, sexual selection, local mate competition (LMC), and reduction in variance in reproductive success should be sufficient to stabilize SH in certain phyla and classes, such as Platyhelminthes, Cirripedia, heterobranch gastropods, oligochaetes, and leeches, and not in polychaetes, caenogastropods, decapods, etc. To improve our understanding of sex allocation and the evolution of sexual systems, we need to better understand how both male and female sexual investment trade off with other life-history parameters (see below).

1.6 Conclusions and New Directions

As shown in the chapters of this volume, our current understanding of transitions in sexual system, in those taxa in which sexual systems are diverse within a relatively low taxonomic level, family, genus, and even species, seems consistent with modern developments in theory. There is also some consistency across taxa. Comparison of Figs. 1.1 and 1.2 shows that the basic selective forces that drive the evolution of dioecy from simultaneous hermaphroditism with outcrossing are similar for both angiosperms and metazoans. Dioecy is favored by a need to avoid selfing, a dearth of resources for growth and reproduction, sexual selection, and benefits of specialization in a sexual role. SH is favored by (a) reproductive assurance through either selfing or increased effective population size, (b) decreased variance in reproductive success, (c) sexual selection through reciprocal mating interactions, and (d) increased phenotypic plasticity in sex allocation.

Sexual systems vary in the amount of phenotypic plasticity in sex allocation shown in the life of an individual (Leonard 2013), and here I suggest (Fig. 1.2) that selection for increased or reduced phenotypic plasticity in sex allocation may drive evolutionary changes in sexual systems. That is, the route from dioecy to SH is hypothesized to often run via ESD and then sequential hermaphroditism in animals, whereas the route from SH to dioecy is predicted to involve the reverse path, from SH to sequential hermaphroditism to ESD to dioecy. Detailed phylogenies with information about reproductive traits, such as those available for certain families of teleosts (Erisman et al. 2013; Figs. 1.6, 1.7, 1.8, and 1.9), may be used to test this hypothesis. Further tests will be possible as more detailed phylogenies, and more information on reproductive patterns, in a greater diversity of organisms, become available. Another important issue that has not been adequately addressed is the advantage of fixing sex at an early developmental stage. That is, what is the adaptive advantage for animals, of irreversibly determining sex before first reproduction as is the case in dioecy, whether sex is genetically or environmentally determined? Why wouldn't be it advantageous to be able to change sex in the face of changing social or physical environmental conditions, as is the case in many angiosperms (see above)?

Not all transitions in animal sexual systems can be explained by the scenario in Fig. 1.2. Picchi and Lorenzi (Chap. 5) show that dioecy in the polychaete genus *Ophyrotrocha* has evolved from SH via incomplete hermaphroditism, i.e., the gradual loss of functionality in one sexual role in some individuals and the other sexual role in others. They show that *O. labronica* is functionally dioecious, while some individuals appear to be hermaphrodites, and the degree of hermaphroditism and phenotypic plasticity may vary among individuals and populations. A similar phenomenon may occur in the very few species of dioecious trematodes (Anderson and Cribb 1994; see above). Such a process could not explain the evolution of SH with outcrossing from dioecy. The model also ignores the sexual systems of gynodioecy and androdioecy which are extremely rare in animals (see above), except in barnacles, where androdioecy with dwarf males seems to often be an intermediary between SH and dioecy (see Chaps. 8 and 9). In angiosperms, the paths from SH to dioecy appear to involve gynodioecy, while androdioecy seems to often be an intermediate step from dioecy to SH (Delph 2009, Chap. 3) although monoecy is also a common intermediate in changes in sexual system in angiosperms (Chap. 3). The patterns in other divisions of land plants are less clear and may involve changes in chromosome number (Chap. 2).

In the land plants in general, as in animals, sexual systems are often very conservative (Tables 1.1 and 1.2). While we now seem to be able to understand why sexual systems change when they change, it is not clear why sexual systems don't change more frequently. Kazancioğlu and Alonzo (2009) developed a model that explained the benefits of sequential hermaphroditism but concluded that the model predicted that it would be beneficial to many species that did not show sequential hermaphroditism. While we now have a better understanding of how dioecy and/or SH may remain stable, Williams' Paradox remains. Why are so many phyla and classes so conservative in sexual system?

The first question, as discussed above, is "Are sexual systems more conservative than other life-history or reproductive traits?" Comparative studies, such as those that are beginning to become available in teleost fish and corals (see above), will allow us to answer this. At first glance the answer seems to be "yes." Another line of inquiry that may help in understanding the evolution and taxonomic distribution of sexual systems is to investigate how male and female sexual investment trade off with other life-history parameters, such as somatic growth, longevity, age of first reproduction, etc. Sex allocation theory started with the simplifying assumption that an organism partitioned its energy into that dedicated to somatic growth and that dedicated to reproduction. Reality is seldom so simple. Reproduction through male function (i.e., sperm or pollen production and associated activity) may reduce somatic growth rate less than does reproduction through female function (i.e., production of eggs or seeds, etc.). This has been elegantly shown in the polychaete, *O. puerilis* (Berglund 1986), and in *Crepidula fornicata*, a gastropod (Broquet et al. 2015). Indirect evidence comes from the frequency of protandry in sequential hermaphrodites that lack social hierarchies (see above) and the observation that lamprey hatched in areas with high food availability have a preponderance of females (Johnson et al. 2017), whereas poorer habitats have a sex ratio skewed to males. Understanding the dynamics of the trade-offs, between somatic growth, male reproductive success, and female reproductive success as three variables, may provide insight into the stability of sexual systems. It might be the case that in phyla in which SH is virtually fixed, these three variables trade off with each other in ways that are quite different from the way they trade off with each other in phyla that are essentially exclusively dioecious. Other life history variables that may involve different trade-offs with male versus female reproductive success in SH versus dioecy may include age of first reproduction and longevity, as has been shown by Prevedelli et al. (2006) for *Ophyrotrocha* polychaetes. Understanding the complexity of life history trade-offs with male versus female reproductive success in a wider range of organisms will certainly enhance our understanding of the evolution of reproductive strategies in general and may help us to demystify Williams' Paradox (Williams 1975).

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Chapter 2
Phylogeny, Evolution, and Ecology of Sexual Systems Across the Land Plants

Andrea L. Case and Linley K. Jesson

Abstract Land plants dominate nearly every terrestrial habitat and include some of the largest and longest-lived organisms on earth. They are well known for their sexual diversity, reflecting tremendous variation in sex expression, and elaborate reproductive structures and behaviors. Much of what we understand about plant sexual diversity comes from studies of a single group—the flowering plants. Here, we discuss our current state of knowledge about sexual systems across the land plants and how principles and concepts derived from studies of angiosperms can (or cannot) be applied to mosses, hornworts, liverworts, ferns, fern allies, and gymnosperms. First, we show how variation in the expression and lability of sexual systems across the land-plant phylogeny raises fundamental questions about sexual-system evolution. Second, we discuss selective mechanisms, focusing specifically on polyploidy as a mechanism that may either constrain or facilitate evolutionary changes in sexual systems. Finally, we compare ecological traits that are commonly associated with alternate sexual systems in angiosperms and their (not so obvious) cognates in other land-plant groups.

2.1 Introduction

Land plants dominate nearly every terrestrial habitat and include some of the largest and longest-lived organisms on earth. They are well known for their sexual diversity, reflecting tremendous variation in sex expression, and elaborate reproductive structures and behaviors. Much of what we understand about plant sexual diversity comes from studies of a single group—the flowering plants (angiosperms). Compared to nonflowering plants (i.e., mosses, hornworts, liverworts, ferns, fern allies, and gymnosperms), angiosperms are the most species-rich (>350,000 described species;

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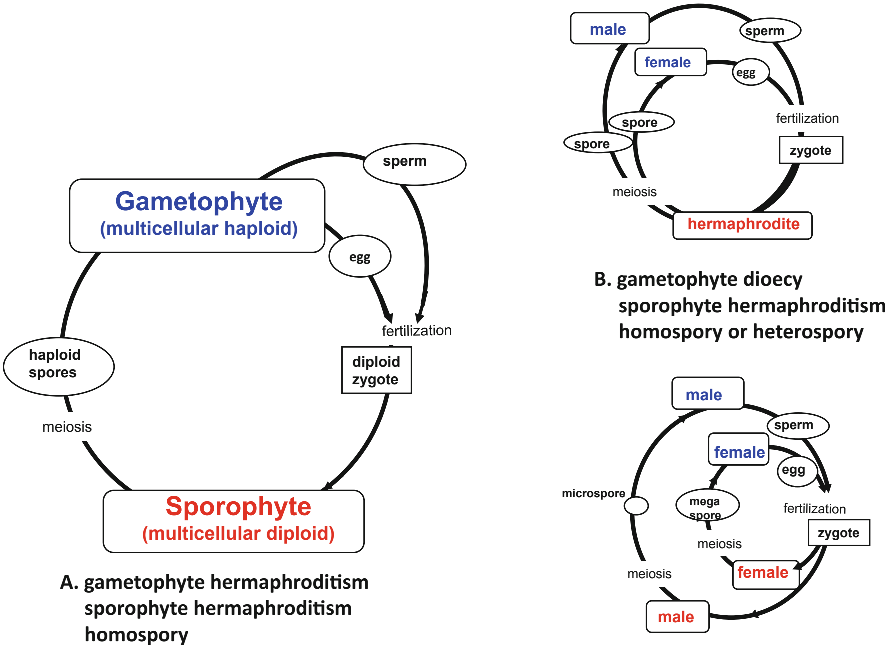
The Plant List v. 1.1, 2013), including the majority of species used for food and structural materials. The "bawdy" nature of flowers also makes their sexual diversity most apparent. Comparative analysis of reproductive biology across the land plants can reveal missing pieces in our understanding of the evolutionary history of plant reproduction and sex expression, as well as factors that generally promote or constrain the evolution of eukaryotic sexual systems in general.

Here, we discuss our current state of knowledge about land-plant sexual systems and how principles and concepts derived from studies of angiosperms can (or cannot) be applied to other land-plant groups. The chapter is organized into three sections: phylogeny, evolution, and ecology. In the first section, we show how variation in both the expression and the lability of sexual systems across the land-plant phylogeny raises fundamental questions about sexual-system evolution. In the second section, we discuss selective mechanisms, focusing specifically on polyploidy as a mechanism that may operate generally across the land plants to either constrain or facilitate changes in sexual systems. In the final section, we compare ecological traits that are commonly associated with alternate sexual systems in angiosperms and their (not so obvious) cognates in other land-plant groups.

To facilitate comparisons across the land plants, we focus on the two most common sexual systems: strict female-male dioecy (sex functions always completely separated between genets) or hermaphroditism (sex functions combined in any form during the life-span and including genets that may be genotypically hermaphroditic but functionally or environmentally dioecious). We use the terms "separate sexes" or "combined sexes" interchangeably with dioecy and hermaphroditism, respectively, acknowledging that although continuous variation exists between these two poles, there are far more dioecious and hermaphroditic plants than any other sexual system (Barrett 2002). The primary reason to dichotomize sexual variation is because it permits the identification of patterns and mechanisms that may be operating in other eukaryotes, such as animals, that also contain dioecious and hermaphroditic species at appreciable frequencies. The primary reason to consider functionally dioecious systems to be hermaphroditic is because the ability to adjust sex expression in response to ecological variation provides advantages not enjoyed by strictly dioecious systems; in other words, functionally dioecious populations can be considered the extreme end of a sexual lability spectrum (Delph and Wolf 2005). We refer readers to the chapter by Pannell for an examination of plant sexual-system transitions that accounts for finer-scale variation.

2.1.1 Duality of Sex Expression in Land-Plant Life Cycles

Unlike animals, land plants have a two-stage (dibiontic) life cycle, and their sexual systems usually differ between their two distinct life stages (Figs. 2.1 and 2.2). The haploid (gametophyte or gamete-bearing) and diploid (sporophyte or spore-bearing) stages in the life cycle can be physiologically independent of one another, so optimization of the sexual system to the environment may occur at either stage.



A. gametophyte hermaphroditism
sporophyte hermaphroditism
homospory

B. gametophyte dioecy
sporophyte hermaphroditism
homospory or heterospory

C. gametophyte dioecy
sporophyte dioecy
heterospory

Fig. 2.1 Diagram of the two-stage (dibiontic) life cycle of land plants for all known combinations of gametophyte (haploid) and sporophyte (diploid) sexual systems. (**a**) Both gametophyte and sporophyte are hermaphroditic, typical of some bryophytes and most ferns and lycophytes. (**b**) Gametophytes are dioecious, and sporophytes are hermaphroditic, typical of many bryophytes, some gymnosperms, and most angiosperms. (**c**) Both gametophyte and sporophyte are dioecious, typical of many gymnosperms and some angiosperms

Thus, in order to fully explore the phylogeny, evolution, and ecology of land-plant sexual systems, we must examine the dynamics of gametophytic and sporophytic stages separately, as well as steps that may be sensitive to selection for sexual specialization at each stage.

In all land plants (Fig. 2.1), haploid (1*n*) egg and sperm fuse to form zygotes that develop into diploid, multicellular sporophytes that produce haploid spores via meiosis; spores develop into haploid, multicellular gametophytes that produce haploid gametes (sperm/sperm cells and eggs) via mitosis. “Gametophyte dioecy” (a.k.a. dioicy) occurs when gametophyte genets produce only one type of gamete (either sperm or eggs), which contrasts with “gametophyte hermaphroditism” (a.k.a. monoicy) when gametophytes are at least capable of producing both eggs and sperm (whether or not they actually produce both). Spores can develop into female gametophytes, male gametophytes, or hermaphroditic gametophytes. For comparison across the land plants, we designate the sex of sporophytes (and spores)

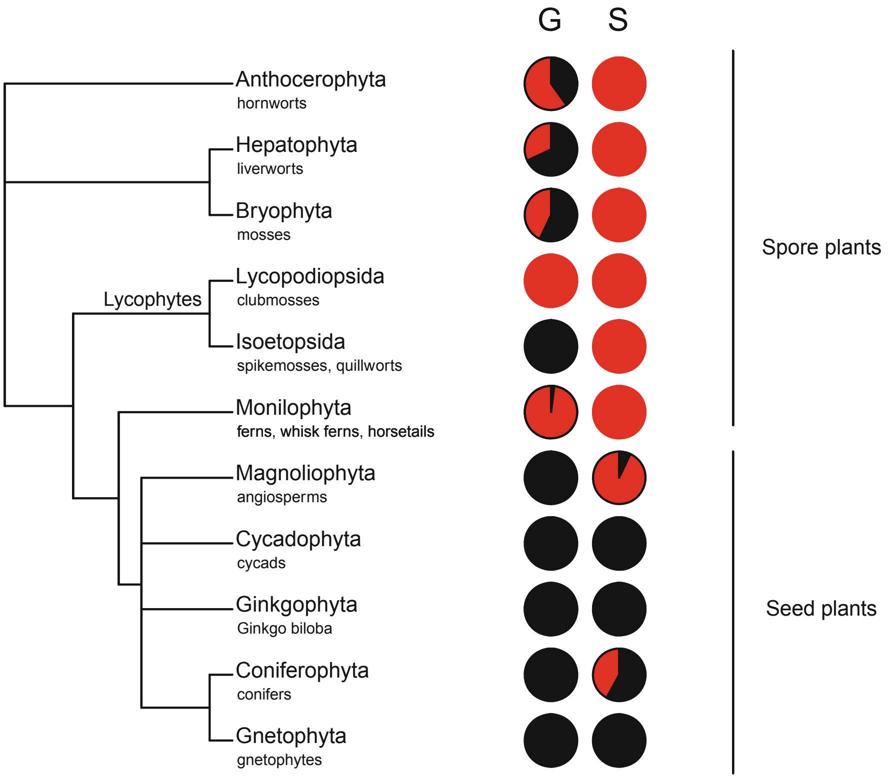


Fig. 2.2 Gametophyte (G) and sporophyte (S) sexual systems for the ten major clades of land plants. Proportion of species that are dioecious (black) or hermaphroditic (red) are indicated for each group and life stage. Lycophytes are split into its two major clades that show alternate gametophyte sexual systems. In all other groups, variation in sexual system occurs among or within families

Table 2.1 Sexual system definitions used throughout this chapter

Life stage	Hermaphroditism	Dioecy
Gametophyte	Gametophytes are capable of producing either sperm or eggs; includes groups with environmental sex determination that are functionally dioecious	Gametophytes are only capable of producing sperm (male) or only eggs (female) under any condition
Sporophyte	Sporophytes are capable of producing spores (gametophytes) of more than one sex	Sporophytes are capable of producing spores (gametophytes) of only one sex

based on the sex of gametophytes that arise from spores, as it has always been done for the seed plants (Jesson and Garnock-Jones 2012; cf. Cronk 2009). Thus, "sporophyte dioecy" occurs when sporophytes produce only female or only male spores, and "sporophyte hermaphroditism" occurs when sporophytes produce hermaphroditic spores and/or combinations of female and male spores (Table 2.1).

Table 2.2 Contrasting characteristics of the life cycles of seed plants (gymnosperms and angiosperms) and spore plants (mosses, hornworts, liverworts, ferns, and lycophytes)

Characteristic relevant to sexual reproduction	Seed plants	Spore plants
A. Establishment of new individuals (dispersal)	Dispersal of multicellular sporophyte embryos by sporophyte parent All sexes disperse equally as embryos, and only males disperse as gametophytes	Dispersal of unicellular spores (new gametophytes) by sporophyte parent All sexes disperse equally as spores/gametophytes
B. Opportunity for selection on gametophyte	Gametophytes matured within sporophytes, precluding independent selection on gametophytes	Gametophytes free-living, selection on sex occurs at both gametophyte and sporophyte stages
C. Control of gametophyte sex expression	Sporophyte	Genotype in bryophytes Environment in homosporous ferns and lycophytes Sporophyte in heterosporous ferns and lycophytes
D. Gametophyte sex expression	Gametophytes female or male	Gametophytes female, male, or hermaphrodite
E. Mode of fertilization	Fertilization takes place within sporophyte Entire male gametophyte (pollen) is dispersed to the female gametophyte Egg embedded within female gametophyte tissue embedded within sporophyte tissue	Fertilization takes place within egg-bearing gametophyte Male gametophyte releases sperm into environment Egg open to environment
F. Effect of selfing on heterozygosity	Selfing can only occur *between* non-identical (sibling) gametophytes within sporophytes, resulting in 50% reduction in heterozygosity (on average) in zygote	Selfing can occur either *within* hermaphroditic gametophytes with genetically identical gametes, resulting in 100% homozygosity in the resulting sporophyte or *between* sibling gametophytes (as in seed plants)

The ten extant major clades of land plants can be split into two groups based on the specific form of the diaspore—the stage of the life cycle that establishes physiologically independent individuals that are capable of mating (Fig. 2.2; Table 2.2A; see Cronk 2009). Adult *spore plants* are dispersed primarily as unicellular spores: Anthocerophyta (hornworts), Bryophyta (mosses), Hepatophyta (liverworts), Lycopodiophyta (clubmosses, spikemosses, quillworts), and Monilophyta (ferns, whisk ferns, horsetails). *Seed plants* establish after dispersing as multicellular embryos within seeds: Coniferophyta, Cycadophyta, Gnetophyta, and Ginkgophyta (all gymnosperms) and Magnoliophyta (angiosperms). These major clades are likely monophyletic and display several important features relevant to selection on sexual systems (Table 2.2). Many of these features derive from two key evolutionary events that transformed the life cycles of plants. First, most spore plants are homosporous (their spores are not sexually dimorphic), while some lycophytes, some ferns, and all seed plants are heterosporous, producing large female megaspores and small male microspores (Fig. 2.1). Second, in the seed plants, spores develop into gametophytes completely enclosed within sporophyte tissue. The retention of the gametophytes by the sporophyte effectively shifted control of sexuality to the sporophyte in seed plants (Table 2.2B, C) precluding selection at the gametophyte stage independent of the sporophyte, including selection on sex (Bateman and Dimichele 1994; Cronk 2009). The consequences of this shift for sex expression, fertilization, and mating (Table 2.2D–F) create vastly different evolutionary dynamics for spore-dispersed and seed-dispersed plant sexual systems.

2.2 Phylogenetic Distribution of Land-Plant Sexual Systems

Dioecy and hermaphroditism are found throughout land plants, but there is variation in whether separate or combined sexes occur predominantly at the gametophyte stage or at the sporophyte stage (Figs. 2.1 and 2.2). The frequency and distribution of sexual systems at both stages is well known in seed plants, and most of the hypotheses for the causes of sexual-system variation are based on studies of angiosperms (reviewed in Renner 2014). Sex expression is less well documented in spore plants, likely because of their smaller stature, infrequent production of sexual structures, and homospory (making it difficult to determine sporophyte sex). Because of these difficulties, variation in sexual systems of spore plants that we describe here is undoubtedly underestimated.

2.2.1 Phylogenetic Distribution of Gametophyte Sexual Systems

Gametophyte dioecy is far more common in plants than is gametophyte hermaphroditism, because all seed plants and a moderate majority of spore plants have separate-sexed gametophytes (Fig. 2.2; Table 2.2D), and seed plants are the most numerous land plants. In spore plants, evolutionary transitions between gametophyte dioecy and hermaphroditism have likely occurred numerous times. Gametophyte sexual systems are variable within the hornworts, liverworts, and mosses, indicating a high degree of evolutionary lability in these groups relative to ferns and lycophytes, which primarily segregate hermaphroditism and dioecy at higher taxonomic ranks (Longton and Schuster 1983; Villarreal and Renner 2013; Devos et al. 2011; McDaniel et al. 2012; Haig 2016; Fig. 2.2).

The ancestral sexual-system state of land-plant gametophytes is not well supported, in large part because of topological uncertainty at the base of the land-plant tree. Recent genomic evidence supports either a clade of mosses and liverworts or only the hornworts as sister to all other land plants (Wickett et al. 2014; Fig. 2.2). One reason to argue for gametophyte dioecy as the ancestral sexual system in land plants is that all gametophyte-dioecious species of hornworts, liverworts, and mosses share the same system of haploid chromosomal sex determination (males carry a single U chromosome, and females carry a single V; Bachtrog et al. 2011), consistent with a single evolutionary origin of dioecy in the earliest spore plants and independent transitions to gametophyte hermaphroditism within each major clade. Phylogenetic reconstructions of sexual systems show gametophyte dioecy to be the most likely ancestral condition in mosses and liverworts (McDaniel et al. 2012; Laenen et al. 2016), while the evidence in hornworts is equivocal, with gametophyte hermaphroditism having a slightly higher likelihood of being ancestral (Villarreal and Renner 2013). Knowing the ancestral sexual condition for land-plant gametophytes is key to elucidating how transitions between dioecy and hermaphroditism have occurred and why gametophytic sexual systems are labile in some groups and not others.

All seed plants are gametophyte dioecious, and it is unclear if gametophyte hermaphroditism is even possible in seed plants. The ancestral condition of the seed plants is most certainly gametophyte dioecy, and canalization of gametophyte dioecy could reflect a phylogenetic constraint related to the production of sexually dimorphic spores (heterospory) and/or a physiological or selective constraint imposed by retention of gametophytes by the parent sporophyte. In terms of phylogenetic constraint, all heterosporous ferns and seed plants are strictly gametophyte dioecious, so reversals to gametophyte hermaphroditism may be constrained (or even impossible) after the establishment of heterospory (Bateman and DiMichele 2002). The retention of spores by the parent sporophyte has both physiological and selective consequences related to sex expression. First, the immediate physiological environment of the gametophytes is tightly controlled by the sporophyte, such that there may be a physiological constraint on gametophyte sex by regulation of the environment during development. Second, reduction of the gametophyte as well as presentation of gametophytes by the sporophyte precludes independent selection on sex ratios in gametophytes (Bateman and Dimichele 1994). Gametophyte dioecy may be fixed in seed plants because evolutionary advantages of gametophyte hermaphroditism are unlikely to outweigh the evolutionary advantages of sporophyte hermaphroditism. Common advantages of hermaphroditism, such as reproductive assurance, are shared regardless of whether gametophytes are dioecious or hermaphroditic. On the other hand, as we discuss in Sect. 2.3, the consequences of selfing for heterozygosity and inbreeding depression differ based on gametophyte sex (Table 2.2F), which could reduce selection for hermaphroditic sex expression in gametophytes.

2.2.2 The Phylogenetic Distribution of Sporophyte Sexual Systems

Sporophyte hermaphroditism is more common in land plants than sporophyte dioecy, the former being fixed for all spore plants and the vast majority (>90%) of angiosperms (Fig. 2.2). In spore plants with gametophyte dioecy and haploid U/V sex determination, sporophytes must have a diploid UV genotype and so are always hermaphroditic (i.e., will always produce both male and female spores). However, the absence of sporophyte dioecy in spore plants is potentially illuminating because it should be possible (Jesson and Garnock-Jones 2012). Biased secondary sex ratios are common at the gametophyte stage among mosses (Stark et al. 2010; Bisang et al. 2014), but it is more difficult to assess bias in primary sex ratio (i.e., at the spore production stage). Sex-differential abortion or death of spores has been documented in at least one moss genus (*Ceratodon*), and this abortion has been shown to have a significant genetic component (McDaniel et al. 2007; Norrell et al. 2014), suggesting that spore sex ratios could evolve. The advantages of producing all-male or all-female gametophytes may be similar to that hypothesized for angiosperms—that it reduces local mate or resource competition (Lloyd 1982; see Sect. 2.4). If so, sporophyte dioecy may exist but remains unknown because of the difficulty in identifying spore-plant sporophytes that produce only one sex of spore. If sporophyte dioecy is truly absent, there may be important differences in the selection pressures experienced by spore versus seed plants at the sporophyte stage or constraints on sporophyte sex expression that have yet to be identified (e.g., effects of polyploidy, see Sect. 2.3).

In seed plants, evolutionary transitions between dioecious and hermaphroditic sporophytes have occurred numerous times (Leslie et al. 2013; Renner 2014). Sporophyte dioecy is very likely ancestral in at least three of the five extant seed-plant clades (Fig. 2.2)—Cycadophyta (~300 spp.), Gnetophyta (~80 spp.), and Ginkgophyta (1 sp.; species numbers from Villereal and Renner 2014). Sporophyte hermaphroditism (always monoecious) has evolved numerous times within the Coniferophyta, where sexual systems are variable within and among families (Leslie et al. 2013). For example, Pinaceae and Araucariaceae are nearly fixed for hermaphroditism; Taxaceae and Podocarpaceae are predominantly dioecious; sexual systems of Cupressaceae vary among lineages within the family. Although sporophyte dioecy (or at least single-sexed flowers) characterizes many major angiosperm clades outside eudicots and monocots, sporophyte hermaphroditism is predominant, with ~94% of flowering species exhibiting some form of hermaphroditism (Bateman et al. 2011), and sporophyte hermaphroditism is likely to be ancestral in this clade (Endress and Doyle 2015; Sauquet et al. 2017). Hundreds to thousands of recent transitions to dioecy have been inferred in flowering plants, mostly within genera that vary among species in sexual system, with a small number of strictly dioecious families accounting for ~10% of dioecious genera (Renner 2014). However, all ferns are uniformly sporophyte-hermaphroditic (including heterosporous ferns), and it is unknown for early seed plants how and when either sporophyte dioecy or lability in sporophyte sexual system was initially established.

2.3 Evolution of Land-Plant Sexual Systems

In the previous section, we identified two broad patterns of sexual-system lability across the land plants that require explanation (Fig. 2.2). First, gametophytic and sporophytic sexual systems shifted from being "labile gametophyte/fixed sporophyte" in spore plants to "fixed gametophyte/labile sporophyte" in seed plants. Second, some lineages have variable sexual systems within families (mosses, liverworts, hornworts, angiosperms, conifers), while others are fixed for a particular sexual system at higher taxonomic levels (ferns, lycophytes, gnetophytes, cycads), implying longer-term maintenance of sexual states.

In this section, we address intrinsic factors that may be associated with evolutionary lability of land-plant sexual systems. There is unlikely to be a single mechanism that explains the origin and maintenance of derived sexual systems across all groups. However, we focus here on the consequences of changes in ploidy/genome size as being potentially general, because genome size has been statistically associated with sexual-system transitions in each lineage of spore plants and in several genera of angiosperms (Ashman et al. 2013).

Increases in genome size, including whole genome doubling, have occurred repeatedly in plants and have contributed significantly to diversification and evolutionary innovation (Otto and Whitton 2000; Jiao et al. 2011). It is yet unclear how often changes in genome size promote sexual-system change or *vice versa* (Miller and Venable 2000; reviewed in Ashman et al. 2013). There are at least two ways that polyploidy can affect sexual-system evolution: *directly* by disrupting sex determination and altering sex expression or *indirectly* by changing the selective advantages of hermaphrodites, males, and females in particular contexts. Alternatively, factors favoring sexual-system transitions might actually result in polyploidy if these factors also affect the production or fusion of unreduced gametes. Below, we review evidence of links between genome size and sexual system in seed plants and then apply these patterns to what is currently known for spore plants.

2.3.1 Polyploidy and Sporophyte Sex in Seed Plants

Angiosperm sporophytes appear to have a greater likelihood of being hermaphrodite when diploid and separate-sexed when polyploid (Ashman et al. 2013), although transitions in both directions have been well documented. One probable case of *direct* transition from sporophyte dioecy to hermaphroditism with polyploidy is *Mercurialis annua* (Euphorbiaceae; Pannell et al. 2004; Obbard et al. 2006). Sexual systems and polyploidy have evolved multiple times within the genus and among populations of *M. annua* (Durand and Durand 1992; Obbard et al. 2006). Based on a recent study of sex determination in *M. annua*, likely scenarios for the evolution of hermaphroditism with polyploidy involve unreduced gamete formation either before or after hybridization between populations or species with different ploidy and, importantly, a change in the regulation of sex expression in polyploid individuals (Russell and Pannell 2014). The authors raise the possibility that sex determination in hexaploid individuals becomes sensitive to extrinsic factors (in this case, population density; Pannell 1997; Vilas and Pannell 2012). These potential effects of polyploidy on individual sex expression are particularly intriguing, as a similar process may account for the shift from genetic to environmental (density-dependent) gametophyte sex determination in ferns and lycophytes, which often have much higher chromosome numbers compared to bryophytes (see below).

An angiosperm example of an *indirect* transition from sporophyte hermaphroditism to dioecy with polyploidy is *Lycium* (Solanaceae). As in *Mercurialis*, sexual-system transitions associated with polyploidy have evolved multiple times within *Lycium* and among populations of *L. californicum* and *L. carolinianum* (Miller and Venable 2002; Yeung et al. 2005; Blank et al. 2014). However, in this case, diploid species are hermaphroditic, and polyploid species have separate sexes. Extensive work in this group implicates polyploidy in the breakdown of physiological self-incompatibility, followed by selection for dioecy to restore obligate outcrossing (Miller and Venable 2002). A recent study in *L. carolinianum* documented several diploid dioecious populations, making this species an exception from the strict association between polyploidy and separate sexes in the rest of the genus (Blank et al. 2014). Whether this means that dioecy has evolved first in general or just in some populations of species remains to be assessed. However, this pattern of exceptional species mirrors observations from several bryophyte genera (see below).

We found no published analysis of ploidy variation in the context of sexual-system evolution for gymnosperms. However, a recent survey of gymnosperm genome size revealed significantly larger genomes in Pinaceae, specifically in the genus *Pinus*, compared to all other groups (Burleigh et al. 2012). Pinaceae is almost exclusively hermaphroditic (Leslie et al. 2013). Three other well-sampled lineages in the Burleigh et al. (2012) study are predominantly dioecious and have small genomes relative to Pinaceae (i.e., Podocarpaceae, Cupressaceae, Gnetales). This may indicate a tendency for polyploid gymnosperms to be hermaphroditic, although it is worth noting that the majority of species included in the genome size survey were Pinaceae. It would be helpful to conduct this comparison explicitly in a phylogenetic context and to include more members of the other predominantly hermaphroditic family (Araucariaceae).

2.3.2 Polyploidy and Gametophyte Sex in Spore Plants

Increased chromosome numbers and polyploidy in spore plants are associated with shifts from dioecy to hermaphroditism (Klekowski and Baker 1966; Engel and Glenny 2008; Ranker and Geiger 2008; Devos et al. 2011; McDaniel et al. 2012; Cargill et al. 2013; Haufler 2014; Perley and Jesson 2015; Laenen et al. 2016), but not the reverse. In groups with haploid U/V sex determination (mosses, liverworts, and hornworts), this most likely represents direct shifts when polyploidy results in tetraploid UUVV sporophytes that produce UV spores if only homologous chromatids pair. In the moss genus *Atrichum* (Perley and Jesson 2015) and liverwort *Megaceros* (Engel and Glenny 2008; Cargill et al. 2013), allopolyploidy is associated with shifts to gametophyte hermaphroditism in multiple species. Gametophyte-dioecious hornworts also have fewer chromosomes (four autosomes and one U or V) compared to gametophyte hermaphrodites (five autosomes plus numerous accessory chromosomes; Villarreal and Renner 2013). However, as with the angiosperms, there are examples of dioecious allopolyploid species of mosses (*Atrichum crispulum*, Perley and Jesson 2015; *Sphagnum subsecundum*, Ricca and Shaw 2010), liverworts (*Radula*; Devos et al. 2011), and hornworts (*Phaeoceros laevis*; Villarreal and Renner 2013), suggesting that either polyploidy does not always result in direct shifts to hermaphroditism or that polyploids can revert to dioecy.

The evolution and maintenance of gametophyte hermaphroditism in ferns and lycophytes may also have been influenced by polyploidy, but the mechanism is likely different from that of mosses, hornworts, and liverworts. Considering gametophytes, hermaphroditic homosporous ferns do have higher chromosome numbers than gametophyte-dioecious heterosporous ferns, which was proposed to result from hybridization followed by genome doubling (Klekowski and Baker 1966; but see Haufler 2014). However, this shift to gametophyte hermaphroditism probably does not result from disruption of sex determination, as homosporous ferns and lycophytes have "extrinsic" sex determination of gametophytes (affected by abiotic conditions, population densities, or neighborhood gametophyte sex ratios; Table 2.2; DeSoto et al. 2008; Vega-Frutis et al. 2014). Klekowski and Baker (1966) hypothesized that polyploidy in homosporous ferns has contributed to the maintenance of hermaphroditism, as genetic variation lost via selfing could be compensated by heterozygosity at the level of the homologous chromosome pairs. However, there is considerable evidence that gene expression in ferns is "diploidized," suggesting that masking of deleterious alleles no longer occurs (reviewed in Haufler 2014). Additionally, most fern gametophytes are genetically hermaphroditic but functionally dioecious, reducing selfing and its associated costs (Ranker and Geiger 2008; Haufler 2014). Although sheltering of genetic load in polyploids may not be important in the maintenance of gametophyte hermaphroditism in homosporous ferns and lycophytes, polyploidy may have played a role in the breakdown of genetic sex. Hermaphroditic homosporous lycophytes also have higher chromosome numbers than dioecious heterosporous lycophytes (Barker 2013; heterosporous *Selaginella*, base chromosome number $1n = 8{,}9$, median chromosome number $= 18$; heterosporous *Isoetes*, base $= 22$, median $= 34$; homosporous Lycopodiaceae, base $= 23$, median $= 68$; data from www.tropicos.org, downloaded Feb 2016). However, this evidence is circumstantial, and polyploid series are present in all groups regardless of sexual system. Thus, there remains no general explanation why higher chromosome numbers should promote gametophyte hermaphroditism in spore plants.

Why are sexual systems not as labile in ferns and fern allies as they are in other spore plants? In homosporous ferns, environmental sex determination and the hormonal regulation of sexual system permit extreme gender plasticity, making them able to respond rapidly to environmental change and the density and distribution of neighboring individuals (Korpelainen 1998). Homosporous ferns are gametophyte hermaphroditic because they are capable of producing sperm or eggs or both (Table 2.1). However, in populations of ferns where gametophyte sex has been documented, gametophytes frequently produce only one gamete type when gametophyte densities are high (Ranker and Geiger 2008). Functional gametophyte dioecy at high densities and hermaphroditism at low densities would limit within-gametophyte selfing to situations of low mate availability. This means there may be little selective advantage for strict, genetically determined gametophyte dioecy, because this would mean the loss of environmental sex determination and the loss of reproductive assurance at low densities.

The lack of sexual lability in lycophyte gametophytes follows heterospory: heterosporous spikemosses and quillworts are fixed gametophyte dioecious, and homosporous clubmosses are fixed gametophyte hermaphroditic (Fig. 2.2). Little is known about the intrinsic or extrinsic control of sex expression in these groups. It remains to be seen whether genetic sex determination constrains transitions or whether there are other evolutionary or ecological explanations for infrequent transitions. For example, Bateman and Dimichele (1994) suggest that the stable aquatic habitats that allow sperm dispersal between individuals of heterosporous *Isoetopsida* minimize selection for reproductive assurance. Invoking ecological constraint to explain the lack of sexual system diversification in one group seems like an evolutionary "just-so" story, not likely to explain differences in evolutionary lability among groups (Korpelainen 1998).

2.4 Ecology of Land-Plant Sexual Systems

Ecological factors may mediate selection affecting the maintenance of sexual systems by influencing the relationship between sexual investment and fitness. In this section, we summarize the ecological factors long associated with transitions between sporophyte hermaphroditism and dioecy in the seed plants. We then discuss how these factors may manifest in in spore plants, which can experience selection on sexual systems of both gametophyte and sporophyte.

2.4.1 Ecology of Seed-Plant Sexual Systems

Hermaphroditic sporophytes in seed plants comprise the full spectrum of body types, life histories, and modes of seed and pollen dispersal. In contrast, dioecious angiosperms and gymnosperms are almost exclusively perennial and are typically also woody; large-bodied with numerous, inconspicuous flowers or cones; tropical or island inhabitants; pollinated by wind, water, or generalist insects; and dispersed by animals as seeds within fleshy fruits (reviewed in Sakai and Weller 1999). Two long-standing hypotheses interpret these correlates as traits that enhance the selective advantages of dioecy—avoiding the costs of selfing and inbreeding and specializing on limited resources available for growth, reproduction, or defense (Lloyd 1982). However, such advantages must outweigh the inherent costs of being unisexual—reduced mate availability compared to hermaphrodites and no reproductive assurance—and some ecological traits may be correlates of dioecy because they mitigate these costs (Eppley and Jesson 2008). Whether or not each of these correlates actually affects selection on sexual systems depends on the extent to which these traits are correlated with each other (Vamosi et al. 2003) and the extent to which combinations of traits affect rates of speciation or extinction (Donoghue 1989; Heilbuth 2000; Leslie et al. 2013; Renner 2014; Käfer et al. 2014). Spurious inter-trait correlations and/or changes in diversification rates make it challenging to identify key traits facilitating sexual-system evolution (Vamosi and Vamosi 2004; Käfer et al. 2014; Sabath et al. 2016).

Three common characteristics of sporophyte-dioecious seed plants may enhance the advantages of separate sexes while mitigating some of the costs (summarized in Table 2.3). One such trait is large body size, which is inherently accompanied by longevity and iteroparity and very often associated with a woody habit, clonality, and a large number of flowers produced per season (Sakai and Weller 1999). Size-related traits are associated with increased likelihood of within-sporophyte selfing (especially flower number) and greater inbreeding depression in plants (especially woody plants; Igic et al. 2008; Duminil et al. 2009), potentially favoring sporophyte dioecy as an obligate outcrossing mechanism. These same traits also increase the number of mating opportunities for females and males, which have fewer compatible mating types within populations compared to hermaphrodites. A second trait is fleshy fruits, which facilitate biotic seed dispersal and increase the cost of reproduction for female plants. The production of fleshy fruits is almost perfectly associated with dioecy in gymnosperms (Leslie et al. 2013). Dioecy in angiosperms permits resource specialization in the face of costly fruit and seed production (reviewed in Obeso 2002), and biotic dispersal enlarges seed shadows, increasing the availability of compatible mates by reducing the clumping of related co-dispersers that results when only one sex morph disperses seed (Heilbuth et al. 2001). A third trait is generalist or abiotic modes of pollen dispersal (Sakai and Weller 1999; Friedman and Barrett 2009), which include "generalist" insects, water, and wind as possible pollen vectors. Abiotic and generalist insect pollen vectors are less discriminating than specialized vectors, the latter of which may reduce pollen transfer between dioecious plants if they avoid a particular sex (e.g., females that do not offer pollen as a reward). High visitation and low discrimination come with a cost of increased self-pollen deposition, selecting for dioecy (Sakai and Weller 1999). Whether or not these common correlates of dioecy result from causal associations or not remains an active area of investigation (Käfer et al. 2014; Sabath et al. 2016).

Table 2.3 Comparison of traits associated with the advantages and costs of separate sexes in land plants

		Traits correlated with dioecy in angiosperms	Manifestation in sporophyte-dioecious seed plants	Manifestation in gametophyte-dioecious spore plants
Advantages of dioecy	Avoidance of selfing and inbreeding	Large body size, clonality, generalist pollination/fertilization increase selfing and its associated costs, favoring dioecy to enforce outcrossing	Large (woody) sporophyte with many flowers increases within-sporophyte selfing Generalist or abiotic pollination increases within-sporophyte selfing Large, long-lived sporophytes accumulate more mitotic mutations, increasing inbreeding depression	Clonality increases within-gametophyte selfing, rapidly reducing heterozygosity; dioecy precludes within-gametophyte selfing Water fertilization or movement of sperm by insects increases selfing within gametophytes or between sibling gametophytes Large, long-lived gametophytes or sporophytes accumulate mitotic mutations; deleterious gametophyte-expressed alleles readily purged; sporophyte-expressed genes increase inbreeding depression
	Resource specialization	Costly reproductive/dispersal structures favor specialization of resource investment	Fleshy fruits/seeds, biotic dispersal increases costs of seed production, favors resource specialization Long-lived, slow-growing species have more time to acquire resources	Larger sporophytes result in larger spore shadows Costly attractive chemicals released by female mosses enhance fertilization success Long-lived, slow-growing species have more time to acquire resources
Costs of dioecy	Reduced mate availability	Large body size, many flowers Costly dispersal structures compensate for reduced	Many, small flowers increase number of mating opportunities Fleshy fruits/seeds,	Attractive chemicals released by female mosses enhance fertilization success, splash

(continued)

Table 2.3 (continued)

		Traits correlated with dioecy in angiosperms	Manifestation in sporophyte-dioecious seed plants	Manifestation in gametophyte-dioecious spore plants
		proportion of population dispersing seed Generalist or abiotic fertilization agents less discriminating	rewards for biotic dispersal agents, increase seed shadows, favor increased mate densities Pollination by wind/water/generalist insects	cups in males increase gamete dispersal and mating opportunities Larger sporophytes and many, small spores enhance spore dispersal and gametophyte density, increasing mate availability; high gametophyte density increases ratios of male/female to hermaphrodite gametophytes in ferns Fertilization by water/generalist insects
	No reproductive assurance	Longevity and iteroparity increase opportunities to reproduce Generalist or abiotic fertilization agents more abundant and available	Large, long-lived (woody), clonal sporophytes have more time to reproduce Pollination by wind/water/generalist insects	Longer-lived or clonal gametophytes have more time to reproduce Fertilization by water/generalist insects

Generalized traits are derived from known correlates of dioecy in angiosperms and applied as specific traits to other plant groups

2.4.2 *Ecology of Spore-Plant Sexual Systems*

Are there similar ecological associations of gametophyte dioecy with body size and dispersal mode in spore plants, and are they likely to have similar costs and benefits to sporophyte dioecy? In Table 2.3, we propose several characteristics of spore plants that might be cognates of those listed for seed plants. These include clonality, large body size, longevity, and traits enhancing dispersal and fertilization success. Recent phylogenetic analyses in mosses, hornworts, and liverworts have enabled tests of some correlates of gametophytic sexual systems. Below, we provide a rationale for proposing these traits as correlates of dioecy in spore plants (Table 2.3), taking into account their unique dynamics of in dispersal, mating, and fertilization (Table 2.2A, E, F).

In spore plants, large body size in both life stages should be associated with gametophyte (but not sporophyte) dioecy. Spore-plant gametophytes typically achieve large body size via cloning, while large sporophytes are typically tall to facilitate spore dispersal. If large (clonal) gametophytes are hermaphrodite, then they should have greater potential for within-gametophyte selfing among clones (or branches) compared to smaller gametophytes, potentially favoring gametophyte dioecy in larger plants as a means of avoiding inbreeding. In addition, if mitotic mutations occur in genes that are expressed in the sporophyte (increasing inbreeding depression), then conditions where mitotic mutations are more frequent, such as large plant size and clonal reproduction, should also be associated with dioecy (Scofield and Schultz 2006; Haig 2016). It is often assumed that having an independent haploid life stage means inbreeding avoidance should be less important as a selective factor in spore plants than in seed plants, because haploid gene expression should rapidly purge deleterious alleles (Cronk 2009; Haig 2016). However, inbreeding depression has been shown in the diploid phase of several species of dioecious mosses (Taylor et al. 2007; Szovenyi et al. 2014), suggesting that selfing could have deleterious fitness effects that maintain gametophyte dioecy. Note that gametophyte dioecy still permits mating between male and female gametophytes produced by the same sporophyte, which is genetically identical to within-sporophyte selfing in hermaphroditic angiosperms (Table 2.2F).

It is likely that traits influencing spore and gamete dispersal shadows also affect selection on the sexual system in spore plants (Table 2.3); however, the predictions here are less clear. Limited (local) dispersal results in clumped distributions of sibling diaspores (spores or seeds). Thus, small dispersal shadows can contribute to lower outcrossed mating opportunities compared to larger dispersal shadows. Dioecy compounds the problems of small shadows for two reasons: first, because only one sex is responsible for dispersal (females for seeds or spores; males for sperm), and second, because males and females have fewer compatible mates than hermaphrodites. Thus, dioecy should not be associated with traits limiting dispersal shadows at any life stage. Sporophyte dioecy in seed plants has long been associated with fleshy fruits (reviewed in Vamosi et al. 2003; Leslie et al. 2013), which increases the overlap in dispersal shadows of unrelated seeds. A similar argument could be used to predict dioecy in spore plants with large spore dispersal shadows (Jesson and Garnock-Jones 2012). Although sporophyte dioecy has never been documented in spore plants (and Sect. 2.2), both Crawford et al. (2009) and Villerreal and Renner (2013) found correlations between smaller spore size and gametophyte dioecy, potentially because smaller spores result in larger dispersal shadows and more outcross mating opportunities for dioecious gametophytes. If inbreeding depression is high in dioecious populations, selection to reduce clumping of offspring gametophytes could reduce the expression of inbreeding depression caused by sib matings. However, Villarreal and Renner (2013) rightly point out that the advantage of long-distance spore dispersal is likely to be beneficial for hermaphroditic gametophytes also. Instead, Villarreal and Renner (2013) suggest that small spores may permit the production of more spores, resulting in a denser spore shadow and allowing sperm to more easily reach a gametophyte of the opposite sex. This form of reproductive assurance would be advantageous to dioecious gametophytes if sib matings were reduced; otherwise the evolution of gametophyte hermaphroditism should be favored. Thus, while there is some evidence for a correlation between enhanced spore dispersal and dioecy in spore plants, the evolutionary mechanism driving this association is not completely clear.

With respect to gamete dispersal shadows, one critical difference between spore plants and seed plants is the potential for sperm dispersal (Table 2.2E). Pollen grains (which bear sperm or spermatogenous cells) can travel farther than sperm; they can survive in air and are often carried by biotic vectors. Thus, seed plants can potentially mate over much longer physical distances than spore plants. If sperm dispersal results in selection on gametophyte sex, we would predict an association between gametophyte dioecy and traits that influence dispersal of gametes in spore plants, similar to the way wind pollination enhances pollen shadows in dioecious seed plants. Recent demonstrations in dioecious mosses and liverworts document traits specifically enhancing sperm dispersal (Table 2.3). In two dioecious moss species, sperm dispersal is enhanced by the movement of insects (specifically, *Collembola*) through moss beds. Intriguingly, these insects move in response to attractive volatile compounds produced by female gametophytes (Cronberg et al. 2006; Rosenstiel et al. 2012). Attractive chemicals may represent increased costs of reproduction for females, maintaining dioecy for resource specialization (Table 2.3). But insect-assisted fertilization may mean that moss sperm can sometimes move farther than previously thought. In the liverwort genus *Radula* (Devos et al. 2011), sperm dispersal is enhanced by epiphytism. Obligate epiphytism in *Radula* is associated with both increased sperm shadows in this genus and a lower likelihood of evolving hermaphroditism compared to facultative epiphytes (Devos et al. 2011).

2.5 Conclusions and Future Directions

There are two specific topics highlighted in this chapter that warrant further study. First, we still have an incomplete understanding of what kinds of events caused transitions between hermaphroditism and dioecy and what kinds of events created novel contexts that select for sexual-system shifts (i.e., direct vs. indirect mechanisms). For example, polyploidy may directly and immediately impose hermaphroditism on spore-plant gametophytes and seed-plant sporophytes by combining both sets of sex-determining genes within individuals. Alternatively, polyploidy may indirectly facilitate selection on sexual systems by altering the expression of inbreeding depression. Ecological traits that are correlated with dioecy are unlikely to occur simultaneously with sexual-system shifts. However, if they precede the evolution of dioecy, they may have altered the context for selection; if they follow the evolution of dioecy, they may contribute to its long-term maintenance. Second, we have an incomplete understanding of why lineages of plants differ so strikingly in the evolutionary lability of sexual systems and why lability manifests at different taxonomic scales (Korpelainen 1998).

Understanding how plant sexual systems change with genome size and with major life-cycle transformations requires deeper understanding of factors influencing gametophyte sex expression in spore plants and sporophyte sex expression in seed plants. The transition to hermaphroditism with increased genome size is more common than a transition to dioecy. However, it appears that there are multiple mechanisms in operation beyond the distinction of direct versus indirect effects described in the previous paragraph. For example, polyploidy has never resulted in hermaphroditic gametophytes in seed plants. This presumably reflects a shift to sporophyte control of gametophyte sex expression. But is the mechanism enforcing gametophyte dioecy a result of a phylogenetic constraint, constant physiological environment, or a lack of independent selection on seed-plant gametophytes (e.g., hypotheses by Bateman and Dimichele 1994, 2002)? More work dissecting the genetic and developmental pathways to separate sexes will help here (Renner 2016), particularly if they focus outside of the seed plants. Ferns and lycophytes hold a "central" evolutionary position within the land plants and are characterized by intermediate states in several key elements of sexual-system evolution. Their gametophyte sexual systems are less labile/polymorphic than bryophytes but more labile than seed plants. Control of gametophyte sex expression in ferns and lycophytes is also intermediate between genetically determined bryophytes and sporophyte-controlled seed plants and variable at high taxonomic levels. These characteristics permit a more nuanced understanding of the mechanisms affecting plant sex expression.

We highlight several important differences between seed-plant and spore-plant life cycles that may be keys to deriving general processes affecting sex expression across the land plants (Tables 2.2 and 2.3). We argue that the opportunity for within-gametophyte selfing in spore plants but not seed plants (Table 2.2E) makes a particularly important difference. First, within-gametophyte selfing results in complete and immediate sporophyte homozygosity rather than a 50% reduction in heterozygosity in plants with gametophyte dioecy. Thus, the success of gametophyte dioecy as a mechanism to avoid the expression of inbreeding depression will be affected by this complete reduction in heterozygosity with selfing. Second, polyploidy (and its consequences for the evolution of sexual systems) should be more likely to arise in lineages with hermaphroditic gametophytes. This is because the production of unreduced gametes occurs during meiosis. Any such event that occurs in hermaphroditic gametophytes should result in unreduced eggs and sperm produced in the same meiotic event and in close proximity to each other. Accounting for these patterns will require fuller understanding of the causes and consequences of life cycle changes for sex expression and selection on sex across the land plants.

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Chapter 3
Transitions Between Combined and Separate Sexes in Flowering Plants

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Abstract Most flowering plants have combined sexes and are functionally hermaphroditic. However, dioecy has evolved frequently and occurs in about half of flowering plant families. In this chapter, I consider reasons for the high frequency of hermaphroditism in flowering plants, drawing particularly on economic arguments that relate investment toward male and female function to the fitness gained through each function, separately or together. I then summarize two leading hypotheses for the evolution of dioecy from hermaphroditism: the potential advantages of sexual specialization and to the role that separation of the sexes may play in avoiding inbreeding. Finally, I review the major evolutionary paths that have likely been followed in transitions from hermaphroditism to dioecy and back again.

3.1 Introduction

The great majority of flowering plant species are hermaphroditic (Yampolsky and Yampolsky 1922; Barrett 2002; Charlesworth 2006), but separate sexes (dioecy) have evolved repeatedly. Although dioecious species occur in about 40% of the approximately 400 angiosperm families, only about 6% of all species have fully separate sexes (Renner and Ricklefs 1995; Weiblen et al. 2000; Case et al. 2008; Renner 2014). Few large clades are entirely dioecious, and most dioecious lineages are scattered as single species or small clades through the angiosperm phylogeny (Kafer et al. 2014; Renner 2014).

The phylogenetic distribution of dioecy among flowering plants suggests that separate sexes have evolved frequently but that dioecy may ultimately represent an evolutionary dead end. Early comparative analysis indeed pointed to increased extinction and reduced diversification rates of lineages that evolve dioecy (Heilbuth 2000; Vamosi et al. 2003; Vamosi and Vamosi 2004). However, recently it has been shown that if speciation typically precedes the evolution of dioecy in one of two

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diverging lineages, derived dioecious lineages become *less* likely to face extinction than their hermaphroditic counterparts (Kafer et al. 2014). If dioecy is not a dead end, its scattered phylogenetic distribution might be due to more frequent reversion to hermaphroditism than has been thought (Kafer et al. 2014, 2017), i.e., it might be the result of frequent transitions between hermaphroditism and dioecy in both directions.

In this chapter, I explore how and why hermaphrodite plants have so frequently "been rendered dioecious," as Darwin (1877) put it, and how and why dioecy might later revert to hermaphroditism. The transition to dioecy from hermaphroditism poses a puzzle, because the frequency of hermaphroditism among flowering plants indicates that it must often be a successful sex allocation strategy. I first consider why hermaphroditism might be so frequent in flowering plants in general, and then I examine hypotheses for its evolution toward dioecy and the paths along which the transition has progressed. Finally, I ask to what extent transitions from combined toward separate sexes are unidirectional (Barrett 2013) and whether, why, and how transitions from dioecy back toward hermaphroditism might occur.

3.2 Why Hermaphroditism?

Why should the vast majority of flowering plants have adopted a hermaphroditic allocation strategy for sexual reproduction? To address this question, it is helpful to think about the relative advantages and disadvantages of combined versus separate sexes in terms of so-called fitness gain curves, or fitness sets, that relate the male versus female components of sexual reproduction to investment of limited resources (Charnov 1982; West 2009). Analysis of such fitness sets has established that separate sexes should evolve from hermaphroditism if investment in at least one sexual function yields a greater than linear fitness return, i.e., if the fitness gain curves are an accelerating function of investment—though this prediction much be modified for inbreeding populations (Charlesworth and Charlesworth 1981). By contrast, if the gain curves are a saturating function of investment, we should expect hermaphroditism to be evolutionarily stable (Charnov et al. 1976). The overwhelming prevalence of hermaphroditism in flowering plants (Yampolsky and Yampolsky 1922; Renner 2014) thus points strongly to the likelihood of saturating gain curves.

Why are fitness gain curves in plants more likely to be a saturating function of investment (promoting the maintenance of hermaphroditism) than an accelerating function, and what conditions might cause them to accelerate (promoting gender specialists)? Direct evidence is lacking, as gain curves have seldom been quantified for plants. However, answers to these questions follow intuitively from consideration of how plants disperse their genes through pollen and seeds. The dynamics of pollination, particularly in animal-pollinated species, means that investment in male function is likely to be saturating. Animal pollinators tend to visit many flowers on the same plant (Ohashi and Yahara 2001) and to deliver the pollen that accumulates on their bodies from one plant to a limited number of recipient flowers (Barrett and

Harder 1996). Under such circumstances, the production of more pollen will then tend to lead to more intense competition among pollen grains dispersed by the same individual to sire a limited number of ovules, i.e., to "local mate competition" (lmc, Hamilton 1967); lmc is likely to be intense in animal-pollinated species (Lloyd 1982).

Investment toward increased attractiveness (e.g., more flowers open simultaneously or the production of more nectar) is also likely to flatten fitness gains, because pollinators will tend to visit more flowers on the same plant, transferring pollen among them rather than to other individuals in the population (Klinkhamer et al. 1994). Similar arguments apply to fitness returns through a plant's female function (de Jong and Klinkhamer 2005). The production of increasing numbers of seeds is likely to translate less than linearly into fitness gains if most seeds are dispersed into a limited "seed shadow" around the plant (Heilbuth et al. 2001), because the progeny of a given individual will then compete with each other for space and resources for growth ("local resource competition" or lrc, Clark 1978).

A related likely advantage of hermaphroditism was suggested by Wilson and Harder (2003), who noted that the relative immobility of plants (as well as modular animals, such as corals) must often limit their reproductive opportunities, because immobile organisms are both more likely to mate with their neighbors and to disperse their offspring nearby. If male and/or female gain curves are saturating, variance in mating and dispersal among individuals can reduce their average reproductive success as a result of Jensen's inequality (Wilson and Harder 2003). Because these variances would tend to be greater in populations with separate sexes, dioecy should be at a disadvantage relative to hermaphroditism—unless dioecy were associated with mechanisms for broad dispersal of gametes and/or offspring. In this sense, Jensen's inequality enhances the disadvantage of separate sexes that derive directly from saturating gain curves (Wilson and Harder 2003).

The constraints placed by limited pollen and/or seed dispersal on a plant's ability to translate investment into fitness not only help to account for why so many flowering plant species are hermaphroditic (Fig. 3.1), but they also provide a plausible explanation for many of the adaptations found in flowers and fruits that mitigate against lmc, lrc, and the implications of Jensen's inequality (Wilson and Harder 2003). For example, many plants do not open all their flowers at once, so that a less attractive floral display is presented to pollinators over longer periods (Harder and Wilson 1994; Klinkhamer et al. 1994). Similarly, many species have mechanisms to dispense pollen little by little to pollinators as they visit, rather than all at once (Harder and Barclay 1994). These explanations also suggest why species that do evolve separate sexes are more likely to have mechanisms for the broad dispersal of their pollen and seeds (see below).

Finally, hermaphroditism may be advantageous over dioecy because it represents a more cost-effective way of distributing resources between male and female functions. Specifically, because animal-pollinated plants must invest resources in attracting and rewarding pollinators, hermaphroditism may represent economization of resources if investment toward enhancing one sexual function (e.g., larger, longer-lasting petals or more nectar) also contributes to transmitting genes through the other

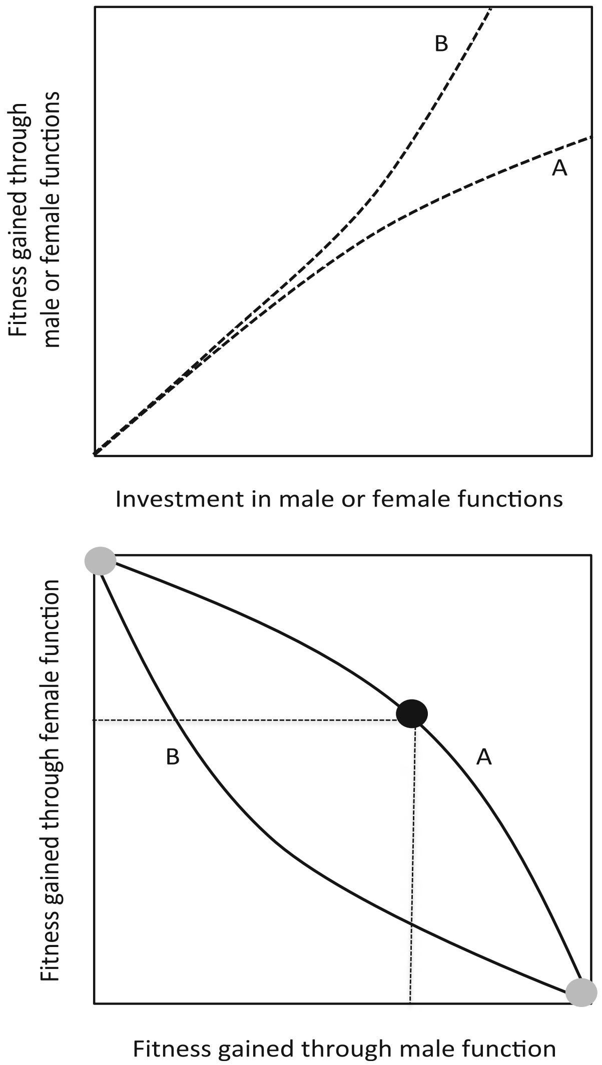

Fig. 3.1 Effects of traits influencing the success of dispersal on fitness gain curves and the evolutionary stability of combined versus separate sexes. Top panel: Expected fitness returns through male or female functions as a function of resources invested in that function. Curve A illustrates saturating returns upon investment, such as when pollen grains produced by an individual compete for mating opportunities (e.g., because of limited dispersal to few mating partners; local mate competition or “lmc”) or when sibling seedlings compete for limiting local resources (because of limited dispersal; local resource competition or “lrc”). Curve B illustrates accelerating returns, which could arise when sexually antagonistic adaptations compromise the fitness of individuals that invest in both sexual functions and favor those that invest all resources in one sexual function, e.g., adaptations that increase dispersal distances, limit male-female interference, and/or cause high investors to attract disproportionately more effective pollinators or seed dispersals than low investors. Bottom panel: “Fitness sets” relating realized male and female fitness. Curves A and B are fitness sets expected as a result of saturating or accelerating fitness gain curves, respectively, such as those indicated in the top panel. The black circle indicates a hermaphroditic evolutionary stable strategy (ess); the gray circles indicate a dioecious ess

(Heath 1977). This hypothesis, which essentially invokes fixed costs in the production of flowers that must be made before any returns on investment can be expected, helps to explain not only why hermaphroditism is common in animal-pollinated plants but also why it is particularly so in species with large, expensive flowers such as those pollinated by birds and bats (Renner and Ricklefs 1995).

3.3 Why Separate Sexes?: The Sexual Specialization Hypothesis

Two non-exclusive broad hypotheses have been put forward to explain the frequent transitions from hermaphroditism to dioecy: the “sexual specialization hypothesis” and the “inbreeding avoidance hypothesis.” The sexual specialization hypothesis posits that individuals should enjoy fitness benefits resulting directly from specializing on only one sex or the other (Charnov et al. 1976; Charlesworth and Charlesworth 1981; Charnov 1982). Thus, individuals producing pollen, say, should focus their efforts on maximizing their siring success, because investment in some female function would compromise fitness more than the gains accrued by producing seeds as well (and *vice versa* for female function). In this sense, it is the antithesis of the “fixed costs” explanation mentioned in the last section, which supposes that investment in one sexual function might enhance fitness accrued through the other. The sexual specialization hypothesis also rests conceptually on the analysis of fitness gain curves and how their saturation might be limited in dioecious species with specialized males and females (see previous section).

If a need to limit the fitness-saturating effects of lmc, lrc, and the implications of Jensen’s inequality is responsible for the evolution of hermaphroditism, then separate sexes should evolve largely in species with adaptations that substantially reduce their effects (Wilson et al. 1994). Male fitness is less likely to be limited by lmc in wind-pollinated species, for which fitness gains should vary more linearly with investment in pollen production, both because the pollen vector is not quickly saturated with pollen and because scramble competition among pollen grains from different individuals should favor the dispersal of more pollen (Klinkhamer et al. 1997). It is thus not surprising that a disproportionate number of dioecious species are wind-pollinated (Friedman 2011). The strong association between dioecy and the possession of fleshy fruits (Renner and Ricklefs 1995), which might be a more effective means of dispersing seeds, could result from selection to reduce lrc. Unfortunately, little direct evidence demonstrates the actual impacts of different modes of pollen and seed dispersal on the intensity of lmc and lrc, and there is a need for studies that test these hypotheses.

A common feature of dioecious plants that underscores the general benefits of sexual specialization is secondary sexual dimorphism, i.e., differences between males and females in vegetative morphology, life history, physiology, and defense (reviewed in Geber et al. 1999; Moore and Pannell 2011; Barrett and Hough 2013).

Although plants rarely show the extremes of sexual dimorphism of some animals (reviewed in Emlen 2008), almost all studied dioecious plants show it to some extent (Lloyd and Webb 1977; Moore and Pannell 2011; Barrett and Hough 2013). The ubiquity of sexual dimorphism in dioecious plants is thus a strong indication that optimization of male versus female functions ultimately requires divergent strategies, and sexual specialization must thus enable real and substantial benefits that are not typically available to hermaphrodites (but see Pickup and Barrett 2012).

Male and female phenotypes are ultimately the expression of the same common genome, so sexual differentiation must require either the evolution of genomic regions or expression patterns of specific genes, which differ between the sexes (Rhen 2000). The first possibility may be realized by the evolution of genetically divergent sex chromosomes. Indeed, the most plausible current model for the evolution of sex chromosomes involves the accumulation of genes in complete linkage to the sex-determining locus that have sexually antagonistic expression, i.e., whose alleles confer different fitness on males or females (Charlesworth and Charlesworth 2005; Charlesworth et al. 2005). Sexually antagonistic genes are precisely those that would underlie secondary sexual dimorphism (Rice 1984). The sex-determining region is enriched for genes implicated for sexual dimorphism in some dioecious plants with well-developed sex chromosomes (Schultheiss et al. 2015), but the genetic architecture of traits expressed differently in males and females is still poorly understood.

Once evolved, it is likely that sexual dimorphism stabilizes separate sexes by contributing to linearization (or even acceleration) of the fitness gain curves (Charnov et al. 1976; Charnov 1982). But could the “possibility” of sexual specialization contribute to the evolution of separate sexes in the first place, e.g., through an inchoate sexual dimorphism in still-hermaphroditic individuals that emphasize one sexual function over the other? This question has not been addressed thoroughly in the theoretical or empirical literature, despite bearing directly on the evolutionary paths between combined and separate sexes; it is thus an area that would repay in-depth analysis.

3.4 Why Separate Sexes?: The Inbreeding Avoidance Hypothesis

The inbreeding avoidance hypothesis posits that dioecy might evolve because unisexual individuals avoid self-fertilization and the associated disadvantages of the expression of inbreeding depression by inbred progeny (e.g., Charlesworth and Charlesworth 1978). Specifically, in partially selfing hermaphroditic populations in which progeny suffer from inbreeding depression, male-sterility mutations may be advantageous because the resulting females can produce only outcrossed progeny (Charlesworth and Charlesworth 1978). Such mutations should theoretically spread if the product between inbreeding depression (the relative fitness of selfed compared with outcrossed progeny) and the selfing rate of hermaphrodites exceeds 0.5 (Charlesworth and Charlesworth 1978; Charlesworth 1999). Above this threshold, male-sterile mutants have a genetic transmission advantage, despite foregoing opportunities for transmitting genes through their male function.

The 0.5 threshold is reduced if male sterility is compensated by the production of a greater number of progeny, e.g., as a result of the reallocation of resources from an abandoned male function to seed production (Lewis 1941; Frank 1989; Bailey et al. 2003; Dufay et al. 2007). Substantial empirical work now supports such compensation or trade-offs, with females in most investigated gynodioecious species (in which females co-occur with hermaphrodites) showing some advantage in their female function (Webb 1999; Shykoff et al. 2003; Dufay and Billard 2012; Spigler and Ashman 2012). Importantly, male-sterility mutations may spread more easily in populations if they are only transmitted via ovules, i.e., if they are in the mitochondrial genome, which is usually maternally transmitted. In such a situation, male-sterile individuals will need only to be marginally more successful as mothers than hermaphrodites (reviewed in Charlesworth 1999). Indeed, many cases of gynodioecy are the result of maternally inherited male-sterility mutations.

The likely importance of inbreeding avoidance in the evolution of separate sexes in flowering plants is illustrated by interesting associations with ploidy in some lineages (see Chap. 2). In *Lycium*, for example, gynodioecy and dioecy have evidently evolved in populations after a transition to polyploidy (Miller and Venable 2000). Hermaphroditic *Lycium* species have a molecular self-incompatibility (SI) system that prevents pollen from fertilizing ovules on the same plant. Importantly, SI in *Lycium* is disrupted by genome duplication, leading to self-compatibility, and it appears that gynodioecy and dioecy have evolved in polyploid lineages as an alternative outcrossing mechanism (Miller and Venable 2000). A similar idea might explain the high frequency of dioecy among plant species on oceanic islands (Baker and Cox 1984; Sakai et al. 1995; Barrett 1996). In this situation, self-incompatibility may first be lost, a transition facilitated by advantages of self-fertility during episodes of colonization when mates are unavailable (Baker 1955), with separate sexes evolving in established populations later in response to selection for inbreeding avoidance (reviewed in Pannell 2015; Pannell et al. 2015).

Perhaps the strongest indirect evidence for the importance of inbreeding avoidance in the evolution of separate sexes is the high prevalence of gynodioecy with nuclear sex determination relative to androdioecy, where males coexist with hermaphrodites (Darwin 1877; Lloyd 1975b). The evolution of gynodioecy and androdioecy requires the spread of male- or female-sterility mutations, respectively, in a hermaphroditic population (Charlesworth and Charlesworth 1978). As noted above, male-sterility mutations will be promoted if hermaphrodites are partially self-fertilizing and produce lower-quality offspring because of inbreeding depression. In contrast, androdioecy cannot readily evolve under the same conditions, because selfing by hermaphrodites (whether it involves inbreeding depression or not) effectively removes a fraction of ovules from the population for which males might have competed to fertilize (Pannell 2002). The rarity of androdioecy compared with gynodioecy (Charlesworth 1984, and see below) is thus fully consistent with inbreeding avoidance being important during early stages in the evolution of separate sexes—though, importantly, gynodioecy in species with self-incompatible hermaphrodites cannot be explained in this way (Dufay and Billard 2012).

3.5 Evolutionary Paths from Hermaphroditism to Dioecy

At least two mutations are required for dioecy to evolve from hermaphroditism, the first rendering some individuals sterile through one sex role and the second rendering the remaining hermaphrodites sterile through the other sex role (Charlesworth and Charlesworth 1978). This sequence can involve two alternative intermediate states in the evolution of fully separate sexes and thus two evolutionary paths via gynodioecy and androdioecy, respectively. Gynodioecious populations would become dioecious when mutations cause hermaphrodites to become male, whereas hermaphrodites in androdioecious populations must eventually become female for dioecy to evolve.

As gynodioecy evolves much more readily than androdioecy (Charlesworth and Charlesworth 1978), the gynodioecious path toward dioecy is also likely followed the more frequently (see Fig. 3.2a). Indeed, males of dioecious species often retain clear signs of a likely hermaphroditic history: they are often “inconstant” or “leaky” in their expression, producing a small proportion of fruits and seeds (Ehlers and Bataillon 2007). That males of dioecious species are more often inconstant in their gender expression than females (Lloyd and Bawa 1984) suggests that dioecy has evolved through the initial spread of a major male-sterility mutation, followed by mutations that gradually modified the hermaphrodites to become increasingly male in their sex allocation (Lloyd 1974; reviewed in Charlesworth 1999; Spigler and Ashman 2012).

In species in which dioecy evolved via the gynodioecious path, the genetics of sex determination suggests that the initial male-sterility mutation was typically recessive (Charlesworth and Charlesworth 1978). Specifically, most dioecious species have an XY sex determination system (Ming et al. 2007), with male function thus suppressed by a male-sterility mutation carried on both X chromosomes (in the case of evolution from cytoplasmic male sterility, a dominant nuclear restorer would occur on the Y chromosome (see Schultz 1994). This is consistent with male-sterility alleles mostly involving loss-of-function mutations, which are typically recessive in their expression (Charlesworth and Charlesworth 1978). If so, mutations that enhance male function at the expense of female function in hermaphrodites must be dominant in their expression. Crosses using genotypes of the dioecious plant *Silene latifolia* with various deletions on the sex chromosomes have revealed patterns of expression consistent with this basic model (Lebel-Hardenack et al. 2002). However, the developmental genetics of sex determination in plants is still poorly understood (Diggle et al. 2011; Renner 2016).

Androdioecy might constitute an alternative intermediate step in the evolution of dioecy from hermaphroditism, but most investigated cases of androdioecy are probably products of the breakdown of dioecy (Pannell 2002, and see next section),

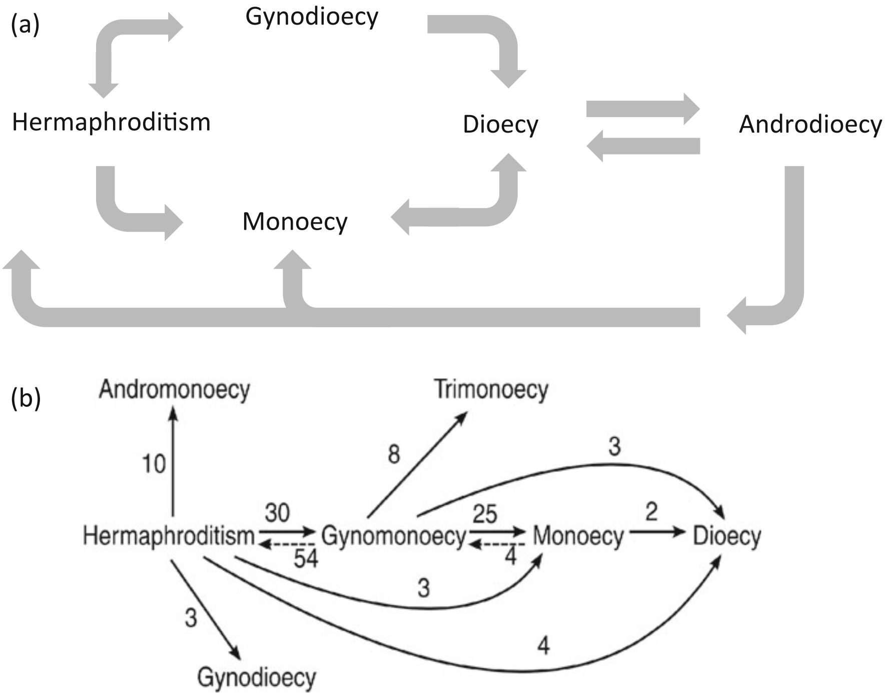

Fig. 3.2 (**a**) A general scheme setting out the possible evolutionary transitions between various configurations of combined and separate sexes in flowering plants, with the arrows indicating the most likely paths on the basis of theoretical considerations and empirical observations. The scheme is somewhat more inclusive than that suggested by Barrett (2002), as it identifies the possible reversion from androdioecy to hermaphroditism with bisexual flowers and to hermaphroditism with unisexual flowers (i.e., monoecy, which is more likely when androdioecy has evolved from monoecious ancestors), as well as reversions from separate to combined sexes for gynodioecy to hermaphroditism and from dioecy to monoecy. (**b**) A scheme setting out transitions between different sexual states in the daisy family (Asteraceae), as inferred by Torices et al. (2011). Dashed arrows indicate reversals, and numbers indicate the absolute number of transitions inferred by phylogenetically informed comparative analysis for the relevant path. Adapted from Torices et al. (2011)

although recent studies of two species in the olive family (Oleaceae) suggest that androdioecy can evolve from hermaphroditism under special conditions (Saumitou-Laprade et al. 2010; Billiard et al. 2015). These latter studies draw attention to the unusual conditions required for the path to be possible, i.e., conditions that allow males to sire more than twice the number of successful offspring in the population than are sired by hermaphrodites (Pannell and Korbecka 2010). In *Phillyrea angustifolia* and *Fraxinus ornus*, woody shrubs or trees in the Mediterranean Basin, androdioecy evolved from hermaphroditism through the spread of female-sterile individuals that nevertheless enjoy a substantial siring advantage, even though they produce little more pollen than the non-male-sterile hermaphrodites. This advantage arises from unusual linkage between the sex-determining locus and a

Fig. 3.3 Two related sexual systems in the olive family, Oleaceae. The top panels show flowers from a distylous population of *Jasminum fruticosum*, with (**a**) a long-styled "pip" morph, showing the stigma at the opening of the floral tube, and (**b**) a short-styled "thrum" morph, showing its two anthers at the (dissected) opening of the floral tube and the stigma deep inside the tube. Bottom panels show two morphs of the androdioecious species *Phillyrea angustifolia*, with (**c**) a hermaphrodite flower, showing a stigma held between two anthers, and (**b**) a male flower, with two anthers but lacking a functional stigma. It is likely that androdioecy in *Phillyrea angustifolia* derives from an ancestral distylous condition. Images in (**a**) and (**b**) by the author. Images in (**c**) and (**d**) by C. Hughes

self-incompatibility locus (Saumitou-Laprade et al. 2010). Remarkably, both species have a self-incompatibility system that is otherwise found only in distylous species (Saumitou-Laprade et al. 2010; Billiard et al. 2015; Vernet et al. 2016). In such species, individuals can neither self-fertilize nor fertilize ovules of unrelated individuals with the same mating type. This incompatibility system probably evolved in association with full distyly (e.g., as found in *Jasminum* species in the same family) and has evidently been retained in *P. angustifolia* and *F. ornus* despite the loss of the morphological manifestation of distyly (dimorphisms in style, anther and stigmatic papilla length, as well as pollen size) (Fig. 3.3). What matters is that hermaphrodites comprise two mating classes in populations of *P. angustifolia* and *F. ornus*, so that a given hermaphrodite can sire ovules on only half the other hermaphrodites in the population, whereas female-sterile individuals can sire offspring on all hermaphrodites (Saumitou-Laprade et al. 2010; Vernet et al. 2016). Thus, the female-sterility locus either renders its pollen grains compatible with the stigmas of individuals that would otherwise be incompatible, or it is linked to such a locus. Functionally, female sterility acts to double the siring prospects of individuals that carry the allele, regardless of whether these individuals produce more pollen (Pannell and Korbecka 2010). Although androdioecy in *P. angustifolia* and *F. ornus* is unusual, it highlights the fact that males can invade and spread in a population only if they are sufficiently competitive sires.

Dioecy may also evolve from hermaphroditism via distyly or "heterodichogamy" in a more straightforward way (Ornduff 1966; Ross 1982; Webb 1999; Pannell and Verdu 2006). In the distyly path, dioecy evolves through the gradual divergence in sex role between the two style morphs, with one becoming more male and the other more female. This has not been a frequent evolutionary path to dioecy, but a number of species have evolved dioecy in this way, and in many distylous species, the average gender differs between the short- and long-styled individuals (with long-styled individuals typically producing more seeds than short-styled ones, which requires that short-styled individuals were better sires) (Darwin 1877; Baker 1958; Ross 1982). In analogy to distyly, "heterodichogamous" populations comprise two classes of hermaphrodites, one protandrous (male function prior to female function) and the other protogynous (female function first) (Renner 2001). Modeling indicates that the temporal segregation of sex roles in heterodichogamous species can facilitate the evolution of dioecy and that, in particular, female-sterility mutations expressed in formerly protandrous individuals are as likely to spread as male-sterility mutations, so that dioecy might evolve via a type of androdioecy (Pannell and Verdu 2006).

The other major evolutionary path to dioecy from hermaphroditism is via monoecy, i.e., hermaphrodites with separate male ("staminate") and female ("pistillate") flowers (reviewed in Charlesworth 1999; Barrett 2002; see Fig. 3.2). Phylogenetic comparative analysis found a particularly strong association between monoecy and dioecy within genera, suggesting that dioecy evolves frequently from monoecious ancestors, rather than in lineages with bisexual hermaphroditic flowers (Renner and Ricklefs 1995). Rather than dioecy evolving via the initial spread of a male- or female-sterility mutation, it arises from increasing sexual specialization, with some individuals producing more male flowers at the expense of their female flowers and *vice versa* for individuals specializing on female function (Barrett 2002). Populations evolving along the monoecy path will tend to pass through an intermediate state of "subdioecy" or "paradioecy" (the path is sometimes referred to as the "monoecy-paradioecy" path), with both males and females producing substantial numbers of flowers of the opposite gender (Lloyd 1980). The monoecy path has received little theoretical or empirical attention, but it seems likely that carefully studied cases would point to sexual specialization as a potentially more important evolutionary driver than inbreeding depression. This is because gradual reduction in pollen production by plants in the sub-female class would not be expected to reduce inbreeding very much compared to a major male-sterility mutation (leading to gynodioecy), which would immediately avoid all inbreeding in the affected individuals.

Finally, it is important to note that dioecy could also evolve by sexual specialization in species in which the separation of sex roles on individual plants is incomplete, e.g., species in which individuals produce bisexual flowers in combination with either male flowers (“andromonoecy”) or female flowers (“gynomonoecy”), respectively. A recent study of the largest family of the angiosperms, the daisy family (Asteraceae; approx. 25,000 species), found comparative evidence for no fewer than nine transitions between various intermediate states and end points in the range of strategies from fully combined to fully separate sexes, including reversions (Torices et al. 2011). The authors found particularly strong evidence for a transition from hermaphroditism to monoecy via the otherwise rare sexual system gynomonoecy, a path in the Asteraceae that may be attributable to the importance of the family’s characteristic floral head (“capitulum”) as both a functional and developmental unit (Torices et al. 2011). The functional contribution to gender by florets in Asteraceae capitula is particularly interesting and revealing. For instance, allocation to the showy petals of the outer ray florets, which may be female, can actually represent a predominantly male component of sex allocation by attracting insects to the floral head as a whole, thereby facilitating the dispersal of pollen from male or hermaphrodite florets in the inner floret whorls.

3.6 Reversions from Dioecy Toward Hermaphroditism

Comparative analyses of shifts among sexual systems have also inferred reversions from dioecy to hermaphroditism (e.g., Gleiser and Verdú 2005). Such studies provide a macroevolutionary context and justification for considering the circumstances and evolutionary processes underlying reversion. Indeed, one hypothesis to explain the scattered distribution of dioecious lineages among angiosperms and their relative failure to diversify proposes that reversions to hermaphroditism occur more frequently than has been presumed (Kafer et al. 2014, 2017).

The most likely explanation for reversions from dioecy is that hermaphrodites with a capacity for self-fertilization can mate in the absence of mates or pollinators, whereas males and females cannot (Baker 1955; Ehlers and Bataillon 2007; Crossman and Charlesworth 2014; Pannell 2015). The evolution of self-fertilization in response to selection when mates or pollinators are absent is a leading hypothesis for transitions from outcrossing to selfing in hermaphrodite plants (Barrett and Harder 1996; Kalisz et al. 2004; Barrett et al. 2014): a possibility also shown for animals (Pannell 2008; Weeks 2012). However, whether mate limitation commonly precipitates the breakdown of dioecy (Baker 1955; Ehlers and Bataillon 2007; Crossman and Charlesworth 2014; Pannell 2015) has barely been tested formally. As noted above, males and females of dioecious species occasionally produce flowers of the opposite sex (Ehlers and Bataillon 2007), and such “leaky” individuals are likely targets of positive selection when mates are limited.

In his seminal work on transitions between combined and separate sexes in *Leptinella* (previously *Cotula*), Lloyd (1975a) considered the breakdown of dioecy in cases when either males or females have inconstant sex expression and can provide reproductive assurance. In the inferred ancestral dioecious population of *Leptinella*, females were likely the heterogametic sex. Lloyd (1975a) thus posited that an isolated female (possessing a ZW genotype) with leaky sex expression was unlikely to found populations that might revert to hermaphroditism, because self-fertilization would lead to the segregation of both males and females in her selfed progeny, immediately reestablishing dioecy in the new population. In contrast, selfing by a single isolated male (with a ZZ genotype) would produce only male progeny. Selection would favor those males with an enhanced female function, so that, over time, the population would evolve a more balanced (or even female-biased sex allocation) (1975a).

Reversion from dioecy has also been invoked to explain sexual system variation in the wind-pollinated annual plant *Mercurialis annua*. In contrast to *Leptinella*, males of *M. annua* are the heterogametic sex (Russell and Pannell 2015). Following Lloyd's (1975a) reasoning, isolated leaky males should thus be less likely to establish a hermaphroditic population, because selfing would yield both sons and daughters. In contrast, leaky females could do so following self-fertilization. Pannell (2001) also argued that because leaky females could produce many more seeds by selfing than could leaky males, they could establish a new population of viable size more quickly. In the absence of males in such a population, selection should increase pollen production by inconstant females. Dorken and Pannell (2009) verified this expectation by removing males from androdioecious populations of *M. annua*, which caused rapid evolution of increased male allocation by the hermaphrodites. Given that fully dioecious populations of *M. annua* often have females with leaky gender expression (Yampolsky 1919; Kuhn 1939), androdioecy could have evolved via initial selection of increased male function in females that become isolated from males (e.g., during colonization), with males subsequently invading evolved hermaphroditic populations as migrants (Pannell 2001). Most of the few cases of androdioecy known in both plants (Pannell 2002) and animals (Weeks et al. 2006) involve species with a colonizing habit, with individuals likely benefiting from an ability to self-fertilize. This pattern provides reasonable support for the hypothesis that androdioecy typically results from the breakdown of dioecy, rather than the spread of female-sterility mutations (as found in the exceptional cases of *Phillyrea* and *Fraxinus* cited above) (Pannell 2002).

3.7 Concluding Remarks

Substantial progress has been made toward answering Darwin's (1877) question on "why hermaphroditism should ever have been rendered dioecious." As reviewed above, much empirical work supports theoretical studies that pointed to the evolution of dioecy as likely response to selection to avoid inbreeding in partially self-fertilizing species. Furthermore, transitions to dioecy driven by selection for inbreeding avoidance usually follow a gynodioecious path, with the initial spread of male-sterility mutations (Dufay et al. 2014). Why gynodioecy gives way to full dioecy in some species (with increased male allocation in hermaphrodites), whereas it is maintained as a female-hermaphrodite polymorphism in others (with the hermaphrodites continuing to allocate substantially to female function) is still poorly understood (Delph and Wolf 2005). More work is needed to understand when gynodioecy should be stable to the evolution of full dioecy or indeed reversion to hermaphroditism.

Although the hypothesis that dioecy evolves as an outcome of selection for sexual specialization too goes back to Darwin (1877) and has been addressed in many theoretical and empirical studies, it continues to be much less well understood or supported than the gynodioecy path. This is perhaps surprising, because plant species with separate sexes almost invariably also show some sexual dimorphism. A key unaddressed question concerns when during their evolution these populations began accumulating sexually dimorphic traits. Does dioecy evolve first, with sexual dimorphism a syndrome that evolves subsequently? Or could dimorphism begin evolving during early stages of gender separation? Variation or even bimodality in the expression of a particular trait could evolve in a population, with subsequent mutations spreading that differentially affect gender. Quantitative genetic analysis of trait associations for sub-dioecious species, particularly those in which gender specialization is limited and in its early stages of evolution, would be helpful in addressing these questions. More attention should be paid to evaluating the frequency with which sexually dimorphic traits arise from the expression of sex-linked genes, as predicted by models for the evolution of sex chromosomes, as opposed to the alternative, where sex-limited expression at autosomal (or pseudoautosomal) loci underlies sexual specialization.

Finally, more work is needed to ascertain the extent to which reversions from dioecy to hermaphroditism could explain the scattered distribution of dioecy among flowering plants and how such reversions might have occurred. Whether they typically begin through the evolution of increased male function in leaky females, as appears to have occurred in *Mercurialis annua*, or of increased female function in males, as proposed for *Leptinella*, also awaits thorough analysis. Although the former path seems more functionally likely, because selfing leaky females already produce many seeds and should be more capable to maintain a new population, the latter might be more prevalent simply because more dioecious species have leaky males than leaky females. Detailed analysis of clades in which transitions between combined versus separate sexes have been frequent will be revealing, particularly if combined with analysis of the ontogeny of sex expression in flowers and inflorescences that vary in gender.

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Chapter 4
The Evolution of Uniparental Reproduction in Rhabditina Nematodes: Phylogenetic Patterns, Developmental Causes, and Surprising Consequences

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Abstract Many groups of eukaryotes with an ancestrally outcrossing sexual system include species that have adopted uniparental reproduction (either parthenogenesis or self-fertility). Nematodes are one such group. Because of recent advances in molecular phylogenetics, the evolutionary patterns of nematode sexual mode are becoming clearer. In this chapter, we first present a molecular phylogeny of over 200 Clade 9 nematodes and map changes in sexual mode upon it. Clade 9 includes both parasites of the order Strongylida and free-living species that include *Caenorhabditis elegans*, the most studied nematode. Uniparental reproduction has evolved a minimum of 15 times in the clade, but none of these are within the clade of parasitic species. We discern two local phylogenetic patterns after the origin of uniparental reproduction. In Pattern 1, parthenogenic or selfing species are isolated taxa that apparently go extinct before speciation. In Pattern 2, uniparental reproduction is ancestral to a diversified clade and is relatively ancient. We next review the evolutionary developmental biology aspects of self-fertility in *Caenorhabditis*, the one taxon for which it has been investigated. Finally, we summarize recent work documenting surprising impacts of self-fertility on genome size and content.

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4.1 Introduction

With a few notable possible exceptions, sexual reproduction through meiosis and syngamy is a fundamental feature of the eukaryotic domain of life (Schurko et al. 2009). Nevertheless, important aspects of sex, such as timing relative to other life events and the identification of a suitable partner, can be implemented in many ways. In multicellular lineages that must produce an embryo (e.g., animals and land plants), anisogamy is the norm, and this introduces the further complexities related to the allocation of male (i.e., sperm-related) and female (i.e., egg-related) function within and/or between individuals. Organisms can also vary in mating system without changing sex allocation. For example, in many angiosperms, an obligately outcrossing hermaphroditic lineage becomes self-fertile (Barrett 2002). Because of the sheer number of possibilities, perhaps we should not be surprised that closely related animals and plants can differ dramatically in how they determine their sexes or allocate sexual function within and between bodies. On the other hand, sex determination and sexual development are typically complex processes, which might instead suggest that stabilizing selection will dominate and change would be slow. The ultimate explanation for this paradox may be that sexual traits can experience tremendously strong directional selection, such that the pace of change is fast despite complex genetic architectures.

The rapid evolution of sexual reproduction is striking and inherently fascinating, but it also offers a chance to examine how development evolves over very short time scales. Changes in sex allocation necessarily involve changes to the pathways that regulate the development of the gonad or flower, secondary sexual characteristics, and neural substrates of sexual behavior. This realization has motivated research in both animals and plants (e.g., Ellis and Lin 2014; Haag 2009; Nasrallah and Nasrallah 2014; Sicard and Lenhard 2011; Young et al. 2008). Changes in sexual mode are also expected to have many consequences, and these are also being studied in a range of taxa (e.g., Thomas et al. 2012b; Wright et al. 2013). This chapter focuses on nematodes, which are a hyper-diverse metazoan phylum that displays a variety of sexual modes (Denver et al. 2011). Most are “traditional” gonochoristic (male-female) species, but uniparental (parthenogenic, self-fertile) and mixed (heterogonic) systems have evolved many times. One of the best-studied animals in any phylum, *Caenorhabditis elegans*, represents one such derived selfing lineage (Kiontke et al. 2004). This provides an important experimental paradigm that can be harnessed to study sexual mode evolution.

Below, we present a new molecular phylogeny of nematodes most closely related to *C. elegans* and use it to characterize the phylogenetic patterns of sexual mode diversity. We then review recent work with the genus *Caenorhabditis*, which exhibits one of the two observed major patterns of sexual mode evolution. Thanks to focused efforts by multiple laboratories, it has become a model genus for exploring changes in sexual mode at the genetic and genomic levels.

4.2 Variation in Nematode Sexual Mode: The Rhabditina (Clade 9) as Example

Many genera of nematodes exhibit species-level variation in sexual mode (Denver et al. 2011), implying that changes can occur rapidly. Denver et al. (2011) also noted that different genera appear to vary with regard to the ability of uniparental lineages to speciate and to revert to gonochorism. To gain a larger-scale view of sexual mode evolution, we have constructed a molecular phylogeny of over 200 species in what has been dubbed Clade 9 by one of us (J.H.) and his colleagues (Holterman et al. 2006; van Megen et al. 2009) and corresponds to the suborder Rhabditina. Several genera within this large taxon employ uniparental reproduction, including the two well-studied model genera *Caenorhabditis* and *Pristionchus* and the entomopathogen *Heterorhabditis*, which has been used as an insecticide in agriculture (Fig. 4.1). We then mapped the sexual mode (available for 178 of these species) onto this tree using ancestral state reconstruction methods (Fig. 4.2). The four types of mode recognized were gonochorism (male and female), androdioecy (males + self-fertile hermaphrodites derived from females), heterogonic (alternating between gonochorism and either selfing or parthenogenesis), and strict parthenogenic (females only). Our tree reconstructs some well-studied groups differently than more focused studies (Felix 2006; Kiontke et al. 2011; Mayer et al. 2007), but this does not impact the general inferences drawn below (see also Denver et al. 2011).

The history of uniparental reproduction in Clade 9 inferred from our analysis suggests the following:

Uniparental Reproduction Evolved at Least 15 Times Both maximum parsimony and maximum likelihood methods broadly agree on the number of independent origins but reconstruct sexual mode evolution slightly differently in the *Oscheius-Heterorhabditidoides* clade.

Vertebrate Parasitic Rhabditina Are Uniformly Gonochoristic Sixty-two of the 178 taxa in our tree are animal parasites, which form a clade belonging to the order Strongylida. These include parasites whose colonization of new hosts would seem to be difficult, such as those of *Halocercus invaginatus*, which infect the lungs of cetaceans (Moser and Rhinehart 1993). Despite the potential benefits of uniparental reproduction for reproductive assurance that might be expected for these species, none have been observed to use any non-gonochoristic mode of reproduction.

Two broad hypotheses for the absence of selfing in the parasitic taxa considered here are (1) a developmental constraint that blocks the evolution of the modifications necessary to achieve uniparental reproduction and (2) lack of benefit to these vertebrate parasites. Support for the constraint hypothesis comes from the existence of vertebrate parasites outside the Rhabditina that are parthenogenic for at least part of their life cycle. For example, *Strongyloides* females use mitotic parthenogenesis to reproduce while in the host gut but can also produce sexual XX females and XO

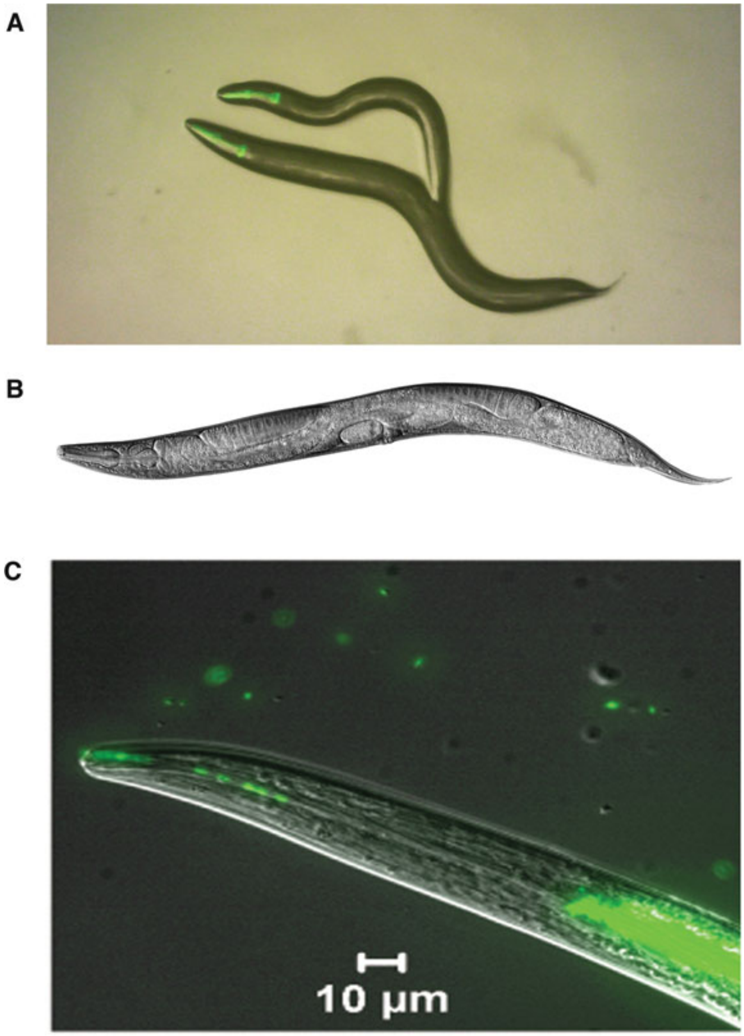

Fig. 4.1 Examples of Clade 9 nematodes with uniparental reproduction. (**a**) *Caenorhabditis briggsae*, an androdioecious species that produces self-fertile XX hermaphrodites (lower) and XO males (upper). This image of mating adults is taken from a stereomicroscope equipped with both transmitted and epifluorescent illumination and reveals expression of a *myo-2:GFP* reporter transgene in the pharyngeal muscles (see Yin et al. 2018). The hermaphrodite is roughly 1 mm long. (**b**) Adult hermaphrodite of *Pristionchus pacificus*, another androdioecious species. Image courtesy of R. Sommer. (**c**) *Heterorhabditis*, an insect parasite in which the egg-laying sex can develop as either a selfing hermaphrodite or a female, which mates with males. Shown here is an infective-stage larva expelling GFP-labelled symbiotic, entomopathogenic *Photorhabdus luminescens* bacteria from its mouth (image modified from Bai et al. 2013)

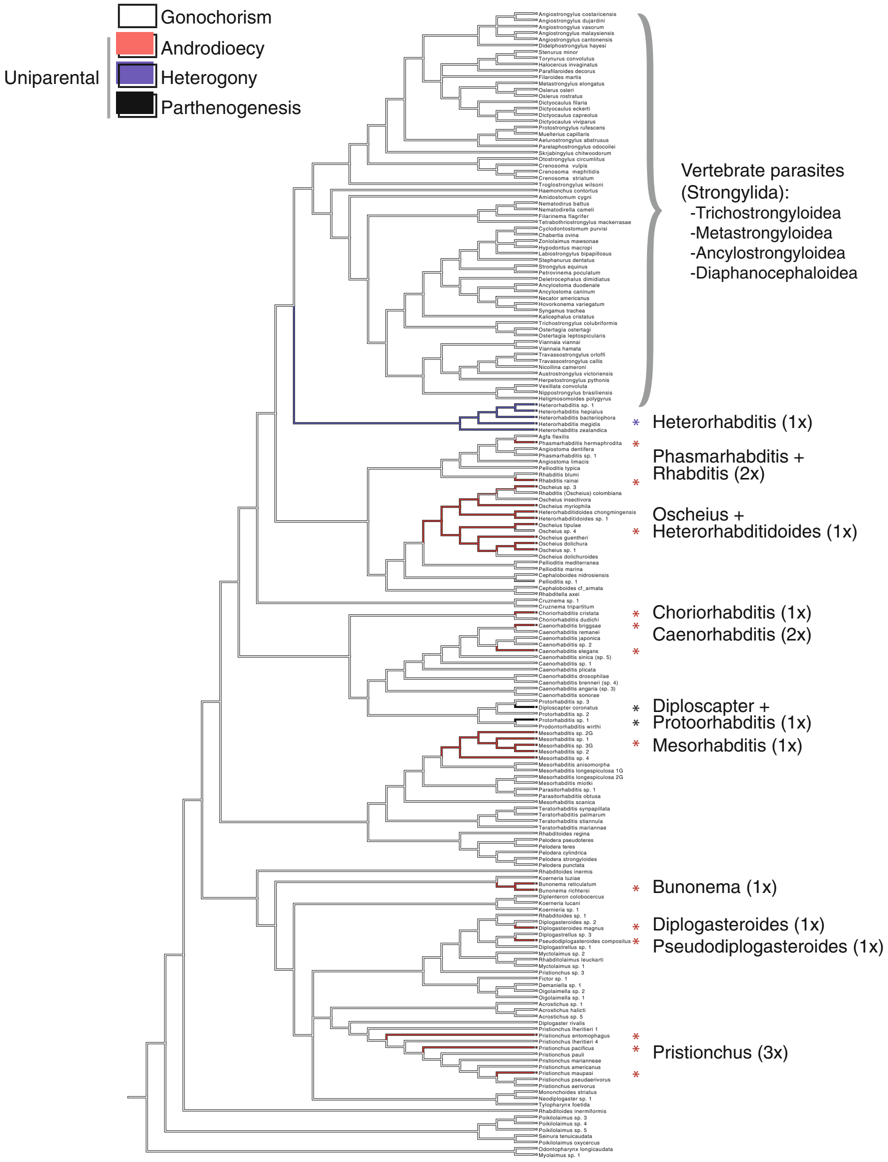

Fig. 4.2 Reconstruction of mating system evolution in Clade 9 nematodes. The cladogram is based on a Bayesian phylogeny inferred from an alignment of 202 small subunit rDNA sequences using methods similar to those of van Megen et al. (2009). The tree was then pruned to 178 non-synonymous taxa for which information about sexual system could be obtained (see Appendix for references). In general, the presence of abundant males was taken to be evidence of gonochorism, though cryptic sperm-dependent parthenogenesis may exist for some taxa. The uniparental reproduction of *Bunonema* is coded here as androdioecy, but has not been characterized, and thus parthenogenesis is also possible. The sexual mode of taxa marked in gray (*Pellioditis* sp. 1 and *Oscheius* sp. 4) is not known. Mesquite (Maddison and Maddison 2015) was used to

males *de novo* via X-specific chromosomal diminution (Nemetschke et al. 2010). The male and female progeny reproduce sexually as free-living organisms when passed into the feces. Similarly, *Rhabdias* species (lungworm parasites of amphibians and ectothermic amniotes) reproduce as selfing hermaphrodites in the blood-feeding, lung-dwelling phase. However, the progeny they pass (as eggs) through the gut often develop into free-living gonochoristic sexual animals (Langford and Janovy 2009; Spieler and Schierenberg 1995). Their sexual progeny, in turn, infect the next host through skin penetration (Baker 1979). A key feature of uniparental reproduction is thus its alternation with sexuality. Perhaps Clade 9 parasites lack the necessary mechanisms to regulate such alternation.

Alternatively, other lines of evidence argue that developmental constraint is not the reason for the lack of uniparental reproduction in Clade 9 parasites. For example, the rhabditid species *Auanema rhodensis* can alternate between selfing and gonochorism (Felix 2004; Kanzaki et al. 2017), the outcome dictated by whether the juvenile develops through a dauer larva (Chaudhuri et al. 2011). This suggests that reproductive plasticity is not lacking in Clade 9 generally. Similarly, *Heterorhabditis*, which is the sister taxon to the clade of vertebrate parasites, is an obligate insect parasite that has a heterogonic lifestyle, in which a gonochoristic phase typically alternates with selfing hermaphrodite (e.g., Ciche 2007; Poinar et al. 1987). The common ancestor of *Heterorhabditis* + Strongylida therefore retained the potential to evolve a new reproductive mode, including one that involves alternation of generations.

Why, then, might sex be the norm in Clade 9 vertebrate parasites? We note that in *Rhabdias* and *Strongyloides*, the parthenogenic or selfing female adults are restricted to sites of attached feeding, whereas sexuality is restricted to sites outside the host body, sites which often persist in close contact with the hosts and facilitate reinfection. This suggests that parasitic feeding modes of these species may be incompatible with mating and that they nevertheless can be adopted when sexual reproduction is facilitated outside the host by deposition of sexual progeny in a confined location (e.g., a pile of dung). Perhaps there is something particular about vertebrate parasitism in Strongylida that makes mating in the host quite reliable, thus reducing selection for uniparental reproduction as a mode of reproductive assurance. Alternatively, the nature of the host immune response may require an unusual amount of genetic variability to maintain successful infection, a version of the Red Queen dynamic described for other systems (e.g., Morran et al. 2011). Distinguishing between these hypotheses will require a deeper understanding of the ecology of these species. Issues to be resolved include the mechanisms that allow infection by multiple juveniles required for outcrossing, a better understanding of how adults

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Fig. 4.2 (continued) perform both maximum likelihood and maximum parsimony tracings of sexual mode evolution, with the latter shown here for simplicity. Inferred character states are color-coded according to the legend, with asterisks marking the inferred transitions from gonochorism to uniparental reproduction. References for sexual mode codings appear in the Appendix manage feed, mate, and remain anchored in host tissues and how they interact with host immune system responses.

Some androdioecious lineages speciate, while others apparently have not. Androdioecy appears to have evolved at least 12 times in Clade 9. In *Caenorhabditis*, *Choriorhabditis*, the clade containing *Diplogastrellus* and *Pseudodiplogasteroides*, *Bunonema*, *Pristionchus*, and the clade containing *Phasmarhabditis hermaphrodita* and *Rhabditis rainai*, selfing appears to be of recent origin, with little cladogenesis. For *Pristionchus* more focused phylogenetic reconstructions (Mayer et al. 2007, 2009) and newly described species (Kanzaki et al. 2012, 2013) support this conclusion, though some details of topology differ. The parthenogenic species *Diploscapter coronatus* and *Protorhabditis* sp. 1 show a similar pattern, which we refer to as Pattern 1. More speculatively, the reconstruction is consistent with extinction of ancient selfing lineages prior to speciation. In contrast to the Pattern 1 lineages, there is strong evidence for speciation after the adoption of selfing in the *Oscheius* + *Heterorhabditidoides* and *Mesorhabditis* clades. Of these, the former also shows signs that species have reverted to gonochorism (e.g., *Oscheius dolichuroides*). Similarly, the origin of heterogonic development in *Heterorhabditis* appears to be ancient. We refer to the deeper origin of uniparental reproduction as Pattern 2.

We considered formal statistical tests that might be able to determine whether uniparental reproduction does generally alter rates of taxon diversification and/or extinction. The most sophisticated of these is Binary State Speciation and Extinction (BiSSE), which uses a maximum likelihood method to estimate the rates of diversification, extinction, and state change for taxa differing in a binary character (Maddison et al. 2007). BiSSE has been used to show that selfing in the plant family Solanaceae reduces net diversification (Goldberg et al. 2010), and this may be occurring in Pattern 1 nematode lineages as well. However, as noted by BiSSE authors, this method is generally reliable only when there are >300 taxa and when the asymmetry in one state vs. the other ("tip bias") is not extreme (Davis et al. 2013). Our tree does not meet these criteria. Other limits include a likely overrepresentation of parasitic taxa and a more general incomplete sampling of extant taxa. Even with complete taxon sampling, however, the presence of Patterns 1 and 2 further suggests that a single model may not be appropriate for Clade 9 nematodes. It therefore remains a future challenge to decisively determine how macroevolutionary patterns may be influenced by adoption of uniparental reproduction.

4.3 Caenorhabditis: An Experimental Genus for Pattern I Selfing

As previously noted by Denver et al. (2011), the marked variation in the evolutionary fates of uniparental lineages remains mysterious. It is likely, however, that it has its roots in taxon-specific aspects of ecology, development, and genome content. To test this general hypothesis, it is necessary to develop experimentally tractable comparative models. Given its unmatched genomic and experimental tools and its mix of gonochoristic and androecious species, *Caenorhabditis* is an obvious choice.

4.3.1 Developmental Genetic Mechanisms of Self-Fertility

C. elegans was chosen over related worms for intense study in large part due to its self-fertile hermaphrodite, which greatly simplifies genetic manipulations (Brenner 1974, 2009). Selfing depends upon the ability of a historically female XX worm to produce a limited amount of sperm in an otherwise female soma. This, in turn, requires local sex reversal in the germ line, so that the chromosomally determined oogenesis fate is briefly overridden. Identification of the global sex determination pathway was an early success of *C. elegans* developmental genetics (Hodgkin 2002; Hodgkin and Brenner 1977) and was followed by molecular characterization that revealed it to be an autocrine signaling system distantly related to the Hedgehog pathway (Zarkower 2006). In parallel to work on the global pathway, both loss-of-function and gain-of-function mutations that specifically impact germ line sexual fate were identified (Ellis 2008). These revealed the existence of posttranscriptional regulatory mechanisms that were essential for XX spermatogenesis (Kimble and Crittenden 2007). Some of the components of these mechanisms, such as the protein FOG-2 and a repeat element in the 3′ UTR of the *tra-2* mRNA, represent strong candidates for innovations that enabled selfing in the *C. elegans* lineage. For example, FOG-2 is the product of a recent gene duplication (Clifford et al. 2000; Nayak et al. 2005), after which it uniquely acquired a binding site allowing it to form a heterodimer with the RNA-binding protein GLD-1. Loss of FOG-2 eliminates XX spermatogenesis, but has no effect on males (Schedl and Kimble 1988). It is thus a new gene that is essential for a newly evolved trait.

With a partial model of how the *C. elegans* hermaphrodite regulates its spermatogenesis, several groups began to examine the independently evolved selfing of *C. briggsae*. Reviews of this work have recently been published (Ellis and Lin 2014; Thomas et al. 2012b), but major points can be summarized here. First, the global sex determination pathway that determines overall sex is conserved in the two species (Haag 2005). Second, loss-of-function studies indicate that somatic phenotypes of orthologous genes are essentially always conserved (Hill et al. 2006; Kelleher et al. 2008; Kuwabara 1996; Streit et al. 1999). An interesting exception is *C. briggsae puf-2*, which has also acquired an essential role in pharyngeal development (Liu and Haag 2014). Third, the germ line role of global sex determiners and the role of germ line-specific regulators of sexual fate are sometimes conserved, and sometimes not. For example, *fog-1* and *fog-3* are conserved sperm-promoting factors in all species examined thus far (Chen et al. 2001; Cho et al. 2004), while *gld-1* (Beadell et al. 2011; Nayak et al. 2005) and PUF family RNA-binding proteins (Liu et al. 2012) play opposite roles in germ cell sex in the two species, despite their similar biochemical activities. Finally, selfing also requires that self-sperm autoactivates without male seminal fluid, and this has been achieved in different ways in different selfing *Caenorhabditis* (Baldi et al. 2009; Wei et al. 2014).

4.3.2 Consequences of Selfing

Beyond self-fertility, the list of other biological attributes that differ between gonochoristic and selfing *Caenorhabditis* species is growing (reviewed by Cutter 2008; Ellis and Lin 2014; Thomas et al. 2012b). These include some that are expected, such as greatly reduced standing genetic diversity (Cutter 2006; Cutter et al. 2006; Dey et al. 2013; Graustein et al. 2002) and relaxed selection on behavioral and gonad-related traits historically associated with outcrossing (Garcia et al. 2007; Palopoli et al. 2008). Others are more surprising. For example, both transcriptome (Thomas et al. 2012a) and genome (Fierst et al. 2015; Yin et al. 2018) assemblies suggest that the genome loses many protein-coding genes and base pairs soon after the transition to selfing. Using a published phylogeny of characterized *Caenorhabditis* (Kiontke et al. 2011), we can formally reconstruct changes in genome size as a quantitative trait using linear parsimony (Fig. 4.3). As with selfing itself, genome contraction appears to have occurred independently in *C. elegans* and *C. briggsae* and in the lineage leading to the less-studied selfer *C. tropicalis*. However, in this latter case, its gonochoristic sister species, *C. wallacei*, also has an unusually small genome. This suggests that either there are other, non-selfing causes of genome shrinkage or perhaps that *C. wallacei* represents a rare case of reversion of a selfing species to obligate outcrossing. Distinguishing between these two may be possible with detailed examination of its genome and reproductive traits, which is ongoing (ESH, D. Yin, E.M. Schwarz, unpublished).

Why should genomes contract after a species becomes self-fertile? Given the reduction in effective population size, one school of thought predicts they should actually acquire selfish DNA elements and become larger (Lynch and Conery 2003), but this is not the case (Fierst et al. 2015; Thomas et al. 2012a). Loss of stabilizing selection alone is also inadequate to explain rapid, reproducible shrinkage, as selfing species lack the thousands of pseudogenes one might expect were this the case. Instead, the most likely explanation involves a recently discovered transmission bias in males that are heterozygous for size-discrepant autosomes (Wang et al. 2010). This mechanism, which we have dubbed indel segregation distortion (ISD), leads males to disproportionately transmit larger homologs to their XX daughters and smaller ones to their XO sons. The cellular mechanism appears to be based on a chromatin-balancing mechanism in meiosis I, such that the side of the spindle lacking an X (destined to form nullo-X spermatocytes) attracts larger autosomes. When ISD acts in a gonochoristic species, it is of little consequence since every embryo has a father and a mother. However, in a partial selfer, occasional mating produces heterozygous males that push larger alleles into their sons, which are less likely to reproduce than hermaphrodites. Computer simulations indeed predict that

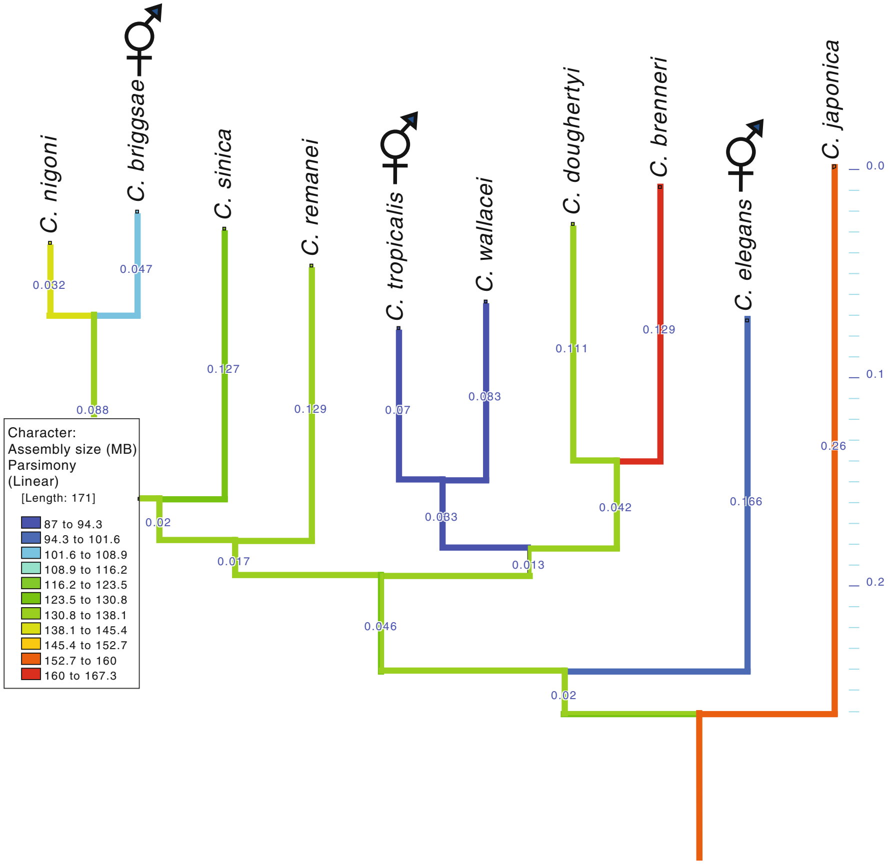

Fig. 4.3 Reconstruction of genome size changes in *Caenorhabditis*. Genome assembly sizes from ongoing projects (E. Schwartz, pers. comm.) were mapped on the phylogeny of Kiontke et al. (2011) and used for ancestral character state reconstruction using linear parsimony, as implemented in Mesquite (Maddison and Maddison 2015)

in a mixed mating system, genome size falls deterministically almost as fast as non-deleterious deletions are created by new mutation (Wang et al. 2010). Further, genes formerly essential for competitive male-male interactions may simultaneously become dispensable. These two phenomena would be predicted to reproducibly shrink the genome to a core that is healthy and optimally self-fertile but potentially weakened in traits historically related to outcrossing.

Another surprising consequence of selfing is the vulnerability of hermaphrodites to sterilization and decreased life span upon mating with males of related outcrossing species (Ting, Woodruff, et al. 2014). Though rapid coevolution of sperm-egg interactions leading to species-specific fertilization is seen in marine invertebrates (Vacquier and Swanson 2011), the cues that attract sperm to the oocytes appear to be conserved in *Caenorhabditis*. Ongoing sexual selection in outcrossers likely favors

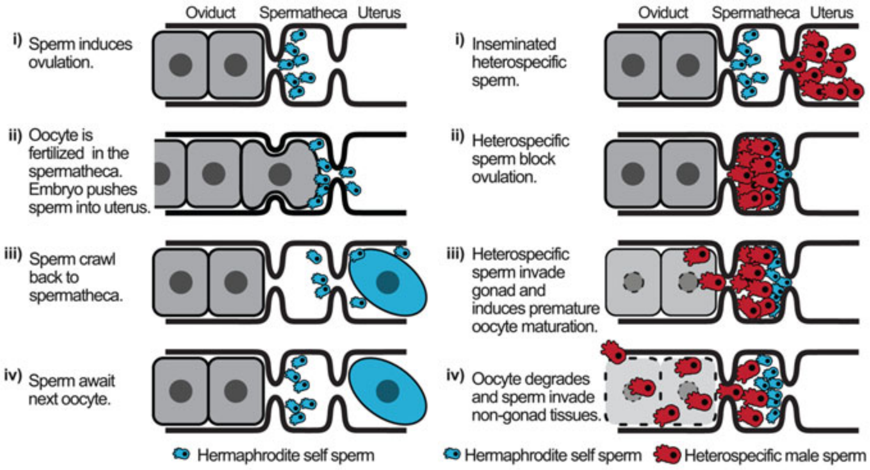

Fig. 4.4 Model for sterilization in interspecific crosses. Left: the sperm of male and hermaphrodite individuals are benign in androdioecious species. They remain confined to the spermathecae prior to ovulation. During ovulation, many of the sperm that do not successfully fertilize the egg are swept into the uterus and then crawl back to the spermathecae (Wu et al. 2012). Right: heterospecific sperm from a related outcrossing species initially home to the spermatheca, as do the conspecific sperm and as the heterospecific sperm would in their own species. Because of their ongoing need to be competitive, however, the heterospecific sperm displace the conspecific sperm from the spermathecae, and subsequently cannot be contained there. Heterospecific sperm then invade the ovary and beyond, causing premature activation of the oocyte cell cycle without fertilization and eventually sterility and early death. Figure adapted from Ting, Woodruff, et al. (2014)

aggressive sperm behavior, to which females are constantly exposed and to which they adapt in a coevolutionary process that is generally cryptic. The rarity of males in selfers means they are rarely competing with other males, and thus selection for both sperm aggressiveness and female tolerance of it is expected to be relaxed. Indeed, when outcrossing males inseminate a hermaphrodite, their sperm rapidly displace conspecific sperm from the sperm storage organ (spermatheca). Further, they often cannot be contained to the spermatheca by the female, such that the sperm invades the ovary and other body tissues (Fig. 4.4). This is reminiscent of the “weak inbreeder, strong outcrosser” (WISO) pattern of asymmetrical pollen tube competition observed in related angiosperms with alternative sexual modes (Brandvain and Haig 2005).

The harmful effects of outcrosser sperm in the context of a self-fertile mate suggest that there are cryptic yet collectively major differences in sperm of selfers and outcrossers. Presumably there are also differences that accumulate between diverged outcrossers, and evidence for this has been found (Ting, Woodruff, et al. 2014). However, the phenomenon of genome shrinkage allows identification of a set of candidate genes whose loss may be responsible for the benign nature of androdioecious male sperm. By using a phylogenomic approach, we identified over 350 genes in the outcrosser *C. remanei* whose expression was greater than tenfold male-biased that were conserved in at least one other outcrosser genome and that were absent from both *C. elegans* and *C. briggsae* (Thomas et al. 2012a). More recent studies in one of the author's laboratory indicate that at least some of these evolutionarily labile, male-expressed genes are directly involved in sperm competition (Yin et al. 2018). For example, the *mss* gene family encodes small, glycosylated sperm surface proteins that are only present in the genomes of outcrossing species. When *mss* genes are deleted from the outcrossing *C. remanei*, mutant males have normal fertility in isolation but sire far fewer progeny when in competition with wild-type males. Further, restoration of *C. nigoni mss* genes to *C. briggsae* males via a transgene confers superior competitive ability upon their sperm *vis-a-vis* wild-type males.

Because restoration of *mss* function to *C. briggsae* males also suppresses hermaphrodite self-fertility, it has the further impact of increasing male frequency (Yin et al. 2018). This impact on sex ratio may be an important clue as to why *mss* genes are lost repeatedly in selfing species. Once males are no longer needed for robust reproduction, the population growth rate reduction they induce (by virtue of the inability to lay eggs) can be avoided by reducing their fertility. However, sterilizing mutations do not generally invade selfing species. This suggests that periodic outcrossing is needed for lineage survival and that the tuning of the male frequency to a level between zero and one half is achieved by selective loss of sperm competition proteins.

4.4 Conclusion and Prospects for Future Research

In their 2011 review, Denver et al. posed a number of important challenges and questions in the evolution of nematode sexual modes. Challenges included lack of clear data on sexual mode for many taxa and incomplete taxon sampling, and clearly these remain 7 years later. Major questions included why species that appear to have a similar ecological niche nevertheless differentiate into a mix of different sexual modes and why some lineages that adopt uniparental reproduction speciate and others do not. Again, these questions remain unanswered.

Another question posed by Denver et al. is why some selfing species revert to gonochorism, while others likely go extinct instead. In the last decade, some progress has been made toward answering this question, at least in the case of *Caenorhabditis*. A combination of ISD and relaxed sexual selection rapidly shrink the genome of selfers like *C. briggsae* and *C. elegans*. Once lost, regain of sex-related genes via hybridization with gonochoristic sister taxa become impossible as WISO-related barriers to interspecies mating evolve. Though levels of outcrossing may remain adequate to block the action of Muller's Ratchet, they may not be adequate to allow rapid adaptation to new environmental stresses, such as pathogens (Morran et al. 2009). Though it may take hundreds of thousands or millions of years, eventually a situation arises to which the selfing lineage cannot adapt. As a result, it blinks out of existence, leaving its outcrossing relatives to carry on. Given the lack of an extensive fossil record for nematodes, a researcher examining diversity just after this point would fail to appreciate that the selfing lineage ever existed, and presumably this ignorance characterizes many extant nematode taxa. It remains to be seen whether other clades exhibiting Pattern 1 distribution of androdioecy show a similar correlation between genome size and sexual mode. Examining this is another worthwhile area of future research.

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Appendix: Sources for Sexual Modes in Fig. 4.2

Species		References
Angiostrongylus	*costaricensis*	Spratt (2015), Morera (1973)
Angiostrongylus	*dujardini*	Spratt (2015), Drozdz et al. (1971), Eira et al. (2006)
Angiostrongylus	*vasorum*	Spratt (2015), Costa et al. (2003)
Angiostrongylus	*malaysiensis*	Spratt (2015), Bhaibulaya and Cross (1971)
Angiostrongylus	*cantonensis*	Spratt (2015), Mackerras and Sandars (1955)
Parafilaroides	*decorus*	Dailey (2009)
Halocercus	*invaginatus*	Moser and Rhinehart (1993)
Stenurus	*minor*	Faulkner et al. (1998)
Torynurus	*convolutus*	Baylis and Daubney (1925)
Filaroides	*martis*	Ko and Anderson (1972)
Metastrongylus	*elongates/salmi*	Morita et al. (2007)
Oslerus	*rostratus*	Varcasia et al. (2015), Bowman et al. (2002)
Oslerus	*osleri*	Varcasia et al. (2015), Bowman et al. (2002)
Dictyocaulus	*eckerti*	Divina et al. (2000)
Dictyocaulus	*capreolus*	Gibbons and Hoglund (2002)
Dictyocaulus	*viviparus*	Divina et al. (2000)
Dictyocaulus	*filaria*	Urquhart et al. (1996)
Protostrongylus	*rufescens*	Jabbar et al. (2013)
Muellerius	*capillaris*	Kuchboev et al. (2015)
Aelurostrongylus	*abstrusus*	Urquhart et al. (1996)
Parelaphostrongylus	*odocoilei*	Kontrimavichus et al. (1985)
Skrjabingylus	*chitwoodorum*	Kontrimavichus et al. (1985)
Otostrongylus	*circumlitus*	Lehnert et al. (2010)
Crenosoma	*vulpis*	Kontrimavichus et al. (1985)
Crenosoma	*striatum*	Kontrimavichus et al. (1985)
Crenosoma	*mephitidis*	Kontrimavichus et al. (1985)

(continued)

Species		References
Troglostrongylus	*wilsoni*	Kontrimavichus et al. (1985)
Viannaia	*viannai*	Monet-Mendoza et al. (2005)
Viannaia	*hamata*	Gomes et al. (2003)
Herpetostrongylus	*pythonis*	Durett-Desset (2009)
Austrostrongylus	*victoriensis*	Beveridge and Durett-Desset (1986)
Nicollina	*cameroni*	Durette-Desset and Cassone (1983)
Travassostrongylus	*callis*	Cameron and Myers (1961)
Travassostrongylus	*orloffi*	Cameron and Myers (1961)
Heligmosomoides	*polygyrus*	Reynolds et al. (2012)
Nippostrongylus	*brasiliensis*	Camberis et al. (2003)
Vexillata	*convoluta*	Gardner et al. (1994)
Deletrocephalus	*dimidiatus*	Taylor et al. (2000)
Ancylostoma	*duodenale*	Schneider et al. (2011)
Ancylostoma	*caninum*	Schneider et al. (2011)
Hovorkonema	*variegatum*	Krone et al. (2007)
Syngamus	*trachea*	Urquhart et al. (1996)
Necator	*americanus*	Schneider et al. (2011)
Cyclodontostomum	*purvisi*	Hasegawa and Syafruddin (1994)
Chabertia	*ovina*	Umair et al. (2016)
Zoniolaimus	*mawsonae*	Huby-Chilton et al. (2002)
Hypodontus	*macropi*	Beveridge (1979)
Labiostrongylus	*bipapillosus*	Chilton et al. (2003)
Stephanurus	*dentatus*	Romanowski (1982)
Strongylus	*equinus*	Urquhart et al. (1996)
Petrovinema	*poculatum*	Silva et al. (1999)
Kalicephalus	*cristatus*	Chilton et al. (2006)
Trichostrongylus	*colubriformis*	Urquhart et al. (1996)
Ostertagia	*ostertagi*	Urquhart et al. (1996)
Ostertagia	*leptospicularis*	Suarez and Cabaret (1992)
Nematodirus	*battus*	Urquhart et al. (1996)
Nematodirella	*cameli*	Sharifiyazdi et al. (2015)
Filarinema	*flagrifer*	Mawson (1964)
Tetrabothriostrongylus	*mackerrasae*	Mawson (1960)
Amidostomum	*cygni*	Kavetska et al. (2011)
Haemonchus	*contortus*	Urquhart et al. (1996)
Heterorhabditis	*megidis*	Ehlers et al. (1998)
Heterorhabditis	*bacteriophora*	Ciche (2007)
Heterorhabditis	*hepialus*	Stock et al. (1996)
Heterorhabditis	sp. 1	Strauch et al. (1994)
Heterorhabditis	*zealandica*	Grewal et al. (2002)
Rhabditis	*colombiana*	Stock et al. (2005)
Oscheius	sp. 3	Felix (2006)
Oscheius	*insectivora*	Felix (2006)

(continued)

Species		References
Oscheius	*myriophila*	Felix (2006)
Heterorhabditoides	*chongmingensis*	Zhang et al. (2008)
Heterorhabditidoides	sp. 1	Felix (2006)
Oscheius	*dolichura*	Felix (2006)
Oscheius	sp. 1	Felix (2006)
Oscheius	*dolichuroides*	Felix (2006)
Oscheius	*guentheri*	Felix (2006)
Oscheius	*tipulae*	Sudhaus (1993)
Pellioditis	*mediterranea*	Fitch (2016a)
Pellioditis	*marina*	Andrássy (1983)
Cephaloboides	*nidrosiensis*	Fitch (2016a)
Cephaloboides	*cf. armata*	Sudhaus and Fitch (2001)
Curviditis	sp. 1	
Rhabditella	sp. 1	
Rhabditella	*axei*	Fitch (2016a)
Agfa	*flexilis*	Ribas and Casanova (2002)
Phasmarhabditis	*hermaphrodita*	Stock and Hunt (2005)
Phasmarhabditis	sp. 1 (neopapillosa)	Hooper et al. (1999)
Angiostoma	*dentifera*	Morand (1992), Morand and Barker (1995)
Angiostoma	*limacis*	Morand (1992), Morand and Barker (1995)
Pellioditis	*typica*	Kiontke and Fitch (2005)
Rhabditis	*blumi*	Kiontke and Fitch (2005)
Rhabditis	*rainai*	Carta and Osbrink (2005)
Cruznema	sp. 1	L. Carta (personal communication)
Cruznema	*tripartitum*	L. Carta (personal communication)
Caenorhabditis	*briggsae*	Kiontke et al. (2011)
Caenorhabditis	*remanei*	Kiontke et al. (2011)
Caenorhabditis	*japonica*	Kiontke et al. (2011)
Caenorhabditis	sp. 2	Kiontke et al. (2011)
Caenorhabditis	*elegans*	Kiontke et al. (2011)
Caenorhabditis	*sinica*	Kiontke et al. (2011)
Caenorhabditis	sp. 1	Kiontke et al. (2011)
Caenorhabditis	*plicata*	Kiontke et al. (2011)
Caenorhabditis	*drosophilae*	Kiontke et al. (2011)
Caenorhabditis	*brenneri*	Kiontke et al. (2011)
Caenorhabditis	*angaria*	Kiontke et al. (2011)
Caenorhabditis	*sonorae*	Kiontke et al. (2011)
Diploscapter	*coronatus*	Lahl et al. (2006)
Protorhabditis	sp. 3	Kiontke and Fitch (2005)
Protorhabditis	sp. 2	Fitch (2016b)
Prodontorhabditis	*wirthi*	Fitch (2016a)
Protorhabditis	sp. 1	Fitch (2016a)
Choriorhabditis	*cristata*	Kiontke and Fitch (2005)

(continued)

Species		References
Choriorhabditis	*dudichi*	Fitch (2016a)
Mesorhabditis	sp. 2	L. Carta (personal communication)
Mesorhabditis	sp. 1	L. Carta (personal communication)
Mesorhabditis	sp. 2	L. Carta (personal communication)
Mesorhabditis	sp. 3	L. Carta (personal communication)
Mesorhabditis	sp. 4	L. Carta (personal communication)
Mesorhabditis	sp. 2G	L. Carta (personal communication)
Mesorhabditis	sp. 3G	L. Carta (personal communication)
Mesorhabditis	*longespiculosa*	Fitch (2016a)
Mesorhabditis	*anisomorpha*	Sudhaus (1978)
Mesorhabditis	*miotki*	Sudhaus (1978)
Parasitorhabditis	sp. 1	Tomalak et al. (1989)
Parasitorhabditis	*obtusa*	Tomalak et al. (1989)
Mesorhabditis	*scanica*	L. Carta (personal communication)
Teratorhabditis	*synpapillata*	Fitch (2016a)
Teratorhabditis	*palmarum*	Gerber and Giblin-Davis (1990)
Teratorhabditis	*stiannula*	Fitch (2016a)
Teratorhabditis	*mariannae*	Fitch (2016a)
Pelodera	*pseudoteres*	Schulte (1989)
Pelodera	*teres*	Schulte (1989)
Pelodera	*punctata*	Fitch (2016a)
Pelodera	*strongyloides*	Saari and Nikander (2006)
Pelodera	*cylindrica*	Hesling (1966)
Rhabditoides	*regina*	Fitch (2016a)
Rhabditoides	*inermis*	Kiontke and Fitch (2005)
Rhabditidoides	sp. 1	Fitch (2016a)
Diplogasteroides	*magnus*	Kiontke et al. (2001)
Diplogasteroides	sp. 2	M. Hermann (personal communication)
Diplogastrellus	sp. 3	Fitch (2016a)
Pseudodiplogasteroides	*compositus*	Korner (1954)
Diplogastrellus	sp. 1	Fitch (2016a)
Rhabditolaimus	*leuckarti*	V. Susoy (personal communication)
Myctolaimus	sp. 1	P. De Ley (personal communication)
Myctolaimus	sp. 2	P. De Ley (personal communication)
Pristionchus	(all species)	Mayer et al. (2007)
Oigolaimella	sp. 2	N. Kanzaki (personal communication)
Demaniella	sp. 1	Pillai and Taylor (1968)
Oigolaimella	sp. 1	N. Kanzaki (personal communication)
Fictor	sp. 1	Powers (2016)
Acrostichus	sp. 1	Kanzaki et al. (2010)
Acrostichus	sp. 5	Kanzaki et al. (2010)
Acrostichus	*halicti*	Kiontke and Fitch (2005)
Diplogaster	*rivalis*	Abolafia (2006)

(continued)

Species		References
Mononchoides	*striatus*	Calaway and Tarjan (1973)
Neodiplogaster	sp. 1	Kanzaki et al. (2008)
Tylopharynx	*foetida*	Wu et al. (2001)
Koerneria	sp. 1	Mayer et al. (2007)
Koerneria	*lucani*	Kanzaki et al. (2014)
Diplenteron	*colobocercus*	Bongers et al. (1988)
Koerneria	*luziae*	Kanzaki et al. (2011)
Bunonema	*reticulatum*[a]	Holterman and Helder (unpublished observations), Fitch (2016a)
Bunonema	*richtersi*[a]	Van Gansbeke et al. (2004), Nematology Lab at University of Nebraska (2016)
Rhabditoides	*inermiformis*	Kiontke and Fitch (2005)
Poikilolaimus	sp. 4	N. Kanzaki (personal communication)
Poikilolaimus	sp. 3	N. Kanzaki (personal communication)
Poikilolaimus	sp. 5	N. Kanzaki (personal communication)
Poikilolaimus	sp. 1	N. Kanzaki (personal communication)
Poikilolaimus	*oxycercus*	Fitch (2016a), Kiontke and Fitch (2005)
Seinura	*tenuicaudata*	Hechler (1963)
Odontopharynx	*longicaudata*	Chitambar and Noffsinger (1989)
Myolaimus	sp. 1	Cobb (1920)

[a] Though reproduction of this strain is uniparental, it is not clear whether it is via parthenogenesis or selfing

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Chapter 5
Polychaete Worms on the Brink Between Hermaphroditism and Separate Sexes

Laura Picchi and Maria Cristina Lorenzi

Abstract Polychaetes (i.e. non-clitellate annelids) display one of the highest diversities of reproductive traits among marine invertebrates, due to the wide variety of habitats they have colonized and the relative simplicity of their reproductive system. Among polychaetes (i.e. non-clitellate annelids), the genus *Ophryotrocha* is one of the best-studied models in sexual system investigations and is providing several clues as to how the evolutionary transition from hermaphroditism to gonochorism may have occurred. Within the genus, there are both hermaphroditic and gonochoric species, but many species exhibit traits intermediate between the two sexual systems. Hermaphroditic species are able to plastically adjust their sex allocation to current mating opportunities, by diminishing the proportion of resources invested into the female function and increasing those invested into the male function, when mating opportunities increase (although the enhanced investment in the male function is expressed in behavioural traits—such as mate competition—rather than increased sperm production). Occasionally, hermaphrodites specialized in the male function are found in hermaphroditic populations (functional-male hermaphrodites), which might represent a first step towards gender specialization. On the other hand, multiple sexual phenotypes have been described in gonochoric (sexually dimorphic) species, with males which produce oocytes and females which produce sperm, which are likely to be vestigial hermaphroditic traits. The existence of functional-male hermaphrodites in hermaphroditic populations and of males and females which produce both eggs and sperm in gonochoric species suggest that in *Ophryotrocha* the transition from hermaphroditism to gonochorism occurred via androdioecy, that is,

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through an evolutionary trajectory involving an intermediate stage where both hermaphrodites and males are present.

5.1 Introduction

Annelida, commonly known as segmented or ringed worms, is a large (around 17,000 recognized species; Zhang 2011) and ecologically diverse phylum. In the last years, thanks to next-generation sequencing, annelids phylogeny has dramatically changed: polychaetes have turned out to be a paraphyletic taxon and have been mostly included in the two major clades Errantia and Sedentaria, the latter comprising also Clitellata (Weigert and Bleidorn 2016). The placement of Clitellata within the clade Sedentaria basically makes Polychaeta synonymous to Annelida. However, we usually use the term *polychaetes* to distinguish non-clitellate annelids from clitellates, and, for the purposes of this review, we will use the term *polychaetes* to indicate non-clitellate annelids, referring to the traditional name.

Polychaetes (i.e. non-clitellate annelids) are a common annelid taxon of the marine benthos, often dominant both as number of species and as number of individuals. They are important components of hard-bottom and pelagic communities as well. This group is well diversified from a systematic point of view, with more than 85 accepted families (Rouse and Fauchald 1997; Brusca and Brusca 2003), and has a large variety of life strategies. In particular, these worms display one of the highest diversity of reproductive traits among marine invertebrates, probably at least partly due to the relative simplicity of their reproductive system (Wilson 1991; Giangrande 1997). Polychaetes indeed generally lack permanent gonads (or other complex reproductive organs) and gametes usually develop through proliferations of cells from the peritoneum. Gametes are then released as gametogonia or primary gametocytes in the coelom, where they mature before being released by means of gonoducts, coelomoducts, nephridia or by simple rupture of the body wall (Brusca and Brusca 2003). This relative simple reproductive system is subjected to few phylogenetic morphological constraints and thus allows the evolution of a wide variety of reproductive modes (Wilson 1991; Giangrande 1997).

Some species of polychaetes have asexual reproduction (most also with sexual reproduction), which is typically accomplished by subdivision of the body into parts and the subsequent regeneration of the missing body segments (Schroeder and Hermans 1975). The body may be divided in two or more parts, as is the case of some species of the genus *Dodecaceria* (Cirratulidae), which are able to regenerate entire individuals from single segments (Schroeder and Hermans 1975; Petersen 1999; Brusca and Brusca 2003).

The most common form of reproduction in polychaetes is sexual reproduction (George and Hartmann-Schröder 1985). Whereas oligochaetes and leeches are mostly hermaphroditic, polychaetes typically have separate sexes. Both simultaneously and sequentially hermaphroditic species exist in about 25 different families (Giangrande 1997; Schroeder and Hermans 1975). Self-fertilization has been reported for several species (*Neanthes (Nereis) limnicola* Smith 1958; Fong and Pearse 1992; *Laonome albicingillum* Hsieh 1997; *Terebrasabella heterouncinata* Finley et al. 2001; *Capitella* sp. Y Méndez 2006) even if none are known to self-fertilize routinely (Knowlton and Jackson 1993).

The relative simplicity of their reproductive system probably allowed these worms to respond to ecological pressures by evolving a large variety of sexual systems (i.e. the pattern of distribution of male versus female functions across individuals in a species, following Leonard 2013) and reproductive strategies, which often differ even in sibling species (Wilson 1991).

One of the most fascinating examples of the extreme adaptability is the case of the gonochoric species *Dinophilus gyrociliatus*. This small interstitial species likely lives in low-density populations, at least temporarily, as do most of the species belonging to interstitial fauna (Westheide 1984). A peculiar mating system has been described in this species that may be advantageous in low-density populations: *D. gyrociliatus* is sexually dimorphic both in its adult morphology and life history traits, and in egg size; males are dwarf, have a very short lifespan relative to females and develop from eggs smaller than those that produce females (Martin and Traut 1987; Sella and Ramella 1999). Soon after hatching from eggs, males inseminate their immature sisters within the protective egg capsule where male and female develop. Then males die before females leave the egg capsule as larvae (Traut 1969; Sella and Ramella 1999). This peculiar mating system produces one of the most skewed sex ratios in the animal kingdom (Charnov 1987), and the free-living populations are composed uniquely of females. The costs of finding mates are likely high in low-density populations; mating as larvae within the egg capsule eliminates these costs and may represent an evolutionary solution for separate sex animals, enabling them to find mates in very sparse and structured populations. Such a female-biased sex ratio has been described also for many species of fig wasps (Agaoninae) in which there is a low level of competition for mates between non-siblings since brothers usually fertilize their sisters, as for *D. gyrociliatus* (Hamilton 1979; West 2009).

On a broader level, polychaete species living in unfavourable environments (including interstitial species) are generally characterized by having a small body size and a relatively short lifespan, and this is linked to a rapid attainment of sexual maturity, a low fecundity and the presence of parental care; on the other hand, species that are highly competitive and dominate the community are usually characterized by long lifespan, very high fecundity and high dispersal capability, with planktonic larvae able to cover long distances (Sveshnikov 1985, in Giangrande 1997).

Hermaphroditism is relatively common in sessile tubicolous worms such as the Sabellidae and the Serpulidae and is often associated with small body size, as in the case of interstitial species such as those belonging to the genus *Ophryotrocha* (Dorvellidae) (Westheide 1984; Giangrande 1997). However, hermaphroditism is the predominant sexual system in a few polychaete families (for instance, in the Spirorbidae, Schroeder and Hermans 1975; Kupriyanova et al. 2001). More often, polychaetes have separate sexes, and hermaphroditic species are often rare in polychaete families (e.g. *Macellicephala violacea* is the only hermaphroditic species in a family (Polynoidae) rich in gonochoric species, Wirén 1907; Rouse and Pleijel 2001). It can be argued that many of these species are described after morphological investigations of small sample sizes which may underestimate some sexual systems, e.g. sequential hermaphroditism, where individuals produce either sperm or eggs at a given stage of their life and may be wrongly assumed to have separate sexes.

Gonochorism is considered the ancestral sexual system in polychaetes, and hermaphroditism is a secondary acquisition in this perspective (Prevedelli et al. 2006). The presence of a few hermaphroditic species within mainly gonochoric genera suggests that the transition from gonochorism to hermaphroditism occurred several times within the polychaetes and at least once in the opposite direction. In a population of *Parasabella microphthalma* (*Demonax microphthalmus* of previous authors), both hermaphrodites and pure females were collected by Gregory (1905), and the closest ancestor of this species seems to be a hermaphrodite (Weeks 2012); this suggests that in *P. microphthalma*, a pure female mutant evolved from a hermaphroditic ancestor. In contrast, the genus *Ophryotrocha* is typically composed of separate sex species (Sella and Ramella 1999), but recent phylogenetic analyses have proposed that simultaneous hermaphroditism is the ancestral sexual system in the genus, where pure males and pure females may have evolved from hermaphroditic individuals (Heggøy et al. 2007; Thornhill et al. 2009). Therefore, the transition between hermaphroditism and separate sexes occurred repeatedly and in both directions within the polychaetes, as in several genera both sexual systems are documented. Table 5.1 reports a list of genera where at least two different sexual systems are described, highlighting how often the transition between sexual systems occurred in these worms.

The presence of several transitions between hermaphroditism and gonochorism in both directions and the lack of morphological constrains in polychaete reproductive system makes these worms an ideal group to study how different selective pressures shape sexual systems, in animals which have colonized a wide variety of habitats.

5.2 *Ophryotrocha* as a Study Model

The genus *Ophryotrocha* (Dorvellidae) was described for the first time in 1869 by Claparède and Metschnikoff (1869), and since then more than 40 species belonging to this taxon have been described, with several new species discovered in recent years (Wiklund et al. 2012; Ravara et al. 2015).

Among polychaetes, the genus *Ophryotrocha* (Fig. 5.1) is an ideal model for the study of the transition between sexual systems. Some species are simultaneous hermaphrodites, others are gonochoric, and at least one species is a sequential hermaphrodite (*O. puerilis*) (Dahlgren et al. 2001). Recently, viviparous species have been reported, whose sexual systems are still unclear (Paxton and Åkesson 2010; Wiklund et al. 2012). Therefore, a large variety of sexual systems occurs within the same genus. The analyses of the phylogeny of this taxon show that within

Table 5.1 A non-exhaustive list of polychaete genera where both hermaphroditic and gonochoric species are described

Family	Species	Sexual system	Sexual system described through	Selfing in hermaphrodite	Habitat	Population density	Sexual dimorphism in gonochorists	Notes on reproductive biology	References
Capitellidae	*Capitella* sp. S	Gonochorist	Presence of sperm or eggs		Intertidal sediments	NA	Genital spines in males	Larval development in parental brood tube	Linke-Gamenick et al. (2000)
	C. iatapiuna	Trioecious	Presence of sperm or eggs or both	NA	Deep-sea whale-fall inside bones	High	Genital spines in males; females with enlarged Lateral genital pores		Silva et al. (2016)
	C. sp. I	Trioecious	Presence of sperm or eggs or both	No	Disturbed habitats	Rapid population explosions	NA		Linke-Gamenick et al. (2000), Petraits (1985)
	C. sp. M	Trioecious	Presence of sperm or eggs or both	NA	Shallow hydrothermal vent areas	NA	NA		Linke-Gamenick et al. (2000)
	C. sp. Y	Trioecious	Presence of sperm or eggs or both	Yes^{a}	Estuaries	NA	Genital spines in males	Brooding behaviour in females and hermaphrodites	Méndez (2006)
Cirratulidae	*Aphelochaeta* spp.	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA			Petersen (1999)

(continued)

Table 5.1 (continued)

Family	Species	Sexual system	Sexual system described through	Selfing in hermaphrodite	Habitat	Population density	Sexual dimorphism in gonochorists	Notes on reproductive biology	References
	A spp.	Gonochorist	Presence of sperm or eggs		NA	NA	Sexual dimorphism in *A. marioni sensu* Gibbs (1971): cellular differences		Petersen (1999)
Cirratulidae	*Caulleriella parva*	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA			Petersen (1999)
	C. spp.	Gonochorist	Presence of sperm or eggs		NA	NA	No	Viviparity	Petersen (1999)
	C. spp.	Gonochorist	Presence of sperm or eggs		NA	NA	No		Petersen (1999)
Cirratulidae	*Chaetozone vivipara* (as *T. vivipara*)	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA		Viviparity	Petersen (1999)
	C. spp.	Gonochorist	Presence of sperm or eggs		NA	NA	NA	Viviparity	Petersen (1999)
Cirratulidae	*Dysponetus pygmaeus*	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA		Protandrous and presence of penial papilla	Tzetlin et al. (2002)
	D. spp.	Gonochorist	Presence of sperm or eggs		NA	NA	NA		Tzetlin et al. (2002)

Dorvillidae	*Ophryotrocha labronica*	Functional gonochorist[b]	Presence of sperm and eggs, fertility experiment		Interstitial habitat in nutrient-rich water	Clumped distribution	Male with wider prostomium, thicker jaws and more rosette glands	Pseudocopulation	Sella and Ramella (1999), Lorenzi and Sella (2013)
	O. macrovifera	Functional gonochorist[b]	Presence of sperm and eggs, fertility experiment		Interstitial habitat in nutrient-rich water	NA	Male with wider prostomium, thicker jaws and more rosette glands	Pseudocopulation	Sella and Ramella (1999), Meconcelli et al. (2015a)
	O. robusta	Functional gonochorist[b]	Presence of sperm and eggs, fertility experiment		Interstitial habitat in nutrient-rich water	NA	Male with wider prostomium, thicker jaws and more rosette glands	Pseudocopulation	Sella and Ramella (1999), Meconcelli et al. (2015a)
	O. diadema	Simultaneous hermaphrodite	Presence of sperm and eggs, fertility experiment	No	Interstitial habitat in nutrient-rich water	Expected to be low		Pseudocopulation	Sella and Ramella (1999)
	O. gracilis	Simultaneous hermaphrodite	Presence of sperm and eggs	No	Interstitial habitat in nutrient-rich water	NA		Pseudocopulation	Sella and Ramella (1999)
	O. hartmanni	Simultaneous hermaphrodite	Presence of sperm and eggs	No	Interstitial habitat in nutrient-rich water	NA		Pseudocopulation	Sella and Ramella (1999)
	O. socialis	Simultaneous hermaphrodite	Presence of sperm and eggs	No	Interstitial habitat in nutrient-rich water	Clumped		Pseudocopulation	Sella and Ramella (1999)

(continued)

Table 5.1 (continued)

Family	Species	Sexual system	Sexual system described through	Selfing in hermaphrodite	Habitat	Population density	Sexual dimorphism in gonochorists	Notes on reproductive biology	References
	O. puerilis	Protandrous, sequential hermaphrodite	Presence of sperm or eggs, fertility experiments	No	Interstitial habitat in nutrient-rich water	Expected to be low		Pseudocopulation	Sella and Ramella (1999)
Maldanidae	*Micromaldane nutricula*	Gonochorist	Presence of sperm or eggs		Mats of red algae in the littoral zone	NA	NA	Presence of spermathecae	Rouse (1990)
	M. pamelae	Gonochorist	Presence of sperm or eggs		Mats of red algae in the littoral zone	NA	NA	Presence of spermathecae	Rouse (1990)
	M. rubrospermatheca	Gonochorist	Presence of sperm or eggs		Mats of red algae	NA	NA	Presence of spermathecae	Rouse (1990)
	M. androgyne	Simultaneous hermaphrodite	Presence of sperm and eggs	Expected	Mats of red algae	NA		Absence of spermathecae	Rouse (1990)
Nereididae	*Hediste japonica*	Gonochorist	Presence of sperm or eggs		Shallow brackish waters	NA	NA		Sato (1999), Sato and Nakashima (2003)
	H. atoka	Gonochorist	Presence of sperm or eggs		Shallow brackish waters	NA	NA	Female spawning within burrows	Sato and Nakashima (2003)
	H. diadroma	Gonochorist	Presence of sperm or eggs		Shallow brackish waters	NA	NA		Sato and Nakashima (2003)
	H. diversicolor	Gonochorist	Presence of sperm or eggs		Shallow brackish waters	NA	NA	Female spawning within burrows	Sato (1999)

	H. limnicola	Simultaneous hermaphrodite	Presence of sperm and eggs	Yes	Fresh waters	NA		Viviparity	Sato (1999)
Nereididae	*Platynereis dumerilii*	Gonochorist	Presence of sperm or eggs		NA	NA	NA	Broadcast spawning	Helm et al. (2014)
	P. massiliensis	Protandrous sequential hermaphrodite	Presence of sperm or eggs	NA	NA	NA			Helm et al. (2014), Schneider et al. (1992)
Sabellidae	*Oripsis* spp.	Gonochorist	Presence of sperm or eggs		NA	NA	NA		Rouse (1994)
	O. androgyne	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA			Rouse (1994)
	O. androgyne	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA		Spermatheca (=sperm storage organ)	Rouse (1994)
Sabellidae	*Parasabella microphthalma* (*Demonax microphthalmus* of previous authors)	Gynodioecious (simultaneous hermaphrodites and females)	No sperm in some specimens	NA	NA	NA	NA		Gregory (1905), Weeks (2012)
	P. pallida	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	NA		External fertilization[a]	Tovar-Hernández et al. (2009)
	Parasabella sp. cf. *P. aulaconota*	Gonochorist[a]	Presence of eggs in some specimens		Harbour and port environment	NA	NA		Capa and Murray (2015)

(continued)

Table 5.1 (continued)

Family	Species	Sexual system	Sexual system described through	Selfing in hermaphrodite	Habitat	Population density	Sexual dimorphism in gonochorists	Notes on reproductive biology	References
	Parasabella sp. cf. *P. japonica*	Gonochorist[a]	Presence of eggs in some specimens		In dead coral rubble, associated with sponges, bryozoa and algae, or in coarse sand	NA	NA		Capa and Murray (2015)
	P. crassichaetae	Gonochorist[a]	Presence of eggs in some specimens		Tropical and temperate waters	NA	NA		Capa and Murray (2015)
	P. aberrans	Gonochorist[a]	Presence of eggs in some specimens		Fouling communities in wharf piles	Low	NA		Capa and Murray (2015)
	P. bioculata	Gonochorist[a]	Presence of eggs in some specimens		Tropical waters	NA	NA		Capa and Murray (2015)
Sabellidae	*Perkinsiana littoralis*	Gonochorist	Presence of sperm or eggs		Antarctic waters	NA	NA		Gambi et al. (2000)
	P. borsibrunoi	Gonochorist	Presence of sperm or eggs		Antarctic waters	NA	NA		Gambi et al. (2000)
	P. antarctica	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	Antarctic waters	High			Gambi and Patti (1999), Gambi et al. (2000)

	P. riwo	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	High			Gambi and Patti (1999), Gambi et al. (2000)
	P. milae	Asexual reproduction	No sperm nor eggs		NA	NA			Gambi et al. (2000)
Sabellidae	*Sabellastarte spectabilis*	Trioecious	Sperm or eggs in most the specimens (85%), both in few others (15%)	NA	Intertidal and subtidal reefs	High	NA	Spawning	Bybee et al. (2007)
Serpulidae	*Hydroides diramphus*	Gonochorist	Presence of sperm or eggs		Benthic marine assemblages	Highly variable	NA	External fertilization	Allen and Marshall (2014)
	H. elegans	Protandrous sequential hermaphrodite	Presence of sperm or eggs	NA	NA	NA		External Fertilization	Miles and Wayne (2009)
Serpulidae	*Spirobranchus polycerus*	2 morphs (possibly 2 species): morph 1, simultaneous hermaphrodite; morph 2, gonochorist	Presence of sperm/eggs; experimental breeding in lab	Yes	*Gonochorist*, cliffs and boulders exposed to wave action; *Hermaphrodite*, obligate association with fire corals; *Millepora complanata* in patch reefs	Usually found singly or in small groups	NA	Broadcast spawners (both forms)	Marsden (1992)
	S. giganteus	Gonochorist	Presence of sperm and eggs		Tropical and subtropical seas associate with live hermatypic coral	Clustered distribution	NA	External fertilization	Hunte et al. (1990)

(continued)

Table 5.1 (continued)

Family	Species	Sexual system	Sexual system described through	Selfing in hermaphrodite	Habitat	Population density	Sexual dimorphism in gonochorists	Notes on reproductive biology	References
	S. lamarckii (as *Pomatoceros lamarckii*)	Protandrous sequential hermaphrodite with a small percentage of simultaneous hermaphrodites	Presence of sperm or eggs in some specimens, both sperm and eggs in few others	NA	NA	NA			Cotter et al. (2003), Castric-Fey (1984)
	S. triqueter (as *Pomatoceros triqueter*)	Protandrous sequential hermaphrodite with a small percentage of simultaneous hermaphrodites	Presence of sperm or eggs in some specimens, both sperm and eggs in few others	NA	NA	NA			Cotter et al. (2003), Castric-Fey (1984)
Syllidae	*Syllis prolifera*	Gonochorist	Presence of sperm only		NA	NA	No	Stolonization	Musco et al. (2010)
	S. gerlachi	Gonochorist	Presence of sperm only		NA	NA	No	Stolonization	Musco et al. (2010)
	S. vittata	Simultaneous hermaphrodite	Presence of sperm and eggs	NA	NA	Low		Stolonization	Musco et al. (2010)

[a] Doubtful evidence

[b] Four sexual phenotypes are described (pure males, males with oocytes, females with sperm, pure females), but individuals behave only as males or females (see text)

NA No data available

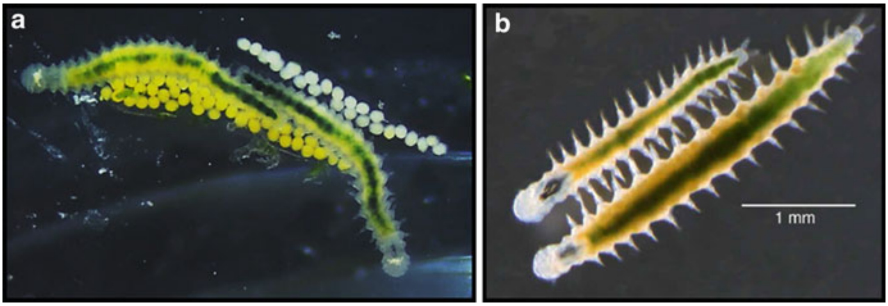

Fig. 5.1 The hermaphroditic species *Ophryotrocha diadema* (**a**) and the gonochoric species *Ophryotrocha robusta* (Paxton and Åkesson 2010; © 2010 Zootaxa, www.mapress.com/j/zt) (**b**)

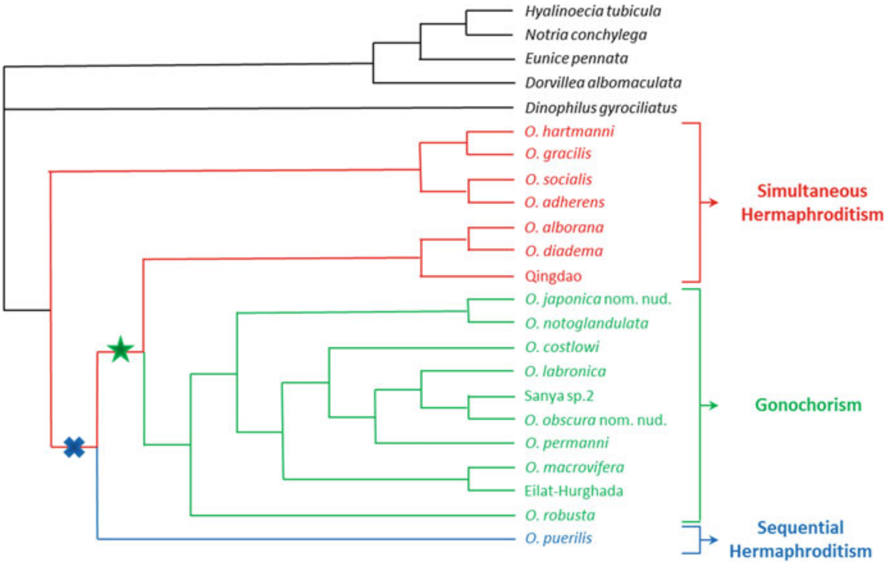

Fig. 5.2 Phylogeny of the *Ophryotrocha* genus, highlighting the diversity of sexual systems. The star represents the hypothetical transition from simultaneous hermaphroditism to gonochorism; the cross represents the hypothetical transition from simultaneous hermaphroditism to sequential hermaphroditism (modified from Dahlgren et al. 2001)

the genus *Ophryotrocha*, the transition between sexual systems happened only once and from hermaphroditism to gonochorism, since the former represents the ancestral situation (Fig. 5.2; Dahlgren et al. 2001).

Comparing the sexual patterns of closely related species allows one to minimize the differences in reproductive traits (e.g. morphology, physiology, etc.) and highlights the effect of ecological variables on the evolution of sexual systems.

Furthermore, all the *Ophryotrocha* species whose sexual system was studied are obligate outcrossers (i.e. they need a partner to successfully reproduce), and thus they are not able to self-fertilize. This could represent an advantage in understanding the transition between sexual systems: avoidance of inbreeding (selfing) indeed has been proposed as one of the main advantages of gonochorism over hermaphroditism both in plants and animals (Ashman 2006; Leonard 2013); the absence of selfing in *Ophryotrocha* species means that the avoidance of inbreeding depression is not a selective force at work in this system and makes it suitable to throw light on other selective forces favouring a sexual system over another.

The worms in this taxon also have technical advantages as study models. Most of them are easy to rear in the laboratory and have relatively short generation times. They release eggs protected in a transparent cocoon, and egg development can be followed at low magnification, which allows for easy measures of individual reproductive success.

In addition, in one simultaneously hermaphroditic species (*O. diadema*), an albino phenotype for the colour of the eggs exists. The presence of the albino phenotype, jointly with the fact that worms have transparent body walls, permits an easy estimate of the reproductive investment in the female role, since maternity can be easily assigned through the colour of the eggs (which are white in albino worms and yellow in wild phenotype worms). A dominant Y allele codes for the yellow egg colour, while the recessive Y allele codes for the white egg colour (Sella and Marzona 1983). Because of their Mendelian inheritance, this genetic marker makes it possible to identify a focal worm in a group and to identify its progeny (cfr. Lorenzi et al. 2014). [Following Åkesson (1976), the egg colour marker is neutral.]

The worms of the genus *Ophryotrocha* are typically small (between 1.5 and 5 mm) and widespread, being distributed from the tropics to the poles. Generally, these worms are part of the infauna and inhabit a large variety of marine habitats (Thornhill et al. 2009): polluted harbours (Åkesson 1976; Simonini and Prevedelli 2003), deep-sea whale-fall and wood-fall habitats (Wiklund et al. 2012), and hydrothermal vents (Blake and Hilbig 1990; Wiklund et al. 2012).

As for their reproductive biology, *Ophryotrocha* worms mate through "pseudocopulation", in which the fertilization is external but the two individuals stay in close contact to each other (Westheide 1984). Pseudocopulation generally follows a long and time-consuming (4.5 h or more) courtship (Åkesson 1973; Westheide 1984; Rouse 1999). One or both parents generally take care of the eggs, which are released in a protective cocoon or a tube of mucus, parchment-like material or loose jelly (Sella and Ramella 1999). Parental care usually consists of the adult(s) resting on the egg cocoon or moving back and forth possibly to clean it (Premoli and Sella 1995).

However, detailed behavioural studies on *Ophryotrocha* worms are still lacking to make it possible a thoroughly description of their mating behaviour.

5.2.1 Ophryotrocha *Simultaneous Hermaphrodites*

Within the genus *Ophryotrocha*, eight species of simultaneous hermaphrodites (often referred to as just hermaphrodites along the text) have been described up to now (Sella and Ramella 1999; Pleijel and Eide 1996). All of them are obligate outcrossers (i.e. they do not self-fertilize), and for a few of them, the mating system has been studied thoroughly (*O. diadema*, Sella 1985, 1988, 1990, 1991, Sella and Lorenzi 2000, Lorenzi et al. 2005; *O. gracilis*, Westheide 1984, Sella et al. 1997; and *O. socialis*, Ockelmann and Åkesson 1990). All these species are part of the interstitial fauna, as they live among the detritus and fouling fauna of polluted harbours. There is no clear information about the density of the populations in the wild, but it is expected to be low, as it is usually in meiobenthic species (Svedmark 1964; Clark 1978; Westheide 1984) (except *O. socialis*, in which several worms live together in a common system of mucous tubes where they perform communal breeding; Ockelmann and Åkesson 1990). Occasionally, population size is expected to increase locally, creating clustered dense populations, at least for short periods (Sella and Ramella 1999). The production of mucus trails, which worms build as they crawl on the substrate, likely favours a clustered spatial distribution, as individuals can probably follow the trails produced by conspecifics and form small groups, at least temporarily (Sella and Ramella 1999).

Living at low population density may represent an important selective pressure in shaping reproductive traits, as is the case of the aforementioned *Dinophilus gyrociliatus*. Low density is also one of the main ecological factors favouring the maintenance of simultaneous hermaphroditism as hermaphrodites have a double likelihood to encounter suitable partners relative to separate sex animals (Tomlinson 1966; Ghiselin 1969; Puurtinen and Kaitala 2002). In the meanwhile, occasional bursts of population density may have favoured the ability to adjust sex allocation (i.e. the ability to modify the proportion of resources allocated to the female and to the male function) in hermaphrodites, and indeed female sex allocation adjustments are easily seen in this taxon (e.g. Lorenzi et al. 2005; Schleicherová et al. 2014).

5.2.1.1 Protandry

Another common trait in hermaphroditic species is a protandrous adolescent phase, during which the worms are still immature but can already produce (and use) sperm. Sella and Lorenzi (2003) have shown that the young worms in their protandrous phase delay the age when they become hermaphrodites if they have the opportunity to fertilize eggs (produced by mature partners). This result was interpreted as a clue that sperm production is costly for these tiny, immature worms (i.e. there is a physiological trade-off between the resources invested in courting and fertilizing egg cocoons on one side and those invested in body growth on the other, Sella and Lorenzi 2003), but it could also be viewed as an adaptive reproductive choice which involves long-term sex allocation adjustments. When the opportunity to fertilize eggs is not zero, young worms may delay the age of full sexual maturity (i.e. the age at which they also invest in the female function), thus spending a longer part of their lives as males, especially if the male role is the preferred sexual role in these hermaphroditic worms (see below).

5.2.1.2 Sexually Mature Hermaphrodites

Up to now, the majority of studies on *Ophryotrocha* hermaphrodites have focused on the sexual system of *O. diadema*. In this species, sexual life begins as soon as the worms reach a body length of six segments and start producing sperm in the third and fourth body segment (Sella 1985). The full sexual maturation will be achieved about 40 days later, when worms will reach a body length of 14–15 segments and will start producing eggs from the fifth segment onwards although continuing to produce sperm as well.

5.2.1.3 Mating

When they mate, hermaphroditic *Ophryotrocha* worms have unilateral mating which means that at each mating, one individual plays the female role—and spawns eggs—and the other plays the male role—and releases sperm. Eggs are protected in a mucus cocoon, and generally both parents stay nearby in the next day taking care of the egg cocoon, creating a kind of nesting site (Premoli and Sella 1995).

Mating was described by Westheide (1984) in the hermaphrodite *O. gracilis* as "pseudocopulation", a relative complex behavioural sequence where two partners stay in physical contact, with the worm in the "male" role attached to the "tail" (i.e. the distal segments) of the worm in the "female" role. The "female" produces a mucous cocoon all around its body, which partially covers the head and neck of the male worm (whose anterior body segments are the sperm-producing segments). Westheide suggested that sperm release might precede the release of eggs within the mucous cocoon, because the worm playing the male role was seen leaving the egg cocoon before the worm playing the female role. However, this was not confirmed in two separate, videorecorded sequences of mating behaviour in *O. diadema*, where the worms playing the male role were the last worms to leave the cocoon (Santi M., personal observation; Lescher A., personal observation; and also Meconcelli S. in *O. adherens*).

One reason why mating requires physical contacts between partners may be that sperm are aflagellate in the genus (Morrow 2004) and thus may need to be "deposited" on the eggs, within the egg cocoon. However, we do not know whether worms mate by pseudocopulation because sperm are immotile or, *vice versa*, if sperm have lost their tail because worms mate by pseudocopulation. Competition for mating may occur, and multiple paternity within a single egg cocoon has been reported (Lorenzi et al. 2014) which makes it difficult to explain why aflagellate sperm have evolved.

In *O. diadema*, pairs are preferentially formed between sexually mature (e.g. ovigerous) hermaphroditic worms, and the two partners regularly alternate their sexual roles for long time periods, behaving once as a female and once as a male (Sella 1985, 1988). This particular mating behaviour was first described by Fischer (1980) in the serranid fish *Hypoplectrus nigricans* and is called “egg trading”.

5.2.1.4 Egg Trading and the Preferred Sexual Role

When simultaneous hermaphrodites mate unilaterally, a conflict arises between partners due to the different pay-offs for the two sexual roles in term of costs and benefits (Charnov 1979; Leonard 2006; Schärer et al. 2014). According to “Bateman’s principle” (Bateman 1948; Charnov 1979; Anthes et al. 2010), an individual should prefer to mate in the male role, as sperm are cheaper than eggs to produce, and mating as a female offers the same fitness returns at higher costs. The preference for the male role should be shared by all the individuals of a population.

The preference for the male role may be not universal among hermaphroditic organisms in general (Leonard 2006), but some clues suggest that *O. diadema* hermaphrodites might have a preference for mating as males. Indeed, mature hermaphrodites (1) abandon their reciprocating partners for worms with riper eggs (Sella and Lorenzi 2000), (2) mate repeatedly in the male role and have a longer lifespan if they lay eggs less often (Di Bona et al. 2010) and (3) make a smaller investment into the female function and aggressively compete for mating in the male role, when mating opportunities are high (Lorenzi et al. 2005, 2006).

If all individuals in the population share the same preference for one of the two sexual roles, a conflict arises when two potential partners meet, as both may aim to play the preferred role. The long courtship which has been described in hermaphroditic species may indeed represent the time two partners need to “agree” on which role they are going to play. An evolutionary solution to the conflict about sexual roles is conditional reciprocity, which occurs when hermaphrodites regularly alternate their sexual roles over successive reproductive bouts (Leonard 2006). Indeed, *O. diadema* mature hermaphrodites are long-lived (about 3 months, according to Di Bona et al. 2010), and they alternate their sexual roles up to 40 times during this period (Di Bona et al. 2015). In this view, egg-trading individuals mate in the less preferred role to get access to mating in the preferred role (Axelrod and Hamilton 1981; Leonard 2005).

5.2.1.5 Why Trading Eggs?

Egg trading is a crucial behavioural mechanism in the study of the evolution of sexual systems in hermaphrodites. This form of cooperation not only solves the sexual conflict that arises between two simultaneously hermaphroditic mates but, as a recent mathematical model by Henshaw et al. (2015) proposes, also permits the maintenance of the hermaphroditism even when the selective pressures favouring this sexual system (i.e. low mating rate) are no longer acting. With this model the authors show that the egg-trading behaviour increases the fitness value of eggs, as eggs can be traded for opportunities to mate in the male role. In this way, egg trading favours a female-biased sex allocation that prevents pure females from invading a trading population.

Empirical support to this model comes from the chalk bass *Serranus tortugarum* in which formation of monogamous pairs that perform long-term reciprocation is maintained in relatively high-density social groups (Hart et al. 2016).

5.2.1.6 Cheating

Reciprocal egg trading is evolutionarily stable only under a low level of cheating (i.e. when individuals attempt to mate repeatedly in the preferred role and avoid mating in the less preferred one only rarely), and therefore some mechanism to guard against nonreciprocating individuals is expected to evolve. In *O. diadema* several mechanisms preventing cheating have been demonstrated. First, individuals are able to recognize the sexual phenotype of their partners (i.e. they behave differently when paired with mature hermaphrodites with ready-to-lay eggs, mature hermaphrodites without ready-to-lay eggs or adolescent males), and, depending on their partners' readiness to lay eggs, hermaphrodites changed their egg production rate accordingly. Sella (1988) and Premoli and Sella (1995) have shown that the time interval between two successive spawnings by the same hermaphrodite is significantly longer when it is paired with a nonreciprocating individual than when it is paired with a mature reciprocating hermaphrodite, which is able to trade eggs. In other words, mature hermaphrodites release egg cocoons more often when they alternate egg laying with their partners (average time interval between two spawnings by the same individual when hermaphrodites are paired with hermaphrodites, 2.97 ± 0.2 days; when hermaphrodites are paired with adolescent males, 5.2 ± 0.2 days; and when paired with nonmature hermaphrodites, 5.4 ± 0.36 days). Furthermore, the study by Sella (1988) also documented that when hermaphrodites can choose between mature hermaphrodites and adolescent males, the former are generally preferred and adolescent males (i.e. nonreciprocating individuals) are rejected as mates.

5.2.1.7 Egg Parcelling

Since egg reciprocation between partners is nonsimultaneous (i.e. at each mating, one individual releases eggs and the other releases sperm), but successive egg layings follow with a delay, there is the risk that the partner fertilizes eggs and then deserts (cheating). A way to minimize fitness losses is to offer only a part of the mature eggs (i.e. egg parcelling). By doing this, not only could cheaters be identified at a lower cost (only a part of the eggs produced are fertilized by the nonreciprocating partner), but also the fitness of reciprocating individuals will be higher. If they continue to reciprocate they have the opportunity to fertilize more eggs.

Egg parcelling has been described in several serranid fishes of the genus *Hypoplectrus* and *Serranus* (Fischer and Petersen 1987; Petersen 2006). A recent field study on the chalk bass *Serranus tortugarum* shows that parcel number is coordinated within pairs, whereas there is almost no correlation between parcel number and fecundity; this suggests that egg parcelling may represent a means to assess equality in resource exchange within a pair and thus a way of communication between partners (Hart et al. 2016).

Egg parcelling has never been described among *Ophryotrocha* hermaphrodites. However, comparing the number of eggs per cocoon and the time interval between two consecutive spawning by the same individual in hermaphroditic and gonochoric species, a strategy similar to the egg parcelling, as described in fish, may occur in hermaphroditic worms. Indeed, simultaneously hermaphroditic species lay smaller cocoons but at a higher rate compared to gonochorist species (Premoli and Sella 1995) (e.g., the simultaneously hermaphroditic *O. diadema* lays about 25 eggs per cocoon every 3 days, while the gonochoric *O. labronica* lays about 130 eggs each 11 days; Premoli and Sella 1995), although hermaphroditic species do not seem morphologically constrained to produce smaller clutches. Simultaneous hermaphroditic *Ophryotrocha* species might parcel eggs by maturing fewer eggs at a time and spawning them relatively more frequently than related gonochoric species.

5.2.1.8 Population Density, Mate Competition and Sex Allocation

As mentioned above, the species of the genus *Ophryotrocha* are expected to live in sparse populations. In such conditions, mate searching is likely to be time and resource consuming, making it relatively disadvantageous for a paired hermaphrodite to desert its partner and break an established pair bond (cfr. Sella and Lorenzi 2000). When pairs are monogamous, any sperm will likely compete with related sperm from the same donor to fertilize the clutch of eggs. Under high rate of local sperm competition (*sensu* Schärer 2009), it is advantageous for sperm donors to bias their sex allocation in favour of the female function, since producing large numbers of sperm does not offer any return in terms of fitness. In *O. diadema*, *O. gracilis* and *O. hartmanni*, the biomass of the body area involved in the female function is 80% of the total gonadal biomass (Premoli and Sella 1995; Sella et al. 1997). This leads to one of the lowest sperm redundancy estimates among those known both for vertebrates and invertebrates (Cohen 1975), as *O. diadema* produces less than one hundred sperm per egg (Sella 1990).

In turn, the female-biased sex allocation reduces the advantages of deserting a partner, since hermaphrodites cannot mate frequently as males due to the relative scarcity of sperm. This stabilizes the egg-trading behaviour. If mating opportunities increase, the competition to fertilize eggs rises between unrelated sperm and hermaphrodites could gain an advantage in switching their sex allocation into investing relatively more resources in the male role at the expense of the female role.

Lorenzi et al. (2005) have shown that *O. diadema* worms adjusted their female sex allocation in response to reproductive competition: the absolute number of laid cocoons significantly decreased when individuals were reared with several conspecific mature hermaphrodites rather than when they were reared in isolated pairs. The diminished female allocation occurred irrespective of density-related stressors (Lorenzi et al. 2005) and was a response to species-specific signals or cues (Schleicherová et al. 2006), supporting the hypothesis that the sex allocation adjustment is a response to mate competition and not a density effect (e.g. competition for food, oxygen depletion, etc.). Additionally, sex allocation adjustments have been shown to be extremely rapid (i.e. worms adjusted their sex allocation to current mating opportunities in 5 days) and have no costs in the short term (Lorenzi et al. 2008).

In contrast, sperm production seems weakly or not at all adjusted to mating opportunities (Lorenzi et al. 2005; Schleicherová et al. 2014). The lack of clear evidence for sperm allocation adjustments can be partially due to the limited amounts of sperm in these species and to the technical difficulties in counting the aflagellate sperm, but it may also be a reasonable finding. Increasing the number of sperm, when sperm are aflagellate and thus immotile, may not be a rewarding strategy: if sperm are immotile, the relative position of the worms during sperm release may have a larger effect on fertilization success than the number of sperm released by each individual. This may explain why worms increase their allocation to the male function allocating more resources to expensive and risky behaviours (such as increasing aggressive interactions for access to mating) that likely enhance male reproductive success rather than barely increasing the number of sperm, which are actually unable to “compete” with other sperm because they cannot move (Lorenzi et al. 2006). As the absolute number of eggs changes depending on mating opportunities, whereas sperm number does not, the relative proportion of resources invested in the two sexual functions, that is, sex allocation, is highly plastic in the species (Lorenzi et al. 2005).

Furthermore, in *O. diadema*, Di Bona et al. (2010) described a particular phenotype with a marked male-biased sex allocation: in a laboratory population, the authors found individuals that were morphologically hermaphrodites (i.e. they had oocytes in their coelom) but behaved as males (i.e. they did not lay eggs) for a long time period (21 days) or (rarely) for their entire life (functional-male phenotype). The frequency of the functional-male worms in the population changed as a function of mating opportunities: functional males were more frequent under a promiscuous mating regime (i.e. when four mature hermaphrodites were reared together) than under a monogamous regime (i.e. when two mature hermaphrodites were reared in isolated pairs).

In many hermaphroditic fishes (e.g. chalk bass), individuals frequently engage in “streaking”, that is, they intrude into other spawning pairs and release sperm attempting to gain more matings in the male role (Fischer 1984; Hart et al. 2010); this behaviour is more frequent in high-density populations (Hart et al. 2010). The presence of functional males in *O. diadema* may suggest the existence of some analogous behaviour in this species, but, at the moment, there is no experimental evidence for it.

This ability to adjust sex allocation to environmental conditions is a key trait in the theoretical speculations as to the evolutionary transition between hermaphroditism and gonochorism (Charlesworth and Charlesworth 1978; Delph and Wolf 2005) and could set the stage for the evolution of separate sexes from hermaphroditic ancestors in *Ophryotrocha* worms (Lorenzi and Sella 2013).

5.2.2 **Ophryotrocha** ***Sequential Hermaphrodites***

Sequential hermaphroditism is expected to be favoured when the reproductive success of an individual as a male—or as a female—is strongly related to its body size (or its age) and the relationship between body size and reproductive success is different for the male and the female sex (Ghiselin 1969, 1974; Charnov 1982; Warner 1988). For example, if individuals with a large body size have a higher reproductive success as females than as males because female reproductive success increases with body size (but male reproductive success does not), it will be advantageous for small individuals to be males and change into females once they will reach a large body size, assuming relatively low costs for sex reversal. Anyway, according with the theory of “quantitative gender” proposed by Klinkhamer and Jong (Klinkhamer et al. 1997; Klinkhamer and de Jong 2002; Cadet et al. 2004), sequential hermaphroditism is rarely more adaptive than gradual changes in sex allocation associated with size/age (e.g. the simultaneous hermaphrodite *O. diadema* has a protandrous adolescent phase and gradually allocates more resources to the female role while aging). Klinkhamer and Jong distinguish between two types of size effects: “direct” effects, which are benefits of size *per se* (e.g. the ability of a tall wind-pollinated tree to spread pollen over a larger area), and “budget” effects, in which benefits are due simply to greater energetic resources (e.g. the ability of a larger fish to produce both more sperm and more eggs). When “direct” effects of size differ for male and female function and are more important than “budget” effects, sex change is expected to be adaptive (Leonard 2013). This seems to be the case for the only known (protandrous) sequential hermaphroditic species of the genus *Ophryotrocha*. Indeed, Berglund (1986, 1990) demonstrated that reproductive success was significantly related to body size for females, but not for males in *O. puerilis*, and that large males fertilized significantly fewer eggs than small males because females preferred to mate with small males (Berglund 1990). Berglund (1990), and Premoli and Sella (1995) made the hypothesis that females prefer small males because of the lower prospective of a conflict with the partner over sexual roles, as large males are more likely to change sex to females. Mating preferences represent indeed a classical example of “direct” effects of size (Cadet et al. 2004) which make sex change adaptative.

The cost of sex reversal in *O. puerilis* is expected to be relatively low (as measured by the time necessary to change from one sex to the other: 2–5 days, Monahan 1988; Premoli and Sella 1995). *O. puerilis* worms start to produce sperm at a body size of about 9 segments and generally change to females when they are 15–20 setigers long (Bacci 1951). Sex reversal is genetically determined (Sella 1980) and controlled by hormones (Pfannenstiel and Grothe 1988), but there is also a social effect: females can inhibit egg production in males and smaller females, and this effect is mediated by hormones (Pfannenstiel 1973; Grothe and Pfannenstiel 1986).

O. puerilis is also one of few sequential hermaphroditic species that change sex repeatedly during their lifetime. This phenomenon has been also described in *Ostrea* oysters where individuals are males when they are brooding and females after egg hatching (Coe 1932; Chaparro and Thompson 1998) and in several species of fishes (Munday et al. 2010; Kuwamura et al. 2011). Among fish the case of the blue-banded goby (*Lythrypnus dalli*) is particularly interesting: in this species individuals are histologically and anatomically simultaneous hermaphrodites, but they behave either as males or as females in relation to their relative body size (St. Mary 1993).

Berglund (1986, 1990) has interpreted alternating sex change in *O. puerilis* in terms of sex allocation theory: males invest few resources in gamete production, as sperm are cheaper to produce than eggs, and thus males can grow faster than females. As a consequence, after some spawnings, a small male and a large female will reach the same body size and will change sex simultaneously, the male changing to female and the female reverting to male (a phenomenon known as “Paar-Kultur”—pair-culture—effect, Hartmann and Huth 1936). Interestingly worms reared together for a long time period (more than 1 month) changed sex so often that they started to behave as simultaneous hermaphrodites which regularly alternated egg laying and egg fertilization on short time intervals (every 3 days, Berglund 1986). We do not know whether pairs are stable for long time periods in the wild, but we do know that population density is often very low in this species, making long-term pair living a realistic option in this species (Premoli and Sella 1995).

5.2.3 Ophryotrocha *Gonochorists*

In the genus *Ophryotrocha* (and generally in polychaetes; Schroeder and Hermans 1975) most species are gonochoric, and only 9 out of more than 40 known species are hermaphroditic (either simultaneous or sequential hermaphrodites). This suggests that, in these animals, male reproductive success does not saturate with increasing investment and expressing only one sexual function often leads to high fitness returns (Charnov 1982); we may try to speculate why.

O. labronica is the best studied gonochoristic species in this genus. It is characterised by a clear sexual dimorphism where males have a wider prostomium and thicker jaws compared to females. Females grow faster than males and become sexually mature at a body length of about 12 segments, whereas males start to produce sperm at a smaller body size (Paxton and Åkesson 2007; Lorenzi and Sella 2013).

O. labronica worms share their habitat with *O. puerilis* and probably eat the same food. So why is one species gonochoric and the other a sequential hermaphrodite? One possible explanation is that in *O. labronica*, both female and male reproductive successes are related to body size, making sex reversal costly. However, Berglund (1991) showed that when worms were reared in pairs, female reproductive success increased with body size in *O. labronica*, whereas male reproductive success was not correlated with body size, as it occurs in *O. puerilis*. If *O. labronica* worms were reared in triplets composed of a female, a small male and a large male, pairs were preferentially formed by a female and a large male. Unfortunately, Berglund's experiment did not make it possible to disentangle the effect of male-male competition (i.e. large males are better competitors) from that of female choice (i.e. females prefer large males), but his results showed that large males had higher fitness returns than small ones and had nothing to gain from changing sex.

In laboratory mass cultures, *O. puerilis* populations typically have strongly male-biased sex ratios (Berglund 1991; Premoli and Sella 1995), whereas usually *O. labronica* populations have even, or slightly female-biased, sex ratios (Åkesson 1970; Sella and Bona 1993). These species-specific sex ratios may contribute to selection in favour or against sex reversal. Skewed sex ratio in *O. puerilis* can favour sex reversal if turning female is advantageous because it will end the competition with the other males. In contrast, the relatively more balanced sex ratios in *O. labronica* populations may impose no pressure in favour of changing sex.

5.3 Intermediate Steps Between Truly Hermaphroditic and Gonochoristic Species in Polychaetes

In the preceding paragraph, we reasoned only about purely gonochoric or purely hermaphroditic species. Although these two sexual systems are the two most common sexual systems in the animal kingdom, they are the two endpoints along a continuum, which includes intermediate steps such as gynodioecy (i.e. hermaphrodites + females), androdioecy (i.e. hermaphrodites + males) and trioecy or subdioecy (i.e. hermaphrodites + females + males) (Delph and Wolf 2005; Charlesworth and Charlesworth 1978; Avise 2012). In their paper, Delph and Wolf (2005) well summarized the evolutionary pathway that leads from a hermaphroditic population to a gonochoric one via gynodioecy, but similar steps could be imagined for the pathway via androdioecy. According to Delph and Wolf (2005), the first step along this pathway occurs when in a hermaphrodite population a male-sterile mutant appears which is able to play the female role only; if female mutants have even a slightly higher fitness compared to hermaphrodites, they will spread in the population. When the population is dimorphic for gender (hermaphrodites + females), hermaphrodites will have higher fitness returns through male function; in this way, hermaphrodites will be favoured if they allocate relatively more resources to the production of male gametes, until they totally suppress the female function, which leads to a gonochoric population. A study from Dorken and Pannell (2009) provided experimental evidence for the androdioecy pathway: they allowed populations of the hermaphroditic plant *Mercurialis annua* to evolve either in the presence of pure male individuals or in populations composed entirely of hermaphrodites. After a few generations, the average male allocation of hermaphrodites had changed in different directions depending on whether pure males were present or not, confirming that pure-sex mutants produce a selective pressure on the hermaphrodite sex allocation.

Identifying and studying populations that display these intermediate sexual systems could not only highlight the selective pressures that favour one sexual system over another (i.e. the adaptive value) but also help to identify the mechanisms underlying the transition (i.e. sexual system evolution).

Unfortunately, relatively few species with sexual systems intermediate between hermaphroditism and gonochorism have been described in animals (Weeks 2012), although recently sexual phenotypes intermediate between hermaphroditism and gonochorism have been reported in *Ophryotrocha* worms.

5.3.1 First Evidence for Intermediate Sexual Phenotypes in **Ophryotrocha** *Worms*

In the oldest literature on *Ophryotrocha* worms, the sexual system of some species was controversial. Now we know that multiple sexual phenotypes exist in several species and that populations differ in the relative frequency of these phenotypes, which clarifies why old descriptions of the sexual system of such species were confused, with authors often contradicting their own findings. For example, evidence for intermediate sexual systems was reported by Bacci (1951) for the species that we now describe as the sequential hermaphrodite *O. puerilis*. Bacci observed that in this species (where small worms are males and switch to female when they reach a large body size), a small percentage of short females and large males existed, and he also reported one male who died at a very large body size without changing to female. In a successive publication, Bacci (1964) argued that small females and large males could actually belong to another species. We will never know which version was the correct one, but both could be correct if populations vary widely in their sexual traits.

5.3.2 Four Sexual Phenotypes in **O. labronica**

In the genus *Ophryotrocha*, androdioecious or gynodioecious populations have not been reported up to now. However, evidence for intermediate steps between hermaphroditism and gonochorism now exist.

Probably one of the most informative cases is *O. labronica*, an apparently gonochoric species that actually has four different sexual phenotypes: pure males, males with oocytes, females with sperm and pure females (Lorenzi and Sella 2013; Meconcelli et al. 2015a). This species was first described as hermaphroditic on the basis of morphological observations (Parenti 1960; La Greca and Bacci 1962) and later as gonochoric with some hermaphroditic individuals (Bacci et al. 1979), which documents that these authors found individuals with eggs and sperm.

Intriguingly, in Lorenzi and Sella (2013), males with oocytes and females with sperm could not be defined as either hermaphrodites or separate-sex individuals because they were sexually dimorphic, functioned as one-sex individuals but produced both male and female gametes (for a functional criterion of gender, cfr. Lloyd 1980; Pannell 2002; Delph 2003) (Lorenzi and Sella 2013). Indeed, females with sperm had the secondary sexual traits that typically identified females in the species (i.e. they had relatively large body size, small head and small jaw size), whereas males with oocytes were phenotypically males (they had relatively small body size, large head and large jaw sizes) (cfr. Paxton and Åkesson 2007). However, a screening for the gametes in the coelom showed that males with oocytes and females with sperm produced both sperm and eggs (Lorenzi and Sella 2013). The special combination of sexual dimorphism and production of two types of gametes suggested Meconcelli et al. (2015a) to classify them as pseudohermaphrodites.

Lorenzi and Sella (2013) discovered these four phenotypes in two American and one Mediterranean populations (i.e. Alamitos Beach, LA, CA; San Diego, CA; Genova, IT), and they documented that each population had distinct proportions of the four phenotypes. Pure females were very rare (virtually absent) in all populations, whereas the frequency of pure males, males with oocytes and females with sperm varied significantly between the American and the Mediterranean populations. Males with oocytes and females with sperm made up nearly the entire Californian populations (95% at Alamitos beach and 92% in San Diego) and were roughly equally represented. In contrast, females with sperm and pure males were the two most common phenotypes in the Mediterranean population, the latter representing about one third of the entire population.

The four sexual phenotypes also differed between each other in another morphological trait, possibly a secondary sexual trait: the number of rosette glands, which are dorsal and epidermal structures located in the posterior segments of adult worms. These glands occur in many *Ophryotrocha* species (Pleijel and Eide 1996) and, in gonochoric species, are typically more abundant in males than in females (Paxton and Åkesson 2010). In *O. labronica*, the number of rosette glands varied among sexual phenotypes and it did so at a different extent across the three populations; rosette glands were significantly more abundant in male than in female phenotypes in the Mediterranean and San Diego populations, whereas they were in roughly similar numbers in the worms in the Alamitos Beach populations (Lorenzi and Sella 2013). The function of the rosette glands is unknown, but they have been associated to mating and spawning and might be involved in the release of sexual pheromones (cfr. Paxton and Åkesson 2007).

From a functional point of view, all sexual phenotypes of *O. labronica* are fertile and are able to reproduce when reared in heterosexual pairs (i.e. when they are paired with a partner exhibiting the opposite sex, based on external morphology). Very few females were able to produce brood when paired with another female (only 8%) or when kept alone (only 2%) for 2 months. In contrast no male in homosexual pairs or in isolation did so. This suggests that sperm in females are functional and may be used to self or fertilize the eggs of another female (at least occasionally), whereas oocytes may be not functional in males (Lorenzi and Sella 2013). Self-fertilization in this species was already reported by Parenti (1960).

Since egg production is reasonably costly (a single egg cocoon accounts for approx. 1/3 of the worm body volume and egg production trade-off with lifespan, Di Bona et al. 2010), Lorenzi and Sella (2013) have also checked whether virgin males with oocytes adjusted their sex allocation in response to mating opportunities, as hermaphrodites typically do. Again, the results of the experiment showed that the degree of plasticity in sex allocation strongly varied among populations. The worms from Alamitos Beach—the most "hermaphroditic-like" population—were able to plastically adjust their sex allocation to current mating opportunities: males reared under high mating opportunities had relative less oocytes in their coelom than males reared with only one partner, while no plastic adjustment was reported for males from the San Diego and the Mediterranean populations, the latter being the most "separate-sex" population.

In conclusion, *O. labronica* is not a strictly gonochoric species, since four different sexual phenotypes exist in at least three geographically separated populations. Yet, this species cannot be defined as either androdioecious or trioecious, as males with oocytes and females with sperm cannot function as hermaphrodites (with the exception of a few females which were able to fertilize their own eggs or those of a female partner). Instead, this species can be viewed as an intermediate step in the transition from hermaphroditism to gonochorism along a hypothetical androdioecious pathway (as defined by Delph and Wolf 2005, see above), where the gametes of the opposite sex in males with sperm and in females with oocytes are vestigial traits of an ancestral hermaphroditic state. However, one observation is not congruent with the expectations from the theoretical models of transitions, namely, the fact that in Delph and Wolf model no more than three sexual phenotypes should coexist, whereas in *O. labronica* four sexual phenotypes coexist. Dorken and Pannell (2009) reasoned that selection on gamete production might weaken towards the end of the pathway leading to separate sexes, when hermaphrodites are already strongly biased towards one sex. This could explain why *O. labronica* females retain sperm: selection for switching off sperm production may be relaxed, because these females already have a disproportionate investment in the female function, compared to their investment in rare sperm (Lorenzi and Sella 2013).

The same four sexual phenotypes have also been reported in two other, so-called gonochoric, species (*O. robusta* and *O. macrovifera*, Meconcelli et al. 2015a) suggesting that this might be a relatively common trait in the genus. In a broader view, examples of intermediate sexual systems have been described also in the mainly hermaphroditic genus *Indodidymozoon* (Platyhelminthes), in which two species (*I. pearsoni* and *I. suttiei*) show traits intermediate between hermaphroditism and gonochorism: female individuals have apparently nonfunctional testis and male individuals have apparently nonfunctional female reproductive organs (Anderson and Cribb 1994). As it is the case in *Ophryotrocha* worms, functional gonochoric species seem to have evolved from a hermaphroditic ancestor (Anderson and Barker 1998). Further examples of intermediate sexual systems have been reported in crustaceans, where individuals with both male and female traits have been reported in gonochoric species, such as *Cherax quadricarinatus*, where individuals, which are functionally male and have male external morphology, have both female and male genital openings (Sagi et al. 1996); similarly, in *Gammarus minus*, functional females may have genital papillae (a male sexual characteristic) (Glazier et al. 2012). Finally, a population entirely composed of individuals with intermediate traits has been described in the crustacean *Parastacus pilimanus*, in which individuals have both female and male gonopores externally but either male or female gonads internally (Rudolph and Verdi 2010).

The three populations of *O. labronica* were largely different from each other in the relative frequency of the sexual phenotypes as well as in the expression of secondary sexual traits (rosette gland numbers) and, although to a small extent, in their degree of plasticity in sex allocation. For this reason, these three populations can be viewed as three different steps along the hermaphroditism-gonochorism continuum. From this perspective, the Alamitos Beach population retained the most hermaphroditic traits; the population was largely composed of individuals producing both type of gametes and having the lowest degree of sexual dimorphism as for the number of rosette glands. Furthermore, males with oocytes were able to plastically adjust their sex allocation in response to mating opportunities, a typical hermaphroditic trait (cfr. Charnov 1982; Schärer 2009). On the other hand, the Mediterranean population retained the least hermaphroditic traits, with a half of the morphological males expressing a pure (i.e. unisexual) male phenotype. Worms in the Mediterranean population were strongly sexually dimorphic and were unable to adjust their sex allocation to current mating opportunities (Lorenzi and Sella 2013). Finally, worms from the San Diego population were intermediate in these traits.

We can imagine the following evolutionary scenario for the "gonochoristic end" of the evolutionary pathway between hermaphroditism and gonochorism. Before a complete separation of sexes is achieved, *Ophryotrocha* worm populations might typically be composed of up to four sexual phenotypes, where the pseudohermaphroditic phenotypes (males with oocytes and females with sperm) have already evolved some degree of sexual specialization in their external morphology (i.e. sexual dimorphism) but may still produce both eggs and sperm and retain some ability to adjust their sex allocation to environmental conditions (mainly social conditions, i.e. mating opportunities). The three *O. labronica* populations described in Lorenzi and Sella (2013) might represent three steps with different degrees of sex specialization. Yet, other questions can be raised. First, we may ask at which extent the sexual phenotype is influenced by environmental factors during development. Second, we can ask where the four sexual phenotypes come from or which hermaphroditic trait enabled the appearance of a minimum level of sexual specialization in the would-be separate sex.

5.3.3 Labile Sex Expression

Sexually labile species have the potential to produce male, female or both kind of gametes depending on the influence of different environmental factors (cfr., for plants, Korpelainen 1998). This form of lability is expected to play a central role in the evolution of sexual systems, with sexually labile species representing an intermediate stage between hermaphroditism and gonochorism (Leonard 2013). Diggle and Miller (2013) have shown that in *Solanum* plants, a fixed sexual expression can evolve from a phenotypically plastic sex expression. Some *Solanum* species were able to produce both hermaphrodite and male flowers and the production of male flowers increased when resources were abundant; however, in other species of the same genus, male flower production was not plastic and did not vary with resource abundance. The authors found that plasticity in sex expression was the ancestral trait and a fixed production of male flowers evolved where resources were predictable and abundant. This suggests that there might be a continuum between plastic sex allocation in hermaphroditic species (see above) and a fixed sex expression in separate-sex species. Indeed, sex expression in gonochoric *Ophryotrocha* species still exhibits some level of plasticity.

5.3.4 Plasticity in Sex Expression During Development

Plasticity in sex expression during the juvenile phase has been found in three so-called gonochoric species of the genus *Ophryotrocha* (i.e. *O. labronica*, *O. robusta*, *O. macrovifera* Rolando 1984; Meconcelli et al. 2015a). Meconcelli et al. (2015a) showed that in these species, juvenile worms which were paired with females developed as males significantly more often than juveniles paired with males or reared in isolation, that is, there was a social effect on sex expression, where the presence of an adult female triggered larvae to develop as males. Intermediate sexual phenotypes (i.e. females with sperm and male with oocytes) were present in all three species and their frequency changed in response to social environment: as expected, juveniles reared in isolation displayed intermediate sexual phenotypes more often than juveniles paired with adults. The presence and the gender of an adult also affected the developmental time of juveniles; in fact, in *O. labronica*, *O. robusta* and *O. macrovifera*, the individuals that developed the same gender as that of their partner needed a significantly longer time period to mature sexually than individuals that matured the sex opposite to that of their partner. In contrast, the effect of the social environment was virtually null during the adult phase, when individuals reared in homosexual pairs did not change to another sex, suggesting that sex expression was labile only during juvenile development (Meconcelli et al. 2015a).

Overall the study by Meconcelli et al. (2015a), jointly with other studies (Rolando 1984; Lorenzi and Sella 2013), shows that, in several, so-called gonochoric species of the genus *Ophryotrocha*, intermediate sexual phenotypes are present and that there is a certain degree of plasticity in sexual expression.

Taking into account all the experimental evidence, it is possible to state that in this genus there is a strong environmental contribution to sex determination but, at the same time, sex determination is also affected by genetic factors.

5.4 The Genetic Basis of Sex Determination in *Ophryotrocha*

In order to understand the selective mechanisms underlying the transition between hermaphroditism and gonochorism, it is important to know the genetic contribution to sex determination.

When the transition occurs from hermaphroditism to gonochorism, generally a polygenic sex determination is expected, since at least two mutations (e.g. the first silencing the female function and the second silencing the male function) are necessary to produce females and males from a hermaphroditic ancestor (Delph and Wolf 2005; Bachtrog et al. 2014). Under a polygenic sex determination system, sex is not determined by a single genetic locus acting as a "master switch" and channelling for either the female or the male—or the hermaphroditic—developmental pathways, but it is instead determined by a quantitative threshold trait controlled by multiple regions in the genome (Bulmer and Bull 1982; Moore and Roberts 2013; Bachtrog et al. 2014). Polygenic sex determination is also known to be sensitive to environmental effects, where abiotic (e.g. temperature) and biotic factors (including social factors, e.g. exposure to the opposite sex) influence gene expression (Falconer 1981; Bull 1983).

We know very little about the genetic basis of sex determination in *Ophryotrocha* worms. A few studies, whose results are based on selection experiments or appropriate crossings, were performed in sequential hermaphrodites (*O. puerilis* Bacci and Bortesi 1961; Bacci 1965; Sella 1969) and in gonochorists (*O. labronica*; Premoli et al. 1996). These experiments suggested that sex determination is probably polygenic in the genus and that environmental factors (i.e. social effects, such as exposure to individuals of the same or the opposite sex, and mating opportunities) have an effect on sex expression.

For example, in the simultaneous hermaphrodite *O. diadema*, Di Bona et al. (2015) showed with backcrossing experiments that the functional-male phenotype is a heritable trait, i.e. the expression of a strongly male-biased allocation, where individuals have a hermaphroditic phenotype but reproduce only as males, is under genetic control. Indeed, in only four generations, the frequency of the male phenotype increased from 5 to 25%. The authors investigated also the variation of life history traits related to sex expression across generations. The hermaphrodites of the lines originating from crosses between functional males and hermaphrodites had a longer protandrous phase (i.e. developed oocytes later) and produced fewer eggs at the first laying compared to hermaphrodites originating from the control lines resulting from hermaphrodite x hermaphrodite crosses. These results suggest that sex allocation is a heritable trait, that there is a genetic association between the functional-male phenotype and a male-biased hermaphrodite sex allocation and that a genetic trade-off may exist between traits associated with male and female functions (Di Bona et al. 2015).

Similarly, Bacci and Bortesi (1961) selected for either a longer or a shorter duration of the initial male phase in the sequential (male-first) hermaphrodite *O. puerilis*. Again, after only 4–5 selected generations, the worms spent almost their entire life either as males or as females. Crosses between individuals with a long male phase and those with a long female phase produced individuals that changed sex at a body length similar to that of the worms in the original population (Bacci and Bortesi 1961). In another study, Sella (1969) confirmed that body size at the moment when the first sex change occurs in *O. puerilis* has a certain degree of heritability (estimated around 0.3–0.4). These studies suggest again a genetic contribution to sex determination in *O. puerilis* where several genes are involved.

Slightly different results were obtained by Premoli et al. (1996) for *O. labronica*. The authors investigated the heritability of sex ratio in a laboratory population in order to propose a model that could explain the sex-determining mechanism; they found that the mean sex ratio of the offspring whose fathers came from families with a male-biased sex ratio significantly differed from the mean sex ratio of the offspring whose fathers came from female-biased sex ratio families, whereas this difference was not significant for the mother lineages. On the basis of their experiment, the authors hypothesised that in this species sex is determined by a multilocus genetic system that combines the effects of a female major sex gene (which could give rise to a form of female heterogamety) and masculinising modifiers (Premoli et al. 1996).

If *O. labronica* represents a taxon towards the endpoint of the transition, we might speculate that polygenic sex determination switched towards a mechanism based on major sex genes at some point along the evolutionary pathways, thus reducing the level of plasticity in favour of a more deterministic (and rigid) system. A genetic sex determination mostly linked to major sex genes (genotypic sex determination) is expected to facilitate the evolution of sex-specific traits (Karlin and Lessard 1986), and, since sex is determined at conception, the individual can begin "developing into" its gender early probably allowing that individual to become "a better male or a better female"; this could represent the major advantage of a genotypic sex determination (Charnov and Bull 1977; Leonard 2013).

5.5 Conclusions

The several experiments conducted in recent years on the genus *Ophryotrocha* allow us to speculate on the evolutionary transition between hermaphroditism and gonochorism in this taxon. As in this genus hermaphroditism is thought to be the basal condition (Heggøy et al. 2007; Thornhill et al. 2009), we discuss the likely pathway that may have led from the hypothetical hermaphroditic ancestors to gonochoric species. On the basis of the experimental evidence available on *Ophryotrocha* species, we may hypothesise that the transition occurred via a pathway similar to that proposed by Delph and Wolf (2005) and via androdioecy even if some significant differences may exist. Indeed, in simultaneously hermaphroditic plants, mutations of mtDNA that cause inability to produce viable pollen grains (cytoplasmic male sterility) have been described in several species (Lewis 1941; Touzet and Meyer 2014) and frequently represent the first step of the aforementioned pathway. Such a mutation has never been reported in animals (Budar et al. 2003), and the first step of the transition is probably longer than in plants, involving gradual changes in sex allocation.

The hermaphroditic species *Ophryotrocha diadema* is able to adjust its sex allocation plastically as a function of mating opportunities, reducing the proportion of resources invested in the female role when mating opportunities increase (Lorenzi et al. 2005)—and this pattern is common to other hermaphroditic species in the genus (Schleicherová et al. 2014). We also know that *Ophryotrocha* hermaphrodites allocate relatively more resources into aggressive behaviours that are likely to increase male reproductive success, when mating opportunities increase (mate competition, Lorenzi et al. 2006). Furthermore, the occasional presence of functional-male-phenotype worms, whose frequency depended on mating opportunities (Di Bona et al. 2010) but was based on a heritable trait (Di Bona et al. 2015), has been reported in hermaphroditic populations. Overall, this evidence suggests that if population density rises in a hermaphroditic population, we could expect that some hermaphrodites may allocate more and more resources to face mate competition, i.e. to male function and aggressive behaviour at the expenses of egg production (and indeed, overall egg production is lower in high- than in low-density populations, Lorenzi et al. 2014). This set the stage for an increase in the frequency of functional males—hermaphrodites “specialized” on the male function. Behaving more often as male is probably advantageous when mating opportunities are large: since finding partners is easier and deserting them is less costly, worms behaving as males have a higher fitness than less male-biased hermaphrodites; they save resources for gamete production (and may have a longer lifespan, Di Bona et al. 2010). If the high population density condition (and the concurrent strong sexual selection) is stable across a sufficiently long time period, the male-biased phenotype will spread in the population and will be selected favourably to the point that some male-biased hermaphrodites will switch off the female function, leading to some sort of androdioecious population (i.e. a population composed of hermaphrodites + pure males). Such a population has never been described in *Ophryotrocha*, but something similar has been described in the Mediterranean population of *O. labronica*, where females are able to fertilize eggs and pure males coexist (see below). In turn, the presence of pure males or strongly male-biased hermaphrodites in the population is expected to impose a selective pressure on the other hermaphrodites in favour of a female-biased allocation. In the presence of pure males or strongly male-biased hermaphrodites, the female-biased hermaphrodites will get a higher reproductive success than hermaphrodites with relatively even sex allocation, and this selective pressure will act to the point that the population will be entirely composed of pure males and pure females—as gonochorist populations are. The three *O. labronica* populations studied by Lorenzi and Sella (2013) seem to represent well three intermediate stages of this second step: the worms are indeed functionally gonochoric and exhibit sexual dimorphism, supporting the hypothesis that they “specialized” for either the male or the female function. Yet, these sexually specialised worms maintain some vestigial hermaphroditic traits. One population—the Alamitos Beach population—retained the most hermaphroditic traits, as pure males were absent and functional males were still able to plastically adjust their sex allocation to face mate competition. Another population (San Diego) included individuals that had lost the ability to make sex allocation adjustments, but pure males were still absent. Eventually, in the most truly gonochoric population—the Mediterranean one—pure males were quite frequent and functional males were no longer plastic in their sex allocation.

Such an evolutionary scenario is depicted in Fig. 5.3 where the experimental evidence for intermediate steps is highlighted.

The selective pressure behind this whole evolutionary process is the one emerging from competition for mating, suggesting that sexual selection may impose disruptive selection on hermaphroditic populations favouring the emergence of specialized sexual phenotypes and ultimately males and females (Lorenzi and Sella 2008; Anthes et al. 2010).

This scenario assumes a simultaneous hermaphroditic ancestor, from which gonochoric species have evolved. According to the phylogenetic tree proposed by Dahlgren and coauthors (2001; Fig. 5.2), the most parsimonious hypothesis predicts

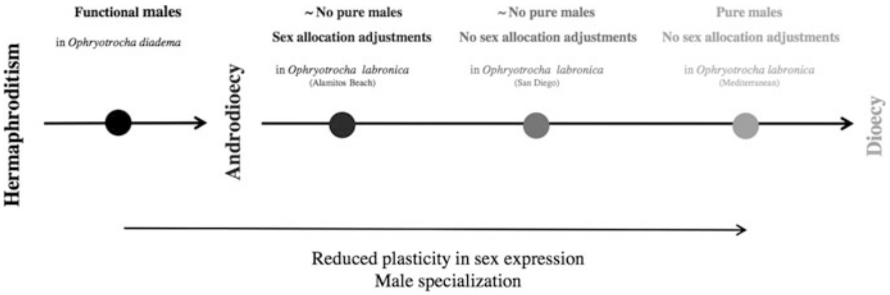

Fig. 5.3 Scheme of the hypothetical transition from hermaphroditism to gonochorism in *Ophryotrocha* worms

that also sequential hermaphroditism has evolved from simultaneous hermaphroditism, if the ancestor is a simultaneous hermaphrodite.

Following the theory of “quantitative gender” (Klinkhamer et al. 1997; Klinkhamer and de Jong 2002; Cadet et al. 2004), sequential hermaphroditism is favoured over simultaneous hermaphroditism when “direct” effects of size (i.e. the benefits of size *per se*) exceed “budget” effects (i.e. the benefits derived from greater energetic resources). This could be the case of the genus *Ophryotrocha*. The preference of *O. puerilis* females for smaller males may represent a strong “direct” effect of size: this mating preference makes disadvantageous for larger individuals to continue to produce sperm while they are producing also eggs, since the mating success of large males is probably low (Berglund 1990). Therefore, if, in populations of simultaneous hermaphrodites, mutants that prefer to have their eggs fertilized by smaller individuals appear and spread, this may set the stage for the evolution of sequential hermaphroditism from simultaneous hermaphroditism.

How and why a preference for small males could emerge is still not clear, and further experiments will be necessary to throw light on the mechanisms underlying this evolutionary pathway.

However, this is not the only scenario that has been proposed. Thanks to the experimental work on *O. puerilis*, it was also hypothesised (Berglund 1991; Premoli and Sella 1995) that both simultaneous hermaphrodites and gonochorists evolved from protandrous sequential hermaphrodites. This hypothesis was based on the observation that sequential hermaphrodites (such as *O. puerilis*) synchronized their sex change to the point that they behaved as simultaneous hermaphrodites when they were reared in pairs for long time periods (Berglund 1986). In Berglund’s view, this suggests that there is the potential for simultaneous hermaphrodites to evolve from sequential hermaphrodites, if sequential hermaphrodites have the opportunity to form relatively long-term pair bonds in natural populations, as it may happen if they live at a low-density population and partners meet at mucus nesting sites.

In the view of Berglund (1991) and Premoli and Sella (1995), separate sexes could originate as well from sequential hermaphrodites. In sequential hermaphrodites (e.g. *O. puerilis*) small males have a higher reproductive success than large ones, since females prefer them as mates (Berglund 1986, 1990). In such populations, if mutant females appear, which exhibit a preference for large males, sex changers might be selected against and populations with pure males will appear. This is especially true if population density increases, which results in increased mate competition where large males may be better competitors.

Although reasonable, this second hypothesis gets weak support from the phylogenetic analyses of the genus *Ophryotrocha*, where sequential hermaphroditism originated recently, relative to the point where simultaneous hermaphrodites and gonochorists separated (Dahlgren et al. 2001).

Although the androdioecious pathways seem more likely to explain the transition from hermaphroditism to separate sexes among *Ophryotrocha* polychaetes, more work is needed to confirm whether the transition really occurred along this way. For example, to list some points which we see as crucial for our understanding of the mechanisms of this biological transition, we need to know in more detail which safeguards exist against nonreciprocating worms (i.e. against functional-male-phenotype worms) in hermaphroditic populations and how such safeguard mechanisms, which prevent cheaters from spreading, may be disrupted in populations evolving towards separate sexes. We need to know how sexual dimorphism emerged in hermaphroditic populations and how dimorphic traits are linked to fitness—where recent work has failed to find any association (Meconcelli et al. 2015b). We also need to know whether higher mate competition abilities confer higher fitness returns to "sexually specialised" hermaphrodites in high-density populations.

Merging data on phylogeny, sexual systems and mating systems have been useful for our understanding of the transition between sexual systems in several families of teleost fishes (reviewed in Erisman et al. 2013). In particular this kind of study made it possible to appreciate how life history traits, such as mating systems (e.g. group spawning, spawning of pairs) and sperm competition, contribute to shape sexual systems, and their study provides experimental support to theoretical models, like the size-advantage model proposed by Ghiselin (1969) (Erisman et al. 2013).

Similarly, working on a genus with such a wide variety of sexual systems has proved fruitful. The presence of several species that can be defined neither as fully simultaneous hermaphroditic nor as clearly gonochoristic offers the opportunity to increase our understanding of a fine-scale evolutionary transition, highlighting the likely intermediate steps of this pathway and throwing light on how sexual selection works in driving sexual systems.

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Chapter 6
Sex-Determining Mechanisms in Bivalves

Sophie Breton, Charlotte Capt, Davide Guerra, and Donald Stewart

Abstract In this review, we provide an overview of the current knowledge on the different sexual systems and sex-determining mechanisms in bivalves, with a focus on the various epigenetic and genetic factors that may be involved. The final section of the review provides recent discoveries on sex-specific mitochondrial genes in bivalves possessing the unconventional system of doubly uniparental inheritance of mitochondria (which is found in several members of the orders Mytiloida, Unionoida, Veneroida, and Nuculanoida). The genes involved in this developmental pathway could represent the first sex-determination system in animals in which mitochondrially encoded genes are directly involved.

6.1 Introduction

More than 70 years have passed since the pioneering review of W.R. Coe on sexual differentiation in bivalve mollusks (Coe 1943). His review summarized information on the diverse modes of sexual reproduction found in bivalves, ranging from functional (simultaneous) hermaphroditism, alternative sexuality (sequential hermaphroditism), to strict gonochorism or dioecy (i.e., species that exist as separate males and females). Coe concluded that bivalves are predominantly of two sexes and that both genetic and environmental factors are probably responsible for the astonishing variability of sexual conditions observed in the group. Twenty-five years after Coe's review, Purchon (1968) proposed that an ancestral gonochoric condition in bivalves is superimposed on an underlying totipotency of developmental pathways, which facilitated the wide range of sexual forms in a variety of environmental conditions. Haley (1977, 1979) was the first to provide evidence that such a system,

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i.e., the coexistence of multiple possible sexual conditions within one species, is primarily under genetic control in the American oyster, *Crassostrea virginica*. In many respects, it seems that all these conclusions remain unchanged to this day. That is, the determinants of sex in most bivalve species studied to date appear to be both genetic and environmental, although no systematic overview on this subject has appeared since Coe's seminal work, even if a great deal of new literature has considerably extended our knowledge in this area. For example, Chávez-Villalba et al. (2011) provided an extensive summary in their review of sex determination in the black-lip pearl oyster, *Pinctada margaritifera*.

Such studies are important for several reasons. First, with ~25,000 living species [14 orders and ~105 families (www.bivatol.org)], the class Bivalvia constitutes the second largest class in the Mollusca, which in turn is the second largest animal phylum. Hence, the biology and ecology of bivalves are sufficiently diverse to provide a rich source of material to better understand the evolution of sex and sex determination in general and to provide unique examples of sex-determining mechanisms, which include the only possible example of mitochondrial genes influencing sex-determination pathways in animals (e.g., Breton et al. 2011).

A second reason for studying sex determination in bivalves relates to their intensive use as bioindicators of environmental health (e.g., Campos et al. 2012). If the health status of bivalve populations appears to be compromised, with obvious failure or loss of reproductive capacity, information about the factors that affect them can be gained from knowledge of life history characteristics and population dynamics of the species involved. This implies, to some extent, knowledge of the strategies and mechanisms involved in reproduction and sex determination.

A third and perhaps more immediate practical reason to investigate the mechanisms of sex determination in bivalves relates to their economic and nutritional importance. Millions of people rely on fish and shellfish production as an important source of protein (e.g., Naylor et al. 2000). The bivalve aquaculture industry, i.e., oysters, clams, scallops, and mussels, has been steadily increasing over the last 20 years (from ~7 to 14.5 million tons, global production; FAO Global Aquaculture Production Statistical Database) and is projected to increase significantly during the coming decades (Shumway 2011). A better understanding of the reproductive biology of bivalve species is thus of crucial importance for their conservation and for maintaining and restocking populations. As in finfish aquaculture, understanding and controlling bivalve sex and reproductive function can help to reach several important goals, such as producing effectively sterile domesticated shellfish to permit genetic improvement while greatly reducing or eliminating negative interactions with wild stocks (Shumway 2011).

In this chapter, we provide an overview of the current knowledge on the different sexual systems and sex-determining mechanisms in bivalves, with a focus on the various epigenetic and genetic factors that may be involved. Epigenetic sex determination (or differentiation) occurs when both sexes can be produced from the same genotype (Beukeboom and Perrin 2014). For our purposes, hermaphroditism will be considered as the product of an epigenetic sexual determination system because within a hermaphroditic individual, genetically identical cell lineages develop into either sperm or eggs. We discuss the different approaches that have been undertaken to elucidate the mode of sex determination in bivalve species including cytogenetic studies, analyses of progeny sex ratios in controlled crosses, experimental manipulation of factors such as ploidy level or environmental rearing conditions, and a variety of more recent "omics" studies. The final section of this chapter provides an overview of sex-specific mitochondrial genes in bivalves possessing the unconventional system of doubly uniparental inheritance of mitochondria [which is found in several members of the orders Mytiloida, Unionoida, Veneroida, and Nuculanoida (Breton et al. 2007; Passamonti and Ghiselli 2009; Boyle and Etter 2013; Zouros 2013)]. The genes involved in this developmental pathway could represent the first sex-determination system in animals in which mitochondrially encoded genes are directly involved.

6.2 Epigenetic Determination of Sex

6.2.1 Hermaphroditism

6.2.1.1 A Multifaceted Minority?

Hermaphrodite bivalves function both as a female and a male (i.e., egg and sperm producing, respectively) at least once during their life cycle. This twofold sexual role can be achieved at the same time (simultaneous or functional hermaphroditism) or at different times (sequential hermaphroditism, i.e., sex change, sex reversal, alternating sexuality). The common ground between the two situations is the innate capacity of a single individual, thus a single genotype, to produce both sperm and eggs (see Box 6.1). This single broad definition of hermaphroditism, however, covers a diverse spectrum of fascinating reproductive strategies and physiological adaptations that are outlined below.

Box 6.1 Hermaphroditic Gonad Structure

The hermaphroditic ability to produce both sperm and eggs is accompanied by specific gonadal organizations that allow for this double capacity. In simultaneous hermaphrodites, the gonad includes the cell precursors of both female and male gametes, and these may be arranged in different ways (Coe 1943; Mackie 1984). In the most common organization, the gonad contains distinct female and male acini in varying proportions, producing eggs and sperm in separate compartments (Sastry 1979): a recent example of such organization was found in hermaphrodite specimens of *Anodonta anatina* (Unionidae) by Hinzmann et al. (2013). In their work on *Atrina seminuda* (Pinnidae), Soria et al. (2002) named this gonad organization as "type 1" and also described an

(continued)

Box 6.1 (continued)
additional “type 2” gonad where acini generate both eggs and sperm, with the first observed at the periphery and the latter at the center of the acinus (a situation also found in the arcid *Arca noae* by Bello et al. 2013). A more clear-cut possibility is to have two distinct female and male gonads (Coe 1943; Mackie 1984): in some species the color of the two sections can be different, allowing for an easy discrimination of female and male tissues. When discrete female and male gonads or acini are present, gametes can be released through a shared duct or through independent ones (Mackie 1984). Sex changers show only one type of acini during the different sexual phases, and when a transient bisexual condition is present between two single-sex ones, a mixed gonad can be observed, with variable proportions of male and female tissues (Coe 1943). Examples and variations of these basic morphologies enlisted above are described in detail by Mackie (1984).

As mentioned above, the vast majority of bivalve species are reported to be strictly gonochoric with hermaphroditic species thought to be rather uncommon (Coe 1943; Gosling 2015). Coe (1943) suggested that fewer than 400 of the 10,000 described species of bivalves deviated from strict gonochorism and Heller (1993) stated that hermaphroditism is present in only 13 out of 117 bivalve families (taxonomy sensu Vaught 1989). These two reviews, as well as Morton (1991), pointed out that freshwater bivalves are more prone to this reproductive strategy than are marine species. That said, some marine groups, such as oysters, appear to be particularly plastic in their reproductive strategies and show many different variants of hermaphroditism; e.g., at any one time, some oyster populations may be made up of simultaneous hermaphroditic individuals, which also have the potential to change sex between seasons (sequential hermaphroditism) (see Collin 2013). Exceptional hermaphroditic individuals also sometimes appear in otherwise strictly gonochoric species at extremely low frequencies (see Morton 1991 for references) because of what are usually thought to be natural errors during development or the result of chemical pollution that alters the developmental pathway (e.g., Ciocan et al. 2012), neither of which is likely to result in heritable changes in sex determination. However, as we will see, gonochoric populations do sometimes transition to hermaphroditism under certain conditions that, for example, reduce the opportunities for mating, such as low population density or restricted dispersal (e.g., Ghiselin 1969). The true number of species exhibiting a hermaphroditic reproductive strategy (in at least some populations) is inevitably higher than previous estimates. The discovery of new cases of hermaphroditism is hindered by the technical challenges of detecting hermaphrodites within a population. These challenges range from the high degree of invasiveness of sexing techniques on single individuals that is required to determine the type of gonad and gametes produced throughout the life cycle to the considerable investment required to monitor the sex ratio of a population that is necessary to register gender changes in size- and age-related classes in the case of sequential hermaphrodites.

6.2.1.2 How to Be Hermaphroditic

Perhaps the simplest situation of hermaphroditism occurs when each individual in a population behaves concurrently as both a functional male and female bearing a combined sperm- and ova-producing organ, the ovotestis. Such individuals are defined as "simultaneous" or "functional" hermaphrodites. Many species of scallops (family Pectinidae) exhibit this hermaphroditic strategy (Merrill and Burch 1960; Shumway and Parsons 2011). Other examples include giant clams (family Tridacnidae; McKoy 1980) and saltwater clams (order Anomalodesmata; Morton 1981). In these taxa, both types of gametes are produced and discharged simultaneously, but not infrequently a single animal can spawn sperm first and then eggs, a temporal delay that is likely a strategy to avoid self-fertilization (Coe 1943; Gosling 2015). Johnston et al. (1998) demonstrated a fascinating adaptation in the hermaphroditic freshwater mussel *Utterbackia imbecillis*, namely, a negative correlation between rates of selfing and proportion of reproductive tissue devoted to sperm production. This observation is consistent with the classic sex allocation theory, which predicts that individuals should reduce proportional male allocation as self-fertilization increases because the pool of eggs available to be fertilized by outcrossed sperm diminishes and each sperm (or unit of male investment) is competing for fewer available eggs (Charnov 1982).

Another option is what is usually termed sequential hermaphroditism. For example, "sex change" or "consecutive sexuality" refers to a situation where all or some individuals of a species change their functional sex once during their life cycle (Coe 1943). The outcome is comparable to gonochorism (i.e., separate sperm- and egg-producing individuals coexisting within a population at any given point in time) but with the difference that single animals switch from producing one type of gamete to the other at some stage of their life. Protandry, which is the case when an individual is born as or first develops into a male and then becomes female, is more common than protogyny, i.e., when an individual first expresses the female phenotype and then later switches to the male phenotype (Oldfield 1961). The scallop *Chlamys varia* (Pectinidae) is a classic example of a protandric species (Lubet 1959; Lucas 1965). Burnell (1995) studied two geographically separate populations of *C. varia* on the west coast of Ireland that exhibited different growth rates and found that male-to-female sex change was correlated with age and not size (i.e., height): indeed, cohorts of the same age had different sizes in the two localities, but the sex ratio was comparable, with younger individuals being mostly males. He suggested that the sex change occurs during the resting stage in the second growing season, possibly triggered by the accumulation of some energetic storage products during winter.

Protandric sex change can also be related to the size of an individual and to the resources it can allocate to produce gametes, rather than to its age (see Fig. 6.1).

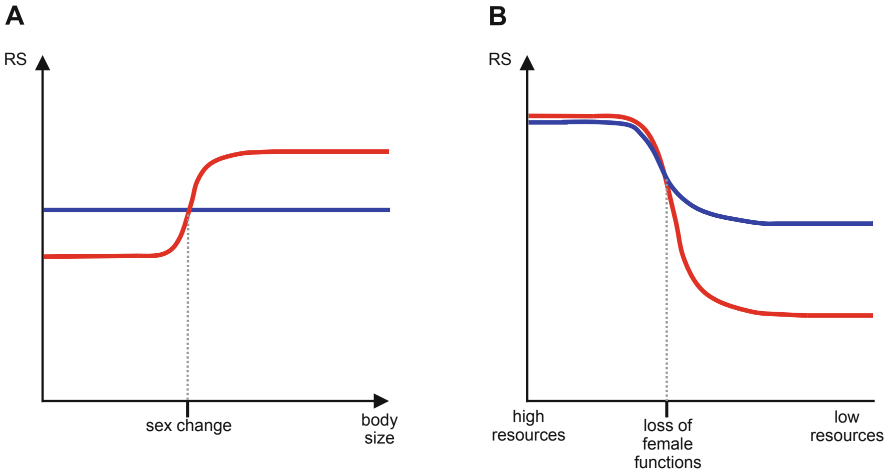

Fig. 6.1 Two models for sex change in bivalves. RS, reproductive success; red line, female RS; blue line, male RS. The trends of RS in both panels are not based on actual experimental data and are solely an interpretation made by the authors meant to give a visual idea of the models. (**a**) Size advantage model as described by Policansky (1982). Male RS is independent of body size, while female RS is proportional to dimension. Small males have a higher RS compared to females of the same size, since the cost of producing sperm is lower than that of eggs, but after a certain threshold size females have higher RS than males, since they can produce eggs more efficiently than the smaller ones. In protandric sex changing bivalves, an individual born as a male can thus switch its sex to female when it reaches the threshold size to increase its own RS. It is not specified however if the RS of smaller females after the sex change is lower than that of bigger ones, or if also male RS increases with size, so we depicted the first reaching a plateau value after the sex change and the second being constant to focus on the differences in RS around the sex change size. (**b**) Resource availability model inferred from the results of works cited in Sect. 6.2.2.3. RS of females and males is proportional to the available resources (food, body nutrients) or to the capacity to accumulate them (e.g., fully functioning feeding ability). When resource availability decreases, RS also decreases for both sexes, but the female falls faster than the male one since eggs require more resources per unit to be produced compared to spermatozoa. Beyond a threshold value of resource accessibility, females have lower RS than males: under these conditions, a female can switch sex and become male to maintain a better RS. In simultaneous hermaphrodites, female functions can be stopped in favor of only the male ones when resources are lacking

Below a certain size, being female can result in a lower reproductive success compared to being a male, in that a small individual can allocate limited reserves into gamete production that can be better used in generating sperm, which require less energy-demanding resources than eggs. Above a certain size, the reproductive success for being a female becomes higher (larger females have a higher capacity for egg production), and a male can then change sex (Ghiselin 1969; Charnov 1982; Policansky 1982). In this scenario, the overall sex ratio of a population can be ~1:1, but that of single cohorts can be skewed toward one sex or the other (i.e., initially more males in smaller, younger individuals and subsequently more females in larger, older individuals). Finally, intermediate phases of simultaneous hermaphroditism can occur during the protandric sex change, as observed in the Noah's ark shell *Arca noae* (family Arcidae) (Bello et al. 2013).

As noted above, the phenomenon of protogyny is rare, but it is the rule in a few species such as the galeommatoids *Kellia suborbicularis* (Laseidae) and *Montacuta substriata* (Montacutidae) (Oldfield 1961). Exceptional cases of protogyny have also been reported in scallops, specifically within the genus *Pecten* (Pectinidae) (Coe 1943). Female-to-male sex change may be driven by low available resources intake (Fig. 6.1 and below for more details). Hermaphroditic species of freshwater *Corbicula* clams (Corbiculidae) are also protogynous, with eggs present year-round and sperm produced in response to seasonal changes in temperature (Pigneur et al. 2012). *Corbicula* clams, together with the little fire ant, *Wasmannia auropunctata*, are the only two cases of obligate androgenesis in animals, and the only one for which the cytological mechanisms of androgenetic reproduction have been well described (see Pigneur et al. 2012 for a review). In this atypical mode of asexual reproduction, the maternal nuclear genome fails to participate in zygote development, and offspring are paternal nuclear clones. Cytological studies show that in androgenetic *Corbicula*, a diploid spermatozoon fertilizes the oocyte, the maternal nuclear contribution is extruded as two polar bodies, and the maternal organelles are retained (Pigneur et al. 2012). Although the mechanism that causes unreduced sperm still remains unknown, the authors suggest that androgenetic reproduction might have been partly responsible for the invasive success of freshwater *Corbicula* clams (Pigneur et al. 2011, 2012). Because androgenetic *Corbicula* are hermaphroditic and capable of self-fertilization, a single individual can establish a new population in any new suitable niche, and each individual can have up to 90,000 offspring per reproductive season (McMahon 1999). With sufficient clonal diversity due to new mutations or to colonization by different genotypes, a successful clone associated with high levels of plasticity and broad environmental tolerance could be selected for, and in the long term, a "general-purpose" genotype could evolve (Van Doninck et al. 2002; Pigneur et al. 2012).

Protogynic sex change is also an integral element of two other categories of sequential hermaphroditism. The oyster *Ostrea edulis* is an example of what Coe (1943) defines "rhythmical consecutive sexuality." Individuals in this species usually complete a male and a female phase, i.e., they release sperm and eggs at different times, each reproductive season. After sperm release has been completed, egg production is started, but, depending on the time of the year, their release can follow immediately or be delayed until the next reproductive season. This means that the sexual phases are not perfectly synchronized in all individuals, which results in a mixed sex population throughout the breeding season. Again, as seen for other strategies above, the first functional phase is male, although some exceptions have been observed (Coe 1943). Oviparous oysters such as *Crassostrea virginica*, on the contrary, act only as one sex during a single reproductive season, and in the following year, the functional sex can change or remain the same, a strategy called "alternative sexuality" by Coe (1943).

The effects of the environment on gender determination have mostly been studied in hermaphroditic species. Indeed, because sacrificing the animal is often required to determine its sex and because sexual maturation, which varies among species, can take several months or years (Gosling 2015), most studies have investigated the environmental effects on sex differentiation in adults in sequential hermaphroditic species, principally because they can undergo sexual reversion. Therefore, the possible influence of the environment on determining sex before first gonadic differentiation is still poorly known, and the underlying mechanisms remain virtually unstudied. The following sections present an overview of recent information that relates the effects of the environment on sex determination before and after first gonadic differentiation in bivalves.

6.2.2 Environmental Effects on Sex Determination

Several bivalve species have been investigated through sex ratio studies in which the stability of sex determination or differentiation was tested using a variety of abiotic and biotic factors known to affect the process of sex determination in other mollusk or invertebrate species (e.g., Heller 1993; Chávez-Villalba et al. 2011; Collin 2013). For example, exogenous steroids, temperature, food availability, and pollutants have all been tested and been shown to affect sex ratios in bivalves (a summary of which is presented in Table 6.1).

6.2.2.1 Exogenous Steroids

Following the discovery of a role of sex hormones in the hormonal regulation of bivalve reproduction (Mackie 1984; Chávez-Villalba et al. 2011; Teaniniuraitemoana et al. 2016), a role that was suggested to be similar to that in vertebrate endocrine systems, several studies have attempted to control the reproductive state of cultured bivalves using exogenous steroids (see Croll and Wang 2007 for a review). However, only a small number of these studies demonstrated that the sex of bivalves can be affected by steroid hormones (i.e., by the demonstration of sex reversal of adult individuals), and none of them explored the underlying mechanisms (Table 6.1). To our knowledge, only one study by Wang and Croll (2004) investigated the possible effects of steroid hormones on sex determination at the juvenile stage before first gonadic differentiation. These authors injected adductor muscles of juvenile sea scallops *Placopecten magellanicus* with 17β-estradiol, testosterone, progesterone, or dehydroepiandrosterone (DHEA) and showed that these treatments accelerated gonadal differentiation, and in some cases shifted observed sex ratios toward males. These results were interpreted as evidence that sex steroids may be involved in sex determination in bivalves as they are in vertebrates (Wang and Croll 2004; Croll and Wang 2007); however, more research is obviously needed to better understand the precise mechanisms underlying these developmental pathways.

Table 6.1 Environmental effects on sex ratios

Factor/species	Treatment and/or main results	References
Exogenous steroids		
Crassostrea gigas *Mulinia lateralis* *Placopecten magellanicus*	17β-estradiol: feminizing effect (with observed sex reversals) Methyltestosterone: masculinizing effect 17β-estradiol, testosterone, progesterone, dehydroepiandrosterone (DHEA): masculinizing effect (with observed sex reversals)	Mori et al. (1969) Moss (1989) Wang and Croll (2004)
Temperature		
Crassostrea corteziensis *Crassostrea gigas* *Crassostrea virginica* *Pinctada margaritifera*	↑ males at 18 °C; ↑ females at 9 °C ↑ females at higher temperatures; ↑ males at lower temperatures (8 °C) ↑ females at higher temperatures ↑ males at higher temperatures 28 °C (combined with low food availability)	Chávez-Villalba et al. (2008) Rodríguez-Jaramillo et al. (2008), Fabioux et al. (2005) Lango-Reynoso et al. (2006) Santerre et al. (2013) Coe (1936) Teaniniuraitemoana et al. (2016)
Food availability		
Argopecten irradians *Crassostrea gigas* *Mytella charruana* *Pinctada margaritifera*	↑ males with low food availability (combined with low temperatures) ↑ males with low food availability ↑ males with low food availability ↑ males with low food availability (combined with high temperatures)	Sastry (1968) Lango-Reynoso (1999) Stenyakina et al. (2010) Teaniniuraitemoana et al. (2016), Chávez-Villalba et al. (2011)
Pollution		
Gomphina veneriformis *Mya arenaria*	↑ males with tributyltin ↑ males with tributyltin	Park et al. (2015) Gagné et al. (2003)

6.2.2.2 Temperature

An effect of temperature on sex determination has been observed in multiple animal species including vertebrates (e.g., reptiles, amphibians, and fishes) and invertebrates (e.g., rotifers, nematodes, insects, crustaceans, and mollusks; Korpelainen 1990). Temperatures above or below a threshold value appear to affect gender determination during a critical time window called the "thermosensitive period" (TSP), and thus progeny developing within a particular temperature regime during this TSP consists solely of males or females (Manolakou et al. 2006). The TSP occurs once during the period of sexual maturation in gonochoric species and during each sex reversion period in hermaphroditic taxa. In bivalves, a temperature effect on sex has only been studied in sequential hermaphroditic oysters, in both adults (in natural populations and under controlled conditions) and spat (i.e., before sexual maturation; under controlled conditions) (Table 6.1). For example, Coe (1936) observed a female-biased sex ratio at high temperature in natural populations of the Eastern oyster, *Crassostrea virginica*. Similarly, Lango-Reynoso et al. (2006) reported that the sex ratio in wild populations of *C. gigas* changed from heavily female-biased during the summer to male-biased in winter, although the proportion of undifferentiated individuals significantly increased over the fall and winter months. In this species, a 1-year conditioning at low temperatures (8 °C) led to a male-biased sex ratio (Fabioux et al. 2005), consistent with the field data of Lango-Reynoso et al. (2006). Finally, a different pattern of sex ratio effects related to temperature was reported for the tropical Cortez oyster *Crassostrea corteziensis* in which higher proportions of males were observed at or above ~18 °C (Chávez-Villalba et al. 2008), whereas more females were observed below ~9 °C (Rodríguez-Jaramillo et al. 2008).

Again to our knowledge, just as only a single study has been conducted on the effects of sex hormones on immature bivalves, only one study has investigated the effects of temperature on sex determination on juveniles before the first signs of gonadic differentiation. Specifically, Santerre et al. (2014) investigated the effect of four different temperature regimes (18 °C, 22 °C, 25 °C, and 28 °C, respectively) on sex determination in Pacific oyster *C. gigas* spat and observed a significant increase in the frequency of males at 25 °C. Interestingly, the authors assessed in parallel mRNA expressions of five putative actors of the molecular cascade of sex-determination/gonadic differentiation pathway previously characterized in this species (i.e., the genes *Cg-Foxl2*, *Cg-Foxl2os*, *Cg-DMl*, *Cg-SoxE*, and *Cg-β-catenin*; Naimi et al. 2009a, b; Santerre et al. 2012) and showed that this increase in males was associated with a change in the balance of expression of male and female genes, in favor of male orthologs such as *Cg-DMl* and *Cg-SoxE* (Santerre et al. 2014). These mRNA expression profiles also suggested a time window of sex determination in spat at around 40–44 days post fertilization (dpf) at 18 °C, in agreement with previous studies which suggested that sex determination may occur at around 45–60 dpf (Naimi et al. 2009b; Santerre et al. 2014). Such effects, i.e., an influence of temperature on the mRNA expression profiles of sex-determining genes, had already been demonstrated in species with temperature-dependent sex determination (TSD), such as in reptiles and fishes (e.g., Rhen et al. 2007; Yamaguchi et al. 2007; Shoemaker-Daly et al. 2010). However, the case of *C. gigas* spat differs slightly because the influence of temperature on the oyster sex determination is apparently responding directly to genetic control (see below), suggesting a mixed sex-determination system (genetic sex determination [GSD] + TSD; Santerre et al. 2013). Additional "omics" studies of this kind will help to further decipher the system of sex determination in oysters (and other bivalve species) and enrich the current information available on the mechanisms involved in temperature-dependent determination in invertebrate species.

6.2.2.3 Food Availability

Recently, Teaniniuraitemoana et al. (2016) examined the effect of environmental combinations of temperature (24 and 28 °C) and food availability (trophic level;

10,000 and 40,000 microalgae cells mL^{-1}) on gender determination in adult black-lip pearl oysters, *Pinctada margaritifera*. Their results showed a significant effect on sex ratio for oysters conditioned at high temperature and low food concentration with a significant proportion of females (50%) changing to males under those conditions (Teaniniuraitemoana et al. 2016). The authors invoked an energetic deficit as the potential cause of the female-to-male sex change; they specifically argued that the increase in oxygen consumption and metabolic rate at high temperature depleted the energetic reserves required for the production of female gametes, which is hypothesized to be more energetically costly than the production of male gametes in mollusk species (Russell-Hunter 1979). The ability to switch to being male in poorly fed female oysters is presumably an adaptive response. This was the conclusion of a previous study by Stenyakina et al. (2010) that demonstrated changes in sex from female to male in adult marine Charru mussels, *Mytella charruana*. These authors showed that mussels collected from different natural populations and maintained in the laboratory with or without food exhibited a male-biased shift in sex ratio under starvation conditions within a month (Stenyakina et al. 2010). This is also in agreement with previous observations in natural populations of hermaphroditic scallops (Sastry 1968) that showed a correlation between a higher proportion of males and a combination of low food supply and cooler temperatures. Similar patterns have also been demonstrated in laboratory reared and natural populations of Pacific oysters (Lango-Reynoso 1999; Chávez-Villalba et al. 2011); i.e., a sex ratio in favor of females was noted when food availability conditions were favorable. Apparently, any situation that lowers body nutrient reserves and/or food intake (e.g., removal of gills, starvation, parasitic infections) always leads to a rise in the percentage of males in populations of bivalves capable of changing sex (Amemiya 1935; Egami 1953; Bahr and Hillman 1967; Davis and Hillman 1971; Cox and Mann 1992).

As part of their study on the effects of temperature and food availability, Teaniniuraitemoana et al. (2016) also investigated patterns of mRNA expressions for nine marker genes of the sexual pathway (*pmarg-foxl2*, *pmarg-c43476*, *pmarg-c45042*, *pmarg-c19309*, *pmarg-c54338*, *pmarg-vit6*, *pmarg-zglp1*, *pmarg-dmrt*, and *pmarg-fem1-like*). According to their results, only *pmarg-foxl2* and *pmarg-fem1-like* were significantly differentially expressed between male and female *P. margaritifera*, suggesting that these two genes are involved at the top of the molecular cascades of sex determination in black-lip pearl oysters (Teaniniuraitemoana et al. 2016). As was proposed for *C. gigas* by Santerre et al. (2014), Teaniniuraitemoana et al. (2016) also suggested that there is a mixed sex-determination system in *P. margaritifera* that involves both genetic and environmental factors. However, because the latter work focused on sex change in adults, additional studies will need to be conducted on juvenile spat before expression of first signs of gonadic differentiation. The genes *pmarg-foxl2* and *pmarg-fem1-like* are likely the best candidate genes to be first explored in further genomic studies of the mechanisms of sex determination in pearl oysters.

6.2.2.4 Pollution

Relatively little information exists in the literature on the effects of environmental pollutants on bivalve sex determination and differentiation or reproductive patterns generally (e.g., Gauthier-Clerc et al. 2002; Gagné et al. 2003; Park et al. 2015). However, the complexity of cellular processes involved in sex change and/or gonad differentiation and maturation certainly provide an opportunity for chemical interference in the pathways leading to the development of females or males. Indeed, several pollutants, such as heavy metals, organophosphates, and organochlorines, are known to interfere with the natural role of hormones and consequently affect sex determination, gonad morphology, gametogenesis, and reproduction in mollusks (Park et al. 2015). One infamous example is tributyltin (TBT), an organotin compound that has been largely used as a biocide in antifouling agents for boats and which is now well-established as a xenoandrogen that skews sex ratios toward males in both wild and reared populations of several mollusk species, including bivalves (Table 6.1). These observations suggest that pollutants currently found in aquatic habitats are able to affect sex determination and differentiation pathways in bivalves. As with the case for the effects of food availability, additional studies are needed to identify the precise biochemical mechanisms and genes involved and also to identify other chemicals that might be involved in the process of endocrine disruption by environmental pollutants in bivalves.

6.2.2.5 Influence of Other Abiotic or Biotic Factors

Other examples of abiotic or biotic effects on sex ratios in bivalves have been noted. For example, some studies of oysters have reported an influence on sex by social factors, i.e., by other individuals living in close proximity probably through secretion of pheromone-like compounds (e.g., Kennedy 1983). Some other studies have reported pronounced phenotypic plasticity of sexual systems in bivalves in response to environmental conditions (Collin 2013). Bivalves are generally sessile animals. Many species do not move after settlement, and they simply reproduce by releasing gametes into the water column (Gosling 2015). This is the rule at least for sperm but not necessarily eggs, because females of some species retain them in dedicated body parts and fertilize them with sperm extracted from the water column (or from their own body in the case of self-fertilizing hermaphrodites) (Gosling 2015). In broadcast-spawning species, spermatozoa actively swim (guided by chemical signals, e.g., Evans and Sherman 2013) until they encounter an egg in the open water (external fertilization, as in the majority of marine species) or until they are taken in passively through the inhalant opening by a female (internal fertilization, as in freshwater mussels of the order Unionoida) (Gosling 2015). There may be situations in which the probability that eggs and sperm can meet will be low: for instance, at low population densities the physical distance among individuals may be relatively large (Ghiselin 1969; Charnov et al. 1976). Environmental variables, such as extremely weak or extremely strong water currents, etc., may also limit fertilization success regardless of population density. In these instances, natural selection may favor hermaphrodites, as their mating chances are improved compared to single-sex individuals (Downing et al. 1989), as will be discussed in the next few case studies.

The numerical decline of some Mexican populations of the gonochoric chocolate clam, *Megapitaria squalida* (Veneridae), might have triggered a rise in the incidence of hermaphroditism. Romo Piñera et al. (2009) investigated sex ratio and occurrence of hermaphroditism in two populations of this commercially exploited species, which has been subjected to an approximately 30 times increase in fishing activity in recent years. An unusually large proportion of hermaphrodites was found (21.8 and 23.5%), much higher compared to earlier reports from different localities (0, 0.25, and 2.6%; see references in Romo Piñera et al. 2009) and to classical estimates (0.1–7%) of incidental hermaphroditism in other gonochoric species by Morton (1991). Also, a sex ratio skewed toward females was observed for the first time in this species. The reduction in population density might have given an advantage to hermaphrodites, according to Ghiselin (1969) and Charnov et al. (1976), and a female-biased sex ratio may be an additional strategy adopted to maximize population reproductive success (Avise 2011).

Hinzmann et al. (2013), on the other hand, provided instead a putative example of environment-driven increased hermaphroditism in the gonochoric freshwater mussel *Anodonta anatina* (Unionidae). Iberian populations from standing waters showed a higher incidence of hermaphrodites than those of river environments (an observation comparable to one made on Ukrainian populations of the same species; Yanovych et al. 2010). This may be caused by differences in gamete dispersal (freshwater mussels do not release eggs) between the two environments, with stronger currents in rivers diffusing sperm more efficiently than is possible in lagoons. In other words, being a hermaphrodite may be a better reproductive strategy for *A. anatina* in standing waters where sperm dispersal can be limited. The cause-effect link among decreasing populations, changing/different environmental conditions, and hermaphrodite percentage in freshwater mussel populations may be difficult to disentangle. Galbraith and Vaughn (2011) studied the negative effects of human-adulterated regimes of water currents and temperature in dam reservoirs on the reproduction and population dynamics in three *Quadrula* species (Unionidae). The most altered environment showed the most sparse populations and the highest proportions of hermaphrodites: these effects were mainly linked to the different temperatures (i.e., colder temperatures than usual), which could have affected sperm motility, leading to a decrease in population density and the “normal” gonochoric sex-determination pattern. However, a density-dependent response to a changed environment similar to that suggested by Hinzmann et al. (2013) for the increase in the incidence of hermaphroditism should also be taken in account.

From these examples it appears that bivalves are able to respond to a changing environment by adapting their sexuality on a microgeographic scale, suggesting a highly plastic reproductive capacity even at population and individual levels. At the macroevolutionary level, natural selection might have molded this reproductive capacity of bivalves in an environment-specific way: gonochorism is almost the rule, but hermaphroditism has evolved several times resulting in a number of different simultaneous and sequential variants. Morton (1991) proposed a model linking reproductive strategy of bivalves and environment after having found a general pattern by comparing incidence of hermaphroditism and sex ratio variations in whole populations and cohorts of diverse ages in several bivalve species from various habitats in Hong Kong. In small river habitats, species showed a tendency toward simultaneous hermaphroditism and brooding, whereas other freshwater environments such as lakes and large rivers were home to gonochoric species with populations biased toward females. Sequential hermaphroditic species that showed juvenile male-to-female sex change were present in estuarine environments, with varying overall sex bias, and intertidal marine habitats hosted mostly gonochoric species without evidence of simultaneous or sequential hermaphroditism. All these strategies are adopted to maximize reproductive success of individuals within a population to their own particular environment. Obviously, a “wrong” strategy in the “wrong” habitat could lead to local extirpation, or even species-level extinction, and would not persist. The tendency of diverse bivalve species to use comparable strategies in similar environments might be an indication of the highly responsive capacity of these animals to changing habitats, a quality that might have played a crucial role in their evolutionary success. The “Hong Kong model” by Morton (1991) surely deserves more consideration if we want to understand the basis of bivalve reproductive biology.

6.3 Genetic Determination of Sex

The genetic mechanisms of sex determination in bivalves have been better clarified by several types of experiments, such as cytogenetic and sex ratio studies, identification/isolation of sex-specific DNA markers using differential expression analyses, and surveys and comparisons of genomes and/or transcriptomes. Several of these putative mechanisms are discussed below.

6.3.1 Cytogenetic or Molecular Evidence for Sex Chromosomes in Bivalves

The quickest approach to elucidate the genetic determinants of sex in a species is to examine the karyotype of male and female individuals in an attempt to identify sex chromosomes. To date, more than 150 species of bivalves have been cytogenetically characterized (Menzel 1968; Ahmed 1973; Wada 1978; Cornet and Soulard 1989, 1990; Thiriot-Quiévreux and Insua 1992; Thiriot-Quiévreux 2002; Leitão and Chaves 2008; Jenkinson 2014), and none has been found to possess heteromorphic sex chromosomes. Had such chromosomes been found, their mode of sex determination could have been undeniably defined as genetic. One bivalve species, though, has been suggested by Guo and Allen (1994) to have an XX-female, XY-male sex determination with Y-domination, namely, the dwarf surf clam, *Mulinia lateralis*. The authors came to this conclusion after having induced diploid gynogenetic as well as triploid *M. lateralis* and observed that all gynogenetic diploids were female and that there was no significant difference in sex ratio between “regular” diploids and triploids. Similarly, Allen et al. (1986) produced triploid soft-shell clams *Mya arenaria* but based on the observed sex ratio (triploids were 77% females) concluded that sex determination in this species best fits the model of an X-autosome balance mechanism as found in insects. Overall, the absence of heteromorphic sex chromosomes in bivalves, together with the environmental factors discussed above and known to contribute to sex determination, illustrates the underlying complexity and lability of the process in this group and points to a polygenic architecture of sex determination, which is discussed below.

6.3.2 (Poly)genic Sex Determination in Bivalves

The genetic determinants of sex in bivalves have also been investigated through analysis of family sex ratios, leading to the development of genetic models without knowledge of molecular mechanisms of sex determination (e.g., Haley 1977, 1979; Guo et al. 1998; Hedrick and Hedgecock 2010). For example, based on observations of sex ratio variation among five families of the Eastern oyster *Crassostrea virginica*, Haley (1977, 1979) proposed a three-loci model for sex determination, with two additive alleles at each locus, one for maleness (m) and one for femaleness (f), and the m:f ratio determining sex. Guo et al. (1998) analyzed sex ratios in 86 pair-mated families of the Pacific oyster *C. gigas* and provided evidence for a single-locus model of primary sex determination with a dominant male allele (M) and a protandric female allele (F), so that FM oysters are true males and FF are protandric females that are capable of sex change. The authors also suggested that the rate of sex change of FF individuals could be influenced by secondary genes and/or environmental factors (Guo et al. 1998). However, because this two-genotype model could not explain observed heterogeneity of sex ratios in half-sib families with a single male parent and different female parents, Hedrick and Hedgecock (2010) proposed an alternative three-genotype model with two kinds of females, fixed FF and protandric FM; the model involves an “f” parameter, which is the probability that FM individuals mature as females, explaining heterogeneity in sex ratios.

Owing to their importance in aquaculture and fisheries, the interest in bivalve genomics and transcriptomics to identify the genes involved in reproduction, sex differentiation, and sex-determination processes has significantly increased in recent years (e.g., Dheilly et al. 2012; Ghiselli et al. 2012; Zhang et al. 2014; Shi et al. 2015; Tong et al. 2015; Teaniniuraitemoana et al. 2014, 2016). However, because this is beyond the scope and goals of the present review, studies relating genes involved in reproduction or in later steps during sexual differentiation are not discussed. Here we provide an overview of recent information on genes known to act in sex determination in other animal species (invertebrates and vertebrates) and/or during early gonadal differentiation and thus potentially might play a role in sex determination in bivalves (because no genes are currently known with certainty to be involved in the initial sex-determination process in this group).

Ghiselli et al. (2012) published the first whole transcriptome analysis by RNA-Seq performed to identify genes involved in bivalve sex determination. By comparing transcriptomes of males and females in the Manila clam *Ruditapes philippinarum*, they identified 1575 genes with strong sex-specific expression including the male-biased gene *Sry* (sex-determining region-y)-box 30 (*Sox30*), a transcription factor involved in the differentiation of developing male germ cells in mammals (Wallis et al. 2008; Ghiselli et al. 2012). Because *Sox30* was not only highly expressed in testis but also in females producing male-biased progenies, the authors suggested that this gene was expressed in eggs of male-biased mothers to induce the development of future embryos toward maleness (Ghiselli et al. 2012).

Genes homologous to sex-determining pathway genes in model species have also been identified in several other bivalve species. In scallops, for example, a homologue of *Dmrt1* (doublesex and mab-3 related transcription factor 1), which is well known for its conserved role in male sex determination and differentiation in a large panel of animal taxa including fruit flies, *C. elegans*, and vertebrates (Kopp 2012), has been identified in the hermaphroditic scallop species *Nodipecten subnodosus* (Llera-Herrera et al. 2013). Jiao et al. (2014) reported in the hermaphrodite scallop, *Chlamys farreri*, the existence of *Znfx1* (Nfx1-type zinc finger-containing 1), a gene tightly linked with *Amhr2* (anti-Mullerian hormone receptor type II), which is involved in early steps of male sex determination in mammals and in sex determination in the tiger pufferfish *Takifugu rubripes* (Kamiya et al. 2012).

In freshwater mussels, an in-depth study of sex-specific genes has recently been conducted on sperm and ovary tissues of the gonochoric species *Hyriopsis schlegelii* (Shi et al. 2015). A total of 45,422 unigenes were found to be differentially expressed between the ovary and sperm, and among them, key genes reported to govern sex-determination pathways in mammals were identified, including *Sry*, *Dmrt1*, *Dmrt2*, *Sox9*, *GATA4*, and *WT1*, which were upregulated in males, and *Wnt4*, *Rspo1*, *Foxl2*, and *β-catenin*, which were upregulated in females (Shi et al. 2015). These results suggest that *H. schlegelii* and mammals use similar gene regulatory mechanisms to control sex determination (Shi et al. 2015). Broadly, male sex determination in mammals is initiated by the expression of *Sry* (sex-determining region on Y), which suppresses ovarian promoting genes and activates *Sox9* (Sry-box 9), a key element of the testis-determining cascade leading to the activation of *Dmrt1* and differentiation of Sertoli cells, whereas female sex determination is initiated by the forkhead box transcription factor *FoxL2*, *β-catenin*, and *Wnt4*, which promotes and maintains ovarian development while suppressing *Sox9* (Veitia 2010). Because hermaphroditism has already been noted in *H. schlegelii*, Shi et al. (2015) also focused their efforts on searching for key genes known to regulate sex determination in other hermaphroditic model species, such as

Caenorhabditis elegans. Interestingly, genes associated with hermaphrodite phenotypes in the roundworm *C. elegans*, i.e., *Tra-1*, *Tra-2α*, *Tra-2β*, *Fem1A*, *Fem1B*, and *Fem1C* (reviewed in Gamble and Zarkower 2012), and genes associated with dosage compensation mechanisms in the fruit fly *Drosophila*, such as *Msl1*, *Msl2*, and *Msl3* (Legube et al. 2006), were also identified in *H. schlegelii*, suggesting that diverse regulatory mechanisms regulate sexual polymorphism in this species (Shi et al. 2015).

The genetic mechanisms of sex determination have been studied more in detail in oysters than in any other group of bivalves. Among the genes that are known to act in sex determination in other animal species and/or during early gonadal differentiation, and thus potentially in sex determination in oysters, there are *Oyvlg* (*Crassostrea gigas*; Fabioux et al. 2004, 2009), a homologue of *vasa*, which is a gene involved in primordial germ cell development and early sex differentiation in eukaryotes; *Cg-DMl* (*C. gigas Dmrt*-like; Naimi et al. 2009a, b), a homologue of *Dmrt1* (other homologues of *Dmrt1* have also been reported in the oysters *Pinctada martensii* and *P. margaritifera*; Yu et al. 2011; Teaniniuraitemoana et al. 2014); *Cg-SoxE*, a homologue of *Sox9* and *β-catenin*, which are, respectively, expressed when sex is still not distinguishable or in mature females and vitellogenic oocytes in *C. gigas* (Santerre et al. 2014); and *CgFoxL2*, *CgSoxH* (or *Sry*-like), and *CgDsx* (*C. gigas*; Zhang et al. 2014), the two latter genes being linked to the triggering of male development. *Vasa*, *FoxL2*, and *β-catenin* have also been reported in the species *Crassostrea hongkongensis* (Tsuda et al. 2003; Tong et al. 2015). In addition, *FoxL2* has been reported in *Pinctada margaritifera* (Teaniniuraitemoana et al. 2015), and *vasa* in *Pinctada fucata*, together with other sex-specific genes acting on early steps of the sex-determination process, including *nanos*, *doublesex*- and *mab*-3-related transcription factor (Matsumoto et al. 2013). All the above genes are thought to be involved in sex determination and early oyster gonadic differentiation. Their conservation among vertebrates and mollusks suggests a deeply evolutionary conserved role in the sex-determination process.

Zhang et al. (2014) proposed a working model for sex determination in the Pacific oyster *Crassostrea gigas* (Fig. 6.2). This species is characterized by protandry, sex change, and rare but consistent hermaphroditism, and the initiation of sex determination is thought to involve both genetic and environmental factors (Zhang et al. 2014). Based on sequence homology and functions inferred from transcriptome data, Zhang et al. (2014) speculated that *CgSoxH*, a *Sry*-like gene that is strictly expressed in testis, may play a leading role in the sex-determining pathway of *C. gigas*. *CgSoxH* would directly or indirectly activate *CgDsx*, a DM domain gene like those (e.g., *Dmrt1*) that have been identified as master switches for testis development in all metazoans studied so far. Both *CgSoxH* and *CgDsx* would interact with, or inhibit, *CgFoxL2*, which is usually specifically expressed in ovaries. The possible interaction among these male- and female-promoting genes is consistent with the reported interaction among *Sry*, *Sox9*, *Dmrt1*, and *FoxL2* in mammals (Veitia 2010; Zhang et al. 2014) (Fig. 6.2). This working model provided by Zhang et al. (2014) will certainly stimulate further investigation on sex-determining pathways in mollusks and other invertebrates.

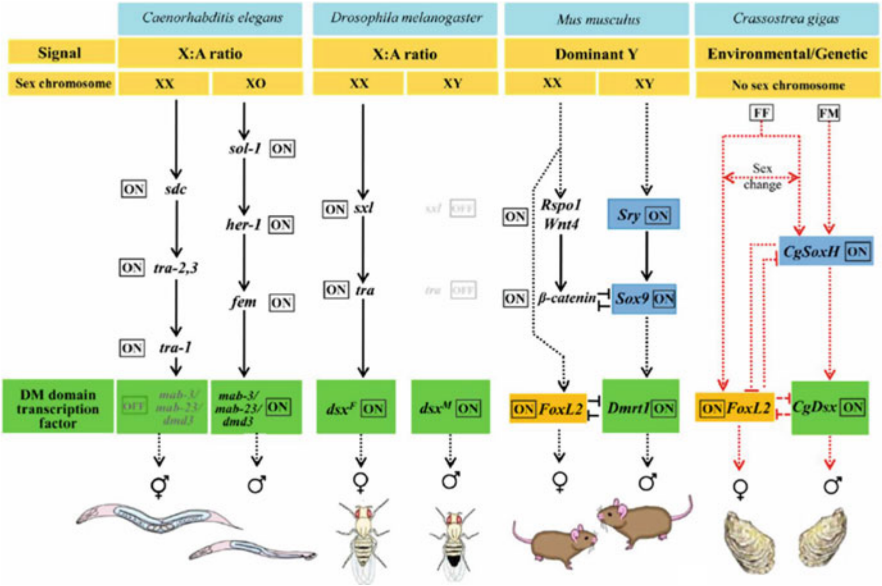

Fig. 6.2 Working model for sex determination in the Pacific oyster *Crassostrea gigas*. Sex-determining pathway in *C. gigas* is compared with that in model organisms as summarized by Gamble and Zarkower (2012). Only selected key sex-specific regulators are shown. Dashed black lines indicate temporal relationships, and dashed red lines indicate hypothetical relationships based on expression data only. [Based on Zhang et al. (2014)]. FF genotype permits sex change (Guo et al. 1998)

6.4 An Unconventional Sex-Determining Mechanism in Bivalves?

As mentioned above, many bivalves including members of the orders Mytiloida, Unionoida, Veneroida (see reviews in Breton et al. 2007; Passamonti and Ghiselli 2009; Zouros 2013), and Nuculanoida (Boyle and Etter 2013) exhibit an unusual system of mitochondrial DNA transmission known as doubly uniparental inheritance (DUI). Under this system, two highly divergent sex-linked mitochondrial DNAs exist, transmitted, respectively, only through eggs and sperm. Females are normally homoplasmic for the female-transmitted mitochondrial genome (F type), whereas males are heteroplasmic for the F and the male-transmitted (M-type) mitochondrial genomes (see Fig. 6.3). Indeed, this unusual association between a male-transmitted genome and maleness is what prompted interest in the possible relationship between mitochondrial genomes and sex-determination mechanisms in bivalves. One of the leading hypotheses to explain the origin and maintenance of DUI is that a male-transmitted genome might have evolved because it plays a role in sex determination (Breton et al. 2011; Zouros 2013), and this could benefit a “selfish” paternally transmitted cytoplasmic genome (Burt and Trivers 2006).

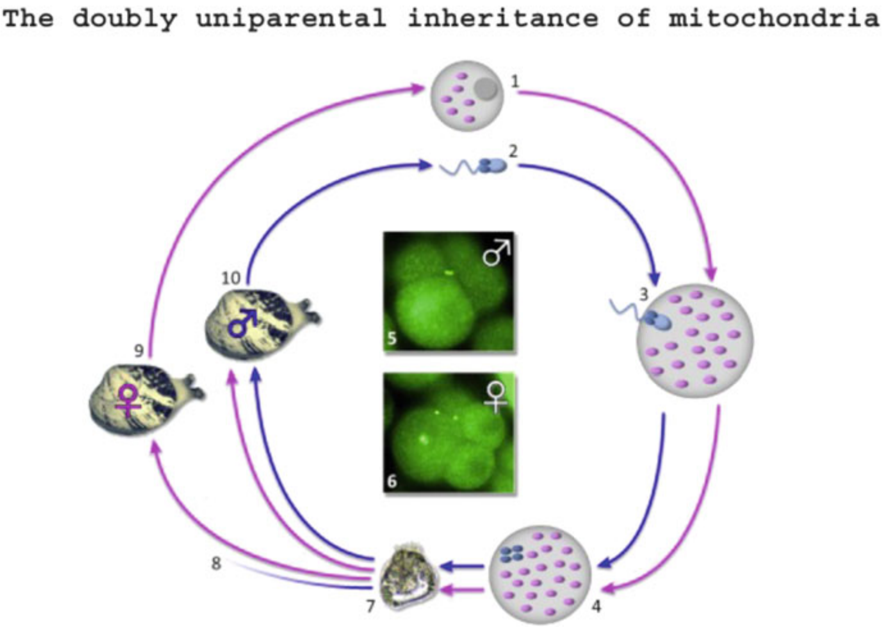

Fig. 6.3 Scheme of mitochondrial transmission in species with doubly uniparental inheritance (DUI) of mitochondria. Pink ovals, egg mitochondria containing F mtDNA; blue ovals, sperm mitochondria carrying M mtDNA; pink arrows, transmission route of F mtDNA; blue arrows, transmission route of M mtDNA. Bright green spots in panels 5 and 6 are sperm mitochondria stained with MitoTracker® Green FM staining. The F mtDNA follows a normal transmission pattern, while the M follows exclusively a father-to-son one: (1) eggs are homoplasmic for F mitochondria, (2) sperm is homoplasmic for M mitochondria, (3) the spermatozoon carries M into the egg, (4) zygotes are heteroplasmic, (5) sperm mitochondria are aggregated in males, (6) sperm mitochondria are dispersed in females, (7) embryos continue development, (8) M is often lost in adult females, (9) adult females transmit only F with eggs, (10) adult males transmit only M with spermatozoa. [Based on Breton et al. (2014)]

Much of the best experimental work on sex ratio biases and theoretical modeling of sex-determination mechanisms in bivalves has been done on the genus *Mytilus*, which was the first organism shown to exhibit DUI (reviewed in Breton et al. 2007; Passamonti and Ghiselli 2009; Zouros 2013). For example, Saavedra et al. (1997) and Kenchington et al. (2002) used controlled crosses to show that offspring sex ratio is a characteristic dictated by the female parent, with individual females producing (a) all female progeny, (b) a roughly 50:50 sex ratio, or (c) a heavily male-biased sex ratio, regardless of the male to which they are mated. Fertilization experiments performed using mussel sperm stained with a mitochondrial dye (i.e., MitoTracker) from these same controlled crosses demonstrated that the sperm-transmitted mitochondria cluster together for the first several cell division cycles following fertilization of eggs from mothers that produce heavily male-biased crosses, whereas the paternally derived mitochondria disperse and rapidly disappear in crosses involving mothers characterized by female-biased progeny (Cao et al.

2004a; see Fig. 6.3). Kenchington et al. (2009) suggested that the data from these controlled crosses demonstrate that paternal mtDNA and maleness are co-inherited, but they argued that maleness is not caused by the male mtDNA genome directly. Zouros (2000) originally proposed a three-factor (i.e., W, X, and Y) genetic model for how the mother's nuclear genotype controls the sex of the offspring. According to the model, the W factor is expressed on the surface of spermatozoa, and it interacts with a complementary X factor expressed in the cytoplasm of the egg, which is coded for by the female parent. These two factors are the default system that potentially lead to the elimination of the sperm mitochondria and ensure uniparental transmission of the mother's mtDNA. However, whether this occurs is affected by a third factor, Z, which has two alleles: an active Z form that suppresses the X factor and facilitates paternal transmission of the M-type mtDNA or an inactive z form, which does not interfere with expression of the X factor, enabling destruction of the paternal mitochondria (Zouros 2000). A refined version of an oligogenic sex-determination model that incorporates an additional factor, S, is presented in Zouros (2013).

Female-dependent sex ratio biases have also been demonstrated in other DUI species [e.g., the venerid clam *Ruditapes philippinarum* (Ghiselli et al. 2012) and the freshwater mussel *Unio delphinus* (Machordom et al. 2015)], suggesting that sex ratio bias may be a general characteristic of taxa exhibiting this unusual system of mtDNA inheritance. Ghiselli et al. (2011) summarized the sex-specific segregation of the M and F genomes in DUI species in terms of a "three-checkpoint" process. The segregation of the M-type mitochondria in fertilized eggs and early-stage male embryos (e.g., Cao et al. 2004a; Ghiselli et al. 2011) is Checkpoint #1, and the destruction/dilution of the M type in early-stage female embryos (Sutherland et al. 1998; Cao et al. 2004a; Guerra et al. 2016) is Checkpoint #2. The sequestration of the M type in primordial germ cells in developing male gonads, and of the F type in the female developmental pathway, is characterized as Checkpoint #3 (Ghiselli et al. 2011).

Some progress has been made with respect to identifying the molecular basis of these critical checkpoints for the fate of the M genome during sexual maturation. Kyriakou et al. (2015) used an electrophoretic mobility shift assay (EMSA) to demonstrate that a small DNA segment of the variable domain 1 (VD1) of the control region of a male-transmitted mitochondrial genome in *Mytilus galloprovincialis* forms a complex with a protein expressed in male gonad, and, more specifically, that this interaction is strongest for perinuclear mitochondria. Kyriakou et al. (2015) refer to this 23-bp fragment as a sperm transmission element (STE) and suggest that the resulting protein-mitochondrial DNA complexes allow these male-derived organelles to resist degradation during spermatogenesis, thereby ensuring the paternal transmission of these genomes.

The hypothesis of a critical role for mitochondrial STEs based in the control region of male-transmitted mitochondrial genomes of *Mytilus* mussels comes from several studies demonstrating recombination of M and F genomes in members of that genus (reviewed in Stewart et al. 2009). Various species of *Mytilus* mussels exhibit two distinct categories of male-transmitted genomes: one that tends to be highly divergent from the F type (e.g., 15–20% sequence divergence for protein-coding genes) and one that tends to be quite similar (e.g., <2–3% sequence divergence; Hoeh et al. 1996). The divergent M type is referred to as a "standard male" or SM type, whereas the other M type is referred to as a "recently masculinized" or RM-type genome (Stewart et al. 2009). Sequencing studies have affirmed that *Mytilus* RM types are recombinant genomes (e.g., Cao et al. 2004b; Burzynski et al. 2006). These RM types are composed of an F genome protein-coding, ribosomal and transfer RNA genes and, significantly, the control region from an SM-type genome. The presence of the sperm transmission element elucidated by Kyriakou et al. (2015) from an SM genome control region appears to be a critical factor effecting the subsequent sequestration of the sperm-derived mitochondria in the germ cells. Recent comparative work on *Mytilus* sp. and the closely related horse mussel, *Modiolus modiolus* (Mytilidae) by Robicheau (2016), suggests that additional conserved sequence motifs in the mitochondrial control region could also function as molecular signals for the cellular mechanisms that govern paternal transmission.

One question that remains to be elucidated is whether this unusual system of DUI had its origin as a cytoplasmic sex-determination system that benefitted a selfish genetic element (Breton et al. 2007; Passamonti and Ghiselli 2009; Zouros 2013). Milani et al. (2013, 2014) recently conducted extensive in silico analyses of molecular signatures and motifs of novel bivalve ORFan genes in the venerid *R. philippinarum* and suggested that the causative agent that lead to the creation of the DUI system may have been a selfish viral element. Although the mitochondrial genomes of some DUI taxa may no longer determine gender directly (e.g., *Mytilus*), it is possible that an ancestral cytoplasmic sex-determination system was subsequently usurped by a nuclear-encoded system. Such an evolutionary arms race between the nuclear and cytoplasmic genomes would be in keeping with the demonstration by Bachtrog et al. (2014) that sex-determination systems are extremely plastic evolutionarily and subject to a variety of selective pressures. Given that sex-determining mechanisms are diverse and can evolve rapidly even among closely related species (see Bachtrog et al. 2014 for a review), it would not be unreasonable to hypothesize that a selfish mitochondrial DNA element could have evolved the ability to affect a species' sex-determination pathway, only to have the nuclear genome evolve a restorer locus that wrested sex determination back from the influence of a selfish mitochondrial element. For example, mitochondrially encoded elements that distort sex ratios are well known in plants with cytoplasmic male sterility (CMS) (Chase 2007). Specifically, CMS typically involves mitochondrial genes causing sterile or inviable pollen, a situation that has been repeatedly followed by the evolution of nuclear restorer genes (Burt and Trivers 2006; Chase 2007).

In bivalves, evidence for a more direct and ongoing role for mitochondrial genomes in sex-determination pathways comes from recent studies of freshwater mussels of the order Unionoida, which also exhibit M- and F-transmitted mitochondrial DNA lineages (e.g., Breton et al. 2009, 2011; Doucet-Beaupré et al. 2010). Whereas the M and F genomes in the marine mussel family Mytilidae and the marine clam family Veneridae appear to experience occasional recombination between the two gender-associated mtDNA genomes leading to the creation of a new male-transmitted genome (reviewed in Stewart et al. 2009), the M and F genomes in the family Unionidae have been distinct from one another for more than 200 million years and may exhibit as much as ~50% amino acid sequence divergence (Doucet-Beaupré et al. 2010). Analysis of complete M and F mitochondrial genomes from several dioecious (gonochoric) species of unionoid taxa (Doucet-Beaupré et al. 2010; Breton et al. 2011) has demonstrated the presence of two unique putative ORFan genes in these genomes, one in the F genome and one in the M genome. The M and F-*orf* genes share no obvious sequence homology to one another or to any other genes on GenBank (Breton et al. 2011). A further fascinating observation is what happens to these genes in closely related hermaphroditic species of unionoid mussels. Breton et al. (2011) showed that hermaphrodites (a) retain a genome that is derived from the F genome of their dioecious ancestor, (b) evolve a divergent "H-*orf*" gene derived from the F-*orf* gene, and (c) lose the M genome altogether. Immunohistochemical probing for the F-ORF protein has also shown that it is localized to areas outside of the mitochondrion, specifically in the nuclear membrane and nucleoplasm (Breton et al. 2011). The presence of the distinctive "ORFan" genes with no homology to known genes of the oxidative phosphorylation pathway suggests a novel role for the proteins they encode, broadening the functional repertoire of mitochondrial genomes (Breton et al. 2014). The rapid evolutionary divergence of the H-*orfs* (which are independently derived in several hermaphroditic species of freshwater mussels) suggests a considerable change in function of these genes associated with the reproductive switch from dioecy to hermaphroditism and, consequently, an implication of a role in sex determination or sexual development (Breton et al. 2011, 2014).

6.5 Conclusion and Perspectives

The sexual strategies and sex-determining mechanisms in bivalves are remarkably diverse. Mixed sex-determination systems, involving both genetic and epigenetic factors, have been described in many species. Bivalve species richness and diversity of sexual systems offer an excellent opportunity for addressing questions about the underlying mechanisms of sex determination. Future sex-determination research in bivalves also promises to yield useful tools for selective breeding programs of economically important species. Recently developed "omic" technologies are now capable of identifying many of the key molecular components involved in sex determination in bivalves and other animals, i.e., they enable the examination of how the expression of these key molecular components affects the physiology of the developing embryo, how this process can be affected by environmental factors, and how it can be reprogrammed in hermaphroditic species. Such approaches and studies will allow us to test hypotheses relating to the underlying factors shaping the evolution of sex-determining systems.

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Chapter 7
Transitions in Sexual and Reproductive Strategies Among the Caenogastropoda

Rachel Collin

Abstract Caenogastropods, members of the largest clade of shelled snails including most familiar marine taxa, are abundant and diverse, and yet surprisingly little is known about their reproduction. In many families, even the basic anatomy has been described for fewer than a handful of species. The literature implies that the general sexual anatomy and sexual behavior do not vary much within a family but for many families this hypothesis remains untested. Available data suggest that aphally, sexual dimorphism, maternal care, and different systems of sex determination have all evolved multiple times in parallel in caenogastropods. Most evolutionary transitions in these features have occurred in non-neogastropods (the taxa formerly included in the Mesogastropoda). Multiple origins of these features provide the ideal system for comparative analyses of the required preconditions for and correlates of evolutionary transitions in sexual strategies. Detailed study of representatives from the numerous families for which scant information is available and more completely resolved phylogenies are necessary to significantly improve our understanding of the evolution of sexual systems in the Caenogastropoda. In addition to basic data on sexual anatomy, behavioral observations are lacking for many groups. What data are available indicate that mate choice and sexual selection are complicated in gastropods and that the costs of reproduction may not be negligible.

7.1 Introduction

The difficulty in understanding evolutionary transitions between sexual systems is not due to a lack of theories or potential selective pressures that could be responsible. Instead the difficulty lies in finding common causes for the diversity of evolutionary patterns observed among divergent groups, when the relevant questions for each group may differ. For example, in heterobranch gastropods, which are simultaneous hermaphrodites (see Table 7.1 for definitions), the question may be why dioecy has

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Table 7.1 Definition of terms as used in this chapter

Term	Definition
Aphallic	Lacking a penis
Protandry	A sequential hermaphrodite where the male phase precedes the female stage and the two sexes do not occur simultaneously
Consecutive hermaphrodite	A sequential hermaphrodite where sex can change more than once
Dioecy	Separate sexes
Environmental sex determination	When sex is determined by environmental conditions like temperature or social interactions and is not set by sex chromosomes
Parthenogenesis	Asexual reproduction which proceeds from an egg without fertilization with a sperm
Protogyny	A sequential hermaphrodite where the female phase precedes the male stage and the two sexes do not occur simultaneously
Sexual dimorphic	Males and females show differences in characteristics other than primary sexual characters
Simultaneous hermaphrodite	Any individual that has functional male and female structures at the same time. In many species maturity of one sex may develop prior to maturity in the second sex, but during most of the adult life, both sexes occur simultaneously

not evolved more often, while in their primarily dioecious sister group, the caenogastropods, the question may be why protandry has evolved so often and simultaneous hermaphroditism so seldom. To answer such questions about real organisms rather than theoretical constructs, it is important not only to know the phylogenetic distribution of sexual systems and to have a firm basis in evolutionary theory but also to understand the comparative biology of the organisms. With such understanding, it is possible to tackle questions like the following: Do developmental constraints limit the evolutionary transitions between sexual systems or make certain transitions more likely than others? Do ecological factors like habitat type or diet impact population density, thus influencing the relative importance of sexual selection versus reproductive assurance? Do costly taxon-specific behaviors like competition for mates or parental care unduly influence the male or female reproductive gain curves, shifting the pattern of optimal sex allocation? There are few non-model invertebrate taxa for which we have sufficient knowledge to begin to address these questions.

Instead this chapter reviews the diversity of sexual systems and reproductive biology among caenogastropods. I highlight the evidence from phylogenetic distributions that sexual dimorphism, aphally, maternal care, and various systems of sex determination have all experienced evolutionary transitions in caenogastropods. I also review the scattered literature on caenogastropod mating behavior, sexual selection, and costs of reproduction. The limited information is insufficient to draw detailed conclusions, but it is vitally important to recognize the diverse behavioral context in which sexual systems evolve and to recognize that snails may incur significant costs of courtship and reproduction.

7.2 Caenogastropoda

Gastropoda, the most diverse class within the Mollusca, with an estimated 120,000 or more species (Appeltans et al. 2012), is often broken into three major groups for discussions of evolutionary patterns (basal gastropods, caenogastropods, and heterobranchs). The basal grade of snails including patellogastropods and vetigastropods generally have simple reproductive anatomy, are free spawners, show little evidence of complicated mate choice mechanisms, and are seldom sexual dimorphic (Beesley et al. 1998 but see Lindberg and Dobberteen 1981). The monophyletic Heterobranchia includes sea slugs and pulmonate slugs and snails, as well as some basal shelled marine forms. Heterobranchs have complex reproductive anatomies, are almost always simultaneous hermaphrodites, and show a wide array of complicated mating behaviors, which are relatively well-studied (Davison and Mordan 2007; Chase 2007; Valdés et al. 2010; Jarne et al. 2010; Baur 2010).

The subclass Caenogastropoda, the monophyletic sister clade to the Heterobranchia, includes approximately 60% of described gastropod species and more than 100 families. Caenogastropods are mostly sea snails, but the group includes some terrestrial and freshwater families. They include herbivores and carnivores of varying levels of specialization, as well as filter feeders, kleptoparasites, blood-sucking parasites, internal parasites, and numerous families of deep-sea and infaunal micro-snails whose ways of life are poorly known (Fretter and Graham 1962; Beesley et al. 1998). Caenogastropods occur in the sea from the deep ocean to the interidal zone and on land from lush rainforests to dry desertic habitats. They display a wider range of reproductive anatomies and systems of sex determination than do heterobranchs, but little is known about sexual selection, mate choice, and parental care behaviors in most caenogastropods.

The diversity of sexual systems in the Caenogastropoda makes them ideal for the application of comparative methods to understand the factors associated with evolutionary transitions between sexual systems and reproductive strategies. Unfortunately, data available from only one or a few “representative” species, uncertain phylogenetic relationships, and dynamic taxonomy of some families and superfamilies make generalizing difficult. Generalizations (including those made here) should be viewed critically in light of the number of species that have been examined and the systematic stability of the group.

The higher-level taxonomy of caenogastropods is still being refined, but the group is divided into three orders, Architaenioglossa (3 superfamilies, 11 families), Littorinimorpha (16 superfamilies, 66 families), and Neogastropoda (7 superfamilies, 44 families), and 6 as yet unassigned superfamilies containing 34 families (WoRMS Editorial Board 2016). The best available phylogenies include fewer than half of the caenogastropod families, but the most complete analysis to date concludes that (1) the three architaenioglossan superfamilies are basal to all other caenogastropods but may not be monophyletic, (2) neogastropods are monophyletic within the Sorbeoconcha (i.e., the non-architaenioglossan caenogastropods), and (3) the relationships among the non-neogastropod Sorbeoconcha remain unclear (Ponder et al. 2008) (see Fig. 7.1).

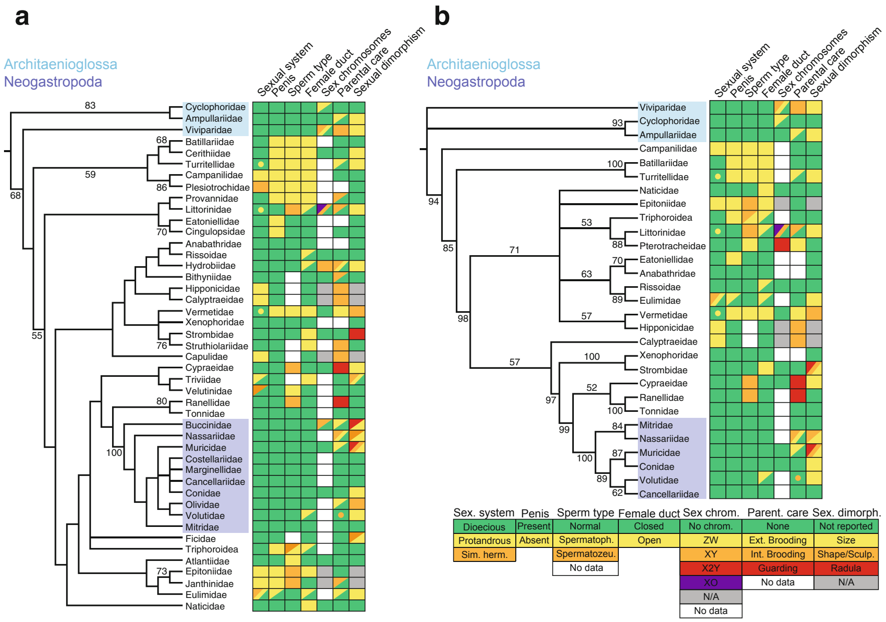

Fig. 7.1 Aspects of the sexual systems and reproductive biology mapped onto phylogenetic trees of select caenogastropods generated by Ponder et al. (2008). (**a**) Strict consensus tree based on morphological characters, with bootstrap values shown on branches and (**b**) strict consensus tree from a Bayesian analysis of morphological characters and molecular data with clade credibility values shown on branches. The states of seven characters in each family are indicated in color. Sexual system: dioecious, protandrous, and simultaneous hermaphrodite as indicated by sexual anatomy. Penis: present/absent in males. Sperm type: spermatophore present; spermatozeumata present; normal, eusperm and usually parasperm are present; no data, sperm have not been examined in detail. Female ducts: open/closed. Sex chromosomes: no chromosome, sex chromosomes have not been detected in karyotypes; N/A, not applicable to protandrous or simultaneous hermaphrodites; no data, published studies are not available. Parental care: external brooding, brooding on the outside of the shell; internal, under the shell, including under or alongside the foot, in the mantle cavity, and inside the body; guarding, adults remain near or on egg capsules but can move around independent of the capsules. Sexual dimorphism includes the characters for which dimorphism has been reported if present in any species in the group; solid squares do not indicate that all species display dimorphism. Diagonal stripes indicate multiple states present in the group. A central spot indicates that this state appears to be an unusual derived state in only one or a few species. See text for relevant citations

7.3 Evolutionary Patterns of Dioecy, Protandry, and Parthenogenesis

Dioecy is inferred to be ancestral in the Gastropoda and the Caenogastropoda (Heller 1993; Ponder and Lindberg 1997; Kay et al. 1998; Fretter et al. 1998; Ponder et al. 2008). Evolutionary transitions to protandry are common among the caenogastropods (Fig. 7.1), while transitions to simultaneous hermaphroditism and parthenogenesis are uncommon. Protogyny is unknown. Protandry occurs in scattered species or genera within primarily dioecious families, suggesting a recent evolutionary origin in these taxa. Other families are entirely protandrous suggesting that this strategy is ancient and evolutionary stable either because it is advantageous or because ontogenetic canalization makes it difficult to revert to the ancestral state. It is likely that protandry is underreported, as protandry is more difficult to detect than simultaneous hermaphroditism (Calvo and Templado 2005; Collin 2013).

The Architaenioglossa, including freshwater ampullariids (apple snails) and viviparids, and the terrestrial Cyclophoroidea are dioecious. Hermaphroditism is not known to occur, but parthenogenesis has arisen via hybridization in the viviparid *Campeloma* sp. (Johnson and Bragg 1999).

Neogastropods are generally dioecious with the exception of some coralliophilid species which are protandrous (Chen and Soong 2002; Richter and Luque 2004; Johnston and Miller 2007). Protandrous sex change in coralliophilids is similar to sex change in patellogastropods and calyptraeids in that it is socially mediated and closely related to individual size (Chen and Soong 2002; Richter and Luque 2004; Johnston and Miller 2007; Collin 2013). Circumstantial evidence suggests that some species of *Vitularia* (Muricidae) and *Iphinopsis alba* (Cancellariidae) might be protandrous (Warén 1984; Bouchet and Warén 1985; Herbert et al. 2009). One turrid *Propebela* (=*Oenopota*) *turricula* has been reported as protandrous with a simultaneous hermaphroditic phase (Smith 1967). However the lack of relationship between sex and size is unusual and imposex (masculinization of females by endocrine disruptors) cannot be ruled out in this case. Imposex is particularly well documented for neogastropods (Oehlmann et al. 1996) but also occurs in ampullariids (Takeda 2000) and some littorinimorphs (Li and Collin 2009). Neither simultaneous hermaphroditism nor parthenogenesis has been reported in the Neogastropoda.

The non-neogastropod Sobreoconcha ("mesogastropods") contain the most diverse assortment of sexual systems and sexual morphologies in the Caenogastropoda (Fig. 7.1). The majority of the families including the well-known Naticidae, Strombidae, Buccinidae, and Nassariidae appear to be exclusively dioecious. A number of other families, including Calyptraeidae, Capulidae, Epitoniidae, Hipponicidae, and possibly Janthinidae, are probably exclusively protandrous (Calvo and Templado 2005; Churchill et al. 2011; Collin 2013).

Two other families contain a mix of dioecious and protandrous species. Eulimids are notable for the diversity of their sexual systems, which includes protandry, dioecy, and simultaneous hermaphroditism. Species of *Melanella*, *Peasistilifer*, and *Vitreolina* are dioecious, and species of *Eulima*, *Stilifer*, and *Haliella* are sequential hermaphrodites (Warén 1980, 1984; Bouchet and Warén 1986). Since many of these specialized parasites show extreme sexual dimorphism and appear to have environmental sex determination, it is difficult to fully confirm protandry without either detailed histological analysis or longitudinal observations of individuals (Elder 1979; Matsuda et al. 2013). Triviids can also be dioecious or protandrous, with four South African species reported as protandrous and ten as dioecious (Gosliner and Liltved 1982, 1987).

Other families are primarily dioecious but include one or a few protandrous species. For example, littorinids are exclusively dioecious except for the two species of *Mainwaringia* (Reid 1986). Likewise, only a single species each of the tiny marine assimineids (*Rugapedia androgyna*) and tornids (*Cyclostremiscus beauii*) have been reported as protandrous (Bieler and Mikkelsen 1988; Fukuda and Ponder 2004). More species need to be examined to determine if protandry is unusual for these groups. Three species of the vermetid genus *Serpulorbis* change sex, one of which, *S. arenarius*, has consecutive hermaphroditism, where animals change from male to female and back again (Calvo and Templado 2005). This is the only known case of consecutive hermaphroditism in a caenogastropod. Sex change may also occur in one species of *Ceraesignum* (as *Dendropoma*) as evidenced by the increased frequency in females in the large size classes (Phillips and Shima 2010). The turritellids *Vermicularia spirata* (Bieler and Hadfield 1990) and *Gazameda gunni* (Carrick 1980) and the planaxid *Fossarus ambiguus* (Houbrick 1990) are also protandrous. The suggestion that *Pedicularia* (Ovulidae) species are protandrous was contested by Goud (2001) but needs to be investigated further. Finally the deep-sea family Laubierinidae may be protandrous based on limited data from one species of these deep-sea tonnoideans (Warén and Bouchet 1989).

Protandry in caenogastropods has often been inferred from size distributions of the sexes, and few details on the natural history of sex change are available for groups other than calyptraeids (reviewed in Collin 2013). In calyptraeids sex change depends on the size of the animal and its social circumstances (Collin et al. 2005; Mérot and Collin 2012a, b). The impact of nearby conspecifics is mediated via touch not waterborne chemicals (Carrillo-Baltodano and Collin 2015). In *Crepidula* cf. *marginalis*, large individuals suppress growth and sex change in smaller animals, while small animals increase the growth rate of larger animals (Carrillo-Baltodano and Collin 2015). It would be interesting to know if similar interactions occur in other protandrous groups.

Simultaneous hermaphroditism is rare in the Caenogastropoda. The "living fossil" *Plesiotrochus crinitus* (Plesiotrochidae) is a simultaneous hermaphrodite (Houbrick 1990; Healy 1993), as are species in the eulimid genera *Pelseneeria*, *Pisolamia*, *Goodingia*, and *Ophioarachnicola*. The eulimids, which generally live in pairs, have a very small testis thought to produce the minimal sperm necessary to fertilize their partner in the absence of sperm competition (Warén 1984). Velutinids also include simultaneous hermaphroditic genera (*Velutina* and *Marsenina*) and dioecious genera (*Lamellaria*, *Coriocella*, and *Marseniopsis*) (Fretter and Graham 1962; Wilson 1998).

Parthenogenesis occurs in a number of estuarine and freshwater caenogastropods, especially thiarids and tateids. Parthenogenesis in these groups has been linked to their large geographic ranges and success as invaders (Facon et al. 2003; Alonso and Castro-Diez 2008; Miranda et al. 2011). In the tateid *Potamopyrgus antipodarum*, diploid sexual populations have numerous males, while parthenogenic populations are polyploid and rarely have males (Wallace 1992). The cochliopid *Heleobia* may also be parthenogenic (Martín 2002).

7.4 Sexual Anatomy of Caenogastropods and Aphally

The sexual anatomy of gastropods has been described and reviewed in detail (Fretter and Graham 1962; Hyman 1967; Fretter et al. 1998; Kay et al. 1998; Voltzow 1994; Strong 2003; Hodgson 2010). In male caenogastropods, a gonoduct connects the gonad to the prostate gland and then extends as a pallial gonoduct or vas deferens to a penis or a simple gonopore. The gonoduct and prostate can be open over part or all of their lengths. In females the oviduct runs past accessory structures for sperm storage and egg packaging as it connects the single ovary to the female genital opening. The bursa copulatrix, where the sperm are deposited, is usually distal to the seminal receptacle or receptaculum seminalis, where the sperm are stored. The albumin or gel gland and, most distally, the capsule gland make secretions associated with packaging eggs. The morphology and arrangement of these structures can vary between species, genera, and families, and the structures may not be homologous across families. During sex change in protandrous caenogastropods, the pallial gonoduct usually transforms from the vas deferens to the albumin and capsule glands, and the penis (if present) is absorbed. In simultaneous hermaphrodites, there may be a separate testis and ovary, each with its own duct, but in *Velutina*, eggs and sperm are produced in acini in the same gonad (Fretter and Graham 1962; Fretter 1998).

One particularly variable character among caenogastropods is the presence or absence of a penis. Most basal gastropods lack an intromittent organ, but a penis similar to those found in caenogastropods has evolved independently several times in the Neritimorpha and other basal gastropods (Hickman 1992; Kano 2008). Basal caenogastropods either lack a penis (e.g., cerithioideans), or it appears to have evolved independently (e.g., Architaenioglossa). Therefore the ancestral state for caenogastropods is ambiguous. Comparative analyses of the diversity of penis morphologies, assessment of homologies, and close examination of ecological and morphological correlates of aphally could be a fruitful area for future research.

Male architaenioglossans all possess an intromittent organ. However the homology of this organ is unlikely as the organ is a modified right tentacle in the viviparids, derived from the mantle edge and innervated by the right pleural ganglion in ampullariids (Berthold 1989) and derived from cephalic tissue in cyclophoroids (Kretzschmar 1919). During the development of ampullariids, both sexes begin to develop a penis, but in females, its growth is arrested as the ovary develops (Andrews 1964). This mechanism explains why female ampullariids often have a small "pseudopenis" (Takeda 2000) and may also explain pseudopenes in some other groups (e.g., Kuwamura et al. 1983; Avise et al. 2004). The functional morphology of the penis has been described using snap-frozen mating pairs in the ampullariid *Pomacea canaliculata* (Giraud-Billoud et al. 2013). This study is unique in demonstrating that the tip of the penis penetrates to the seminal receptacle rather than inferring the depth of penetration from the location of sperm in the female reproductive system. Little information is available on the function of the intromittent organs in other architaenioglossan taxa.

Many sorbeoconchans have a solid muscular penis behind the right cephalic tentacle. The penis is usually derived from pedal tissue with pedal innervation (Voltzow 1994; Hodgson 2010). Sperm are transferred in an open groove or closed duct that runs along the ventral surface of the penis. The penis may range from long and tapered to broad and paddle-shaped, and diverse morphologies are present in many groups. For example, in rissooideans, the penis can be tapered with an open groove, bifid with the vas deferens in one branch and a flagellum in the other, or can have seven or more circular suckers (Szarowska 2006). When not in use, the penis is usually folded back over the head in the mantle cavity. In collumbellids, the penis is often held in a special pouch in the mantle roof under the hypobranchial gland (Marcus and Marcus 1962; deMaintenon 1999).

Penis morphology sometimes correlates with female genital morphology. In calyptraeids, genera in which a long tapering penis is tipped with a long terminal papilla are those in which the females have a distinct genital papilla (Collin 2003). Similarly, the presence of simple lobes on the left edge of the rissooidean penis is correlated with the presence of two female receptacles (Szarowska 2006). Penis morphology is relatively stable within genera in some groups (Szarowska 2006; Georgiev 2012). In others it differs among closely related species (Reid 1989; Reed 1995) suggesting that penis morphology may be one of the first characters to diverge during or after speciation.

A number of superfamilies (including cerithioideans, cingulopsoideans, vermetoideans, triphoroideans, and epitonioideans) lack a penis (Ponder et al. 2008). Hodgson (2010, p. 127) included capulids and hipponicids in this group, but their penes are similar to other caenogastropods (e.g., Graham 1954; Poulicek et al. 1997; Collin 2003). The overall similarity of caenogastropod penes suggests that aphally may represent parallel losses of the penis. However the penis of anabathrids and emblandids is innervated by the cerebral ganglion and may have evolved in parallel to other caenogastropods (Ponder 1988), and it is possible that other groups have evolved a penis independently. Aphally is not closely related to characteristics of the female system. For example, the female pallial gonoduct can be open in both phallate and aphallate families suggesting that evolutionary transitions to and from aphally are not tightly constrained by female morphology (Fretter et al. 1998; Hodgson 2010; Fig. 7.1).

The female reproductive system is also evolutionarily dynamic, including mesodermal and ectodermal structures that vary in number and form across taxa (Fretter 1984; Fretter and Graham 1994; Ponder and Lindberg 1997). Comparative analyses could contribute significantly to our understanding of the evolutionary transitions in the arrangement of these structures. One major impediment to such an analysis is the lack of clear homologies, as parts of the female reproductive system are named based on their function rather than on homologies. These may represent convergent morphologies that have evolved to solve the problems of copulation, sperm storage, and embryo packaging (Fretter et al. 1998). Ontogenetic information could be useful in untangling these homologies. For example, comparative development shows that the posterior gonoduct develops from a gonadal and a renal primordia in *Viviparus* and *Littorina* and from a single primordium in *Nassarius* and *Crepidula* (Drummond

1903; Moritz 1939; deMaintenon 2001). Ontogenetic information like this could be particularly important in understanding the evolutionary transition from dioecy to simultaneous hermaphroditism.

7.5 Sperm, Parasperm, and Postcopulatory Selection

Studies of insects show that the contents of ejaculates (seminal fluids as well as sperm cells) in polyandrous animals are often active in altering fertilization success or female reproductive output (Simmons and Fitzpatrick 2012). Sperm cells from the same male might work together to ensure fertilization by one of their cohort, sperm may actively hinder sperm from other males, and chemicals in seminal fluids may also be active in competition between ejaculates (Holman and Snook 2008; Holman et al. 2008; Higginson and Pitnick 2011). Little is known about the role of these mechanisms in caenogastropod reproduction.

Sperm competition is likely to play an important role in caenogastropod reproduction. Parasperm, sperm that are not used to fertilize eggs, may have been present ancestrally in caenogastropods, as very simple parasperm are present in nerites and some vetigastropods (Buckland-Nicks 1998). Within caenogastropods they have diversified in form and function, although they have been reduced or eliminated in some neogastropods and atlantids (Buckland-Nicks and Hodgson 2005; Jamieson and Newman 1989; Buckland-Nicks 1998; Hodgson 2010). Most kinds of parasperm contain glycoproteins which may be released into the seminal fluid (Buckland-Nicks 1998). In many aphallic species and some other groups (*Littorina* and *Fusitriton*), large parasperm (spermatozeugmata) play a role in transporting eusperm (Buckland-Nicks et al. 1999; Buckland-Nicks and Tompkins 2005). In aphallic species, spermatozeugmata enter the female, and in other species, secretions from the prostate gland cause the eusperm to detach from the parasperm before it leaves the male reproductive tract making their role unclear (Buckland-Nicks et al. 1999). Another kind of parasperm, lancet parasperm, is present in many neogastropods and some caenogastropods. These long cells make a plug in the bursa copulatrix that may inhibit penetration by later males (Buckland-Nicks 1998). Experiments confirm the role of parasperm in sperm competition. When male *Viviparus ater* are exposed to rival males or to a male-biased population sex ratio, they produce a lower ratio of eusperm to parasperm (Oppliger et al. 1998) and produce larger parasperm (Oppliger et al. 2003). When males from male-biased or female-biased environments were mated with virgin females, parasperm length accounted for 15% of male success in siring offspring (Oppliger et al. 2003).

Spermatophores also occur in a number of caenogastropod families (Robertson 1989, 2007). These are comprised of encapsulated packages of eusperm and are distinct from spermatozeugmata (Hadfield and Hopper 1980; Glaubrecht and Strong 2004). Like spermatozeugmata, spermatophores appear to have evolved independently a number of times among caenogastropods (Glaubrecht and Strong 2004;

Robertson 2007) and are not obviously correlated with other transitions in the sexual systems of caenogastropods (Fig. 7.1).

Female anatomy sets the stage for sperm competition. Most caenogastropods can store sperm, using the seminal receptacle, the ovary, or the spermatophore bursa (Buckland-Nicks and Darling 1993; Whelan and Strong 2014). Many species can store sperm for several months or more. Calyptraeids can store sperm for 6 months or a year (Brante et al. 2011; pers. obs.), and *Viviparus* can store sperm for up to 2 years (Trüb 1990). The muricid *Dicathais orbita* and the littorinid *Littorina saxatilis* store sperm for more than a year (Westley et al. 2010; Panova et al. 2010; Johannesson et al. 2016). However, in some temperate species, the pallial oviduct atrophies during the nonreproductive season, and sperm are not stored between seasons (e.g., Whelan and Strong 2014).

Stored sperm from multiple males result in multiple paternity in many caenogastropods. Genotyping of offspring demonstrates that single broods can have 15–23 sires (*Littorina saxatilis*: Panova et al. 2010; Johannesson et al. 2016), 10–15 sires (*Solenosteira macrospira*: Kamel and Grosberg 2012), 1–7 sires (*Rapana venosa*: Xue et al. 2014), 2–8 sires (*Busycon carica*: Walker et al. 2007), and 1–5 sires (*Crepidula* spp.: Le Cam et al. 2014; Brante et al. 2011). In some species the proportions of paternity vary among males but are roughly equal across capsules in a single brood (Walker et al. 2007; Xue et al. 2014). In *Neptunea arthritica* the last male to mate achieves greater paternity than previous males by removing sperm they deposited inside the female (Lombardo et al. 2012). In *Rapana venosa* the last males to mate also sires the largest proportion of offspring, but the mechanism behind this is not known (Xue et al. 2016). In contrast, earlier mates sire a greater proportion of the offspring in *Littorina obtusata* (Paterson et al. 2001), and larger males sire more offspring in *L. saxatilis* (Johannesson et al. 2016).

Copulatory courtship, activities that may impact postcopulatory cryptic female choice, may also occur in caenogastropods. For example, mating in *Pomacea canaliculata* may take 10–20 h. Approximately 2 h is necessary for final courting and genital connection (Burela and Martín 2011), and dissection of snap-frozen animals shows that it takes 2–4 h for the penis to reach maximal penetration (Giraud-Billoud et al. 2013). The remaining time is used for sperm transfer, and those snails where copula was limited to 4–6 h had reduced fecundity after 10 days if they were not allowed to copulate again. A behavior that has been interpreted as nuptial feeding where the female licks a secretion produced by the outer gland of the muscular penis sheath may occur during copulation (Burela and Martín 2007, 2014). The function of such feeding is not clear. The increased incidence of this behavior in high-density populations suggests that it may function to encourage females to continue mating in the presence of other potential mates (Burela and Martín 2014) but it could also act as copulatory courtship which may influence postcopulatory female choice. Models of sperm storage (e.g., Manier et al. 2013), a better understanding of the role played by parasperm, and application of methods developed to track sperm in the reproductive tract of terrestrial gastropods (e.g., Kupfernagel et al. 2013) could help interpret results from mating experiments and paternity analyses.

7.6 Sex Determination

The mechanism of sex determination is currently unknown for most caenogastropods. In protandrous groups, sex is labile, and sex change is environmentally mediated. Genetic effects on sex change have so far not been examined. In dioecious groups, sex is thought to be genetically controlled, and the impact of environmental conditions has not been examined. Cytoplasmic sex factors have not been detected in any caenogastropod (Yusa 2006).

A 50:50 sex ratio, which is commonly reported for dioecious marine gastropods, is consistent with the expectation of heterogametic genetic sex determination. There is karyotype evidence to support sex chromosomes in a few of the 291 gastropod karyotypes reviewed by Thiriot-Quiévreux (2003) and subsequent work. Among the outgroups of caenogastropods, no sex chromosomes are reported for vetigastropods or simultaneously hermaphroditic opisthobranchs, but an XO system has been detected in several nerites (Thiriot-Quiévreux 2003). Among architaenioglossans, XY and ZW sex determination systems both occur in the freshwater snail genus *Viviparus* (Barsiene et al. 2000), and ZW has been reported in cyclophorid species (Kongim et al. 2006). Among non-architaenioglossans, an XO system has been reported in the pomatiopsid *Neotricula aperta*, the rissoid *Rissoa ventricosa*, and littorinid *Melarhaphe neritoides*. *Littorina saxatilis* has an XY system (Rolan-Alvarez et al. 1996), as do three species of neogastropods (*Fasciolaria lignaria*, *Pisania striata*, and *Pisania maculosa*), one carinariid (*Pterosoma planum*) and one hydroibiid (*Benedictia baiacalensis*) (Thiriot-Quiévreux 2003; Odierna et al. 2006). Another carinariid and four pterotracheids have a system in which males are determined by XY_1Y_2 (Thiriot-Quiévreux 2003). In each of these groups, there are also species for which sex chromosomes were not evident in karyotypes (Fig. 7.1).

Breeding experiments with the apple snail *Pomacea canaliculata* have shed light on the genetics of sex determination. The population sex ratio at hatching is not biased, but the sex ratio of individual broods is highly variable both in field-collected broods and in the lab (Yusa and Suzuki 2003). Food availability, temperature, and age do not affect sex ratio, but broods with heavier eggs tended to include more female offspring (Yusa 2004; Yusa and Suzuki 2003). The brood sex ratio is determined by genetic contributions from both parents but with a larger contribution from the mother (Yusa 2006, 2007a, b). Sex is multigenic and probably involves at least four genes (Yusa 2007a, b).

A heritable, sex-linked, microsatellite polymorphism has been demonstrated in *Busycon carica* and *B. canaliculatus* (Avise et al. 2004). This locus is heterozygous in females and hemizygous in males. Alleles are transmitted from mothers to both sons and daughters, but fathers only transmit alleles to their daughters. Using this genetic marker, Avise et al. (2004) demonstrated that embryonic sex ratios do not deviate from 50:50 despite the fact that adult sex ratios are often highly biased.

Overall these studies suggest that sex-determining systems in caenogastropods are evolutionarily flexible. This view is supported by the presence of different systems of sex chromosomes in species from the same genus (*Viviparus*) or the same family (Carinariidae), as well as the apparent multiple evolutionary origins of sex chromosomes in groups that generally lack them. In addition multigene sex determination, like that observed in *Pomacea canaliculata*, is hypothesized to occur during evolutionary transitions between different genetic systems of sex determination (Yusa 2007a). New data, covering a more taxonomically diverse set of species, would contribute significantly to a more complete picture of the evolution of sex-determining systems in gastropods.

7.7 Sexual Dimorphism

Sexual dimorphism is common in many dioecious animals and has been used to infer characteristics of mating systems in organisms for which direct observations are not feasible. As such it could be a useful tool for inferring evolutionary transitions in sex allocation or mating behavior in taxa for which we lack direct observations. Sexual size dimorphism can result from competition for mates (males are larger) or from fecundity advantage (females are larger). Other secondary sexual characteristics may be related directly to offspring production (e.g., hip morphology in human females), male-male conflicts (weapons), or female mate choice (male ornaments). When viewed in this context, sexual dimorphisms in caenogastropod shells appear to be primarily related to female fecundity or egg mass production, while radula dimorphisms are suggestive of weaponry.

Sexual size dimorphism in shell length is relatively straightforward to discover using preserved specimens and is widely documented in caenogastropods. In size-dimorphic caenogastropods, the female is almost invariably larger than the male. For example, in 14 out of 19 species of littorinids studied by Reid (1986), females were larger than males. In freshwater families like viviparids, hydrobiids, and ampullariids, larger female size is also common (Estebenet and Cazzaniga 1998; Jakubic 2006), as it is in cypraeids (Irie and Morimoto 2008; Katoh 1989), strombids (Ueno 1997), muricids (Son and Hughes 2000), nassariids (Avaca et al. 2013), and eulimids (Matsuda et al. 2013) as well as in the volutid *Voluta ebraea* (Matthews-Cascon et al. 2010), and the melongenid *Pugilina morio* (Matthews-Cascon et al. 1990). In *Assiminea japonica*, females are larger than males, but in the sympatric *Angustassiminea castanea*, the males are larger (Kurata and Kikuchi 2000). Males are also larger than females in the cypraeid *Umbilia hesitata* (Griffiths 1961). Detailed study of these species that do not fit the general pattern could be informative.

Dimorphism in shell shape has been detected using geometric morphometrics (Avaca et al. 2013; Minton and Wang 2011). In most cases the female shape produces a larger volume and therefore a larger space for increased female reproduction. For example, in *Viviparus subpurpureus*, *Littoraria variegata*, *Nucella lapillus*, and *Buccinanops globulosus*, females are more globose or stouter and have larger apertures than males (Son and Hughes 2000; Riascos and Guzman 2010; Minton and Wang 2011; Avaca et al. 2013). In the terrestrial

C. septemspirale, females are taller and have wider whorls than males, resulting in 50% more volume in the shell (Reichenbach et al. 2012). In *B. globulosus* and *C. septemspirale*, the sex of >80% of the snails can be distinguished using multivariate analysis of morphometric data, which could be useful for determining sex ratios of shells in museum collections (Reichenbach et al. 2012; Avaca et al. 2013). Female body size usually correlates with fecundity suggesting this dimorphism may be the result of fecundity selection (Erlandsson and Johannesson 1994; Collin 2000). However in some species, large females are also preferred by males (Erlandsson and Johannesson 1994; Zahradnik et al. 2008), suggesting that sexual selection could also play a role.

Sexual size dimorphism can develop in a variety of ways. Female littorinids grow more quickly than males (Johannesson et al. 1997; Riascos and Guzman 2010). In an apple snail and a eulimid, females grow more rapidly than males only after maturation (Estebenet and Cazzaniga 1998; Matsuda et al. 2013), and females of a cowrie grow for longer than males before adding the terminal shell lips (Irie and Morimoto 2008). Independent of growth, females may also allocate more energy to reproduction. For example, females of dimorphic *Strombus canarium* allocate more energy to body tissue and gonads compared to males, which allocate more energy to shell deposition (Cob et al. 2008). Reproduction can also cost more for females than for males. Compared to males and pre-spawning females, post-spawning *Buccinum undatum* females have lower digestive gland index, lower carbohydrate and protein content of the foot, decreased activity of glycolytic enzymes in the foot, a decrease in foot contortions associated with escape response, and a decreased ability to recover from escape exercise (Brokordt et al. 2003). This suggests that the significant metabolic demands of reproduction as a female may select for larger female size.

Sexual dimorphism in shell sculpture, when present, seems to be related to female egg deposition or capsule formation. In the *Olivella plata* (Olividae), the females have a wide, vertical groove adjacent to the parietal callus, which is not present in males or juveniles. This may be related to attaching egg capsules to the parietal callus (Borzone 1995; Pastorino 2007). Mature females of the turrids *Aforia circinata* and *Gemmula lordhoweensis* have a tertiary apertural notch that is not present in juveniles or males (Shimek 1984; Kantor and Sysoev 1991). The notch of *G. lordhoweensis* appears and disappears during the life of each female and could reflect distinct reproductive seasons or events (Kantor and Sysoev 1991). In reproductive female bursids in the genera *Crossata* and *Tutufa*, the normally digitate aperture margin is flared and circular (Beu 1998). This may be associated with the way brooding females hold the egg mass over the aperture (Beu 1998). In the nassariid *Buccinanops globulosus*, the shell callus is slender in males and immature females. In large females it is inflated, generating increased space for the attachment of egg capsules to the callus (Márquez and Averbuj 2016). In the vermetid *Serpulorbis arenarius*, the presence of a pallial slit, a feature thought to be related to the presence of brooded egg capsules, is significantly but not exclusively linked to females (Calvo and Templado 2005). In some cases the possible function of shell dimorphism is not clear. In a species of *Lambis*, female shells have a single high knob which is longer than the paired knobs of males. The spines of males are also shorter and flatter than those of females (Ueno 1997). It is tempting to imagine that these differences are related to male-male competition.

Radula teeth are sexually dimorphic in a number of groups. Male *Rapana venosa* have a wider tooth base and wider and taller central cusps on the rachidian than do females (Harding et al. 2008). A similar pattern in rachidian morphology has been reported for other muricids (Fujioka 1982, 1984). In the collumbellids *Euplica varians* and *E. versicolor*, the radula is dimorphic in adults (deMaintenon 2004). Females and juveniles of both species have a flat-tipped outer secondary lateral cusp, while adult males have a long pointed cusp. Adult males also have more tooth rows (deMaintenon 2004). In some cowries the radula is smaller but has more rows in females than in males (Schilder and Schilder 1961). In the strombid *Conomurex persicus*, the outer and inner marginal teeth of females have more cusps than do males (Mutlu 2004). In the buccinid *Pisania pusio*, the inner cusp of the lateral tooth is longer and thinner in males than the females (Matthews-Cascon et al. 2005). The function of these radula dimorphisms are unknown, but those where males differ from juveniles and adult females could be related to aggressive interactions during reproduction and courtship.

Sexual dimorphism has occasionally been reported in the color and the soft anatomy of caenogastropods. The dimorphic lateral projections on the propodium are sexually dimorphic in the tonnoidean *Ficus subintermedia* (Arakawa and Hayashi 1972; Fretter 1984). In cowries *Cypraea gracilis*, females are red, and males are brown (Griffiths 1961), and in *Monetaria annulus*, the golden ring on the shell is paler in females than in males (Schilder and Schilder 1961). Some species of the terrestrial *Cochlostoma* show differences in pigmentation between males and females (Gofas 2001). Despite reports of sexual dimorphism from many families of gastropods, no comprehensive studies have been undertaken to determine how sexual dimorphism varies among closely related species and how this relates to mating system.

7.8 Mating Behavior, Mate Choice, and Sexual Selection

Sexual selection may play an important role in evolutionary transitions between sexual systems as well as maintaining the stability of any system (reviewed in Leonard 2005, 2006, 2010). Aspects of sexual selection including pre-copulatory mate choice, sexual conflict over mating decisions, and postcopulatory choice have all been incorporated into theories in this field, but they have been poorly studied in caenogastropods. In many groups mating is difficult to observe under natural conditions, and observations are sorely lacking. However data from easily observed intertidal species and experiments with a few model species have shown that snails show male-male aggression, female choosiness, and sexual conflict over mating.

A number of caenogastropods form mating aggregations and deposit eggs communally. This is especially common in muricids (D'Asaro 1986). The black murex *Hexaplex nigritus* makes especially impressive aggregations, extending up to 900 m^2, weighing approximately 3 metric tons and including 5000 animals (Cudney-Bueno et al. 2008). The pink-mouthed murex *Phylonotus erythrostomus* aggregate into mountains of animals, as high as 2.5 m, all contributing to a common egg mass (Cudney-Bueno et al. 2008). Other caenogastropods, most notably strombids, also aggregate to mate, with densities of *Strombus luhuanus* reaching 20 m^{-2} in a mating aggregation of 200 animals (Catterall and Poiner 1983). Mating aggregations set the stage for sexual selection and mate choice.

Aggression and male-male conflicts have been reported in both aggregating and non-aggregating species. In *Echinolittorina malaccana* and *E. radiata*, males compete to occupy the copulation position. Larger contenders are more likely to succeed in ousting another male from this position on the female's shell (Ng et al. 2016). In *Strombus luhuanus* males also compete for copulations. Males approach a female and then fall behind and approach again until they finally copulate (Kuwamura et al. 1983). When another male approaches a mating pair, the original male moves the operculum up and down and waves the proboscis at the intruder. In *Strombus pugilis*, males guard their mates, sparing with approaching males and using their proboscis to jab at each other (Bradshaw-Hawkins and Sander 1981). The winner copulates with the female and takes up the guarding position, touching the outside of the female's shell lip (Bradshaw-Hawkins and Sander 1981). Males of the ovulid *Cyphoma gibbosum* fight by biting at each other repeatedly and rearing up on the foot and lunging at each other (Ghiselin and Wilson 1966).

Copulation may be risky or costly. Costs of copulation include increased likelihood of dislodgement by waves and increased predation risk in copulating pairs compared to individual littorinids (Koch et al. 2007; Johannesson et al. 2010). Mating snails may also risk damage by their partner. For example, copulating female *Strombus* species sometimes pull the male along by their penis, which may be torn or broken (Reed 1995). However, the most ubiquitous cost of copulation might be in terms of time. Copulation generally takes several hours in caenogastropods [e.g., 3–6 h in *Buccinum isaotakii* (Ilano et al. 2004), up to 20 h in *Pomacea canaliculata* (Burela and Martín 2011)].

Significant costs of mating may lead to sexual conflict. Sexual conflict appears likely in a number of caenogastropods, where females avoid or reject mating attempts. For example, female *Littorina* species generally produce mucus trails that can be distinguished from males' trails. Males follow these trails to find females (Erlandsson and Johannesson 1994; Ng and Williams 2015). In dense populations of *L. saxatilis*, females may avoid encounters with males by making trails that cannot be distinguished from those of males (Johannesson et al. 2010). Females of *L. melanostoma* actively reject mating attempts by bending their heads and extruding their snouts to push away the penis of male suitors (Ng and Williams 2015). Female apple snails rotate their shells and shake off 60% of courtship attempts before copulation, and another 30% of males attempting copulation are dislodged in this way (Burela and Martín 2009). *Neptunea arthritica* shows the most extreme sexual conflict over mating reported for any caenogastropod (Lombardo and Goshima 2011). Females routinely try to avoid mating by running away and resist males by biting the penis and foot (Miranda et al. 2008). In one study more than 80% of copulations were terminated by female aggression (Lombardo and Goshima 2010). Female resistance increases with the number of males she has previous copulated with, suggesting that the cost of multiple matings outweigh the benefits for females (Lombardo and Goshima 2011). After copulation, males guard the female and may bite or flip her when she tries to resume normal activity (Lombardo and Goshima 2011). In a related species, *Buccinum undutum* receptive females lie on their backs with their foot extended. This behavior has been observed in the lab where the male touches or strokes the female foot with his proboscis during their 2–6 h copulation (Martel et al. 1986).

Further evidence of active mate choice is provided by patterns of size preference. Male preference for larger females has been reported in *Viviparous ater* (Staub and Ribi 1995), *Buccinanops globulosus* (Avaca et al. 2012), and a number of littorinid spp. (e.g., Hollander et al. 2005; Erlandsson and Johannesson 1994; Johnson 1999) but has not been detected in golden ring cowries (Katoh 1989) or *Cerithidea rhizophorarum* (Ohtaki et al. 2001). In *Littoraria ardouiniana* male snails prefer larger females at two stages in the mating process; they prefer to follow the trails of larger females, and they copulate for longer with larger females (Ng and Williams 2014). A surprising male preference not related to female size in *Neptunea arthritica* is that males prefer previously mated females to virgins (Miranda et al. 2008).

7.9 Parental Care

Parental care is relevant to discussions of sexual systems, as it can significantly alter the relative investment in reproduction made by males and females. Protection of offspring is energetically expensive, and the costs are almost always incurred by females. Caenogastropods generally make elaborate capsules and often protect their broods. Among outgroup taxa, vetigastropods generally protect eggs with only a simple membrane and jelly coat; opisthobranchs produce gel masses with embedded eggs. Nerites produce blister-shaped egg capsules that are well-defended by calcospherites embedded in the dorsal wall (Kano and Fukumori 2010). Brood protection or brooding is very rare in all of these groups. In caenogastropods, material incorporated into capsule walls and other protective structures can represent up to 50% of the investment in a brood (Perron and Corpuz 1982). The process of egg laying and molding the capsules is also expensive, sometimes taking several days during which the females do not feed and during which they may be vulnerable to predation (Brokordt et al. 2003). In many caenogastropod species, parental care extends past this initial investment and ranges from egg guarding and external brooding to eu-vivipary. It is noteworthy that parental care occurs infrequently in basal gastropods (Lindberg and Dobberteen 1981) and is also limited among opisthobranchs and pulmonates (reviewed by Baur 1994).

Caenogastropods from soft-bottom habitats have evolved the strategy of attaching eggs to adult shells (Fig. 7.2). Female nassariids *Bullia melanoides* and *Buccinanops* spp. (Averbuj et al. 2014; Averbuj and Penchaszadeh 2010, 2016),

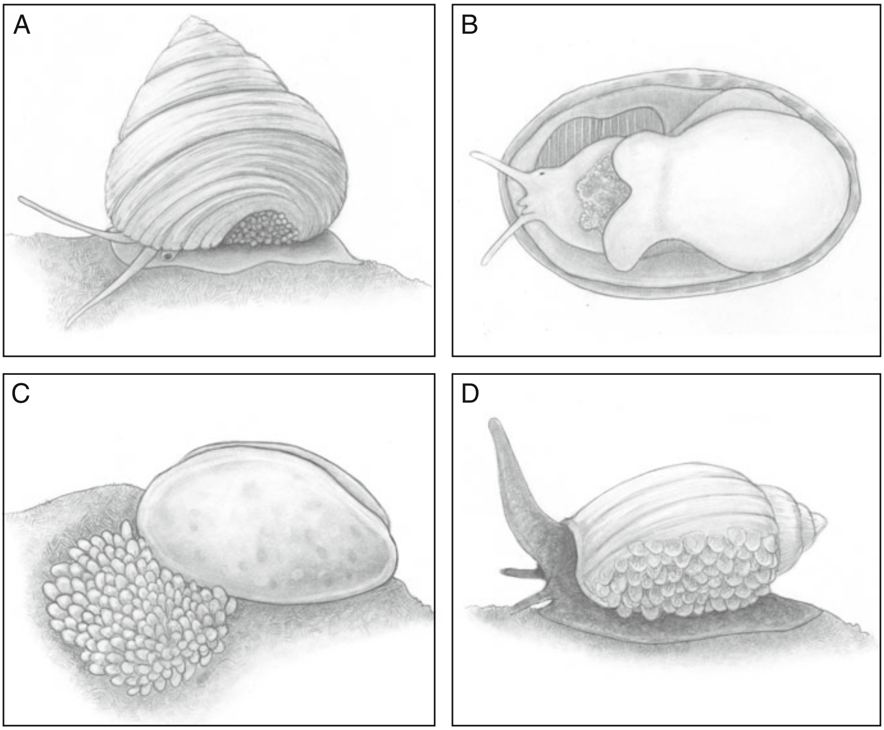

Fig. 7.2 Examples of maternal care in gastropods. (**a**) *Margarites vorticiferus*, a vetigastropod broods in the umbilicus of the shell (after Lindberg and Dobberteen 1981). (**b**) *Crepidula* species brood their transparent capsules between the propodium, the neck, and the substrate. (**c**) Cowries, *Cypraea* species, deposit compact masses of capsules that they guard by sitting on or near. (**d**) *Buccinanops* species lay their eggs on their own shells (after Averbuj and Penchaszadeh 2010)

collumbellids *Bifurcium bicanaliferum* and other species in the *Strombina*-group (Fortunato et al. 1998; Fortunato 2002), and the olivid *Olivella plata* (Pastorino 2007) deposit egg capsules on their own shells. The muricid *Hexaplex nigritus* and the hydroibid *Peringia ulvae* also carry egg capsules, but if they are their own is unclear (Thorson 1946; Cudney-Bueno et al. 2008). Female *Solenosteira macrospira* (Buccinidae) deposit capsules on their male partners (Kamel and Grosberg 2012). Such parental care has a significant cost. Experiments attaching capsules to both male and female *S. macrospira* demonstrate reduced growth in both sexes compared to controls without capsules (Kamel and Grosberg 2012).

In some families, females guard their benthic egg masses until they hatch (Fig. 7.2). In aquaria, females of *Buccinum isaotakii* guard their eggs until another female deposits on the mass (Ilano et al. 2004). *Fusitriton* spp. and other ranellids guard their eggs by sitting on or adjacent to the capsules (Gallardo et al. 2012; Beu 1998; Ramón 1991), as do female cowries (Ostergaard 1950; Wilson 1985; Osorio et al. 1999). Mother cowries may try to deter threats by pushing them away, by lifting the shell up and suddenly bringing it down to the substrate, by biting with the radula, or in one species flashing eyespots on the mantle (Ostergaard 1950). Female snails probably do not feed while guarding their eggs (Wilson 1985). Many mothers only leave the mass when the eggs have hatched, and one species of cowrie has been observed to assist hatching by chewing off the tops of the capsules (Katoh 1989).

In caenogastropods egg capsules can be brooded external to the body under the foot (e.g., calyptraeids; hipponicids) and in the mantle cavity (e.g., coralliophilids; vermetids). Eggs can also be brooded internally within the female reproductive ducts (littorinids; cerithioideans), in the gonad (*Janthina janthina*), in subhemocoelic pouches (cerithioideans, planaxids) and novel pouches in the pedal gland (a provannid; Reynolds et al. 2010), or in dorsal pouches behind the head which open via a pore in the sole of the foot (ovulids in the genus *Pedicularia*; Simone 2005). In general internally brooded embryos are lecithotrophic and are often encapsulated inside the brood pouch. Matrotrophic vivipary, where nutrients secreted from maternal epithelium are provided directly to the embryos, is uncommon and has been demonstrated unambiguously only in some thiarids (Glaubrecht 2006). However, the size difference between the 40–45 μm eggs and 250 μm hatchling Warén's larvae suggests that the hydrothermal vent gastropod *Ifremeria nautilei* may also be matrotrophic (Reynolds et al. 2010).

Brooding is phylogenetically conserved in some groups (e.g., all calyptraeids brood), but it is evolutionarily labile in others. In cerithioideans brooding has evolved multiple times, involving different morphological modifications in association with transitions to freshwater (Strong et al. 2011; Köhler et al. 2004; Strong and Glaubrecht 2002, 2007; Glaubrecht and Strong 2007; Glaubrecht et al. 2009). An increase in egg size, subsequent to the invasion of freshwater habitats, may be a preadaptation for the evolution of brooding in pachychilid (Köhler et al. 2004). *Potamopyrgus* (Tateiidae) species have evolved ovovivipary at least twice in parallel, both times preceded by a transition from marine to freshwater habitats (Haase 2005). It is interesting to note that the evolution of parthenogenesis is also associated with transitions to freshwater (Auld and Jarne 2016). In littorinids, another primarily oviparous group, ovovivipary, has evolved in at least three genera (*Littorina*, *Littoraria*, and *Tectarius*) but is not associated with any obvious shifts in habitat or lifestyle (Reid and Geller 1997).

7.10 Future Directions

It is customary to conclude by highlighting major unanswered questions that current circumstances suggest could be fruitfully addressed. The situation for caenogastropods defies this kind of summary. In no group is there such a broad diversity of sexual strategies spread across such a large number of families, studied by so few researchers. The rich diversity of caenogastropods and the large number of independent evolutionary transitions between different reproductive strategies mean that caenogastropods provide unique opportunities to study the complex interplay of sexual selection, reproductive anatomy, habitat, behavior, and sex allocation in the evolution of sexual systems. More basic data must be accumulated before caenogastropods can provide powerful tests of important evolutionary theories. Some areas ripe for study are:

- Many anatomical studies of caenogastropods are old and oft cited. Comparative morphology using modern methods, strategic taxon sampling, and phylogenetic interpretation could provide valuable new insights into homologies among structures and the changes in sexual anatomy that accompany transitions in sexual systems.
- Systems of sex determination are variable among caenogastropods. Strategic taxonomic sampling, inheritance studies, and next-generation sequencing could transform the way we view the evolution of sex determination and the mechanisms behind evolutionary transitions from genetic dioecy to environmental sex determination.
- Caenogastropods exhibit diverse mating behaviors with unexplored impacts on sex allocation and reproductive success. Behavioral studies are needed to obtain even a preliminary understanding of the prevalence and importance of sexual selection, cryptic female choice, and copulatory courtship in gastropod reproduction.
- Reproduction is costly, yet fewer than a handful of studies have examined the costs of reproduction over and above the direct investment in the gonads or gametes. Comparisons of the costs of egg guarding, brooding, and the production of protective structures will provide important insights into sex allocation.

Switches among diverse strategies occur in caenogastropods at population, species, genus, or family levels. Therefore sampling must be dense, and phylogenies resolved at the relevant taxonomic scales. If this can be done, caenogastropods offer an unparalleled opportunity to understand evolutionary transitions between dioecy and hermaphroditism, the origins of maternal care, and the molecular evolution of sex determination.

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Chapter 8
Hermaphrodites, Dwarf Males, and Females: Evolutionary Transitions of Sexual Systems in Barnacles

Yoichi Yusa

Abstract Ever since Darwin (A monograph on the sub-class Cirripedia. The Ray Society, 1851), the diverse array of sexual systems in barnacles and rich transitions between them have attracted researchers. Here, I review recent theoretical and empirical studies on barnacle sexuality. First, the distribution of sexual systems among barnacles (in the broadest sense) is explained. Next, I introduce the concept of "the barnacle prototype" to explain the pattern of sexual systems and their transitions. Sex allocation theory and its extensions follow, and resource allocation model is explained to integrate sex allocation and life history models. The empirical evidence, both from phylogenetic comparisons and intraspecific studies, is reviewed. Lastly, I summarize knowns and unknowns about barnacle sexuality. Most likely, barnacles will provide topics for further study on the diversity and unifying theory of sexual systems.

8.1 Introduction

Darwin (1851) was the first to find diverse sexual systems in barnacles. He found, after 8 years of intensive scrutiny, that barnacles are basically simultaneous hermaphrodites but that there are tiny (dwarf) males attached to a restricted area of large hermaphrodites in several species (e.g., *Scalpellum scalpellum*) (Fig. 8.1). The coexistence of males and hermaphrodites within a population is termed androdioecy (Charlesworth 1984). Still, in other species (e.g., *Ibla cumingi*), Darwin found dwarf males attached to pure females rather than hermaphrodites, i.e., their sexual system is dioecy (or gonochorism; separate sexes).

Such diverse sexual systems in barnacles fascinated Darwin. In a letter to his botanist friend J. D. Hooker, he even noted a plausible scenario for the evolutionary transition from hermaphroditism to dioecy. "I never shd have made this [i.e., finding of dwarf males in barnacles] out, had not my species theory convinced me, that an hermaphrodite species must pass into a bisexual species by insensibly small stages,

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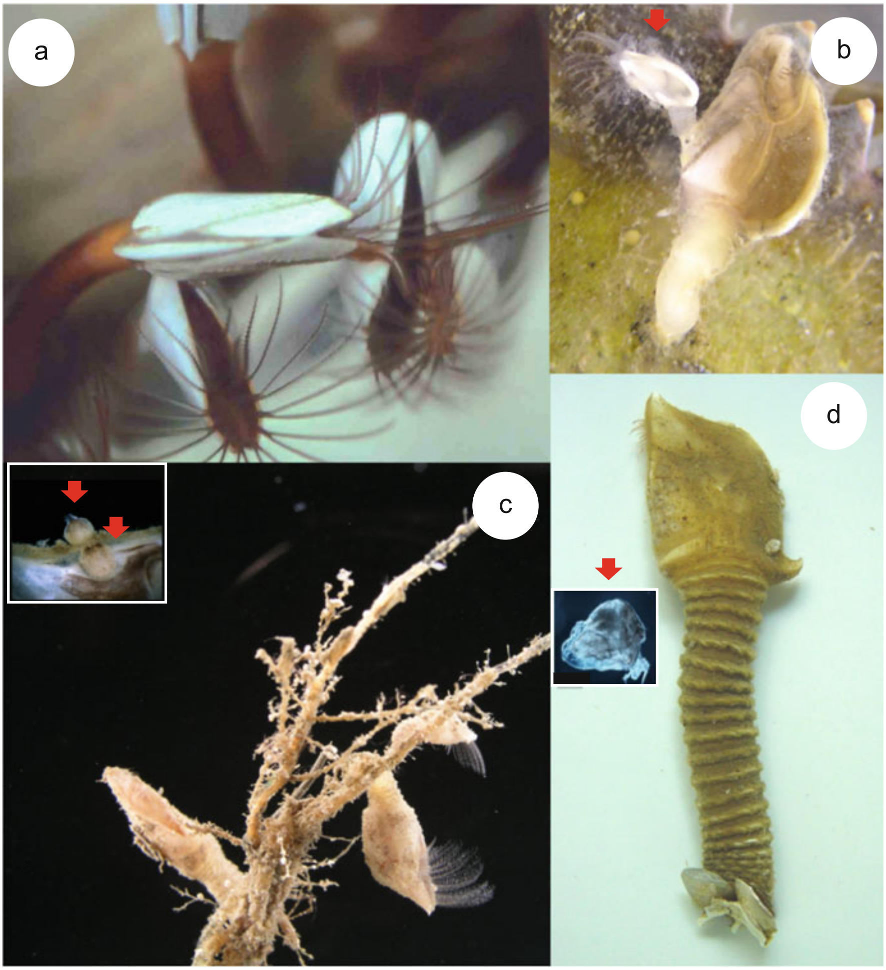

Fig. 8.1 Various barnacle species with different sexual systems. (**a**) *Lepas anserifera* (20–50 mm hermaphrodites), (**b**) *Octolasmis warwickii* (a 15 mm hermaphrodite with a 5 mm dwarf male), (**c**) *Scalpellum scalpellum* (30–50 mm hermaphrodites with 0.3–0.4 mm males), and (**d**) *S. stearnsii* (a 150 mm female with a 0.4 mm male). Dwarf males are indicated by red arrows. Photos by M. Ishimura (**b**) and J. T. Høeg (**c**)

and here we have it, for the male organs in the hermaphrodite are beginning to fail, and independent males ready formed" (note added by Yusa; Darwin Correspondence Project 2016, see also Høeg et al. 2015). He believed that the sexual transitions were shaped by his "species theory," i.e., evolution by natural (perhaps including sexual) selection. However, as was often the case (e.g., for the equal sex ratio; see West 2009), he was too deliberate to express his speculation about the ultimate cause of the sexual system diversity in barnacles. In this respect, he only wrote "Regarding the final cause, both of the simpler case of the separation of the sexes,... and of the much more singular fact of the existence of Complemental[1] males, I can throw no light" (P. 291 in Darwin 1851).

Much later, some cirripedologists, including Newman (1980), Crisp (1983), and Høeg (1995a), noted plausible scenarios for the evolution of sexual systems. They all believed that the ancestor of barnacles was hermaphroditic and that both dwarf males and pure females evolved from hermaphrodites. Above all, our understanding of the transitions between sexual systems in barnacles was greatly advanced by Charnov (1982, 1987). He formalized the problem from a modern evolutionary perspective and made the first theoretical model for the evolution of dwarf males in hermaphrodites, based on his sex allocation theory (Charnov 1982; West 2009). The basic idea is very simple: organisms should optimize resource allocation to male and female functions to maximize fitness.

So, if you are interested in the diversity of sexual systems in animals and want to study it, barnacles are one of the choices in terms of both rich transitions and tradition. Barnacles are sessile animals that live on various types of substrata (such as rocks, driftwood, living animals, and algae) from shallow (intertidal or neustonic) to deep-sea habitats (Anderson 1994). Their sessile nature makes it easy to record the distribution of individuals over time if necessary. Moreover, the rich fossil record facilitates reconstruction of the long-term morphological, and to some extent ecological, changes (e.g., Gale 2016).

In this chapter, I review recent theoretical and empirical studies on barnacle sexuality. One goal is to solicit researchers to study the sexuality of barnacles. Another goal is to build a general framework for studying the evolution of sexual systems using barnacles as model organisms. First, let us see how sexual systems are distributed among barnacles. Next, I will describe how to view the transitions by explaining "the barnacle prototype." Then, we will explore how to approach the issues by using sex allocation theory and its extensions. The resource allocation model is explained in the hope of integrating sex allocation and life history models. The empirical evidence for and against the theories follows. Lastly, in the Discussion, I will review what we know and what we do not know about barnacle sexuality, with implications for the evolution of sexual systems in general. Further details of barnacle general biology can be found in Anderson (1994) and Høeg et al. (2015). The sexuality of barnacles has been reviewed by Høeg (1995a; on barnacles in the broadest sense, in the subclass Thecostraca), Yamaguchi et al. (2012; on recent theoretical advancement), and Yusa et al. (2013, on the diversity of sexual systems among barnacles).

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[1] Note: Although Darwin himself gave different terminology for males on hermaphrodites ("complemental males") and those on females ("dwarf males"), in this chapter, I call both types "dwarf males" following recent literature (Høeg 1995a; Yusa et al. 2012, 2013) because these males are often morphologically indistinguishable and the males on hermaphrodites exist not to "complement" the reproduction of hermaphrodites but to maximize the males' own fitness.

8.2 Phylogenetic Character Distribution

Figure 8.2a shows the distribution of sexual systems and the mode of sex determination in the subclass Thecostraca. At the base of Thecostraca, an enigmatic taxon called Facetotecta branched off, the adult forms of which are probably endoparasites of yet unknown hosts (Glenner et al. 2008). We know nothing about their sexuality. Next, the Ascothoracida is a small group (with approximately 100 species), with the majority of individuals being dioecious (females + dwarf males) (Grygier and Høeg 2005). At least one species has sexually dimorphic larvae, suggesting genetic sex determination (Høeg et al. 2009). However, simultaneous hermaphroditism has evolved secondarily in the family Petrarcidae (Ascothoracida). We know so little about the sexual systems of ascothoracidans that the cause of the transition between different sexual systems is unknown.

Cirripedia is the sister taxon to Ascothoracida and contains Acrothoracica, Rhizocephala, and Thoracica. The burrowing barnacles, the acrothoracicans, are mostly symbiotic, living inside burrows excavated in calcareous substrata, including molluscan shells, corals, and calcareous rocks (Chan et al. 2014). They are all dioecious, with large females and dwarf males (Høeg 1995a). Although little is known about their reproduction, Gotelli and Spivey (1992) suggested that there is competition between dwarf males judging from their attachment patterns.

The rhizocephalans contain approximately 250 species and are all highly specialized parasites on crustaceans (Høeg and Lützen 1995; Walker 2001; Høeg et al. 2005). They are all dioecious (females with dwarf males). They include at least one group (Kentrogonida) with genetic sex determination and larval sexual dimorphism (Yanagimachi 1961) and another group (Akentrogonida) with possibly environmental sex determination and without larval dimorphism (Høeg and Lützen 1995). A recent molecular phylogenetic study has shown that Akentrogonida is a monophyletic group nested within paraphyletic Kentrogonida (Glenner et al. 2010), indicating that environmental sex determination might have evolved from the ancestral state of genetic sex determination. Yamaguchi et al. (2014) constructed a theoretical model and suggested that the mode of competition among dwarf males, as determined by attachment site restrictions, ultimately determines the mode of sex determination.

The true barnacles, the thoracicans, contain many (approximately 800) species (Foster and Buckeridge 1987) and a diverse array of sexual systems (Darwin 1851; Charnov 1987; Høeg 1995a; Yusa et al. 2013). They comprise the paraphyletic taxon Pedunculata (or goose barnacles) and the monophyletic taxon Sessilia (mainly Balanomorpha, the acorn barnacles), which is nested within the pedunculates (Pérez-Losada et al. 2008; Lin et al. 2015). Hermaphroditism, androdioecy, and dioecy are all known in the pedunculates, whereas dioecy is unknown in the balanomorphs. In this chapter, I only discuss thoracicans in detail, although sometimes I refer to other taxa when necessary. Please consult Charnov (1987) and Høeg (1995a) for the sexual systems of Thecostraca and Høeg (1995b) and Høeg and Lützen (1995) for those of Rhizocephala in particular.

The distribution of sexual systems among Thoracica was thoroughly studied by Yusa et al. (2012) and Lin et al. (2015). These studies suggest that the ancestral barnacle (at least after the *Ibla* lineage branched off) was hermaphroditic and that males, and to a lesser extent, females, have evolved several times in the thoracican phylogeny (Fig. 8.2b). *Ibla* contains both dioecious and androdioecious species; thus, their ancestral sexual system cannot be definitively determined.

In thoracicans, the size of males differs greatly among species (Fig. 8.1). In some species such as the androdioecious species *Scalpellum scalpellum*, the males cannot feed and must rely totally on resources given from the mother. In fact, such males are "rudimentary to a degree, which I believe can hardly be equaled in the whole animal kingdom; they may, in fact, be said to exist as mere bags of spermatozoa" (p. 23 in Darwin 1851). A related dioecious species, *S. stearnsii*, probably represents one of the largest sexual size dimorphisms in animals. The males are less than 0.5 mm in length, whereas the females can reach 150 mm, showing a >300 times difference in length (likely >1 million times in volume) (Ozaki et al. 2008). In contrast, in other species such as the androdioecious *Octolasmis warwickii*, the males are virtually small hermaphrodites that mature as male much earlier and retard female development (Yusa et al. 2010). Such males have functional cirri and can actually grow (e.g., Wijayanti and Yusa 2016). Among Thoracica, we can find any intermediate state between these two extremes (Yusa et al. 2013).

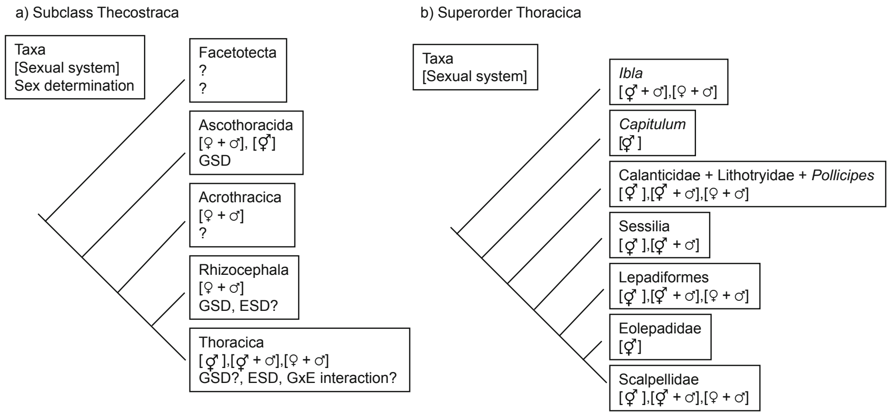

Fig. 8.2 Phylogenetic distribution of sexual systems and mode of sex determination in the subclass Thecostraca (**a**) and in the superorder Thoracica (**b**). The signs ♂, ♀, and ⚥ indicate male, female, and hermaphrodite, respectively. *GSD* genetic sex determination, *ESD* environmental sex determination. Phylogeny by Pérez-Losada et al. (2008) and Lin et al. (2015), and sexual systems mainly by Yusa et al. (2012) and Lin et al. (2015) (see text for details)

8.3 The Barnacle Prototype

Figure 8.3a shows a hypothetical model organism, here termed “the barnacle prototype,” that illustrates how to consider the transitions among sexual systems in barnacles (Yusa et al. 2013). Each individual allocates resources to growth, male function, and female function, and the allocation pattern changes with time. The prototype first allocates all resources to growth. Next, it starts to allocate some resources to male function and then to female function simultaneously. Thus, it is a simultaneous hermaphrodite with a tendency for male function to mature earlier than female function (i.e., a protandric simultaneous hermaphrodite, or PSH). Many extant barnacles have this sexual expression (Newman 1980; Crisp 1983; Kelly and Sanford 2010; Inatsuchi et al. 2010), and this is the sexual expression theoretically predicted for typical barnacles (Charnov 1987; Yamaguchi et al. 2013a). In addition, PSHs are common in simultaneously hermaphroditic animals (Chaine and Angeloni 2005; Vizoso and Schärer 2007; Schärer 2009; Leonard 2013).

Using the idea of the barnacle prototype, the diversity in the sexual systems of barnacles can be attributed to (1) changes in each individual’s temporal allocation of resources to growth, male function, and female function, resulting in dwarf males and females and (2) the presence or absence of the coexistence of more than one type of allocation pattern within a population, resulting in androdioecy and dioecy.

The transition from PSH to dwarf male is achieved by earlier maturation as male and more resource allocation to male function instead of growth and female function (Fig. 8.3b). The extent to which resources are allocated to growth (G) differs among species, which explains the variation in the relative size of males on the continuum from *Scalpellum scalpellum* (sperm bags; $G = 0$) to *Octolasmis warwickii* (miniature hermaphrodites; $G \gg 0$). Although some “males” may later become hermaphrodites, they can be distinguished from typical hermaphrodites because they are attached to a conspecific hermaphrodite and cannot change their position (Yusa et al. 2010, 2013; but see Kugele and Yule (2000) for the possibility that barnacles gradually change locations). In addition, the life history of males differs from that of conspecific hermaphrodites in that the males mature at a smaller size, remain smaller, and are much less likely to have female function than hermaphrodites (Crisp 1983; Yusa et al. 2010).

The transition from PSH to female is fulfilled by retarding male maturation and allocating more resources to female function instead of male function, to the extent that virtually no resources are allocated to male function (Fig. 8.3c). Female barnacles do not tend to be smaller than hermaphrodites of closely related species (e.g., *Scalpellum stearnsii* females are even larger than *S. scalpellum* hermaphrodites). This suggests that the allocation to growth function does not differ much between female and hermaphrodite life histories.

Transitions in sexual systems can also be described using this resource allocation pattern. The transition from hermaphroditism to androdioecy indicates the invasion of individuals who stress male allocation in the hermaphrodite population. The transition from androdioecy to dioecy indicates the abortion of male function in hermaphrodites.

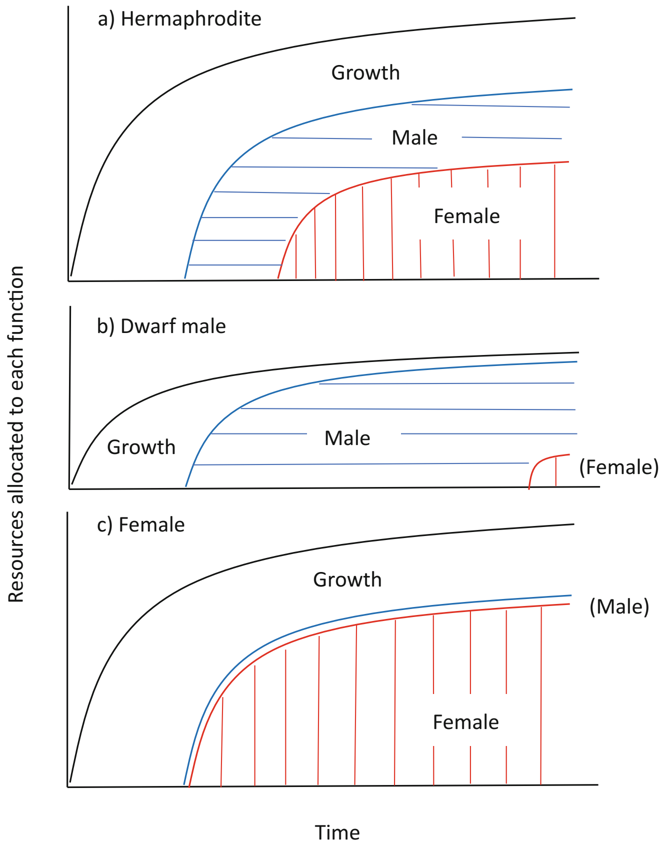

Fig. 8.3 Resource allocation pattern of (**a**) the typical barnacle, called the barnacle prototype, (**b**) the dwarf male, and (**c**) the female. Note that the resources allocated to each function (growth, male, and female) are shown as the area between two curves here. Modified from Yusa et al. (2013)

In the broadest sense, the barnacle prototype is only a schematic representation showing that all barnacles (and, in fact, all organisms) potentially allocate resources to growth, male function, and female function over time to maximize fitness (the resource allocation model; see below). In a narrower sense, the prototype insists that in the case of barnacles, male function typically matures earlier than female function as predicted by theory (Charnov 1987; Yamaguchi et al. 2013a). Therefore, such an allocation pattern supplying resources to growth, male function, and female function should have been shaped by natural and sexual selection. Thus, the questions to be asked here are (1) why the time-dependent allocation pattern is adaptive and why individuals with different allocation patterns can coexist (i.e., the adaptive significance of sexual expression and the sexual system, respectively), and (2) how they are maintained in barnacles (mechanism). In the next section, I will address the "why" questions. The "how" question will be briefly reviewed in Sect. 8.7.

8.4 Sex Allocation Theory

Charnov (1980, 1982) first formalized the optimal resource allocation to male and female functions (sex allocation). It is worth noting that he used barnacles as a model for hermaphrodites. The basic idea is simple, in which the optimal sex allocation is the one that maximizes the fitness of the individual in a given environment. First, let us consider the relationship between resource input to female function (mainly egg production and, if present, maternal care) and fitness in a hypothetical animal (Fig. 8.4a). This relationship is linear if the number of offspring produced is proportional to the resource input into egg production, as is the case in most invertebrates with little or no maternal care. In the case of brooders, the number of offspring may not increase linearly with resource input (i.e., diminishing returns) due to limitations in brooding space (Heath 1979) or sperm supply (Henshaw et al. 2014). In contrast, the cost of maternal care, such as ventilation and protection from predators or diseases, may become less costly per egg as the number of eggs increases, making the fitness curve increase exponentially (increasing returns). On balance, the female fitness curve may show either diminishing returns, increasing returns, or be nearly linear. Although they are brooders, empirical studies on barnacles showed that the relationship between body size and fecundity is nearly linear in them (Hines 1978; Zann and Harker 1978; Yusa et al. 2001; Ozaki et al. 2008), and let us assume here the linear relationship for the sake of simplicity.

Next, we consider the relationship between resource input to male function (sperm production, male copulatory organ, and secondary male characteristics) and fitness. The shape of the male fitness curve differs depending on the situation. For instance, if mating occurs within a group with a limited number of individuals, as

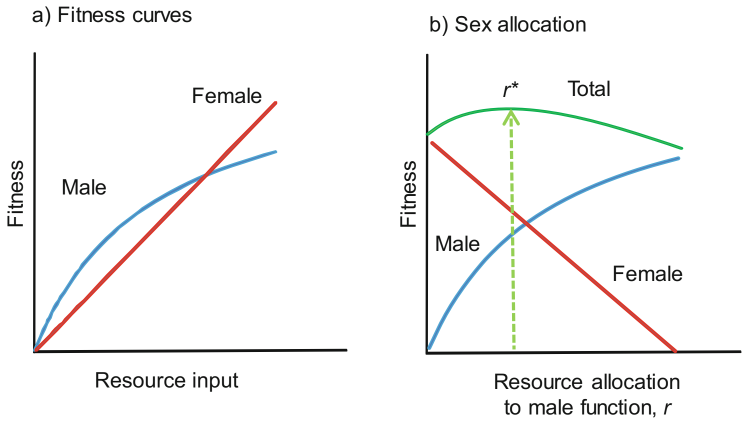

Fig. 8.4 Sex allocation theory. (**a**) Male and female fitness curves as a function of resource input, (**b**) fitness curves as a function of the proportion of resource input allocated to male function (sex allocation, *r*). In (**b**), the optimal sex allocation (r^*) is shown as the point with the total maximum fitness in both male and female functions. Modified from Charnov (1982)

expected in sedentary animals such as barnacles, male fitness does not increase linearly with resource input (Fig. 8.4a). This is because the number of eggs a male potentially fertilizes is limited by the number of eggs that the neighboring individuals possess.

Then, we convert the x-axis to the proportion of resources allocated to male function relative to the total allocation to male and female functions (r, which changes from 0 to 1) (Fig. 8.4b). The optimal allocation, r^*, is the point that maximizes $m \times f$, where m is the fitness through male function and f is the fitness through female function (Charnov 1982). If the female fitness curve is linear and the male curve shows a pattern of diminishing returns, as we supposed for barnacles, then the total fitness through male and female functions is maximized at a point between 0 and 1. This indicates that it should allocate resources to both male and female functions (i.e., a simultaneous hermaphrodite). A similar conclusion can be derived when the female curve saturates (Henshaw et al. 2014).

Thus, sex allocation theory predicts that sedentary animals that form small mating groups should become simultaneous hermaphrodites. Barnacles are sedentary animals with functionally internal fertilization (although the male-acting individual deposits the sperm in the mantle cavity—outside the body—of the female-acting individual) (Anderson 1994). Therefore, the mating partners of an individual are those within the reach of its penis (except for so-called sperm casting; Barazandeh et al. 2013, 2014). Note that the shape of the male fitness curve is determined by the size of the mating group. Small mating groups cause the male fitness curve to saturate earlier, and large groups make the curve straighter. Charnov (1982) derived the following equation for the optimal allocation to male function:

$$r^* = (K - 1)/(2K - 1),$$

where K is the number of individuals in the mating group except for the focal individual (thus, $K + 1$ is the mating group size).

Why, then, have dwarf males and females evolved? First, let us consider the evolution of dwarf males. If the mating group is large, the optimal allocation to male function (r^*) is large, which indicates that many hermaphrodites each produce a large amount of sperm. In this case, a mutant small individual that specializes in male function cannot invade the population with hermaphrodites due to intense sperm competition among large hermaphrodites. However, if the mating group is small, so is r^*, and sperm competition among hermaphrodites is less intense. Then, a small male may be able to compete with large hermaphrodites and invade the population (Charnov 1987). Of course, the actual number of offspring a dwarf male produces is smaller than the number produced by a hermaphrodite (especially because the latter can pass its genes through both male and female functions). However, dwarf males are likely to have advantages over hermaphrodites in that (1) they are more likely to survive to maturity due to their small size and likely shorter time to maturity (Newman 1980; Crisp 1983; Ewers-Saucedo et al. 2015) and (2) they may have better access to eggs to fertilize due to proximity (Gotelli and Spivey 1992; Urano et al. 2009).

The evolution of females from hermaphrodites is expected in even smaller mating groups. Consider the extreme case in which there is only one large individual and several dwarf males attached to it. The large individual has no one to fertilize, so there is no value in keeping male function, and it should eschew the male function and allocate the resources to female function ($r^* = 0$), i.e., it should be a female (Charnov 1987). Moreover, a theoretical model shows that hermaphrodites may discard the male function even if there is another hermaphrodite in the group because they can "lose" against dwarf males in sperm competition (Yamaguchi et al. 2013a). In short, sex allocation theory predicts that the size of the mating group determines the diversity of sexual systems in barnacles: simultaneous hermaphrodites in relatively large groups, hermaphrodites with dwarf males in smaller groups, and females with dwarf males in even smaller groups.

Charnov's sex allocation theory was later modified to incorporate factors important for barnacle life histories, such as male growth (Yamaguchi et al. 2007), food availability and settlement (Yamaguchi et al. 2008), and relative advantage of dwarf males over hermaphrodites (Urano et al. 2009) (reviewed in Yamaguchi et al. 2012; Chap. 9).

Although the abovementioned models postulate that the fitness of hermaphrodites and dwarf males is equal, Yamaguchi et al. (2013b, c) considered models with unequal fitness (Chap. 9). Although their average fitness is lower than that of hermaphrodites, dwarf males can evolve if (1) the substratum is ephemeral and thus it is better for later-coming larvae to become dwarf males than simply die while immature and (2) the proportion of individuals choosing one sex type is limited due to a scarcity of substratum. For the latter, some barnacles live only in highly specialized habitats; an example is *Koleolepas avis*, which lives underneath the pedal of certain sea anemones symbiotic with hermit crabs (Yusa et al. 2001). These theoretical models are important in showing that androdioecy evolves even when the fitness of dwarf males is lower than that of hermaphrodites (and *vice versa*). In such cases, dwarf males and hermaphrodites may be regarded as alternative tactics within a conditional strategy, with the tactic that an individual adopts depending on its own environmental or physiological conditions (Gross 1996; Leonard 2013; Yamaguchi et al. 2013b, c). If so, the coexistence of two sex types might be more widespread than equal fitness models suggest.

8.5 Resource Allocation Theory

The success of sex allocation models partly lies in their simplification in considering the optimal resource allocation only to male and female functions. However, in the actual world, organisms allocate resources to growth as well as to male and female functions (ignoring resources for living, such as metabolism, defense, and food searching). Therefore, a natural extension of sex allocation theory is to include resource allocation to growth, as we have observed in the barnacle prototype. Because growth affects fitness by altering future reproductive value (Roff 1992), this extension will inevitably introduce the time axis. Thus, we need to consider the optimal allocation to growth, male function, and female function over time. This is an attempt to combine sex allocation theory and life history theories (Zhang and Wang 1994; Yamaguchi et al. 2013a).

Kakehashi and Harada (1987) considered time-dependent shifts in fitness curves in explaining sex change and other sexual expressions in plants. Although this model does not incorporate allocation to growth, it introduces the time axis to sex allocation theory. Later, in barnacles, Yamaguchi et al. (2013a) developed a model that simultaneously incorporated the proportion of dwarf males and resource allocation (to growth, male function, and female function) of hermaphrodites. In their model, all hermaphrodites become PSHs, as is usual for barnacles (Newman 1980; Crisp 1983; Kelly and Sanford 2010; Inatsuchi et al. 2010). However, in their model, the dwarf male pathway and the hermaphrodite pathway are fixed in advance, and the males are not allowed to grow. No formal model treating the temporal changes in resource allocation to growth, male function, and female function to explain various sexual systems, including PSH, androdioecy, and dioecy, has been developed.

8.6 Interspecific Empirical Studies

Both phylogenetic interspecific comparisons and intraspecific experimental/observational studies exist as empirical studies on sexual systems and sex allocation in barnacles. The phylogenetic studies address the evolutionary patterns and causes of sexual systems. For instance, Kelly and Sanford (2010) posed questions about the evolutionary reasons for the diverse sexual systems in barnacles using modern phylogeny. This line was further promoted by Yusa et al. (2012), who showed that different sexual systems in barnacles can be considered to be largely adaptive in pedunculate barnacles. As we have seen, sex allocation models predict that sexual systems in barnacles will change from hermaphroditism to androdioecy and to dioecy as the size of the mating group decreases. As predicted, the evolutionary transitions from hermaphroditism to androdioecy and from androdioecy to dioecy are both linked to reductions in mating group size. Lin et al. (2015) further tested the evolution of dwarf males in the entire thoracican phylogeny (including both Pedunculata and Sessilia) by making inferences from the DNA sequences of multiple genes. Their study also supported the prediction that dwarf males have evolved in deep-sea species, which tend to have small mating groups. These results suggest that transitions between sexual systems are relatively labile in barnacles on an evolutionary timescale. The complete lack of dioecy in Sessilia suggests the presence of a phylogenetic constraint, although this may also be explained by generally larger mating group sizes in sessilian barnacles than pedunculate barnacles (Chan and Høeg 2015).

Therefore, the pattern of sexual system evolution in the thoracican lineage overall agrees well with the theoretical expectation that mating group size is a determinant of sexual systems. However, mating group size is linked to habitat, and these are difficult to disentangle from each other. For instance, intertidal and neustonic barnacles usually form large groups, whereas epibiotic or deep-sea species tend to form smaller groups. A notable exception to this association is the deep-sea pedunculates of the family Eolepadidae, which inhabit hydrothermal vents. They often form large groups on the vents, and, as predicted, they are all hermaphroditic (Yusa et al. 2012). However, a formal statistical model incorporating phylogenetic information is needed to disentangle the effects of group size and other environmental factors on sexual systems in barnacles.

Although it is logically possible to study sex allocation of hermaphrodites using interspecific comparison, no such study exists in barnacles. This is probably because of our common (but untested) recognition that sex allocation can change in evolutionarily shorter time periods than speciation events.

8.7 Intraspecific Studies on Sexual Systems

The association between mating group size and sexual systems can be studied within androdioecious species. However, intraspecific studies on barnacles have not provided support for the prediction that small mating groups are linked to the presence of dwarf males or females. For instance, Spremberg et al. (2012) showed in a field population of *S. scalpellum* that the proportion of hermaphrodites with males does not differ between solitary (81%) and gregarious (62%) hermaphrodites (nor does the number of males per hermaphrodite). However, they studied only 52 individuals, and a larger data set is needed to draw a firm conclusion.

In agreement with the prediction from sex allocation theory, dwarf males were found in deep-sea or symbiotic species with small mating groups that have been considered to be purely hermaphroditic. For instance, Crisp (1983) found “aperture males” in the balanomorph *Chelonibia patula* (synonymized to *C. testudinaria*; Cheang et al. 2013), which is epibiotic on sea turtles, crabs, and horseshoe crabs (Ewers-Saucedo et al. 2015). The aperture males are small individuals who attach near the aperture of large hermaphrodites and develop relatively larger penises at much smaller body sizes than conspecific hermaphrodites. Because a small proportion of these “males” later become hermaphroditic, Crisp determined that these are basically hermaphrodites that develop male function much earlier than normal hermaphrodites do in response to their attachment site. Similar examples of such “conditional” dwarf males were later found in the pedunculates *Octolasmis warwickii* (Yusa et al. 2010), *O. unguisiformis* (Sawada et al. 2015), and *Alepas pacifica* (Yusa et al. 2015).

Some experimental studies have attempted to demonstrate phenotypic plasticity in sexual expression. Høeg et al. (2016) conducted a transplanting experiment in the pedunculate *Scalpellum scalpellum*. They showed that newly metamorphosed juveniles that settle on the receptacles of hermaphrodites (i.e., future males) can become hermaphrodites when they are removed from the receptacles within 8 h of settlement. In contrast, no juveniles became hermaphrodites, and all became males if they had been in the receptacles of hermaphrodites for 24 h or longer. Likewise, Wijayanti and Yusa (2016) conducted bi-directional transplanting in *Octolasmis warwickii*. Irrespective of the original attachment site (crabs for future hermaphrodites and the shell plate of conspecifics for future dwarf males), the individuals transplanted onto the conspecifics developed longer penises but grew less than those transplanted onto an artificial substratum (plastic sticks). However, the effect of the original site on penis length was also determined. These results indicate that the male and hermaphroditic sexual expressions are at least partially environmentally affected by the attachment site.

These studies inevitably address the issue of sex determination. Sex determination is defined here as the manner in which the different sex types are determined (males and females in dioecious species and males and hermaphrodites in androdioecious species). Strangely, there have been no studies investigating the mode of sex determination in dioecious thoracican barnacles. As explained, sex determination in the rhizocephalans is genetic in kentrogonids and may be environmental in akentrogonids (Høeg and Lützen 1995).

In the androdioecious thoracicans, genetic sex determination was suggested in the balanomorph *Conopea galeata* (Gomez 1975), and environmental sex determination was suggested in *Scalpellum scalpellum* (Callan 1941). In the same *S. scalpellum*, Svane (1986) performed settlement experiments with varying numbers of young hermaphrodites as settlement sites for potential dwarf males. He found that the proportion of individuals who became dwarf males did not exceed 0.5 under any conditions. Based on this, he suggested that half of the larvae are destined to become hermaphrodites but the remaining half can choose between becoming dwarf males and hermaphrodites based on the settlement site. Svane's hypothesis has been tested, although not thoroughly, in Spremberg et al. (2012) and Høeg et al. (2016). These studies gave some support for his hypothesis in the sense that there is an environmental factor and probably a genetic factor in sex determination, although a direct test of his hypothesis is needed.

8.8 Intraspecific Studies on Sex Allocation

Raimondi and Martin (1991) tested adaptive sex allocation in hermaphroditic barnacles. Using the balanomorph *Catomerus polymerus*, they demonstrated that allocation to male and female functions (measured as the dry weights of testis + seminal vesicles and of the egg mass, respectively) differs between individuals in small and larger group sizes in a predicted way (with a greater allocation to male function in larger mating groups). However, similar studies in *Tetraclita rubescens* by Kelly and Sanford (2010) and Kelly et al. (2012) and in *Semibalanus balanoides* and *Balanus glandula* by Hoch and Levinton (2012) all failed to support the prediction from sex allocation theory.

These contradictory results may in part stem from species difference, but confounding factors should also be considered. First, the actual mating group size is smaller than the observed size if individuals mate as female only once or a few times (Schärer 2009). Both Kelly et al. (2012) and Ewers-Saucedo et al. (2016) used microsatellite markers for paternity analysis and showed that the actual number of fathers of a brood is much smaller than the apparent group size. However, in the pedunculate *Pollicipes elegans*, a high level of multiple paternity (up to five fathers) was confirmed, and the number of fathers increased with density (Plough et al. 2014). Second, a distinction between “fixed” and “variable” costs should be made in considering allocation (Schärer 2009; Hoch and Levinton 2012). The fixed costs are those that are not immediately consumed in each mating but are necessary for mating, such as the penis in the case of barnacles. The variable costs are those used in each mating event, such as gametes. The penis is in fact not “fixed” in barnacles, being subject to change depending on environmental factors such as wave action (Hoch 2008, 2009; Neufeld and Palmer 2008) and, more importantly, individual density. Because some balanomorph barnacles develop longer penises at low densities than high densities (Hoch 2008; Neufeld 2011), incorporating penis size into male resource allocation may partly offset the tendency toward a smaller investment in sperm in small groups, which tends to occur at low densities. Moreover, individuals in small mating groups may be able to increase their effective group size by elongating their penis. Third, there may be covariance between group size and individual conditions (Hoch and Levinton 2012). For instance, if large groups tend to occur in relatively food-rich conditions and small groups in food-poor conditions, the members in small groups have worse nutritional conditions and are expected to be less fecund than those in large groups. Such effects might mask the predicted higher female allocation (in terms of proportion) of individuals in small groups. Although theoretically this will not change the optimal sex allocation, in reality, egg and sperm production in barnacles often show different responses to food availability (Hines 1978), making sex allocation variable in response to food availability. In addition, other environmental factors such as temperature, desiccation, and predation or parasite risk may also affect reproductive output as male and as female. Therefore, these environmental factors should be carefully monitored when conducting experiments or observations of sex allocation.

8.9 Mode of Fertilization and Self-Fertilization

Thoracican barnacles use the penis for delivering sperm, as has been reported in detail in *Balanus* spp. (Barnes and Barnes 1977) and *Tetraclita japonica* (Murata et al. 2001). In addition, another mode of sperm delivery was recently found by Barazandeh et al. (2013, 2014). Using genetic markers, they found that, in the pedunculate *Pollicipes polymerus* and, to a lesser extent, in the balanomorphs *Balanus glandula* and *Chthamalus dalli*, fertilization occurs between distant individuals in cases where the penis cannot reach. They suggested that individuals cast sperm for fertilizing distant individuals. Later, it was observed that *P. polymerus* individuals leak sperm mass even while exposed to the air during low tides (Barazandeh and Palmer 2015). However, the generality of sperm casting in other species remains unknown.

Few barnacles have been suggested to self-fertilize, based on brooding in isolated individuals or in distant individuals (Barnes and Crisp 1956; Anderson 1994). However, at least some part of brooding in distant individuals may be due to sperm casting from distant individuals (Barazandeh et al. 2013, 2014). Clear evidence for self-fertilization needs to be found by isolating individuals or genetic paternity analysis as in Kelly et al. (2012) and Ewers-Saucedo et al. (2016).

The scarcity of self-fertilization in barnacles may be due to the lack of strong selective pressure for it. It seems that only a small proportion of individuals are unable to obtain sperm from other individuals because of gregarious settlement patterns (e.g., Dreanno et al. 2006) and, at low densities, the presence of dwarf males. Other modes of sperm delivery, such as sperm casting, might also help.

8.10 Discussion

8.10.1 Sexual System Evolution

The current knowledge on barnacle sexuality indicates that the transition from hermaphroditism to androdioecy (i.e., the evolution of dwarf males from hermaphrodites) has occurred several times, and although less frequent, the transition from androdioecy to dioecy (the evolution of females from hermaphrodites) has also occurred repeatedly in the thoracican lineage. Thus, barnacles have highly labile sexual systems on evolutionary timescales. Moreover, experimental evidence has shown that changes from hermaphrodites to dwarf males occur even within species as a result of phenotypic plasticity. This is impressive if we consider the overall trend that phylogeny rather than adaptation seems to better explain the distribution of sexual systems in animals (Leonard 2010, 2013).

Phylogenetic comparative studies (Yusa et al. 2012; Lin et al. 2015) test the effect of only one explanatory variable, viz., mating group size. Mating group size often covaries with other environmental factors such as food availability or temperature, which can affect sexual systems by altering energy input and metabolic rates (Yamaguchi et al. 2008). Therefore, the evaluation of multiple explanatory variables is needed in interspecific comparisons. Moreover, intraspecific studies on the relationship between group size and sexual system are still scarce.

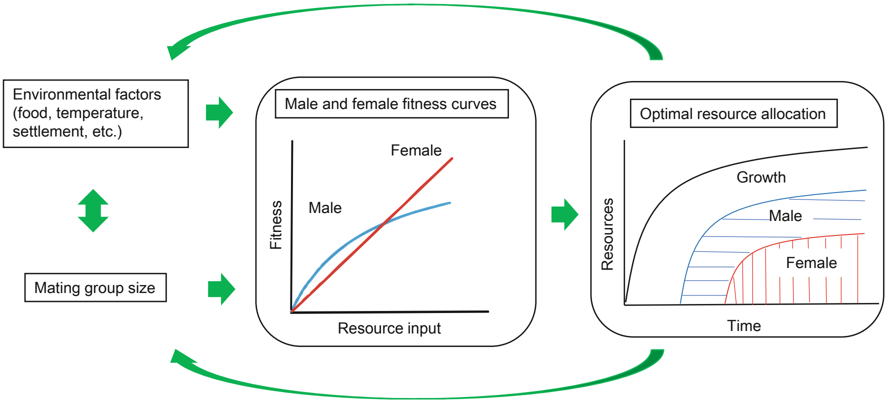

Fig. 8.5 Conceptual framework (the resource allocation model) explaining the relationships among environmental factors, mating group size, male and female fitness curves, and optimal resource allocation

8.10.2 The Resource Allocation Model

One of the goals of this chapter is to build a general framework for thinking about the evolution of sexual systems using barnacles as model organisms (the resource allocation model; Fig. 8.5). I advocate the view that environmental factors determine male and female fitness curves either directly (e.g., through food intake and temperature) or via their effects on mating group size, and these fitness curves in turn determine the optimal allocation to growth, male function, and female function over time. However, a theoretical model that links these components will be needed.

The relationships among these components should be regarded as mutual interactions rather than unilateral relationships. For instance, mating group size affects optimal resource allocation as explained, but this allocation also affects mating group size (i.e., the number of individuals capable of producing eggs) by altering growth rates and female allocation (Yamaguchi et al. 2008). This alters the shapes of fitness curves and then optimal resource allocation again. In addition, on an evolutionary timescale, a reduced chance of mating due to very small mating groups might affect habitat selection. Therefore, the environmental conditions they choose, fitness curves, and resource allocation patterns should be regarded as a coevolutionary unit.

Another example of a supposedly coevolutionary unit is the presence of dwarf males and the general lack of self-fertilization in barnacles at low densities. This speaks against the low-density model (Ghiselin 1974) as an adaptive reason for the evolution of hermaphroditism, at least in barnacles (Yusa et al. 2013).

Their sedentary nature is perhaps the most important ecological factor that affects reproductive characteristics in barnacles. As crustaceans, barnacles have already lost the ability to broadcast their sperm into the water (except for the release of sperm from the penis; Barazandeh et al. 2013, 2014; Barazandeh and Palmer 2015). This forces them to mate within groups, and, ultimately, it is the adaptive reason why they are simultaneous hermaphrodites, then dwarf males, and pure females as the group size decreases. The same sedentary nature also requires efficient sperm transfer, and this may be the evolutionary cause for the development of a long penis in barnacles. Interestingly, the penises of the balanomorphs tend to be longer than those of pedunculates (Neufeld and Palmer 2008; Barazandeh et al. 2014). This is apparently because the pedunculates can use the peduncle as an effective "penis," which can bend and slightly elongate toward the mating partner (Hoch et al. 2016). In contrast, the balanomorphs do not possess peduncles, and they must deliver sperm via their long penises (if they do not spermcast). In fact, they have one of the longest penises in the animal kingdom (up to seven times their body length; Neufeld and Palmer 2008; Barazandeh et al. 2014).

Sperm casting (Barazandeh et al. 2013, 2014; Barazandeh and Palmer 2015) makes the effective mating group large, as it can deliver sperm to more distant individuals than copulation. As a result, it is predicted that the male fitness curve becomes less saturated and the optimal allocation becomes less female biased in species where sperm casting is common. However, it is doubtful whether this mode of sperm delivery can affect the overall pattern of sexual systems observed in barnacles. This is because dioecy and androdioecy tend to occur in species with small mating group sizes, such as those in deep-sea habitats (Yusa et al. 2012; Lin et al. 2015), and casting sperm in the water would be ineffective if the individuals are distantly distributed.

8.10.3 Proximate Factors Affecting Sexual Expression: The How Questions

Although very little is known about sex determination in barnacles, a major message is that a simple distinction between genetic and environmental sex determination is not valid, as in the genetic × environmental interaction model of Svane (1986). A major challenge is the direct testing of Svane's hypothesis in *S. scalpellum*, although raising virgin hermaphrodites may be difficult and time-consuming. Moreover, the sex determination systems in other barnacles should also be explored. Detailed investigations into the genetics of sex determination mechanisms and sexual differentiation (Beukeboom and Perrin 2014) are also needed.

Although less widely recognized, sex allocation patterns can also be affected both by genetic and environmental components (Leonard 2013). Most of the current models implicitly assume that allocation is determined genetically by considering evolutionary strategies, although major results should hold even in the case of phenotypically plastic sex allocation (Yusa et al. 2012). However, the optimal sex allocation pattern and sexual systems may differ if they are conditionally dependent (Yamaguchi et al. 2013b, c). In general, we need to consider the possibility that individuals with different sexualities may not have equal fitness when considering the evolution of sexual systems.

Another question is why sexual systems are plastic in some cases and not in others. In general, the costs and benefits of maintaining sexual plasticity and fixity are suggested to be important (Leonard 2013). In thoracicans, large variation in environmental factors that parents cannot predict might be a selective force for plastic sexual expression in the offspring (Kelly and Sanford 2010; Yusa et al. 2013). However, no experiments have been performed to test this hypothesis.

8.10.4 Implications for Other Organisms

Here, I discuss two further topics in which understanding barnacle sexual systems may broaden our understanding of the evolution of sexual systems in general, namely, male dwarfism and androdioecy.

8.10.4.1 Male Dwarfism

In dioecious organisms, sexual size dimorphism occurs when different selection pressures act on the male and the female sexes (Shuster and Wade 2003; Fairbairn et al. 2007; Hirst and Kiørboe 2014). In many animals, males are larger than females (male-biased size dimorphism), but in many others, females are larger (female-biased size dimorphism). Female-biased size dimorphism occurs when selection pressure (both natural and sexual) on large body size acts more strongly on females than on males (Foellmer and Fairbairn 2005). Male dwarfism can be regarded as an extreme case of female-biased size dimorphism in which males are on average half or less than half the length of females (Vollrath 1998). Thus, the standard theory suggests that in animals with dwarf males, selection based on male body size is expected to be much weaker than female body size. In addition, being small may even have advantages, such as higher mobility and better access to the fertilization site (Ghiselin 1974; Vollrath 1998).

The presence of dwarf males in closely related dioecious and androdioecious barnacles suggests that the males with females do not differ qualitatively from those with hermaphrodites. Moreover, dioecy evolved from androdioecious ancestors in barnacles (Yusa et al. 2012). Therefore, the phenomenon of male dwarfism needs to be considered in potentially hermaphroditic populations rather than being confined to dioecious populations. Thus, the resource allocation model is needed in cases where individuals can allocate to growth, male, and female functions. According to the model, male dwarfism occurs when some individuals mature as male and allocate more resources to male function instead of growth or female function at a much younger age (Fig. 8.3b).

The resource allocation model also allows the consideration of another type of dwarf males. In many organisms, individuals first mature as male and then change their sex to female (protandry). Of course, individuals at the male stage are smaller than those at the female stage in the case of protandry, but extremely (less than half the length) dwarfed males are occasionally found in sex-changing animals such as bivalves and gastropods (e.g., Collin 2013). In many cases, the distinction between protandric sex change and dioecy with environmental sex determination appears to be ambiguous, and these situations need to be treated in the same manner under the resource allocation model. To generalize the question, it is important to ask why individuals allocate to male function much earlier than female function in potential hermaphrodites, rather than why male individuals mature earlier than female individuals.

8.10.4.2 Androdioecy

Androdioecy is a rare sexual system (Pannell 2002) that has been reported only in approximately 120 species in the animal kingdom (Weeks 2012; see Lin et al. 2015; Sawada et al. 2015; Yusa et al. 2015 for new additions in barnacles). In animals, most cases of androdioecy have evolved from dioecious ancestors, with the hermaphrodites having evolved from females (Weeks 2012). They have acquired the ability to produce a small amount of sperm to self-fertilize in case they fail to obtain allosperm (Weeks et al. 2006; Weeks 2012). Unlike these cases, androdioecy in barnacles evolved from hermaphroditic ancestors (Yusa et al. 2012; Lin et al. 2015; Ewers-Saucedo et al. 2015, 2016), as originally postulated in the model for the evolution of androdioecy by Charlesworth (1984). Charlesworth insisted that androdioecy is highly unlikely to evolve in nature because the evolution of males in a hermaphroditic population requires that the males attain twice or greater male reproductive success, as hermaphrodites have both male and female pathways to pass their genes to the next generation (Pannell 2002). This situation is difficult to attain because the male fitness curve is often saturated in hermaphrodite populations (Charnov 1982), meaning that doubling the resource allocation to male function does not double the male reproductive success. Therefore, the evolution of males in hermaphrodite populations is rather paradoxical.

The dwarf males circumvent this paradox. The argument by Charlesworth (1984) implicitly postulates other fitness components being equal. However, being dwarf, the barnacle males have a greater advantage in life history traits such as higher survival to reach maturity and possibly a shorter generation time (Crisp 1983; Yamaguchi et al. 2012; Ewers-Saucedo et al. 2016). In other words, with these advantages, dwarf males do not have to attain two times higher reproductive success as males than hermaphrodites. In fact, approximately 30% of known cases of androdioecy occur in barnacles (Weeks 2012), indicating that this advantage is important in the evolution of androdioecy in the animal kingdom. The presence of such males warrants the study of other androdioecious organisms, as they may also circumvent the paradox of the twofold higher mating advantage by males in some way.

8.11 Conclusion and Future Directions

As we have seen, the thoracican lineage contains many species with diverse sexual systems and rich transitions between them. Sex allocation theory and empirical studies fueled by the theory have advanced our understanding of the evolution of these sexual systems. However, there are still many unknowns. Concerning theoretical advancement, we still lack a formal model that incorporates the temporal change in optimal resource allocation to growth, male function, and female function. This is an important challenge for the future.

Concerning intraspecific experimental and observational studies, no studies have measured all the necessary fitness components of dwarf males and hermaphrodites in androdioecious barnacles under natural conditions. This is important because some theoretical studies (Yamaguchi et al. 2013b, c) predict that dwarf males will appear even if they are less adapted than hermaphrodites. Such empirical studies will require information on long-term (ideally lifetime) reproductive success through male and female functions and the survival rate over time for both hermaphrodites and males. Reproductive success as female can be estimated by counting (or weighing) brooding embryos and therefore is not very difficult. Reproductive success as a male normally requires paternity analysis involving genetic markers because multiple dwarf males and male-acting hermaphrodites can fertilize the eggs of an individual. Ewers-Saucedo et al. (2016) used microsatellite markers for this purpose. Interestingly, they found that dwarf males of *Chelonibia testudinaria* do not have greater fertilization success than conspecific hermaphrodites and that actual number of fathers of each brood is quite small (less than 2). Although not directly measured, survival under natural conditions has been inferred by Ewers-Saucedo et al. (2015). Such an approach is important for the future.

Another research direction is to use barnacles as model organisms to study sexual systems (Spremberg et al. 2012). For instance, studying phenotypic plasticity in sex determination and sex allocation in androdioecious species requires a laboratory supply of many individuals, as most androdioecious species are difficult to collect in nature. An important obstacle for laboratory rearing is larval culture, as most species have lengthy larval periods (normally six nauplius stages and a cyprid stage). However, *Scalpellum scalpellum* is lecithotrophic, and it is relatively easy to obtain larvae ready to settle (Svane 1986; Høeg et al. 2016). In addition, it has a close dioecious relative, *S. stearnsii*, so comparing these two species would be fruitful in considering the transition between sexual systems. However, they are both difficult to obtain (subtidal and deep-sea habitats near Europe and Japan, respectively) and have rather long life spans (years). *Octolasmis* species such as *O. warwickii* and *O. unguisiformis* are other candidates. Although their larval durations are both long (>1 month), techniques for larval rearing have been developed in some congeners (e.g., Yap et al. 2015). Several androdioecious balanomorphs are also candidates for model organisms.

Concerning the mechanisms underlying sexual systems and sex allocation, recent genetic analysis techniques will be a great help in specifying the genes involved in sex determination in both dioecious and androdioecious species. Using transcriptomics and other molecular techniques, the genes involved in male and female functions and factors regulating the variation in resource allocation may be identified.

Lastly, when we turn our eyes to other Thecostraca, there are plenty of peculiarities. Rhizocephalans are a source of insight into sex determination (Yanagimachi 1961; Yamaguchi et al. 2014), mode of fertilization, and sex ratio (Høeg 1995a, b). Investigations into the reproduction of acrothoracicans, ascothoracidans (which have both dioecious and hermaphroditic sexual systems), and facetotectans are almost completely lacking. The diversity of sexuality in barnacles in the broadest sense, which has fascinated Darwin and his successors, will continue to attract the attention of reproductive biologists.

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Chapter 9
Life History Constraints Facilitate the Evolution of Androdioecy and Male Dwarfing

Sachi Yamaguchi

Abstract "Sex allocation" is the allocation of resources between male and female functions, while "life history strategy" is one between growth and reproduction (and survival). Although life history strategy and sex allocation theories have commonly been studied separately, they interact strongly since both study the optimal allocation of resources available for each individual. For example, individuals with different life history schedules may also differ in terms of sexuality. To illustrate how such life history/sex allocation polymorphism evolves to form various sexual systems such as androdioecy (the coexistence of males and hermaphrodites), I introduce simple mathematical models that consider how constraints (temporal or spatial limitations) on the decision-making of life history path facilitate the coexistence of individuals with different schedules of resource allocation (life history and sexuality), focusing on androdioecious barnacles (dwarf males + hermaphrodites) as an example. The temporal limitation model shows that an unlucky individual who enters an old microhabitat should become a dwarf male to make the best of a bad situation. Although the individual's fitness could be higher if it has sufficient time for growth in a young microhabitat, becoming a dwarf male is the optimal tactic for the unlucky individual. The coexistence of different sexualities was also explained by the spatial limitation model, which assumes life history constraints among based on the microscopic environmental conditions.

9.1 Introduction

An individual's sexuality is determined by the allocation of resources toward either male or female reproductive functions. Individuals who invest all their resources in male (female) function are dioecious males (females), while ones that invest in both functions are simultaneous hermaphrodites. The allocation of resources between male and female functions is called "sex allocation" (Charnov 1982). Sex allocation

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sometimes depends on age and/or body size of individuals [age- or size-dependent sex allocation (Angeloni et al. 2002; Sakai and Sakai 2003; Cadet et al. 2004; Yamaguchi et al. 2008)]. For example, protandric sex changers first allocate resources to male function and then to female function as they grow. Note that the above argument assumes that small individuals allocate resources to growth, in addition to reproduction as males. The resource allocation between growth and reproduction (and survival) is called the "life history strategy." Although life history and sex allocation theories have commonly been studied separately (Zhang and Wang 1994), they interact strongly since both study the optimal allocation of resources available for each individual.

Individuals generally obtain more resources as they grow larger and invest these resources in further growth and reproduction. Therefore, it is necessary to consider the adaptive resource allocation schedule while the amounts of resources depend on past allocations (Stearns 1992; Yamaguchi et al. 2008, 2013a, c). In some populations, individuals with different life histories may coexist. While such life history polymorphism may be observed among same-sex individuals, e.g., harem males and sneaker males in fish (Martin and Taborsky 1997), sometimes different life history schedules are observed among individuals of different sexuality, that is, sex allocation correlates with life history. When males and females have different life histories in dioecious (separate sexes) species, sexual size dimorphism occurs (Chou et al. 2016). In addition, males and simultaneous hermaphrodites in androdioecious species may have different life histories. A clear example of such difference is dwarf males in androdioecious barnacles (Zardus and Hadfield 2004; Ewers-Saucedo et al. 2015, 2016).

Then, why do multiple life history/sex allocation schedules coexist within a population to form various sexual systems such as androdioecy? First, if individuals with different strategies have the same fitness, then those strategies can coexist as the evolutionary stable state. A clear example is the 1:1 sex ratio commonly observed in dioecious organisms (Fisherian sex ratio). If one strategy always has a higher fitness than another, a polymorphism of strategies should not be maintained, as individuals should adapt to choose the former strategy. Theoretical studies on the evolution of sexual systems usually look for conditions in which individuals with different strategies have the same fitness.

Second, if individuals who experience different environments have different sexuality and life history as alternative tactics, polymorphism in the population is likely to occur. Interestingly, in this case, the fitness of different tactics is not necessarily identical. For example, when life history choices are constrained, individuals who experience unfavorable conditions have no choice but to adopt tactics that result in lower fitness, even if other tactics would produce higher fitness.

I have studied theoretical modeling of the interaction between sexual systems and life history in marine organisms (Yamaguchi et al. 2007, 2008, 2012, 2013a, b, c). In this chapter, I introduce three theoretical studies that consider how temporal and spatial limitations on the decision-making of life history path facilitate the coexistence of individuals with different schedules of resource allocation (life history and sexuality), focusing on marine organisms as an example.

Various sexual systems are observed in marine invertebrates: simultaneous hermaphroditism, dioecy (separate sexes, coexistence of males and females), androdioecy (males and hermaphrodites), gynodioecy (females and hermaphrodites), and sex changes (male to female or female to male). Barnacles (Arthropoda, Cirripedia), sessile crustaceans, are interesting subjects as they show three sexual systems (hermaphroditism, dioecy, and androdioecy) and diverse life histories (Darwin 1851; Kelly and Sanford 2010; Yusa et al. 2012, 2013; Yamaguchi et al. 2012). In addition, when present, barnacle males are small when compared to conspecific females or hermaphrodites and thus are called "dwarf males" (Darwin 1851; Vollrath 1998; Yusa et al. 2010). A dwarf male attaches to the body of a female or a hermaphrodite throughout his lifetime.

Dwarf males are defined as males with length 50% or shorter than female/hermaphrodite size (Vollrath 1998; Yusa et al. 2012). In addition to barnacles, dwarf males are observed in diverse animals, including marine organisms such as echiurans (Vollrath 1998; Goto et al. 2013), polychaetes (Rouse et al. 2004), parasitic bivalves (Turner and Yakovlev 1983), and ophiuroids (reviewed in Ghiselin 1974) and terrestrial ones such as spiders (Vollrath 1998). Ghiselin (1974) discussed that dwarf males are favored in the following environmental conditions: (1) a low population density, (2) a restricted motility or sessility, and (3) a much larger longevity in females than in males. Conditions (1) and (2) reduce the male-male competition for mates because of small probability of their encounter and enhance the difficulty of finding mating partners. Therefore, males attaching on the body of females or hermaphrodites enjoy a considerable advantage. Condition (3) may favor earlier maturation in males than females, and it leads to male dwarfing. In unproductive environments, the population density tends to be low, and the motility of individuals tends to be small because they must save the energy to look for mating partners. In fact, species with dwarf males are more common in the food-scarce environments such as deepwater habitats than in shallow seas that are more productive (Ghiselin 1974). The relationship between food-limited environments and the evolution of dwarf males was also discussed by Yamaguchi et al. (2008), using a mathematical model. In comparison with other taxa with dwarf males, barnacles have the unique feature that dwarf males are present in both dioecious and androdioecious taxa.

Darwin's finding of dwarf males coexisting with hermaphrodites poses an interesting puzzle, because hermaphrodites can invest far more resources toward male function relative to dwarf males (Darwin 1851; Charnov 1982, 1987; Yamaguchi et al. 2008, 2012, 2013a, b, c). Sperm competition may occur between dwarf males and hermaphrodites during the fertilization of hermaphrodite eggs. Due to their lower volumes of sperm when compared to large hermaphrodites, dwarf males seem to have no chance of winning the fertilization race, although they need to achieve sufficient fitness to invade the hermaphroditic population to allow the emergence of androdioecy in barnacles.

Notably, an interesting clue to understanding the evolution of dwarf males is that species with dwarf males are often parasitic or symbiotic with other animals. For example, in barnacles, some species with dwarf males attach to sea urchins

(Kolbasov and Zevina 1999; Spremberg et al. 2012), crabs (Yusa et al. 2010; Buhl-Mortensen and Høeg 2013; Sawada et al. 2015), turtles (Crisp 1983), and so on. If barnacles attach onto a crab's carapace and thereafter the crab molts its carapace or dies, they also die. Individuals may adaptively choose their life history and sex allocation, according to the remaining life of the microhabitat, e.g., the duration until molting or death of a crab. As a result, it may be possible for dwarf males to emerge with females or hermaphrodites. This is the idea examined by the first and second models described in this paper. A time-independent model is introduced where population dynamics are stationary and the strategies of each individual are independent of time as in the first model; the second model is a temporal limitation model where the longevity of a microhabitat is limited and the strategies of each individual depend on the duration of the microhabitat.

In the third model, the effect of constraint, which directly forces individuals to a particular option, is studied. The limitation of opportunity to be a dwarf male (spatial limitation due to restriction of attachment sites for dwarf males) is incorporated into the first model (i.e., time-independent model).

In summary, three simple models are explored for marine organisms that explain the conditions for different patterns of sexuality and life history, including dwarf males. The effect of life history constraints (temporal or spatial limitations) to sex allocation in the evolutionarily stable state (ESS) population is studied, in contrast to the results with no constraint of life history choice in the time-independent model.

9.2 Models and Results

Let us consider a marine organism living in a microhabitat. Newly settled larvae enter a microhabitat at a constant rate per unit time and become sessile after metamorphosis. For simplicity, I assume that the population consists of two size classes of individuals: small and large. When larvae directly settle in a microhabitat, they first remain small and immature and grow without reproduction. After reaching a large size, they then have the ability to reproduce as potential hermaphrodites, potentially having both male and female reproductive functions. In contrast, larvae that settle on conspecific large individuals are able to reproduce as dwarf males immediately, but they remain small in size throughout their lifetime.

In other words, newly settled larvae are able to choose their life history: whether they become dwarf males or they become potential large hermaphrodites after a small immature growth stage. If larvae choose the latter, they allocate their resources to male function (sperm production) and female production (egg production). This is called the "sex allocation" problem in simultaneous hermaphrodites (Charnov 1982, 1987). Newly settled larvae have two strategies, a proportion to become immature individuals c and sex allocation when achieving a large size m (i.e., allocation ratio to male function), which evolve adaptively to maximize their own fitness (Fig. 9.1).

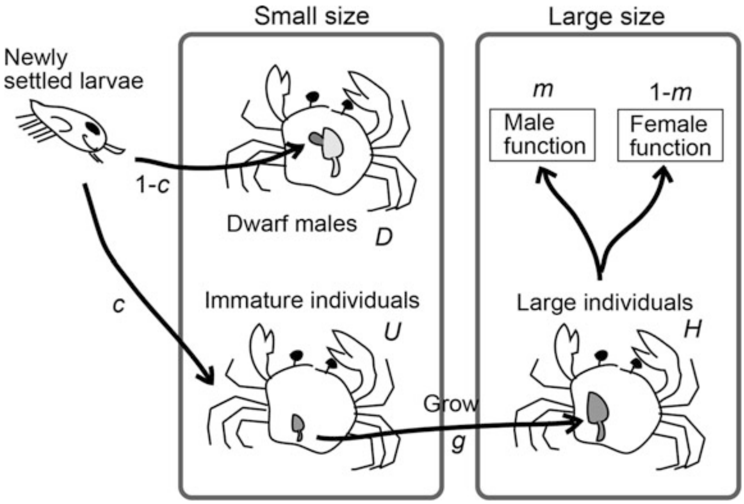

Fig. 9.1 Marine benthic larvae entering a microhabitat. In this figure, it is assumed that barnacle larvae approach the carapace of a crab at a constant rate per unit time. After metamorphosis, they become sessile. When larvae settle on large conspecific individuals, they can reproduce as dwarf males immediately but cannot grow to a large size. Alternately, if larvae settle on the carapace directly, they first stay small and immature and eventually grow to large individuals with investment in reproductive function(s). The problem of allocating resources between male and female (sex allocation problem) is important for large individuals to obtain maximum fitness. The two strategies for each individual are the proportion that settle directly on the carapace (to become an immature individual) c and the male allocation ratio when becoming a large individual m

9.2.1 Time-Independent Model

Next, I calculate the condition in which dwarf males appear in the population. As the simplest case, consider that the numbers of the three types of individuals, i.e., dwarf males, small immature individuals, and large reproductive ones, remain stationary and that the two strategies (c, m) of new larvae are time independent. The microhabitat is stable and persists for a long time. Under these assumptions, can dwarf males evolve in the population?

The information needed to calculate the optimal life history choice by larvae is the expected lifetime reproductive success in each life history. Larvae may choose the life history that brings with it a higher reproductive success.

The expected lifetime reproductive successes for a dwarf male and for a small immature individual are denoted as V_{D} and V_{U}, respectively. The following relationships are satisfied:

$$\text{If } V_{\mathrm{D}} > V_{\mathrm{U}}, \text{ all larvae become dwarf male } (c = 0). \tag{9.1a}$$

$$\text{If } V_{\mathrm{D}} < V_{\mathrm{U}}, \text{ all larvae become small-immature individuals } (c = 1). \tag{9.1b}$$

If $V_{\mathrm{D}} = V_{\mathrm{U}}$, some larvae become dwarf males and the other become small-immature individuals $(0 < c < 1)$. (9.1c)

I here show the expected lifetime reproductive successes for the two life histories (V_{D}, V_{U}). First, the reproductive success of a dwarf male is defined by the number of eggs fertilized by his own sperm:

$$\begin{aligned}&(\text{Reproductive success for a dwarf male } V_D)\\ &= \Sigma\left[\begin{array}{l}(\text{Survival rate until time } t \text{ for a dwarf male})\\ \times(\text{Number of eggs all large individuals produce per unit time})\\ \times(\text{Fraction of number of eggs a dwarf male fertilizes per unit time})\end{array}\right]\end{aligned} \tag{9.2}$$

The symbol Σ indicates that the contents of $[\cdots]$ are summed from time 0 to time ∞ since the longevity of the microhabitat is sufficiently long. "The survival rate for a dwarf male" can be calculated by his mortality rate per unit time. Dwarf males suffer not only their mortality rate u but also the probability of the microhabitat loss μ, because they cannot live without the latter, e.g., the molted carapace of crabs or the death of crabs. Then, the net mortality rate of a dwarf male is $u + \mu$, and his survival rate until time t is given by $e^{-(u + \mu)t}$.

The second component in Eq. (9.2), "number of eggs of all large individuals," is the number of eggs that one large individual produces multiplied by the stationary population of large individuals $\widehat{H}$. Denoting the reproductive resource per large individual per unit time as R and the allocation ratio to male function as m, the number of eggs per large individual per unit time is represented by $(1 - m^{*})R$. Then,

$$\begin{aligned}&(\text{Number of eggs all large individuals produce per unit time})\\ &\quad = (1 - m^{*})R\widehat{H}\end{aligned} \tag{9.3}$$

Note that the male allocation is denoted by m^{*} instead of m, assuming that the allocation strategy achieved evolutionary stability.

The third component in Eq. (9.2), "fraction of number of eggs a dwarf male fertilizes per unit time," is calculated as the ratio of a dwarf male's sperm to the total sperm production within the population. Since dwarf males are small compared to large individuals, the reproductive resources of a dwarf male must be much smaller than that of a large individual R. Here, I introduce "the relative fertility of a dwarf male α" ($\alpha < 1$). The number of sperm from a dwarf male is αR, and then the total amount of sperm in the stationary population of dwarf males $\widehat{D}$ is $\alpha R\widehat{D}$. The total amount of sperm of the stationary large-individual population $\widehat{H}$ is $m^{*}R\widehat{H}$.

$$\begin{aligned}&(\text{Fraction of number of eggs a dwarf male fertilizes per unit time})\\&=\frac{\alpha R}{\alpha R\widehat{D}+m^{*}R\widehat{H}}\end{aligned} \tag{9.4}$$

The expected lifetime reproductive success for a dwarf male V_{D} in Eq. (9.2) is rewritten mathematically, using Eqs. (9.3) and (9.4):

$$V_{\mathrm{D}}=\int_{0}^{\infty}e^{-(u+\mu)t}(1-m^{*})R\cdot\frac{\alpha R}{\alpha R\widehat{D}+m^{*}R\widehat{H}}\mathrm{d}t. \tag{9.5}$$

Second, in a similar way, I explain the expected lifetime reproductive success for a small immature individual V_{U}. In this life history, an immature individual will be reproductively active after achieving a large size.

$$\begin{aligned}&(\text{Reproductive success for a small immature individual } V_U)\\&=\Sigma\left[\begin{array}{l}(\text{Survival rate until time } t \text{ for an immature individual})\\\times(\text{Probability for growth to a large size until time } t)\\\times(\text{Reproductive success per unit time for a large individual})\end{array}\right]\end{aligned} \tag{9.6}$$

"Survival rate for a small immature individual" is calculated using the sum of the mortality rate u and the probability of microhabitat loss μ. Note that, for simplicity, three types of individuals are assumed to have the same mortality rate u. As for "the probability for growth to the large size," I assume that a small immature individual becomes a large one with the probability of growth per unit time g (mathematically, this is called the Markovian jump, and the mean time for an immature individual to stay in the small size class is 1/g).

Finally, consider the expected lifetime reproductive success for a large individual V_{H}.

$$\begin{aligned}&(\text{Reproductive success for a large individual } V_H)\\&=\Sigma\left[\begin{array}{l}(\text{Survival rate until time } t \text{ for a large individual})\\\times\left\{\begin{array}{l}(\text{Number of eggs it produces per unit time})\\+(\text{Number of eggs it fertilizes per unit time})\end{array}\right\}\end{array}\right]\end{aligned} \tag{9.7}$$

Note that a large individual chooses its male allocation m optimally under conditions where other large ones adopt the strategy m^{*}, which is the evolutionarily stable strategy. Equation (9.7) mathematically leads to the following:

$$V_H=\int_{0}^{\infty}e^{-(u+\mu)t}\max_{0\leq m\leq 1}\left[(1-m)R+(1-m^{*})R\widehat{H}\cdot\frac{mR}{\alpha R\widehat{D}+m^{*}R\widehat{H}}\right]\mathrm{d}t. \tag{9.8}$$

The symbol max indicates that larvae choose strategy m, which maximizes the content of $[\cdots]$ in Eq. (9.8). I find that the strategy shown below is evolutionarily stable (to obtain the evolutionarily stable strategy, I should seek a value of m^* that meets $\partial[\cdots]/\partial m = 0$ when $m = m^*$, but note that sometimes boundary values such as 0 or 1 may be a stable solution):

$$\text{If } \alpha\widehat{D} < \widehat{H}, \quad m^* = \frac{1}{2} - \frac{\alpha\widehat{D}}{2\widehat{H}}. \tag{9.9a}$$

$$\text{If } \alpha\widehat{D} > \widehat{H}, \quad m^* = 0. \tag{9.9b}$$

Equation (9.9) shows that the sexuality of large individuals is simultaneous hermaphrodite or female. When large individuals are hermaphrodites, the allocation to male function m^* depends on the ratio of the number of dwarf males $\widehat{D}$ to that of hermaphrodites $\widehat{H}$. Without dwarf males ($\widehat{D} = 0$), the ESS sex allocation is to invest equally in male and female functions ($m^* = 0.5$). As the number of dwarf males $\widehat{D}$ increases, the ESS sex allocation of large individuals becomes biased toward female function ($m^* < 0.5$, Eq. (9.9a)), and finally all large individuals become pure females ($m^* = 0$, Eq. (9.9b)).

Interestingly, the evolutionarily stable state of the population is determined by the relationship among four parameters: the relative fertility of dwarf males α, the growth rate for small immature individuals g, the mortality rate u, and the probability of microhabitat loss μ. Note that all individuals (dwarf males, immature individuals, and large individuals) have the same mortality rate. Following some arithmetic, two evolutionarily stable states for the population can be derived:

1. When $\alpha < \frac{g}{g+u+\mu}$ holds

The right-hand side $g/(g + u + \mu)$ represents the probability for a small immature individual to reach a large size before its death or the loss of its microhabitat. When the probability to reach a large size is greater than the relative fertility of a dwarf male α, all newly settled larvae choose the small immature individual pathway ($c^* = 1$) and later become large individuals. That is, in this case, inequality $V_D < V_U$ in Eq. (9.1b) holds. Since there are no dwarf males ($\widehat{D} = 0$), large individuals are hermaphrodites with equal investment in male and female functions ($m^* = 0.5$ from Eq. (9.9a); see Fig. 9.2).

2. When $\alpha > \frac{g}{g+u+\mu}$ holds

When the relative fertility of a dwarf male α is larger than the probability of growing from small and immature to large size, some larvae become dwarf males, and the others become small immature individuals ($0 < c^* < 1$). The latter grow to a large size and become pure females ($m^* = 0$ from Eq. (9.9b); see Fig. 9.3). In this case, equality $V_D = V_U$ in Eq. (9.1c) holds, and dwarf males coexist with large females.

From the above results, if the strategies of each individual (c, m) are time independent, two cases of evolutionarily stable states in the population appear depending on the difficulty in growing to a large size class before being killed.

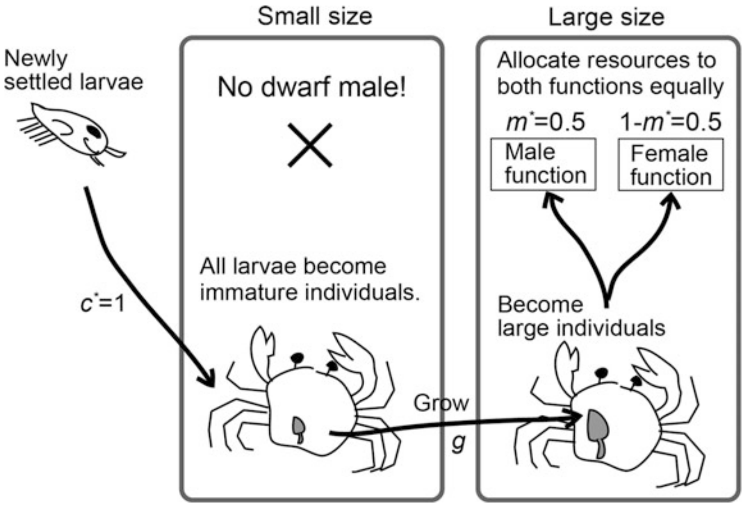

Fig. 9.2 When the probability for immature individuals to grow is greater than the relative fertility of dwarf males. This case corresponds to environments that have a plentiful food resource. All larvae choose the immature-individual life history, since they easily achieve a large size due to the available food. Large individuals invest their resources to male and female function equally. The sexual system in the population exhibits simultaneous hermaphrodites, and there are no dwarf males

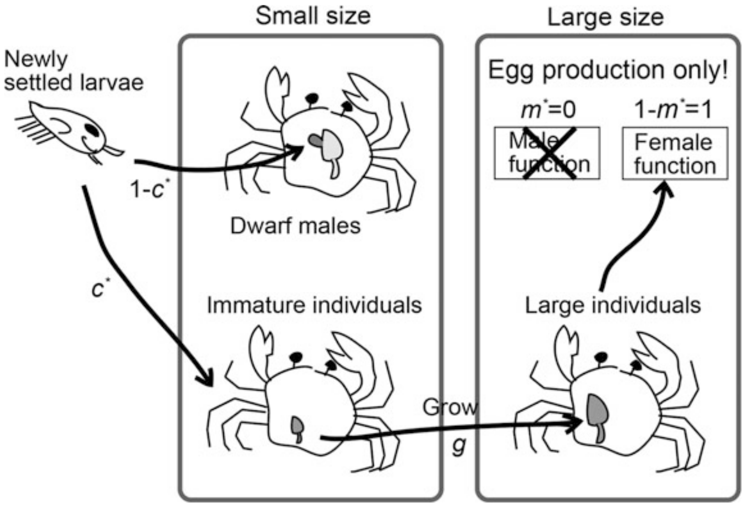

Fig. 9.3 When the relative fertility of dwarf males is greater than the probability of growth for immature individuals. This case corresponds to environments that have a poor food resource. Some larvae (the proportion 1-c^*) become dwarf males, and others (the proportion c^*) become small immature individuals. When immature individuals grow to a large size, they have no male function and become pure female. The sexual system in the population exhibits the coexistence of females and dwarf males

Easy growth, e.g., in a food-rich environment, leads to all larvae choosing to become immature individuals first and to later grow into large hermaphrodites with equal sex allocation. In contrast, if growth is slow because of poor food availability, some larvae become dwarf males, and the others grow into pure females. Note that there is no coexistence of dwarf males and hermaphrodites in an evolutionarily stable state in the population. However, some barnacle species do show the coexistence of dwarf males and hermaphrodites (Crisp 1983; Zardus and Hadfield 2004; Buhl-Mortensen and Høeg 2006; Yusa et al. 2010; Sawada et al. 2015), which cannot be explained by this simple model. How is it possible to improve the model to solve this coexistence?

9.2.2 Temporal Limitation Model with Time-Dependent Strategies

One possible way to explain the observed coexistence of dwarf males and hermaphrodites in some barnacles may be by incorporating the nonstationary nature of the population due to an unstable microhabitat. Here the maximum longevity of a microhabitat is set to age 3650. Most barnacles that show such a coexistence attach to crab carapaces (Yusa et al. 2010; Buhl-Mortensen and Høeg 2013; Sawada et al. 2015), turtles (Crisp 1983), and the spines of sea urchins (Kolbasov and Zevina 1999). If they attach to short-lived microhabitats such as crab carapaces, they need to adapt to the expected loss of the microhabitat following the molting or death of crabs. Thus, in such a situation, it is necessary to calculate a time-dependent optimal strategy instead of assuming a stationary nature as in the previous model. The two strategies of newly settled larvae (c, m) should be affected by the age of the microhabitat t, so the strategies (c, m) should be rewritten to $(c(t), m(t))$. Note that the populations of three types of individuals (dwarf males, immature individuals, and large individuals) are not constant and depend on the strategies of each larva $(c(t), m(t))$. The method for calculating the solution of a time-dependent strategy is called "dynamic optimization" (see Yamaguchi et al. 2013a in detail). Here I illustrate the results of dynamic optimization without the derivation of mathematical equations.

Let us focus on growth rate for an immature individual g, fixing the other parameters (α, u, μ) as constants values. As in the previous model, α is the relative fertility of dwarf males, u is the mortality rate, and μ is the probability of microhabitat loss. When growth rate g is large ($g = 0.5$), three types of sexual systems appear, depending the age of the microhabitat t (see Fig. 9.4a). When the microhabitat is young (until the age is 2000), all newly settled larvae remain small and immature ($c^*(t) = 1$) and then later grow to a large size. Large individuals become hermaphrodites with equal investment in male and female functions ($m^*(t) = 0.5$). The population should exhibit simultaneous hermaphroditism.

As the microhabitat becomes older (age 2000), the composition of the population starts to change drastically, since the choice of life history by newly settled larvae switches from that of immature individuals to that of dwarf males. All newly settled

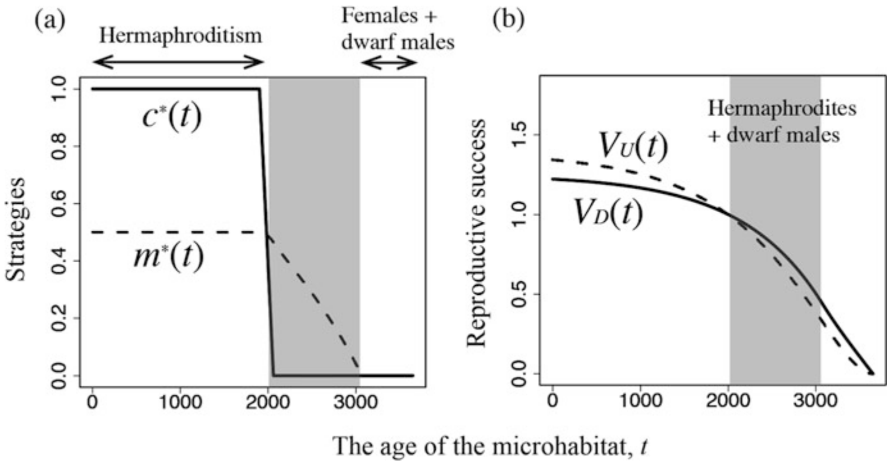

Fig. 9.4 When the growth rate for immature individuals g is large ($g = 0.5$). The horizontal axis is the age of the microhabitat t. Three different sexual systems appear depending on microhabitat age. The meshed region between age 2000 and 3000 shows the coexistence of dwarf males and hermaphrodites. (**a**) The evolutionarily stable strategies $(c^*(t), m^*(t))$, where $c^*(t)$ is the proportion of those larvae becoming immature individuals and $m^*(t)$ is the allocation ratio to male function of large individuals. All larvae become immature individuals when $c^*(t) = 1$, and they become dwarf males when $c^*(t) = 0$. If $0 < m^*(t) < 1$, large individuals become simultaneous hermaphrodites. If $m^*(t) = 0$, they become pure females. Note that the two strategies for each individual depend on microhabitat age t. (**b**) Expected future reproductive success. $V_D(t)$ and $V_U(t)$ are reproductive successes for a dwarf male and for an immature individual, respectively. The two curves of reproductive success intersect at age 2000. The parameters are $\alpha = 0.5$, $u = 0.3$, and $\mu = 0.1$. The maximum longevity of microhabitat is 3650. Modified from Yamaguchi et al. (2013a)

larvae that enter the microhabitat after age 2000 become dwarf males. The appearance of dwarf males affects the sex allocation of large individuals, that is, it induces female-biased sex allocation. This result can be interpreted as follows: large individuals need to produce less sperm because of the existence of dwarf males in the population. This interpretation explains the decrease in sperm production by large individuals. A coexistence of dwarf males and hermaphrodites is observed in the meshed region of Fig. 9.4a. When the age of the microhabitat increased (>3000), large individuals do not invest resources in male function, and they become pure female.

The appearance of dwarf males is an important point in the time-dependent change of sexual systems. If the expected reproductive success for a dwarf male $V_D(t)$ is greater than that for an immature individual $V_U(t)$, newly settled larvae should choose a dwarf male life history. Figure 9.4b shows that the curve of reproductive success for a dwarf male intersects that for an immature individual at age 2000. In a young microhabitat, the inequality $V_U(t) > V_D(t)$ holds, and all newly settled larvae choose the small immature life history. In contrast, when the microhabitat become old, $V_U(t) < V_D(t)$ holds, and all newly settled larvae become dwarf males. Newly settled larvae that enter an older microhabitat choose the dwarf male

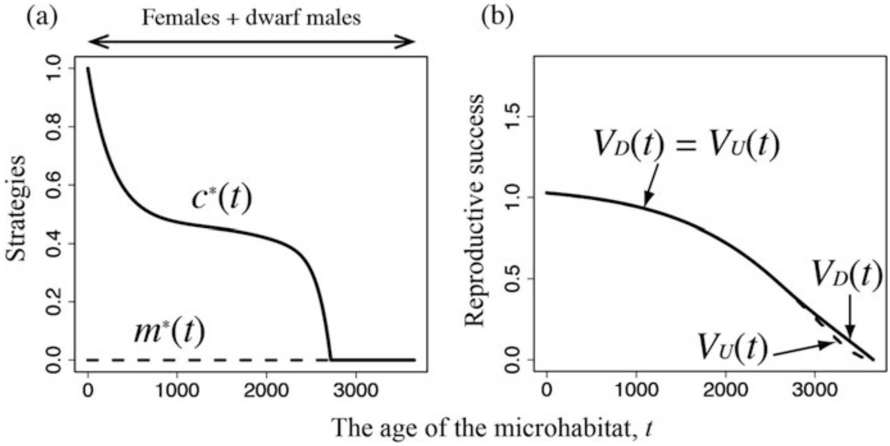

Fig. 9.5 When the growth rate for immature individuals g is small ($g = 0.3$). The horizontal axis is the age of the microhabitat t. The sexual system in the population exhibits only the coexistence of dwarf males and pure females. (**a**) The evolutionarily stable strategy $(c^*(t), m^*(t))$, where $c^*(t)$ is the proportion of those individuals that are becoming immature and $m^*(t)$ is the allocation ratio to male function of large individuals. The proportion $c^*(t)$ decreases with the passage of time. (**b**) The expected future reproductive success. $V_D(t)$ and $V_U(t)$ are reproductive successes for a dwarf male and for an immature individual, respectively. The two curves of reproductive success are equal for a long period ($V_D(t) = V_U(t)$). The parameters are $\alpha = 0.5$, $u = 0.3$, and $\mu = 0.1$. The maximum longevity of a microhabitat is 3650. Modified from Yamaguchi et al. (2013a)

life history as they can be reproductively active immediately, before the disappearance of the microhabitat.

On the other hand, when the growth rate for immature individuals g is small ($g = 0.3$), the sexual system is only the coexistence of pure females and dwarf males (see Fig. 9.5). Since there is a relatively long period of time in which $V_U(t) = V_D(t)$ holds (Fig. 9.5b), some larvae become dwarf males, and others become immature individuals ($0 < c^*(t) < 1$ in Fig. 9.5a). The proportion of newly settled larvae that become small immature individuals decrease with the passage of time, and eventually all larvae choose the dwarf male life history. At this time, the expected reproductive success for dwarf males $V_D(t)$ is greater than that for immature individuals $V_U(t)$ (see Fig. 9.5b).

Results showed that, when the growth rate for immature individuals g is large, an evolutionarily stable sexual system in the population changes from simultaneous hermaphroditism to the coexistence of hermaphrodites and dwarf males and then to the coexistence of females and dwarf males, as the microhabitat becomes older. The balance between the growth rate of immature individuals and the time remaining for the habitat creates a mixture of hermaphrodites and dwarf males. Subsequently, with the approaching demise of the microhabitat, this mixture disappears, and eventually the sexual system changes to separate sexes.

9.2.3 Spatial Limitation Model with Time-Independent Strategies

How is it possible to change the previous two models to obtain a solution where dwarf males stably coexist with simultaneous hermaphrodites? It is an interesting puzzle since Darwin's finding that dwarf males exist stably in the hermaphrodite population, although hermaphrodites invest more resources toward male function relative to dwarf males (Darwin 1851; Charnov 1982, 1987, Yamaguchi et al. 2008, 2012, 2013a, b, c).

In the previous two models that considered time-independent/time-dependent strategies (i.e., the time-independent model and the temporal limitation model), I assumed that newly settled larvae choose between two life histories adaptively in order to maximize their own fitness. Now let us consider the case where this choice is constrained and only a proportion p of new larvae can become dwarf males. As a real-life example, in the barnacle species *Scalpellum scalpellum*, which attaches to hydroids, dwarf males live on limited areas of hermaphrodites (Spremberg et al. 2012). Hermaphrodites prepare special pockets for dwarf males called "receptacles," and therefore if many larvae want to become dwarf males, all of them cannot attach in receptacles, due to a limitation of the number and size of receptacles. Indeed, since this species lives in cold waters (Spremberg et al. 2012), the probability of becoming a large adult may be low, and the large hermaphrodite population may also be low. Due to this, the choice to become a dwarf male may be constrained. Those larvae that fail to settle on large individuals (hermaphrodites or females) are forced to remain small and immature and grow. This is plausible for other marine species as well, such as echiurans (Vollrath 1998; Jaccarini et al. 1983) and molluscs (Turner and Yakovlev 1983; Ó Foighil 1985).

The effect of the limited opportunity to become dwarf males is incorporated into the time-independent model (see Fig. 9.6). In this calculation, I assume that populations of each type of individual do not change at all and the two strategies of each individual are time independent. When the opportunity to become a dwarf male is high, I obtained similar results to the time-independent model (Fig. 9.7a): hermaphroditism or the coexistence of dwarf males and pure females depends on the growth rate of immature individuals g. If the growth rate g is large, all larvae first remain immature and then eventually become large hermaphrodites. Otherwise, some larvae become dwarf males, and the remainders become females without male function. However, when I calculate the evolutionarily stable strategies (c^*, m^*) under the opportunity to become a dwarf male is limited, the coexistence of dwarf males and hermaphrodites appears over a wide area (meshed region in Fig. 9.7b). The region of hermaphroditism is the same as that in Fig. 9.7a. Let us focus on the intermediate value of the growth rate g. In this case, the number of large individuals does not increase much because the growth rate is on the small side. New larvae prefer to attach to large individuals, but unfortunately, the number of receptacles is restricted. The exquisite balance between the above two conditions may promote a mixture of dwarf males and hermaphrodites.

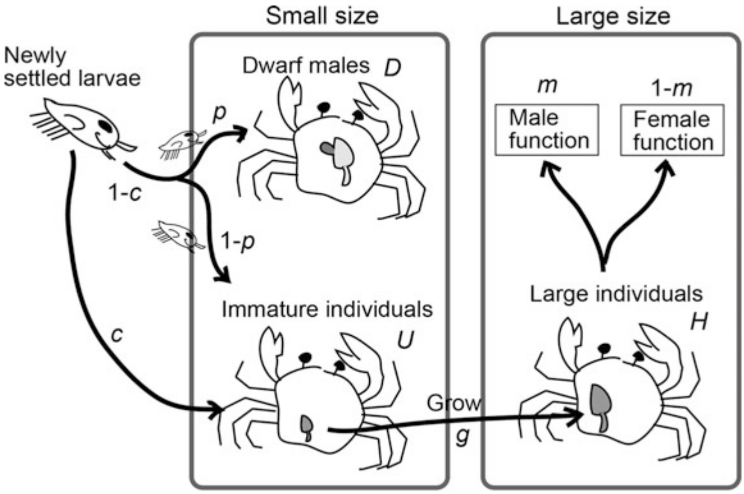

Fig. 9.6 The model scheme when the opportunity to become a dwarf male is limited. The proportion p of larvae that choose the dwarf male life history become dwarf males as desired. Unfortunately, the remaining $1 - p$ cannot become dwarf males and have to be immature individuals. Note that this figure differs from Fig. 9.1 regarding the opportunity limitation p

In general, the theory of evolutionarily stable strategy predicts that all types of strategies should have the same reproductive success when those strategies coexist stably. For example, denoting that the reproductive successes of a dwarf male and that of an immature individual are V_D and V_U, respectively, I have the following relationship:

$$\text{If } V_D = V_U, \text{ some proportion of larvae become dwarf males and the rest become immature individuals.} \tag{9.10}$$

If either life history has a higher reproductive success than the other one, all individuals would choose the former pathway adaptively.

However, in this model, where there is a limited opportunity to become a dwarf male, the reproductive success of a dwarf male V_D is not necessary equal to that of an immature individual V_U (Fig. 9.8). See the region where the growth rate $g < 0.3$ and the opportunity to become a dwarf male $p < 0.5$ in Fig. 9.8. This region shows that all larvae cannot become dwarf males despite $V_D > V_U$. In the current model, it is important to determine the point at which dwarf males stably coexist with hermaphrodites. The answer is that immature individuals have difficulty in growing into large adults and the opportunity to become a dwarf male is limited. Some larvae forcibly choose an immature-individual life history despite the fact that the dwarf male life history would provide a higher reproductive success than the route they have chosen. Interestingly, even if dwarf males coexist with pure females, there is a region where

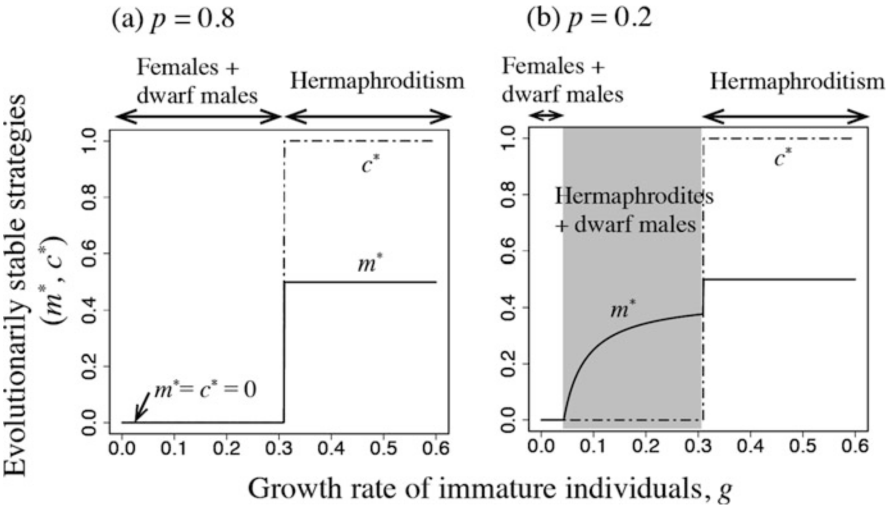

Fig. 9.7 Evolutionarily stable strategies and sexual systems with the opportunity limitation to become dwarf males. The horizontal axis is the growth rate of immature individuals g. The vertical axis is the evolutionarily stable strategy (c^*, m^*), where c^* is the proportion becoming immature individuals and m^* is the allocation ratio to male function of large individuals. When $c^* = 1$, all larvae become immature individuals. Despite all larvae aiming to be dwarf males if $c^* = 0$, actually only a proportion p of larvae can make this transition. (**a**) When the opportunity to become a dwarf male is high ($p = 0.8$). When the growth rate g is large, the sexual system exhibits simultaneous hermaphroditism. Otherwise, the sexual system exhibits the coexistence of dwarf males and females. (**b**) When the opportunity to become a dwarf male is low ($p = 0.2$). The meshed region shows the coexistence of dwarf males and hermaphrodites. The allocation ratio to male function of a large individual m^* increases as the growth rate g increases. The parameters are $\alpha = 0.5$, $u = 0.3$, and $\mu = 0.01$. Modified from Yamaguchi et al. (2013b)

the reproductive successes of the two life histories (dwarf males or immature individuals who will eventually mature as females) are not identical.

Another potential constraint of life history choice is the scarcity of opportunity to become growing immature individuals. If the population is very dense and the available substrata are scarce, some individuals cannot find suitable substrata to grow. Those who failed to become growing individuals have no other choice but to become dwarf males, even if the expected fitness is lower than that of growing individuals. Possible examples of such a constraint are species living in rare microhabitat, such as the parasitic bivalve on the mud shrimps (Kato and Itani 1995), the scalpellid barnacle on deep-sea shell debris or pumice (Ozaki et al. 2008; Yusa et al. 2012), or the bone worm (*Osedax*) on vertebrate bones sunken to the ocean floor (Rouse et al. 2004). As predicted, these animals have females and dwarf males. However, the limitation of microhabitats may enhance local competition for mates; the effect of local mate competition should also be considered.

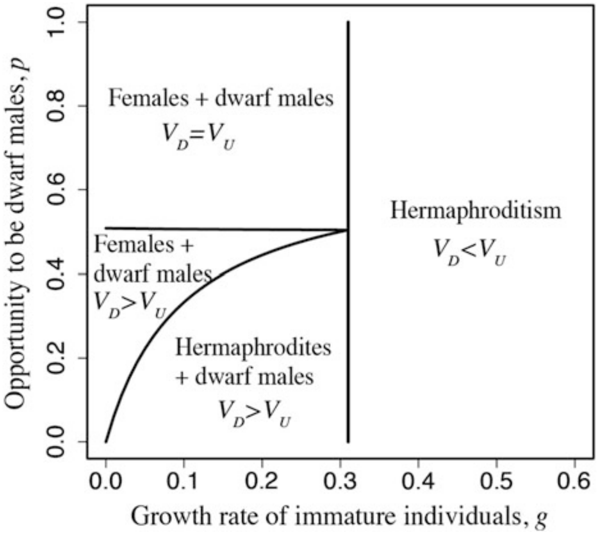

Fig. 9.8 Sexual systems depending on the growth rate for immature individuals *g* and the opportunity probability to become dwarf males *p*. During high growth rates ($g > 0.3$), the sexual system exhibits simultaneous hermaphroditism. When the growth rate *g* is small ($g < 0.3$), dwarf males appear. In this region, if the opportunity to become a dwarf male *p* is large ($p > 0.5$), dwarf males coexist with females, and the reproductive successes of a dwarf male and an immature individual are equal ($V_D = V_U$), while if the opportunity *p* is small ($p < 0.5$), the two reproductive successes are not identical, and the inequality $V_D > V_U$ holds. This means that, although the reproductive success of a dwarf male is higher than that of an immature individual, some larvae (proportion *p*) forcibly remain immature and grow to a large size, because of the severe opportunity limitation to be dwarf male. The parameters are $\alpha = 0.5$, $u = 0.3$, and $\mu = 0.01$. Modified from Yamaguchi et al. (2013b)

9.3 Discussion

Local mate competition in hermaphrodites leads to a decrease in male allocation due to competition among sperm from the same individual. Many previous theories focus on local mate competition as a main factor in the evolution of sexual systems (Charnov 1982, 1987; Yamaguchi et al. 2008, 2012, 2013a, b, c; Henshaw et al. 2014). In particular, local mate competition is supposed to favor the evolution of androdioecy (coexistence of dwarf males and hermaphrodites) among barnacles. If simultaneous hermaphrodites allocate a considerable proportion of available resources to male function, dwarf males cannot achieve sufficient fertilization success, since large hermaphrodites have much greater resources than dwarf males. Strong local mate competition favors a reduction in male allocation by hermaphrodites, allowing dwarf males to obtain sufficient success, despite their small amount of available resources (Charnov 1982, 1987; Yamaguchi et al. 2008, 2012, 2013a, b, c).

The importance of local mate competition is also suggested indirectly in the first model in this chapter (i.e., the time-independent model without temporal and spatial limitations). This model assumes that sperm are fully mixed within a population and thus no local mate competition occurs. For this assumption, dwarf male life history cannot be adaptive unless high mortality lowers the possibility of maturation as a large hermaphrodite. In contrast, the dioecy region in the time-independent model reveals the importance of sexual specificity in life history. The emergence of dwarf males requires conditions where the survival rate until maturation as a large hermaphrodite is smaller than the relative fertility of dwarf males to hermaphrodites. Under such conditions, the fitness of a dwarf male is greater than that of a hermaphrodite until the frequency of dwarf males is so high that a negative frequency-dependent selection based on the Fisher condition lowers their fitness. At this point, it is no longer adaptive for the remaining individuals to invest resources in male function, as dwarf males produce sufficient sperm in the population. According to the above argument, local mate competition is required for the coexistence of dwarf males and hermaphrodites to evolve; although the life history advantage may favor dwarf males without local mate competition, it can only lead to the evolution of a dioecious system, not androdioecious ones.

The evolution of androdioecy without local mate competition was subsequently modeled by considering life history constraints (i.e., the temporal limitation model with time-dependent strategies and the spatial limitation model with time-independent strategies), that is, that dwarf males and hermaphrodites have different life histories and thus have different ecological constraints. In the temporal limitation model, an individual who enters a young microhabitat can achieve higher reproductive success as a large hermaphrodite after staying immature while growing to full size. In contrast, an individual who enters an old microhabitat is likely to die before maturation if it attempts to grow into a large hermaphrodite. Such an unlucky individual who enters an old microhabitat should become a dwarf male to make the best of a bad situation. Although its fitness could be higher if it finds a young microhabitat where it would have sufficient time for growth, becoming a dwarf male is the optimal tactic for the unlucky individual who experiences an inferior environment.

The coexistence of dwarf males and females appeared in both the time-independent model and the temporal limitation model, but the mechanisms of coexistence were different. In the time-independent model, each individual adapts to the average environment in the population, and sexual differentiation in an evolutionarily stable ratio occurs, based on frequency-dependent selection. In contrast, in the temporal limitation model, each individual chooses the optimal tactic under its given environment (e.g., settlement timing to the microhabitat). Therefore, fitness differs between an individual who enters a good environment and one that enters a poor environment. Different life histories can coexist as alternative tactics, in the same way as alternative reproductive behaviors exhibited by male horned beetles that experience different nutritional conditions during larval development (Emlen 1997; Moczek and Emlen 2000).

The mechanism enabling the coexistence of different sexualities (e.g., dioecy, androdioecy) in the spatial limitation model is similar to that in the temporal limitation model. In the spatial limitation model, the difference in life history constraints among individuals is based on the difference of microscopic environmental conditions, instead of the different timing of settlement in the temporal limitation model. Thanks to this assumption, I successfully explained the stable coexistence of dwarf males and hermaphrodites.

The role of life history in the evolution of androdioecy is supported by some previous studies (Zardus and Hadfield 2004; Ewers-Saucedo et al. 2015, 2016: in the turtle barnacle *Chelonibia testudinaria*). If males and hermaphrodites have the same life history, males need to fertilize at least twice as many eggs as hermaphrodites, to coexist with hermaphrodites (Lloyds 1975; Charlesworth and Charlesworth 1978; Charnov 1987). However, dwarf males have a different life history to hermaphrodites (Zardus and Hadfield 2004; Ewers-Saucedo et al. 2015, 2016); they mature earlier (Vollrath 1998), and this is advantageous under low growth rates and high mortality rates. In such environments, dwarf males and hermaphrodites may have comparative fitness. Ewers-Saucedo et al. (2015, 2016) found that the mortality rate of an androdioecious barnacle *C. testudinaria* is higher than in other barnacle species with purely hermaphroditic sexual systems, suggesting that a sex-specific life history equalizes the fitness of males and hermaphrodites.

Notably, both the temporal and the spatial limitation models assumed that individuals chose their sexuality optimally and plastically based on their environment. Sexuality in the androdioecious barnacle *Scalpellum* is at least partly environmentally determined; larvae that attach to specialized pockets (receptacles) on hermaphrodite bodies differentiate into dwarf males (Svane 1986; Spremberg et al. 2012; Høeg et al. 2016). Thus, the opportunity to become a dwarf male may be limited by the availability of receptacles, suggesting that a limitation of life history choice is assumed in the spatial limitation model. Wijayanti and Yusa (2016) also reveal plastic sexual expression in another androdioecious barnacle, *Octolasmis warwickii*.

Androdioecy plays an important role as the evolutionary transitional state between dioecy and hermaphroditism (Weeks et al. 2006; Weeks 2012; Sawada et al. 2015). In this chapter, in addition, I propose that androdioecy may be a transitional state in the ecological time scale. That is, the same population exhibits different sexual systems such as hermaphroditism and dioecy depending on environmental factors, and androdioecy may appear as a transition between two different sexual systems. The description of sexual systems is commonly based on the sexuality of specimens sampled at a particular time. Therefore, such ecological transition of sexual systems may be overlooked. For example, the absence of dwarf males in one sampling is not necessarily an evidence of pure hermaphroditism. Long-term observation of sexuality in a population may help to identify such transitions within sexual systems.

Acknowledgments The models introduced in this chapter were results of collaborative works with Y. Iwasa, K. Sawada, and Y. Yusa. I am very grateful to E.L. Charnov and J.T. Høeg for the valuable discussion about barnacles' sexual systems and H. Kokko for helpful comments. I thank J.L. Leonard for encouraging me to write this chapter.

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Chapter 10
Sexual Systems in Shrimps (Infraorder Caridea Dana, 1852), with Special Reference to the Historical Origin and Adaptive Value of Protandric Simultaneous Hermaphroditism

J. Antonio Baeza

Abstract In this chapter, the diversity of sexual systems in the infraorder Caridea is summarized. Caridean shrimps exhibit six different sexual systems: gonochorism (separate sexes), strict protandry, partial protandry with primary females, partial protandry with primary males, partial protandry with primary males and primary females, and protandric simultaneous hermaphroditism. Within monophyletic clades belonging to the species-rich and ecologically dissimilar order Decapoda, gender expression is the most diverse in caridean shrimps. Additional studies on the life history and sexual system of caridean shrimps coupled with improvements on our understanding of the internal phylogenetic relationships within this species-rich clade are needed for a formal testing of transition asymmetries in the group. The historical origin of protandric simultaneous hermaphroditism, a sexual system that represents the "pinnacle" of evolution with respect to gender expression in the Caridea, remains to be fully understood. The conditions that maintain protandric simultaneous hermaphroditism include sex-dependent time commitments and sex-dependent energetic costs. The role of sexual selection in explaining the adaptive value of protandric simultaneous hermaphroditism needs to be addressed. Caridean shrimps represent excellent model systems to continue improving our understanding about the mechanisms explaining the diversity of gender expression patterns in nature.

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10.1 Introduction

Aquatic and terrestrial animals and plants exhibit remarkable disparity in terms of reproductive behaviors and sexual strategies (Ghiselin 1969; Charnov 1982; Policansky 1982; Klinkhamer et al. 1997; Shuster and Wade 2003; Chiba 2007; West 2009). For instance, hermaphroditism is the dominant sexual system in plants but is also common in various vertebrate and invertebrate animal clades (Ghiselin 1969; Klinkhamer et al. 1997). Classically, hermaphroditism is divided into strictly simultaneous, in which individuals allocate resources to and reproduce as both male and female at the same time, and strictly sequential, in which individuals experience a drastic and irreversible shift in sex allocation (i.e., sex change) at a certain age/size (Charnov 1982). In turn, gonochorism (separate sexes) is the dominant sexual system in animals, particularly among terrestrial vertebrates but is also observed in various invertebrate and plant taxa (Charnov 1982; West 2009). Our knowledge about sexual systems (gender expression patterns) and the conditions favoring and/or constraining them in marine and terrestrial animals and plants has increased substantially during the last decades (Ghiselin 1969; Charnov 1982; Klinkhamer et al. 1997; Shuster and Wade 2003; West 2009). Nonetheless, the sexual system of most animals and plants, particularly aquatic (both marine and freshwater) invertebrates, still remains unknown.

In animals, disparity in sexual systems is not as great as that reported for plants (see Charnov 1982; Policansky 1982; Klinkhamer et al. 1997; West 2009). Still, a number of recent studies have reported animal species featuring sex allocation patterns that do not conform to the classical distinction above among strictly sequential hermaphrodites, strictly simultaneous hermaphrodites, and gonochoric species (e.g., males coexisting with simultaneous hermaphrodites in androdioecious clam shrimps, Zucker et al. 1997; bidirectional sex change in goby fishes, St Mary 1994 and Munday et al. 1998; extreme female-biased sex allocation in simultaneous hermaphrodites, Baeza 2010a). One enigmatic example of mixed sexual systems in animals is protandric simultaneous hermaphroditism, in which individuals consistently mature and reproduce initially as males and later in life become functional simultaneous hermaphrodites. In animals, protandric simultaneous hermaphroditism has been confirmed in a polychaete worm (Sella 1990), an ascidian (Manríquez and Castilla 2005), two gastropod snails (Tomiyama 1996; Dahirel et al. 2016), and in various shrimps belonging to the genera *Lysmata*, *Exhippolysmata*, and *Parhippolyte* (Bauer and Holt 1998; Fiedler 1998; Braga et al. 2009; Baeza et al. 2010; Baeza 2013). Studies examining gender expression in animals, either terrestrial or aquatic, vertebrates or invertebrates, are warranted to improve our understanding regarding the conditions favoring and constraining dissimilar breeding strategies in nature.

Sex allocation theory is the body of models that attempts to explain the evolution and adaptive significance of different sexual systems, including mixed gender expression patterns such as protandric simultaneous hermaphroditism (Charnov 1982; West 2009). Sex allocation theory is not limited only to predicting those conditions explaining the evolution and stability of particular sexual systems. It also attempts to answer and unify questions such as (a) when and how should individuals adjust the sex of their offspring in response to environmental conditions, (b) when and in what direction are individuals selected to change sex, and (c) what is the optimal amount of resources that individuals should allocate to male and female reproduction in simultaneous hermaphrodites? Sex allocation theory, now considered one of the most robust branches of evolutionary biology, has been most useful in predicting and understanding those conditions favoring the evolution of strictly sequential and strictly simultaneous hermaphroditism (Charnov 1982; West 2009). However, theoretical studies conducted in an attempt to explain the evolution of mixed sexual systems, such as protandric simultaneous hermaphroditism, still lag behind (see, i.e., Cadet et al. 2004 for an exception).

In this chapter, my first goal is reviewing the diversity of sexual systems in the infraorder Caridea Dana, 1852, one of the most species-rich clades of decapod (order Decapoda) crustaceans. Caridean shrimps also exhibit remarkable morphological, ecological, and behavioral disparity (Bauer 2004; De Grave et al. 2009) (Fig. 10.1). Revealing transition pathways between dissimilar gender expression patterns is most relevant to understand their adaptive value (Weeks et al. 2009). Thus, after summarizing sexual systems disparity in caridean shrimps, I propose transition scenarios connecting dissimilar gender expression patterns in this clade. Importantly, during the last decade, several studies attempting to understand the (1) historical origin and (2) adaptive value of gender expression patterns in shrimps have been conducted in protandric simultaneous hermaphrodites. Thus, my third and final goal is to review the available literature dealing with the two topics above (1 and 2) in protandric simultaneous hermaphrodites. I decided to focus on protandric simultaneous hermaphrodites because of two main reasons. First, the latter sexual system has provided an opportunity to test predictions at the core of sex allocation theory (Bauer 2000; Baeza and Bauer 2004; Baeza 2006, 2007a, b, 2013). Second, sex allocation theory has been most useful to understand the historical origins and adaptive value of this “mixed” gender expression pattern (see Baeza 2013 and references therein). Indeed, caridean shrimps exhibiting protandric simultaneous hermaphroditism have turned into one of a few biological systems within crustacean decapods that has improved our understanding about the mechanisms explaining the diversity of gender expression patterns in nature (Bauer and Holt 1998; Bauer 2000, 2002a, b, 2004; Baeza and Bauer 2004; Baeza 2006, 2007a, b, 2013). A review of the literature therefore is likely to help guiding future experimental work and comparative studies in this group so to continue improving our understanding of gender expression in animals and beyond.

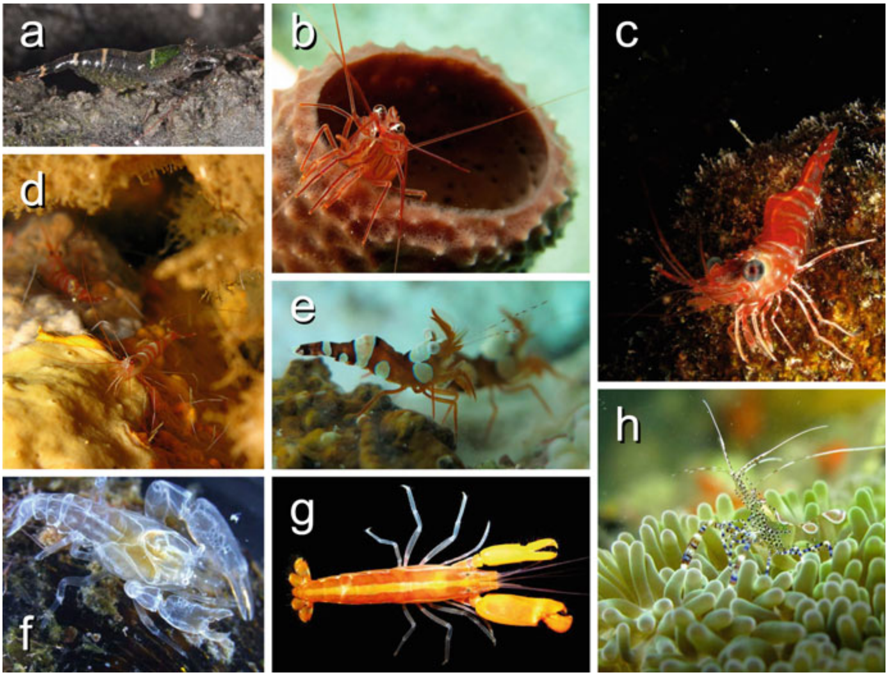

Fig. 10.1 Diversity in shrimps belonging to the infraorder Caridea. (**a**) The semiterrestrial shrimp *Merguia rhizophorae* (fam. Merguiidae) observed crawling off the water in the intertidal zone of a mangrove forest in Brazil. (**b**) The peppermint shrimp *Lysmata pederseni* (fam. Lysmatidae) is most often found as long-term monogamous pairs inside the tubes of the sponge *Callyspongia vaginalis* in Belize and Panama. (**c**) The hingebeak shrimp *Cinetorhyncus* cf. *rigens* is active at night in shallow reefs in the Caribbean Sea (fam. Rhynchocinetidae). (**d**) The enigmatic shrimp *Parahippolyte misticia* (fam. Barbouridae), a common inhabitant of deep marine caves in the Indo-Pacific. (**e**) The squat-anemone shrimp *Thor amboinensis* (fam. Thoridae) is a protandric species most often found living in groups around the column or perching to the tentacles of various sea anemones in tropical latitudes worldwide. (**f**) The Atlantic pearl oyster shrimp *Pontonia manningi* (fam. Palaemonidae, subfam. Pontoniinae) lives in the mantle cavity of various bivalves, including the winged oyster *Pteria colymbus* in the Caribbean and tropical western Atlantic. (**g**) Habitus (dorsal view of the entire animal) and color pattern of the stripped snapping shrimp *Alpheus formosus* (fam. Alpheidae), a common inhabitant of intertidal shores in the tropical and subtropical western Atlantic. (**h**) The shrimp *Periclimenes yucatanicus* (fam. Palaemonidae) living in association with the sun sea anemone *Stichodactyla helianthus* in Panama. Photographic credits: all photographs by J. Antonio Baeza other than (**a**) by Arthur Anker

10.2 Sexual Systems in the Infraorder Caridea

A review of the literature shows that caridean shrimps exhibit six different sexual systems (Fig. 10.2). A brief review of each sexual system highlighting relevant details follows.

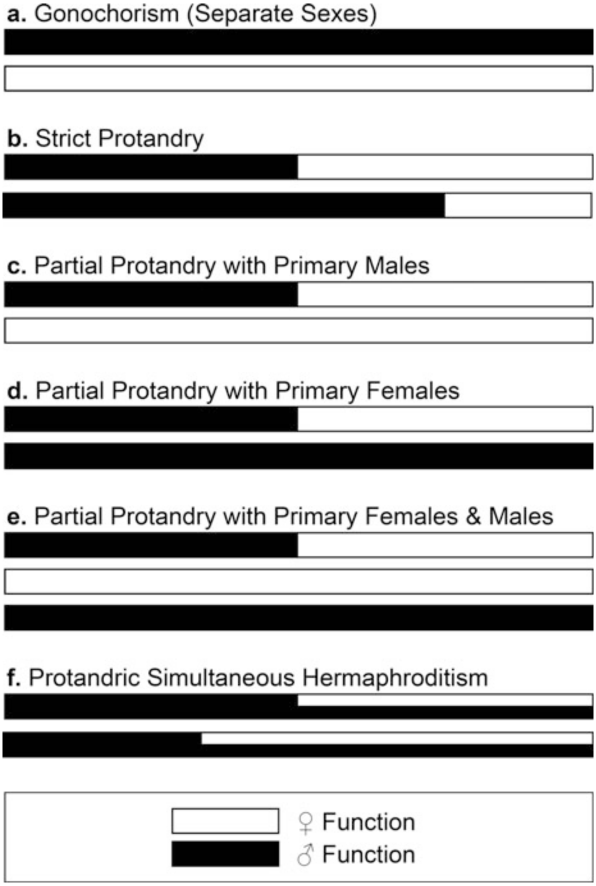

Fig. 10.2 Ontogeny of sexual systems in species belonging to the infraorder Caridea. (**a**) In gonochoric species, populations are made up of individuals expressing either the male or female function during their entire lifetime. (**b**) In strictly protandric species, each individual in the population invariably matures and reproduces as male first and later in life changes sex to female. The timing of sex change can be variable among individuals within the same population or from different populations, and this behavioral flexibility is denoted in the diagram depicting ontogenetic trajectories, one in which an individual changes sex early (top bar) in life compared to another changing sex later in life (bottom bar). (**c**) In species exhibiting partial protandry with primary males, populations are made up of individuals reproducing only as males during their entire life and protandric individuals that change sex from male to female. (**d**) In species exhibiting partial protandry with primary females, populations are made up of individuals reproducing only as females during their entire life and protandric individuals that change sex from male to female. (**e**) In species exhibiting partial protandry with primary males and females, populations are made up of individuals reproducing only as males during their entire life, only as females during their entire life, and protandric individuals that change sex from male to female. (**f**) In species exhibiting protandric simultaneous hermaphroditism, each individual in the population invariably matures and reproduces as male first and later in life becomes a simultaneous hermaphrodite that reproduces as both male and female. The timing of sex phase change can be variable among individuals within the same population or from different populations, and this behavioral flexibility is denoted in the diagram depicting ontogenetic trajectories, one in which an individual turns into simultaneous hermaphrodite later (top bar) in life compared to another changing sex phase earlier in life (bottom bar)

10.2.1 Gonochorism: Separate Sexes

Separate sexes are often assumed to be the most common sexual system in caridean shrimps (Fig. 10.2a). Nevertheless, studies formally testing for gonochorism in this group are rather uncommon. Three recent studies in hippolytid (fam. Hippolytidae) shrimps, *Hippolyte inermis*, *H. niezabitowskii*, and *H. williamsi*, all small species that inhabit shallow seaweed meadows and/or seagrass beds in temperate/subtropical environments, serve as an example to depict the distribution of primary and secondary male and female sexual characters in gonochoric shrimps (Espinoza et al. 2008; Cobos et al. 2005, 2010; Manjón-Cabeza et al. 2009, 2011).

In these three species, males have gonopores on the coxae of the fifth pair of pereopods but no (female, see below) gonopores on the coxae of the third pair of legs (Fig. 10.3a, b). In *H. williamsi*, the male gonopores are clearly defined with a posterior border that protrudes greatly, forming a rounded lip (Espinoza et al.

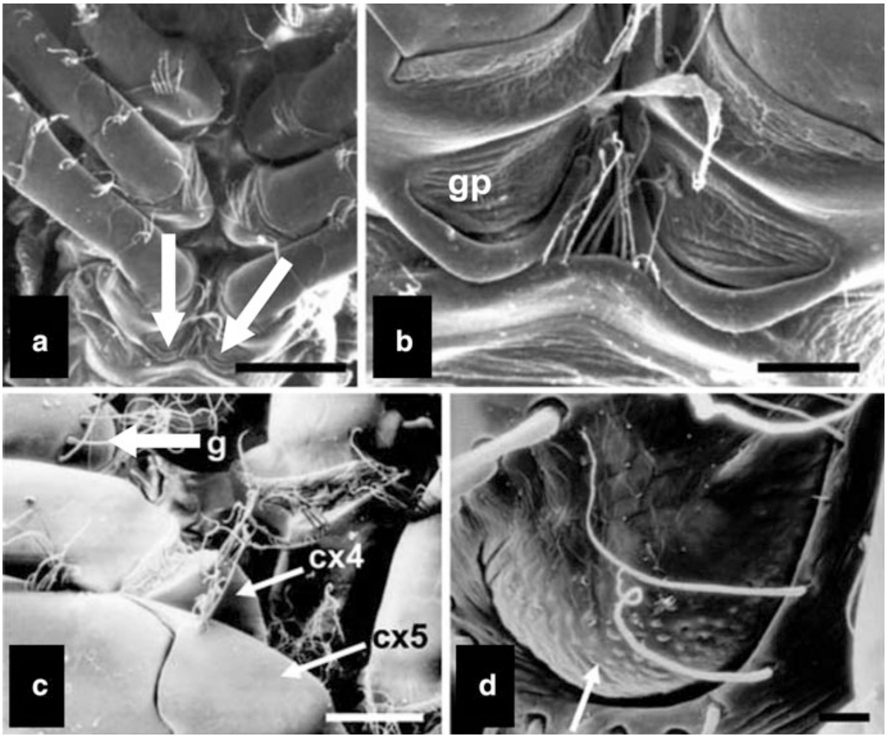

Fig. 10.3 Scanning electron microscopy (SEM) view of gonopores in male and female individuals of the gonochoric shrimp *Hippolyte williamsi*. (**a**) Ventral view of the male gonopores (arrows) on the coxae of the fifth pair of pereopods. (**b**) Detail of the male gonopores (gp). (**c**) Ventral view of the female gonopores (g, arrows) on the coxae of the third pair of pereopods. The fifth pereopod (cx5) coxae shows no sign of male gonopore. cx4 = coxae of fourth pereopod. (**d**) Detail of a female gonopore (arrow). Modified from Espinoza et al. (2008)

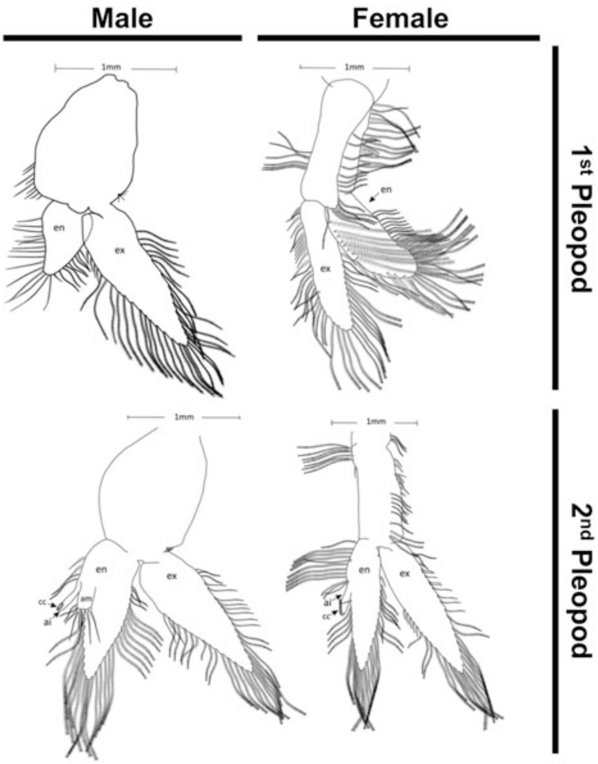

Fig. 10.4 First and second pleopods in mature males and females of the gonochoric shrimp *Hippolyte williamsi*. Left top, endopod (en) and exopod (ex) of second pleopod in male individual. Right top, endopod (en) and exopod (ex) of first pleopod in female individual. Left bottom, endopod (en) and exopod (ex) of second pleopod in male individual. Note the inner margin of the second pleopod showing appendix masculina (am) bearing spines and appendix interna (ai) bearing coupling hooks (cc). Right bottom, endopod (en) and exopod (ex) of second pleopod in female individual. Note the inner margin of the second pleopod showing appendix interna (ai) bearing coupling hooks (cc) but lacking appendix masculina. Modified from Espinoza et al. (2008)

2008). In mature males of *H. williamsi*, the exopods from the first pair of pleopods are approximately twice the length of the endopods (Fig. 10.4). In the three species, the endopods from the first pair of pleopods have no appendix interna and no cincinnuli (tiny hooks or hook-like structures) (Fig. 10.4). By contrast, the endopods from the second pair of pleopods had an appendix interna with numerous cincinnuli and an appendix masculina with long straight setae at the distal end (Fig. 10.4). In all three species, the gonads dissected from male shrimps are paired testes located on the dorsal side of the cephalothorax, above the hepatopancreas, and below the heart (Fig. 10.5a). Vasa deferentia that develop into terminal and expanded ejaculatory ducts, full of spermatophores, connect the gonads to the gonopores (Espinoza et al. 2008; Cobos et al. 2005, 2010; Manjón-Cabeza et al. 2009, 2011). In caridean shrimps, spermatophores are simple: a cylinder with two layers, one with mucous

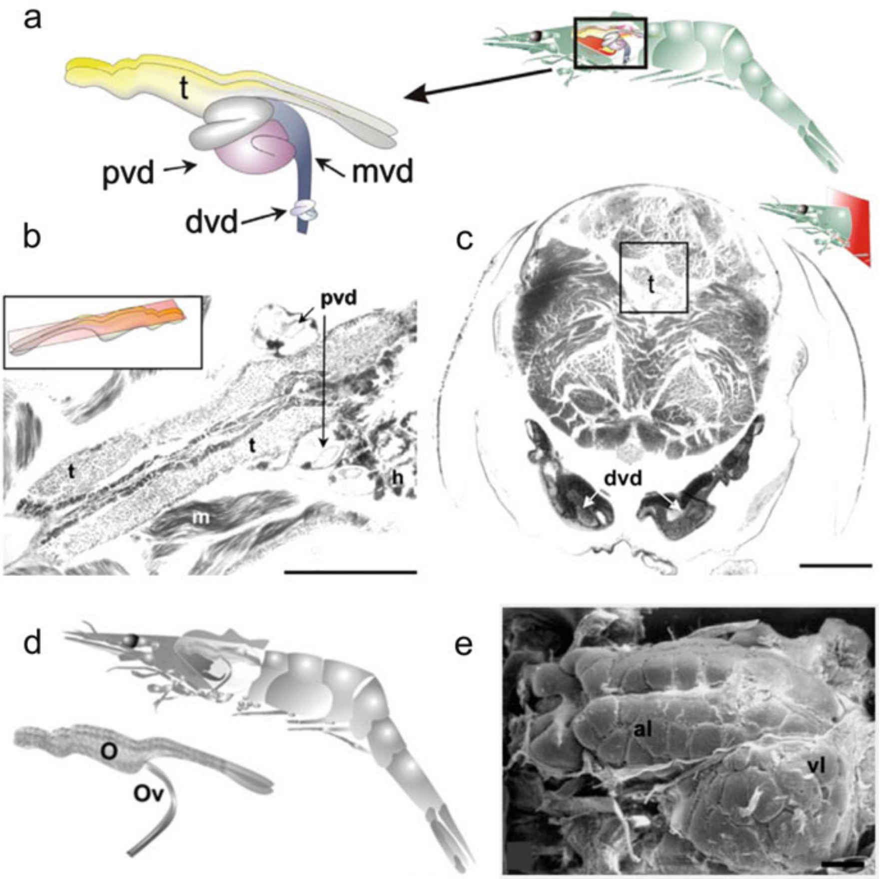

Fig. 10.5 Male and female internal anatomy of the reproductive system in gonochoric shrimp belonging to the genus *Hippolyte* sp. (**a**) Diagram of the male reproductive system in *H. niezabitowskii* and its different structures, their morphology, and their location. (**b**) Dorsal section of the paired testes in *H. niezabitowskii*. Mallory's trichrome stain. Scale bar = 0.5 mm. The inset shows the exact position of the section in the testes. (**c**) Transverse section of the carapace in male individual of *H. niezabitowskii* (at almost the height of the coxa of the fifth pereopod). Toluidine blue stain. Scale bar = 0.1 mm. (**d**) Diagram of the female reproductive system in *H. niezabitowskii* and its different structures, their morphology, and their location. (**e**) Lateral view of mature ovary in female of *Hippolyte williamsi*. *al* anterior lobe, *dvd* distal vas deference, *h* hepatopancreas, *m* muscle, *mvd* medial vas deferens (left), *o* paired ovaries, *ov* oviduct, *pvd* proximal vas deference (left), *t* paired testes, *vl* ventral lobe. Modified from Espinoza et al. (2008) and Manjón-Cabeza et al. (2011)

substance and spermatozoa and another layer composed of structural substances (Bauer 2004; Cobos et al. 2010).

In turn, in females, the gonopores, located in the third pair of pereopods, are not clearly delimited and often consist of a slit-like opening closed by a thickened valve-like flap or cover (Fig. 10.3c, d). Setae surrounding the gonopores are visible in both mature and immature females. By contrast, the male gonopores are not flanked by setae (Fig. 10.3a, b). In females, the endopods from the first pair of pleopods have no appendix interna and cincinnuli (Fig. 10.4). The endopods of the second pair of pleopods have an appendix interna with numerous cincinnuli but lack an appendix masculina (Fig. 10.4). The gonads of both ovigerous and nonovigerous females are paired ovaries lying above the hepatopancreas, below the heart, and extending into the first abdominal segment (Fig. 10.5). Oviducts connect the gonads with the gonopores located in the coxae of the third pereopods (Cobos et al. 2005).

Additional details about the anatomy of the male and female reproductive system can be found in Cobos et al. (2010) and Manjón-Cabeza et al. (2011). Most gonochoric shrimps follow the pattern described above, but some minor variability exists in the Caridea (reviewed in Bauer 2004). For instance, two genera represent exceptions to the general pattern above that are worthy to highlight. In the genera *Synalpheus* (fam. Alpheidae) and *Janicella* (fam. Oplophoridae), male shrimps do not bear appendices masculinae on the second pleopods (Banner and Banner 1975; Dardeau 1984; Bauer 2004; De Grave et al. 2009). Sexing in this shrimps is difficult and relies mostly on the position of the gonopores. In the gonochoric *H. williamsi* and *H. inermis*, as well as in other gonochoric shrimps in which differences between the sexes, both in terms of body size and body parts (see below), are considerable, neither of the two sexes change sex at any moment during their life (Fig. 10.2a).

In most caridean shrimps, a gonochoric sexual system has been inferred based on the study of the population dynamics and a detailed examination of male and female primary and secondary sexual characters in individuals with a wide range of body sizes. In addition to *H. inermis*, *H. niezabitowskii*, and *H. williamsi*, other studies have shown the same gonochoric condition in *Hippolyte obliquimanus* (Terossi et al. 2008), *Nauticaris marionis* (Yaldwyn 1966), *Tozeuma carolinense* (Ewald 1969), and *Heptacarpus pictus* (Bauer 1976), all of the above also belonging to the fam. Hippolytidae. Other gonochoric species include the pandalid (fam. Pandalidae) shrimp *Pandalus propinquus* (Jagersten 1936 in Butler 1964), *P. bonnieri* (Pike 1952), and *Dichelopandalus leptocerus* (Stevenson and Pierce 1985). In the family Thoridae, confirmed cases of gonochorism include *Thor dobkini* and *T. floridanus*, two species that inhabit shallow-water seagrass beds in the Greater Caribbean (Bauer and VanHoy 1996). In the family Processidae, well-studied examples include *Processa bermudensis* and *P. riveroi* (Bauer and Conner 2011). Detailed anatomical and morphological studies have demonstrated that the sexual system in three eusocial alpheid shrimp (fam. Alpheidae), *Synalpheus chacei*, *S. rathbunae*, and *S. regalis*, is gonochory (Toth and Bauer 2007). In other genera belonging to the same family Alpheidae, a well-studied species that is gonochoric is *Betaeus lilianae* (Baeza et al. 2010c). In the family Pontonidae, gonochoric species include various symbiotic shrimps in the genus *Pontonia* (*P. margarita*, Baeza et al. 2008; *P. mexicana*, Baeza et al. 2011; *P. manningi*, Baeza et al. 2016), *Paranchistus pycnodontae* (Baeza et al. 2013), and *Odontonia katoi* (Baeza et al. 2015).

Also, detailed studies on the population dynamics and the distribution of male and female primary and secondary sexual characters in shrimps of different sizes have demonstrated that another hippolytid shrimp, *Hippolyte inermis*, is gonochoric (Cobos et al. 2005; Manjón-Cabeza et al. 2009; see also Reverberi 1950).

Importantly, a conspecific population from Italy has been reported to be protandric (Zupo 1994, 2000, and references therein). The controversy above might well be explained if the two sampled populations pertain to two different cryptic species, each one with a different sexual system (Espinoza et al. 2008). The sexual system of *H. inermis* is still a topic of debate that does need further attention (see Manjón-Cabeza et al. 2009).

Gonochoric shrimps might or not be sexually dimorphic in terms of body size and/or body parts. When dimorphic, the differences between the sexes might be minimal or considerable. For instance, *H. williamsi* and various other gonochoric shrimps exhibit marked "reverse" sexual dimorphism: on average, females attain much larger body sizes than conspecifics males (Espinoza et al. 2008). By contrast, in other species exhibiting "classical" sexual dimorphism, males attain much larger average and maximum body sizes than females (Bauer 2004). In some of this species with classical sexual dimorphism, additional differences between the sexes can be found in the shape and relative size of body structures often used for combat. These structures include claws and/or maxillipeds (Correa and Thiel 2003). Interestingly, in a few species in which males are much larger than females, males exhibit different morphotypes (e.g., the freshwater shrimp *Macrobrachium rosenbergii*, Barki et al. 1991; the marine shrimp *Rhynchocinetes typus*, Correa and Thiel 2003). For instance, in *R. typus*, competitively dominant "robustus" males coexist in the same population with sneaker "typus" males (Correa and Thiel 2003). Dominant "robustus" males monopolize females during mating interactions, while smaller "typus" male morphotypes with less developed chelipeds and maxillipeds sneak mating opportunities when receptive females are released during combats among "robustus" males (Thiel and Hinojosa 2003). A similar pattern has recently been described for two other congeneric species (*R. brucei*, Thiel et al. 2010; *R. durbanensis*, Sanjeevi et al. 2015).

10.2.2 Strict Protandry

In strictly sequential protandric hermaphrodites, shrimps invariably mature first as males and change later in life into females (Fig. 10.2b). Small sexually mature males have typical external male characters (i.e., gonopores on the coxae of the fifth pair of pereopods and appendices masculinae on the second pair of pereopods). Their internal anatomy includes paired testes on top of the hepatopancreas, vasa deferentia, ejaculatory ducts, and associated androgenic glands, leading to the male gonopores. At some point in time during their lifetime, males become "transitionals"; their gonads change from testes to ovaries and develop other primary and secondary female sexual characters (Fig. 10.6a–c). Transitionals are thus individuals in the process of changing sex with a combination of typical male and female internal and external traits. For instance, in the strictly sequential hermaphrodite *Thor amboinensis*, transitionals bear female and male gonopores, respectively, in the coxae of the third and fifth pereopods, appendices masculinae in the second pleopods, and ovaries with vitellogenic (yolky) oocytes (Baeza and Piantoni 2010)

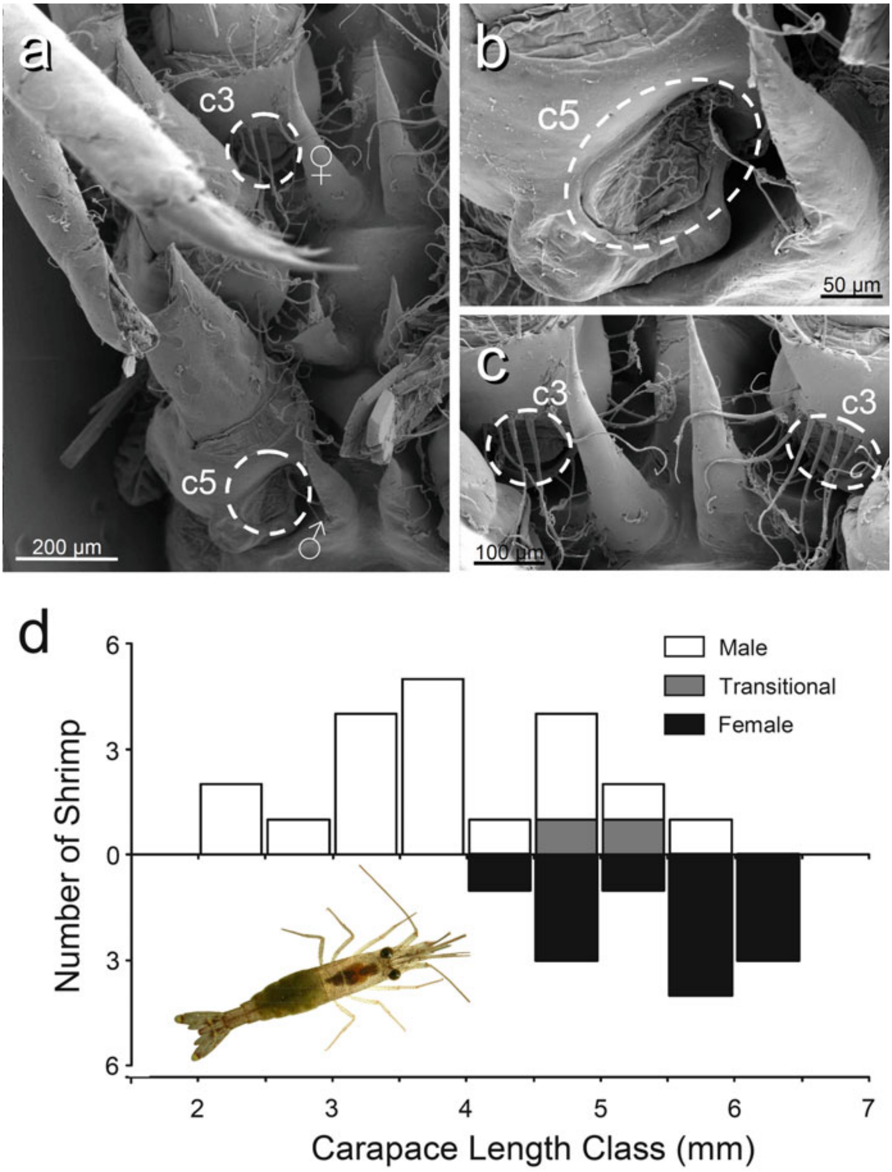

Fig. 10.6 Strict protandric hermaphroditism. Scanning electron microscopy (SEM) view of transitional individual in *Thor amboinensis* and population structure in *Merguia rizhophorae*. (**a**) Ventral view of transitional shrimp in *Thor amboinensis*; male and female gonopores on the coxae of the fifth (c5) and third (c3) pereopods, respectively. The male (♂) and female (♀) gonopores are encircled. (**b**) Ventral view of transitional shrimp in *Thor amboinensis*; detail of the male gonopore on coxae of fifth pereopod (encircled). (**c**) Ventral view of transitional shrimp in *Thor amboinensis*; detail of the female gonopores on coxae of third pereopods (encircled). (**d**) Population structure of the strict protandric shrimp *Merguia rhizophorae* at Isla Colon, Bocas del Toro Archipelago, Caribbean coast of Panama. Note the presence of transitional individuals of intermediate body size compared to males and females in the same population. Modified from Baeza (2010a, b) and Baeza and Piantoni (2010)

(Fig. 10.6a–c). It is not clear yet if sex change in strictly sequential hermaphrodites occurs over a single, a few, or over a longer set of instars (see Bauer 2004).

Strict protandric hermaphroditism was reported early during the 1920s and 1930s (Berkeley 1929) and then confirmed during the 1960s (Butler 1964) for various pandalid shrimps (fam. Pandalidae) belonging to the genera *Pandalus* (i.e., *P. borealis*, *P. danae*, *P. goniurus*, *P. hypsinotus*, *P. jordani*, *P. montagui*, *P. platyceros*, *P. stenolepis*, *P. tridens*) and *Pandalopsis* (i.e., *Pandalopsis dispar*). Protandry was also early recognized in members pertaining to the genera *Campylonotus* in the fam. Campylonotidae (*Campylonotus rathbunae* and *C. semistriatus*, Yaldwyn 1960, 1966) and *Chorismus* in the fam. Hippolytidae (*Chorismus antarcticus*, Yaldwyn 1966). These studies relied on detailed examination of the population dynamics and external male and female primary sexual characters (i.e., presence of appendices masculinae and embryos) in shrimps of different body size classes (Berkeley 1929; Butler 1964, 1980; see also Stevenson and Pierce 1985). In all these sex-changing shrimps, the presence of transitional individuals with a body size intermediate between that of males and females in the population was noticed at some time point during the year (often spring) (e.g., Fig. 10.6d). Later studies during the 1980s inferred strict protandry in representatives of various caridean families (see Table 1 in Brook et al. 1994). Most recently, strict protandry has been recognized in shrimp belonging to the families Merguiidae (i.e., *Merguia rhizophorae*, Baeza 2010a, b), Thoridae (i.e., *Thor amboinensis*, Baeza and Piantoni 2010), and Rhynchocinetidae (*Rhynchocinetes uritai*, Bauer and Thiel 2011). Interestingly, studies conducted during the 1980s incorrectly (see below) reported strict protandry in representatives of the family Lysmatidae (i.e., *Lysmata seticaudata*, Charniaux-Cotton 1965 and references therein).

In a few pandalid shrimps, some authors have mentioned the occasional occurrence of either juveniles that mature directly as females and skip the male phase or juveniles that mature early as females after spending a short nonreproductive time period as males (see Bauer 2000 for a review). Individuals of protandric pandalid shrimp species which begin life as females are referred to as primary females; those which change sex in their first year following the repression of male sex characteristics and never function as males are called secondary females; and those which function first as males and then change sex are called hermaphroditic females (Mistakidis 1957; Stevenson and Pierce 1985). However, more detailed studies in the same species have not find evidence of such primary females [i.e., in *Pandalus borealis*, presence vs. absence of primary females (Allen 1959 vs. Butler 1967); in *Pandalus jordani*, presence vs. absence of primary females (Tegelberg and Smith 1957 vs. Butler 1967)]. In two species, *P. hypsinotus* and *P. montagui*, however, early maturing (primary) females that skipped the male phase were confirmed by detailed analysis of the population dynamics (Butler 1980; Stevenson and Pierce 1985). Whether or not these primary females correspond to shrimps with the same genotype than that in sequential hermaphroditic shrimps but that change sex early in life in order to optimize their fitness according to environmental, including social, conditions (Charnov 1982) or if they exhibit a “pure female” genotype different from that of sex changing individuals remains to be clarified (Charnov et al. 1978; Charnov 1981, 1982; Bergstrom 1997; Bauer 2000).

10.2.3 Partial Protandry with Primary Females

In protandric hermaphroditism with primary females, a portion of the population comprises individuals that develop, mature, and reproduce as females and never change sex. In turn, the remaining portion of the population develop and mature into functional males that later in life change sex and thus turn into functional females (Fig. 10.2c). This sexual system has been inferred for four species (i.e., *Processa edulis*, Noël 1973, 1976; *Crangon crangon*, Boddeke et al. 1991, Schatte and Saborowski 2006; *Crangon franciscorum*, Gavio et al. 2006; *Argis dentata*, Fréchette et al. 1970) (Fig. 10.7). Importantly, the evidence for protandry with primary females in *Processa edulis* has been debated (Labat and Noël 1987; Bauer and Conner 2011), and the question about the sexual system exhibited by this species begs further attention. Various species of *Pandalus* might also exhibit this sexual system (e.g. *Pandalus hypsinotus*, see above) if further experimentation and genomic analyses demonstrate that early maturation of juveniles as females is not due to phenotypic plasticity of the timing of sex change but rather genomic determination (see above and below).

In *Processa edulis* (fam. Processidae) and *Argis dentata* (fam. Crangonidae), the sexual system has been inferred based on studies on the population dynamics and a detailed examination of male and female primary and secondary sexual characters and their distribution (presence/absence) with shrimp body size (Noël 1973, 1976; Fréchette et al. 1970; see also Bauer 2004) (Fig. 10.7). It is worthy to highlight that species belonging to the family Crangonidae are unique among caridean shrimps in that the second pleopods lack the appendix interna, and the appendices masculinae in the second pleopods are relatively small (Butler 1980). Also, in *Processa edulis*, sexual characters present in mature females include a seminal receptacle ventrally located between the coxae of the fifth pereopods and the first abdominal segment (Noël 1976). This receptacle is absent in all other caridean shrimp with the exception of the freshwater atyid shrimp *Atyaephyra desmaresti* (Noël 1976; Descouterelle 1971 in Bauer 2004). In *Crangon crangon*, additional laboratory experiments have been conducted to test for sex change in male individuals. Interestingly, out of 70 males maintained in the laboratory over a period of 8 months, only a single shrimp did experience sex reversal suggesting that *C. crangon* is a facultative rather than an obligate partial protandric hermaphrodite (Schatte and Saborowski 2006). Although herein I have classified *C. crangon* as a partial protandric hermaphrodite, in line with Bauer (2002a, b), the details about the sexual system of *C. crangon* are still a subject of debate and controversy (Schatte and Saborowski 2006; and references therein).

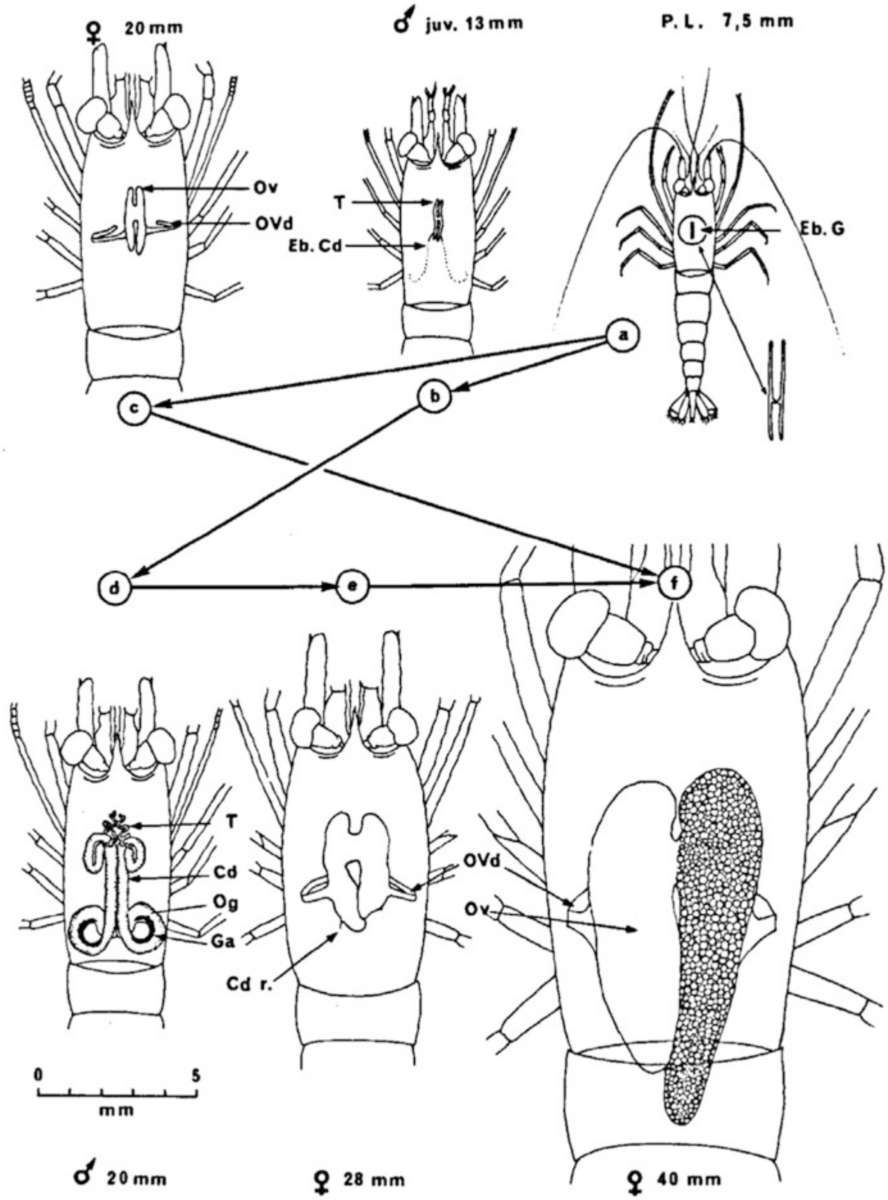

Fig. 10.7 Ontogeny of gender expression in *Processa edulis*, a shrimp exhibiting partial protandry with primary females. (**a**) Postlarval individual, (**b**) juvenile male in the process of sexual maturation, (**c**) primary female (nonreproductive), (**d**) reproductive (adult) male, (**e**) juvenile secondary female, (**f**) reproductive female, either primary or secondary exhibiting vitellogenic ovaries. *Cd* vas deferens, *Cd r* residual vas deferens, *Eb.Cd* vas deferens, *Eb.G.* gonad, *Ga* androgenic gland, *Og* gonopore, *Ov* ovaries, *Ovd* oviduct, *T* testes. Modified from Noël (1976)

10.2.4 Partial Protandry with Primary Males

In protandric hermaphroditism with primary males (or lifetime males), a portion of the population comprises individuals that develop, mature, and reproduce as males and never change sex. In turn, the remaining portion of the population develops and matures into functional males that later in life change sex and turn into functional females (Fig. 10.2d). This sexual system has been inferred in two alpheid species from the genus *Athanas*: *A. indicus*, a species which lives in symbiosis with the sea urchin *Echinometra mathaei* in the Indo-Pacific (Gherardi and Calloni 1993), and *A. kominatoensis*, inhabiting among the spines of the purple sea urchin *Anthocidaris crassispina* in Japan (Nakashima 1987). In these species, male sex types (primary males and sex-changing males) do not differ in their external morphological characteristics (Nakashima 1987; Gherardi and Calloni 1993). Importantly, data on population dynamics, histology, and the rearing of males in the laboratory was used to suggest secondary sex change (from female back to male) in females of *A. kominatoensis* (Suzuki 1970). Nonetheless, a later study by Nakashima (1987) in the same species provided more detailed information against the purported ability of females to revert back to males. Still, the possibility that some females change sex again into males in *A. kominatoensis* remains an open question (Fig. 10.8).

10.2.5 Partial Protandry with Primary Males and Primary Females

In protandric hermaphroditism with primary males and primary females, a portion of the population comprises individuals that develop, mature, and reproduce as males and never change sex. Another portion of the population comprises individuals that develop, mature, and reproduce as females and never change sex. Lastly, the remaining portion of the population develop and mature into functional males that later in life change sex turning into functional females (Fig. 10.2e).

This sexual system has been reported for one species of thorid (fam. Thoridae) shrimp, *Thor manningi* (Bauer 1986; Bauer and VanHoy 1996). In *T. manningi*, the population is composed by ~50% primary males (individuals born male with prehensile third pereopods that do not change sex), ~49% protandrous hermaphrodites (males without prehensile pereopods that change sex to females later in life), and ~1% primary females (born and remaining female during their entire life) (Bauer 1986). Interestingly, in this species, primary males exhibit substantial morphological differences that make them easily distinguishable from protandric males. Primary males remain small and have hypertrophied male appendices and prehensile third pereopods, while male-phase hermaphrodites exhibit relatively small appendices masculinae and do not bear prehensile third pereopods (Bauer 1986) (Fig. 10.9).

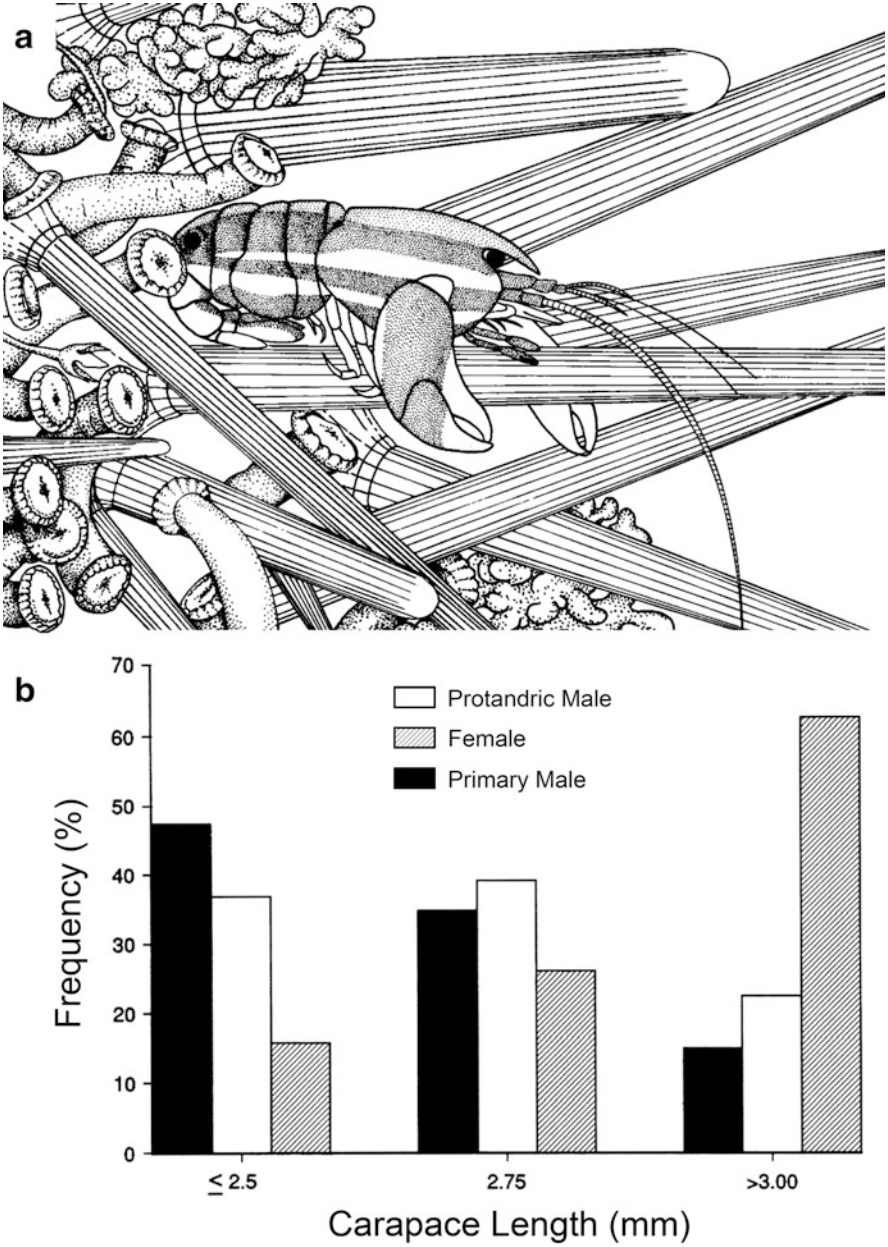

Fig. 10.8 Partial protandry with primary males in *Athanas indicus*. (**a**) Shrimp perching to a spine of its host sea urchin *Echinometra mathaei*. (**b**) Sex ratio at three different body size classes in *A. indicus*. Note that protandric males change sex over a large range of body size. Modified from Gherardi and Calloni (1993)

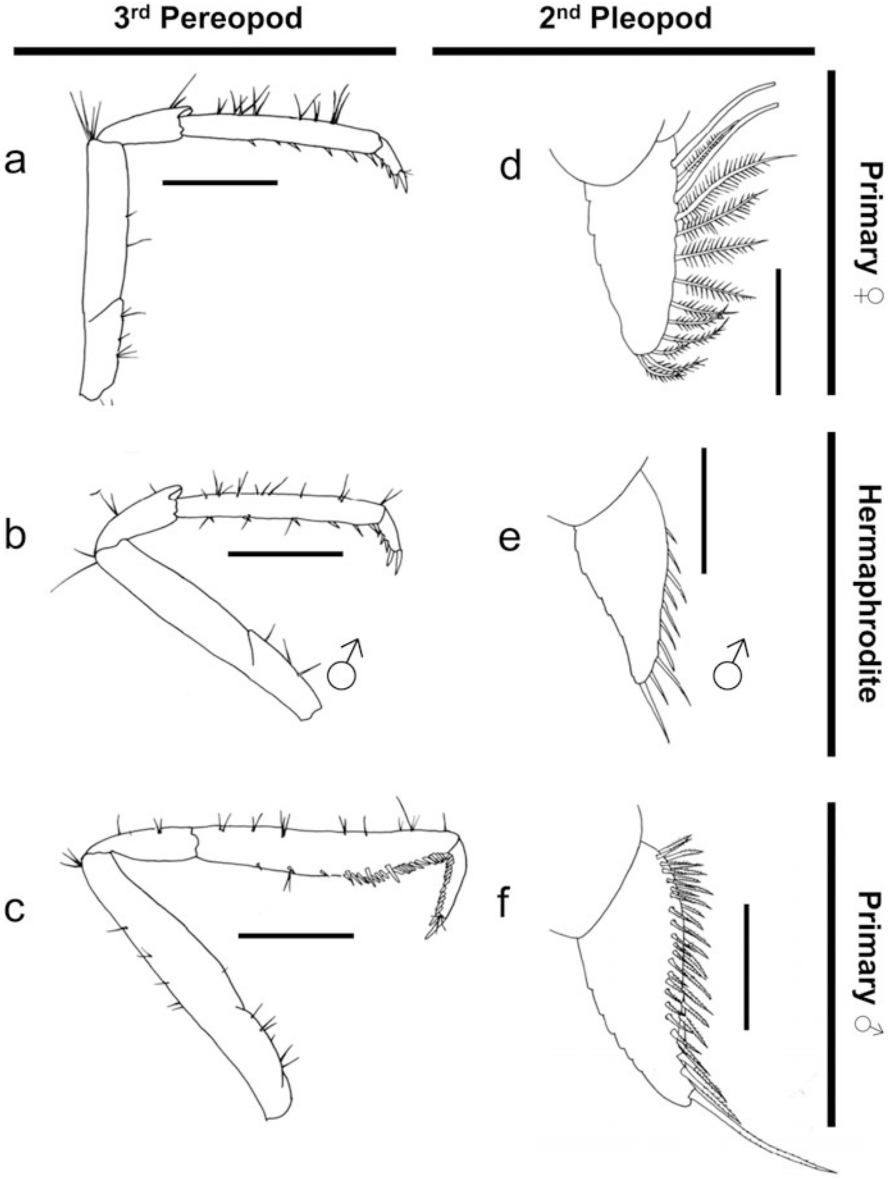

Fig. 10.9 Partial protandry with primary males and primary females in *Thor manningi*. (**a**) Third pereopod (lateral view) of brooding female. (**b**) Third pereopod of male-phase hermaphrodite. (**c**) Third pereopod of primary male, (**d**) endopods of the right first pleopod of brooding female, (**e**) endopods of the right first pleopod of male-phase hermaphrodite, (**f**) endopods of the right first pleopod of primary male. Scale bars represent 0.5 mm in (**a**), (**b**), and (**c**) and 0.2 mm in (**d**), (**e**), and (**f**). Modified from Bauer (1986)

10.2.6 Protandric Simultaneous Hermaphroditism

In protandric simultaneous hermaphrodites, juveniles consistently mature as male individuals which bear “typical” caridean external male secondary characters and are capable of reproducing only as males (Bauer and Holt 1998). Males later attain the female sexual function, developing into functional simultaneous hermaphroditic individuals [herein called hermaphrodites but also called “female-phase” individuals by Bauer and Holt (1998), Bauer (2000, 2002a, b, 2004, 2006), and Baldwin and Bauer (2003) and “euhermaphrodites” by Lin and Zhang (2001) and Zhang and Lin (2004)]. After maturation, hermaphrodites resemble females of caridean gonochoric species, brooding embryos in an abdominal chamber. However, hermaphrodites retain testicular tissue, male ducts (Fig. 10.10), and gonopores and reproduce as both male and female (Bauer and Holt 1998).

Copulation is not reciprocal in these shrimps: i.e., a newly molted pre-spawning hermaphrodite copulates as a female, with a male or another hermaphrodite acting exclusively as a male at that time. Both males and hermaphrodites can mate as male at any time, even minutes before and after molting (Bauer and Holt 1998). A spermatophore is attached to the underside of the pre-spawning hermaphrodite by the mating partner during copulation that lasts a few seconds. Sperm from a mating is used to fertilize only the eggs released during the spawning event that occurs 2–3 h later, because there is no long-term sperm storage in caridean shrimps (Bauer and Holt 1998). After becoming hermaphrodites, individuals do not revert to their initial sexual condition (Bauer 2002a), and self-fertilization has been tested for but not observed in hermaphrodites (Bauer 2002a; Baeza 2013 and references therein). Hermaphrodites likely go through successive cycles of vitellogenesis, molting, mating (as female), spawning, brood incubation, and hatching, serving the male-role when possible, until death (Bauer 2002a, b).

Protandric simultaneous hermaphroditism has been reported before for all species of the genera *Lysmata* and *Exhippolysmata* whose sexual system has been examined (Bauer and Holt 1998; Baeza 2009, 2013 and references therein). Thus, protandric simultaneous hermaphroditism appears to be a fixed trait in the family Lysmatidae (Baeza 2013). In the closely related family Barbouriidae, protandric simultaneous hermaphroditism seems to occur in *Parahippolyte misticia* and *P*. cf. *uveae* (Onaga et al. 2012; Baeza, unpubl. data). Studies on the external morphology and internal anatomy of shrimps also suggest protandric simultaneous hermaphroditism in *Calliasmata* spp. (formerly belonging to the fam. Lysmatidae) and *Barbouria* spp. (Baeza, unpubl. data). Nonetheless, functionality of the hermaphroditic shrimps still needs to be confirmed in the latter two genera as well as in the genus *Janicea*.

Most recently, in *Salmoneus carvachoi* (fam. Alpheidae), a species living solitarily or in pairs within burrows of mud and snapping shrimps, it has been demonstrated that all specimens (brooding or not embryos) bear appendices masculinae on the endopods of the second pleopods. In burrows harboring two shrimps, pairs consist of two brooding shrimps, two non-brooding shrimps, or one brooding and one non brooding shrimp. The presence of appendices masculinae in all collected

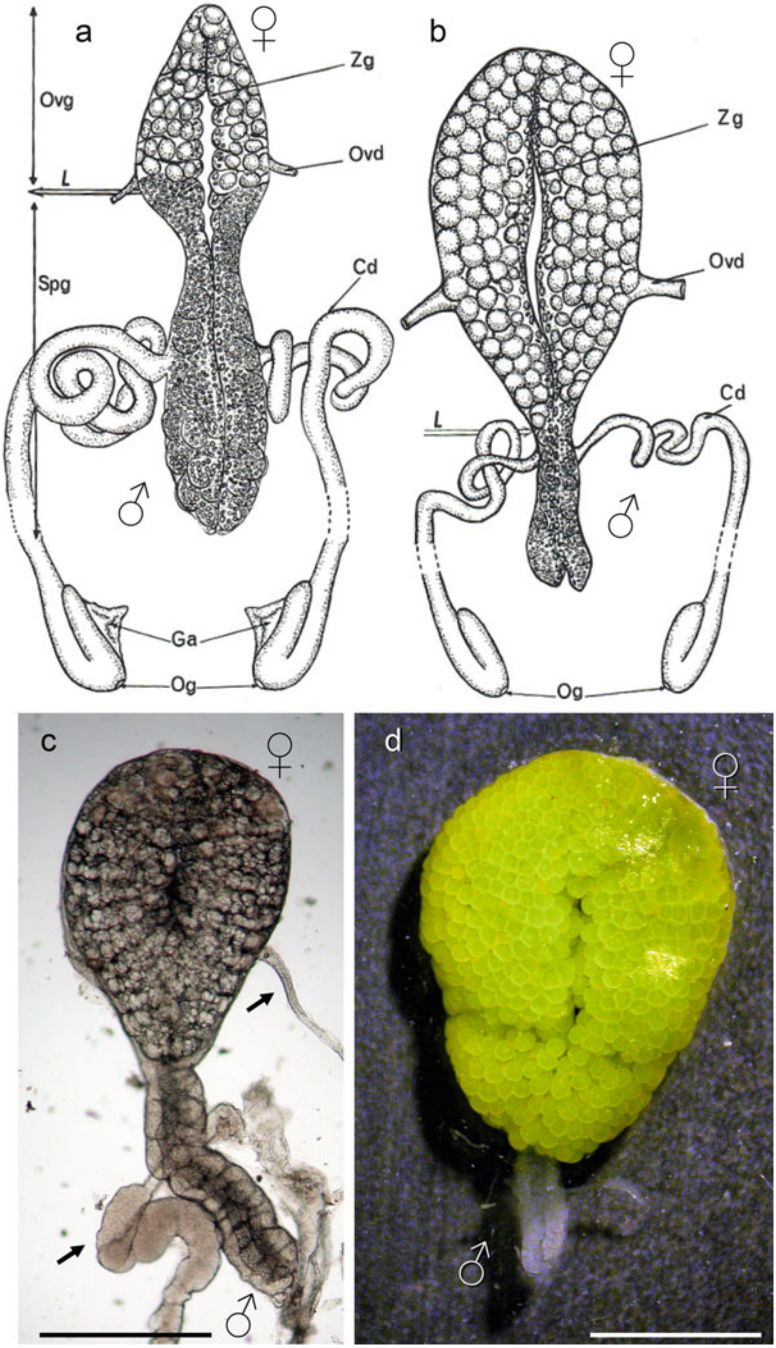

Fig. 10.10 Male (male-phase) and simultaneous hermaphrodite (female-phase) internal anatomy of the reproductive system in protandric simultaneous hermaphroditic shrimps belonging to the genus *Lysmata*. (**a**) Diagram of the male reproductive system in *L. seticaudata*. (**b**) Diagram of the

shrimps, whether brooding or not embryos, and the pairing of brooding shrimps within host burrows suggest that *S. carvachoi* is likely a protandric simultaneously hermaphroditic shrimp (Oliveira et al. 2015).

Lastly, in the two cryptic species of *Synalpheus paraneptunus* (fam. Alpheidae), most individuals in the population have been shown to be intersex, possessing both male and female gonopores. Whether intersexuality is due to functional simultaneous hermaphroditism and sexual immaturity or represents a developmental anomaly without particular adaptive significance in otherwise gonochoric species remains to be addressed in these cryptic species (Tóth and Bauer 2008; see also Toth and Bauer 2007).

10.3 Transitions Among Sexual Systems in the Infraorder Caridea

Understanding the details of transitions between dissimilar gender expression patterns is most relevant to improve our knowledge about the evolution and adaptive value of sexual systems in nature (see Weeks et al. 2009). Transitions among dissimilar sexual systems have been studied before in several plant taxa (Barrett 2013 and references therein) but only in a few marine invertebrate clades (e.g., polychaete worms in the genus *Ophriotrocha*, Heggøy et al. 2007). Unfortunately, with one exception (see below), studies examining transitions between sexual systems in caridean shrimps are lacking. In crustaceans, to the best of my knowledge, this type of study has been conducted before in three groups of non-decapod crustacean (in clam shrimps [Brachiopoda], Weeks et al. 2009; in tadpole shrimps [Branchiopoda], Mathers et al. 2013; in barnacles [Cirripedia], Yusa et al. 2012). In the following, I propose different transition scenarios connecting the different sexual systems observed in the infraorder Caridea.

To explain transitions in gender expression patterns in this group, at least from a heuristic viewpoint, a simple genetic sex-determining model can be used as a framework. In such model, mutations can result either in the activation or inactivation (silencing) of the male and/or female function (e.g., at specific moments during lifetime) in individuals already expressing the opposite sex function (see

Fig. 10.10 (continued) simultaneous hermaphroditic reproductive system in *L. seticaudata*. (**c**) Photograph of the male reproductive system in *L. holthuisi*. Scale bar = 1000 micrometers. The top and bottom arrow points to the female oviduct and male vas deferens, respectively. (**d**) Photograph of the simultaneously hermaphroditic reproductive system in *L. hochi*. Scale bar = 1 cm. *Cd* vas deferens, *Ga* androgenic gland, *L* limit between oogenesis and spermiogenesis, *Og* male genital aperture, *Ovd* oviduct, *Ovg* oogenesis in the anterior part of the gonad, *Spg* spermatogenesis, *Zg* germinative zone. Male and female symbols also indicate the testicular and ovarian part of the gonad in (**a**), (**b**), (**c**), and (**d**). Modified from Charniaux-Cotton (1975), Baeza and Anker (2008), and Baeza (2008)

Charlesworth and Charlesworth 1978; Sassaman 1995; Weeks et al. 2009; and references therein). Depending upon the number of mutation steps and/or events (e.g., extinction of sexual types) in an ancestral population that ultimately result in the progression from one gender expression pattern to another, putative transition scenarios between sexual systems can be more or less parsimonious.

Considering the rationale above, a theoretical transition from partial protandry either with primary males or primary females to strict protandry in the Caridea can occur under a relatively simple scenario that includes one step: the extinction of (e.g., competitively inferior) males or females, respectively, in the partially hermaphroditic ancestral population. The transition from strict protandry to partial protandry, either with primary males or primary females, represents a more complex evolutionary scenario as it requires two mutational steps, one activating the male or female function later or early in life, respectively, together with a second step silencing the male or female function early or later in life, respectively, in strictly protandric individuals belonging to an ancestral population (Fig. 10.11).

Similarly, a direct transition from partial protandry with primary males and females to strict protandry, partial protandry with primary males, partial protandry with primary females, or gonochorism can occur under relatively simple scenarios that include the extinction of (e.g., competitively inferior) both males and females, only females, only males, or only strictly protandric individuals, respectively, in an ancestral populations in which all three morphotypes (sexual types) coexist. The transition to partial protandry with primary males and females from strict protandry, either with primary males or primary females, again involves more complex evolutionary scenarios requiring various mutational steps. For instance, among various possible pathways, transitions from partial protandry, either with primary males or primary females, should be more probable than from strict protandry or gonochorism as the former two transitions entail a mutation in a strict protandric hermaphrodite silencing one of the sex functions. In contrast, transition to partial protandry with primary males and females from strict simultaneous conditions needs two parallel mutations, one silencing only the male function and another silencing only the female function at the same time in different strict protandric shrimps belonging to the same ancestral population (Fig. 10.11).

With regard to species with separate sexes, the transition from gonochorism to partial protandry with primary males or with primary females is expected if a single mutation, either in male or female individuals, activates either the female function or the male function later or early in life, respectively, occurring together with a second mutation silencing the female function later in life or the male function early in life. These mutations can occur theoretically in members of the two sexes and ultimately lead to populations in which pure males or pure females coexist with strict protandric individuals. Direct transitions from either partial protandry with primary males or with primary females rely on the reverse mechanism above. The transition of gonochorism directly to strict protandry is less likely, yet still plausible, as it requires two parallel events, one activating the female function later in life in males together with the elimination of the population of members of the opposite sex.

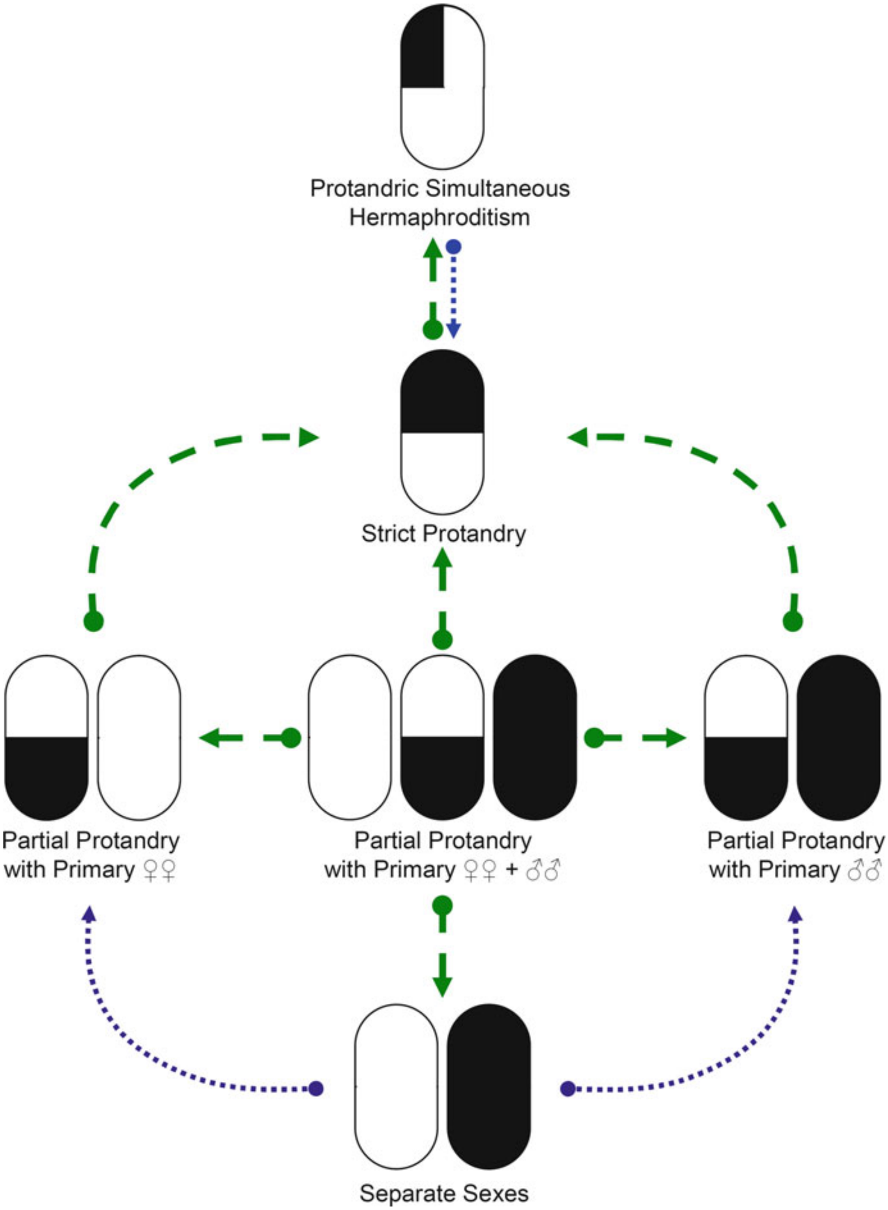

Fig. 10.11 Transition among the sexual systems exhibited by shrimp belonging to the infraorder Caridea. The expression of male and female functions during the ontogeny of shrimp individuals (ovoids) are represented as black and white colors, respectively. Bottom and top of each ovoid represent early and late during adult life of an individual, respectively. Thick dashed (green) lines represent most parsimonious transitions, while thin dotted (blue) lines represent less parsimonious transitions considering a simple genetic model. Transitions between sexual systems not considered parsimonious given the relatively large number of parallel events required for the evolutionary shift from one to another sexual system are not noted but are still plausible

Lastly, the transition from strict protandry to protandric simultaneous hermaphroditism is expected if a single mutation activates the male function later in life in individuals belonging to a population comprised of strict sequential hermaphroditic individuals. The reverse transition from protandric simultaneous hermaphroditism to strict protandry requires the silencing of the female function later in life in an originally protandric simultaneously hermaphroditic individual. Less likely, but still plausible, is the direct transition from gonochorism to protandric simultaneous hermaphroditism. The progression of gonochorism to protandric simultaneous hermaphroditism likely requires the existence of other "transitory" sexual systems serving as intermediate evolutionary stages between the above (Fig. 10.11).

Taking into account the diversity of sexual systems herein shown in the Caridea and the putative scenarios depicted above is possible to predict that particular sexual transitions will be more common than others during the evolutionary history of this group. For instance, transitions from partial protandry with primary males and females to partial protandry with primary males, or with primary females, or strict protandry, should be much more common that transitions between gonochorism and protandric simultaneous hermaphroditism. Unfortunately, the scarcity of studies on the life history and sexual system of caridean shrimps together with the incomplete understanding of the internal phylogenetic relationships within this species-rich clade (but see Baeza 2013; De Grave and Fransen 2011) is precluding, at present, a formal testing of transition asymmetries within a phylogenetic framework (e.g., as in Weeks et al. 2009). To the best of my knowledge, a detailed examination of the historical events correlating with the origin of a particular sexual system has been conducted only in the case of protandric simultaneous hermaphroditic shrimps (Baeza 2013). In short (see below), I discuss the historical origins of protandric simultaneous hermaphroditism in the infraorder Caridea.

It should be noted that limited experimental research suggests that the default sex is female in caridean shrimps (and other crustaceans) and that the "turning" on and off of an "androgenic gland" (AG, located near the ejaculatory ducts in some shrimps, Bauer 2004) produces maleness (AG turned on) or femaleness (AG turned off) in the absence of secretions (hormones) from this AG (Charniaux-Cotton and Payen 1975 and references therein; also see Bauer 2004). Therefore, although I have suggested above various scenarios that involve a minimum of two mutational steps for the transition of a particular sexual system to another, it might well be that only a single mutation is necessary for such transition to occur: either a mutation silencing a putative gene(s) for the androgenic gland function with age (resulting in male to female sex change, e.g., in the pandalid *Pandalus platyceros*, Hoffman 1972) or a single mutation activating the androgenic gland anlage, which all decapods, male and female individuals, possess (e.g., in the thorid *Thor manningi*, Bauer 1986). Certainly, additional studies on the genomics and physiology of sex determination and sex reversal are warranted as they will help improve our understanding of sexual system transitions in caridean shrimps.

10.4 The Historical Origin of Protandric Simultaneous Hermaphroditism

Protandric simultaneous hermaphroditism has been suggested to represent the "pinnacle" of evolution with respect to gender expression in the Caridea (Bauer 2000, 2004, 2006). Although recent studies have shown that the variety of lifestyles in the genus *Lysmata* and allies is greater than originally recognized (Baeza et al. 2008; Baeza and Anker 2008; Baeza 2009), an emerging dichotomy in social organization and ecology was noticed by initial studies (Bauer 2000, 2002a, b). One group of species (named "crowd" species by Bauer 2000) was described as inhabiting warm subtropical environments, occurring as dense aggregations in their refuges and exhibiting no specialized fish-cleaning behavior (i.e., *Lysmata californica*, Bauer and Newman 2004; *L. wurdemanni*, Baeza 2006) (Fig. 10.2). A second group (named "pair" species by Bauer 2000) was described as mostly tropical, occurring at low densities in the subtidal, and dwelling as socially monogamous pairs on sea anemones used as spots for fish-cleaning activities (i.e., *L. grabhami*, Wirtz 1997; *L. amboinensis*, Fiedler 1998). Based on the dichotomy above, Bauer (2000) proposed a "historical contingency hypothesis" to explain the evolution of protandric simultaneous hermaphroditism in the genus *Lysmata*. According to his model, protandric simultaneous hermaphroditism evolved in the tropics from an ancestral strictly protandric species of *Lysmata* that, in response to predation pressure, adopted a symbiotic lifestyle (i.e., associating with sea anemones) and became a specialized fish cleaner. Restricted mobility of individuals due to their association with sessile hosts, and, hence, reduced probability of encountering mating partners, would have favored simultaneous hermaphroditism (also, see Bauer 2006).

Interestingly, Bauer's (2000) hypothesis assumes a transition from strict protandry to protandric simultaneous hermaphroditism in an ancestral species of *Lysmata* and is also a more specific case of the more general model proposed by Tomlinson (1966) and Ghiselin (1969): simultaneous hermaphroditism (with an early male phase in the case of *Lysmata*) is advantageous when population density is low because the probability of finding sexual partners is negligible (Tomlinson 1966; Ghiselin 1969). Importantly, the body of literature on the natural history of the family Lysmatidae and allies (fam. Barbouridae and Merguiidae) accumulated during the last years and advances in ancestral character-state reconstruction using maximum likelihood and Bayesian inference (see Baeza 2013) did make it possible to test whether the ancestral *Lysmata* shrimp featured a symbiotic lifestyle and a socially monogamous mating system as proposed by Bauer (2000).

Recently, Baeza (2013) tested whether the ancestral *Lysmata* was (1) socially monogamous and (2) symbiotic with, e.g., sea anemones [as predicted by Bauer's (2000) model]. To accomplish this goal, Baeza (2013) first developed a multi-locus phylogeny of shrimps from the infraorder Caridea with emphasis in the families Lysmatidae, Barbouriidae, and Merguiidae. Next, the resulting phylogenetic tree was used as a framework to map the lifestyle (symbiosis vs. facultative symbiosis vs. obligatory symbiosis) and socio-ecology (gregarious behavior vs. group-living vs. "socially monogamous" pair-living) of shrimps. Finally, the likelihood of specific traits occurring at particular ancestral nodes in the phylogeny was tested. If the historical contingency hypothesis proposed by Bauer (2000) does explain the origin of SH in caridean shrimps, then, Baeza (2013) predicted that the ancestral Lysmatidae should exhibit a symbiotic lifestyle and a socially monogamous (pair-living) mating system (also, see Bauer 2000).

A phylogenetic tree comprising representatives of the families Lysmatidae, Barbouriidae, and Merguiidae and other genera within the family Hippolytidae that resulted from a combined analysis of three molecular markers, one mitochondrial gene (16S) and two nuclear genes (H3 and Enolase), was sufficiently robust for the exploration of the conditions favoring simultaneous hermaphroditism (Baeza 2013). Mapping of the lifestyle and socio-ecology on the phylogeny of shrimps using maximum likelihood and Bayesian inference suggested that the ancestral Lysmatidae was most likely free-living and either exhibited a gregarious behavior or lived in small groups but was not socially monogamous. The reconstruction also suggested a single historical scenario within the Lysmatidae that included four different transitions from a free-living to a symbiotic lifestyle (either facultative or obligatory) (Fig. 10.12). In this scenario, facultative use of crevices used by other organisms (i.e., moray eels) originated twice independently: once in *L. seticaudata* from the Mediterranean and a second time in *L. californica* from the northeastern Pacific. Also, obligatory symbiosis originated twice independently during the evolutionary history of these shrimps: once in *L. debelius* from the Indo-Pacific (host: sea anemones) and a second time in *L. pederseni* from the Caribbean (host: tube sponges). The strictly symbiotic lifestyle characteristic of the "cleaner" clade (represented by *L. debelius* in Baeza's 2013 study), the neotropical *L. pederseni*, and the before mentioned facultative association of *L. seticaudata* and *L. californica* with moray eels represent four independent origins of a derived symbiotic lifestyle according to the analyses. In general, the analyses unequivocally demonstrated that the most recent common ancestor of the family Lysmatidae was not socially monogamous (Fig. 10.12).

Overall, the ancestral character-state reconstruction analysis conducted in Baeza's (2013) study did not provide support for Bauer's (2000) hypothesis on the evolution of SH in the Lysmatidae; simultaneous hermaphroditism did not evolve in the tropics from an ancestral species of Lysmatidae that became a specialized fish cleaner and develop symbiosis with sessile marine invertebrates (e.g., sea anemones) (Bauer 2000, 2002a, b, 2006). Furthermore, Baeza's (2013) formal testing of sexual system progression in the Caridea suggests that transitions between sexual systems do not necessarily involve the most parsimonious pathway.

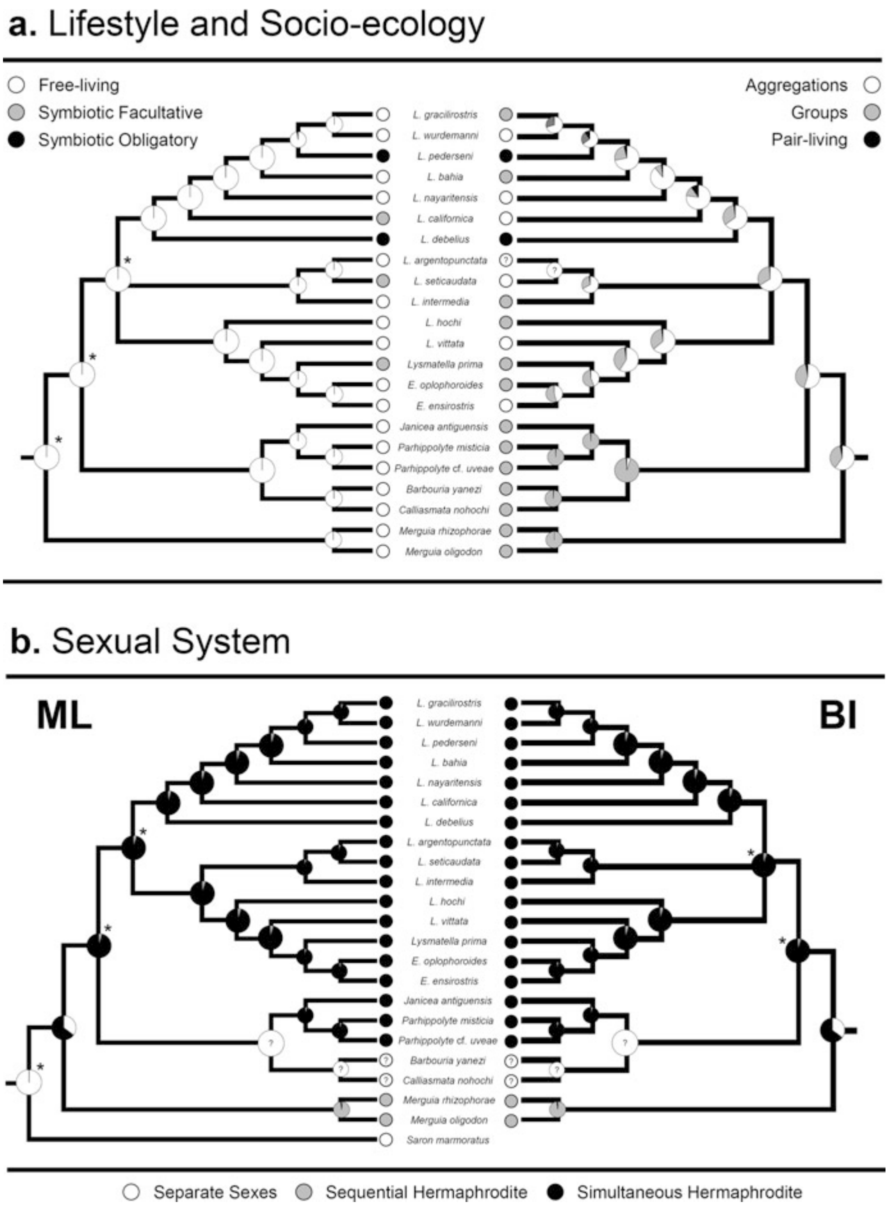

Fig. 10.12 The historical origin of protandric simultaneous hermaphroditism in caridean shrimp *Lysmata* and allies. (**a**) Lifestyles (left) and socio-ecology (right) of broken-back shrimps from the genus *Lysmata* (L.) and allies and maximum likelihood ancestral character-state reconstruction using the phylogenetic hypothesis that resulted from a total evidence maximum likelihood analysis. Column of circles to the right and left of the list of species represents lifestyle (free-living, facultative symbiosis, obligatory symbiosis) and socio-ecological (gregarious, group-living, pair-living) traits exhibited by the species herein studied. Pie charts on the nodes indicate the probabilities for maximum likelihood estimates of the different ancestral character states. Asterisks in the nodes depicting the most recent common ancestor of the Lysmatidae and Lysmatidae + Barbouriidae indicate that the inferred character state is significantly more likely to occur than

10.5 The Adaptive Value of Protandric Simultaneous Hermaphroditism

In addition to understanding the details of transitions between dissimilar gender expression patterns, determination of the selective forces favoring these transitions or maintaining them once they have originated is most relevant to improve our understanding about the origin and adaptive value of sexual systems (see Weeks et al. 2009). Indeed, the selective pressures which favor one sexual system over another among closely related species also represent an outstanding yet unresolved question about sexuality not only in caridean shrimps but beyond (Bauer 2002a; Bauer and Conner 2011; Baeza 2006, 2013). The adaptive value of the different sexual systems present in the infraorder Caridea has been discussed by several authors before (Charnov 1982; Bauer and VanHoy 1996; Correa and Thiel 2003; Bauer 2004; Espinoza et al. 2008; among others). Nonetheless, to the best of my knowledge, a detailed dissection of the different environmental determinants favoring a particular sexual system that has also profited from an experimental approach has been conducted only in the case of protandric simultaneous hermaphroditic shrimps (Baeza 2006 and references therein). In the following, I focus in discussing the conditions that maintain protandric simultaneous hermaphroditism in the infraorder Caridea.

The question to answer here is: once protandric simultaneous hermaphroditism has evolved (see above), what conditions maintain it? Interestingly, given its (putative) rarity, no specific hypotheses have been put forward to explain the adaptive value of mixed sexual systems like protandric simultaneous hermaphroditism. Nonetheless, various hypotheses have been proposed through the years to account for size-dependent sex allocation reported for various strict simultaneous hermaphroditic species (i.e., de Jong and Klinkhamer 1994; Klinkhamer et al. 1997; Day and Aarssen 1997; St Mary 1997; Angeloni et al. 2002; Cadet et al. 2004). Baeza (2006) suggested that the theoretical frameworks above might prove useful for understanding the adaptive value of protandric simultaneous hermaphroditism. Specifically, if protandric simultaneous hermaphroditism is considered as a form of size-dependent sex allocation in which small individuals allocate energy exclusively to the male function while large individuals allocate energy to both male and female functions,

Fig. 10.12 (continued) other character states in the same node. (**b**) Sexual systems of broken-back shrimps from the genus *Lysmata* and allies and maximum likelihood ancestral character-state reconstruction using the phylogenetic hypothesis that resulted from total evidence maximum likelihood and Bayesian Inference analyses. Column of circles to the right and left of the list of species represents sexual systems (separate sexes, strict sequential hermaphroditism, protandric simultaneous hermaphroditism) exhibited by the species herein studied. Pie charts on the nodes indicate the probabilities for maximum likelihood estimates of the different ancestral character states. Asterisks in the nodes depicting the most recent common ancestor of the Lysmatidae and Lysmatidae + Barbouriidae indicate that the inferred character state is significantly more likely to occur than other character states in the same node. Modified from Baeza (2013) then any of three mechanisms may explain the adaptive value of this sexual system. The shrimp *Lysmata wurdemanni* was used as a model organism in a first attempt to experimentally evaluate three hypotheses explaining the stability of protandric simultaneous hermaphroditism (Baeza 2006).

The first mechanism explaining the stability of protandric simultaneous hermaphroditism, named in Baeza (2006) as to the "sex-dependent energetic cost model," argues that due to the differential resource requirements for gamete production, the female function incurs higher energetic costs of reproduction than the male function (Lloyd and Bawa 1984; de Jong and Klinkhamer 1994). This model assumes that large individuals have more resources or greater resource holding power than small individuals (see also Angeloni et al. 2002). Because the growth rate of an individual decreases more when reproducing as a female than as a male, small individuals with the greatest scope for growth that experience limitations on resource acquisition should reproduce only as males. In contrast, because large hermaphroditic individuals have achieved most of their growth potential and feature great resource holding power, these should allocate resources to both the male and female reproductive function (de Jong and Klinkhamer 1994; see also Fisher 1981).

The second mechanism, named the "sex-dependent mortality model," argues that mortality rates are sex-specific (Policansky 1982; Klinkhamer et al. 1997). If the probability of dying when small is greater for hermaphrodites reproducing mostly as females than those mostly reproducing as males, then individuals should emphasize male reproduction when small and female reproduction when large (Klinkhamer et al. 1997). In this case, protandric simultaneous hermaphroditism may evolve because natural selection delays to a larger size reproduction in the sex that pays the higher mortality cost. Small individuals should express the sex function (i.e., male) with the lowest mortality rate (see Charnov 1982, p. 207; Iwasa 1991).

Lastly, the third mechanism, earlier formalized as the "sex-dependent time commitment mode" by Day and Aarssen (1997), considers that mortality rates decrease with increases in size/age of hermaphrodites and that the female function requires a longer reproductive time commitment than does the male function as a result of the time required for gamete production. These two factors combine to create a selective regime promoting the evolution of a positive correlation between female allocation and size. Because the probability of dying early in the reproductive season is high for small individuals, there will not be sufficient time for female reproduction. By contrast, the possibility of successful reproduction as a male when small will be greater than as a female because male gamete production occurs quickly (Day and Aarssen 1997).

Specific assumptions and predictions of each model above were evaluated with manipulative experiments by Baeza (2006). A first laboratory experiment demonstrated that, among small individuals matched in body size, males grow (estimated as mm/day during a total of 45 days) quicker than hermaphrodites, an indication that the female function incurs higher energetic costs of reproduction than the male function in the studied shrimp (Fig. 10.13a). In this experiment, decreased growth rate was chosen as a proxy for energetic costs suffered by shrimps as consequence of both the male and female sexual functions because of (1) the recognized trade-off between growth and reproduction (Stearns 1992) and (2) the ease of measuring

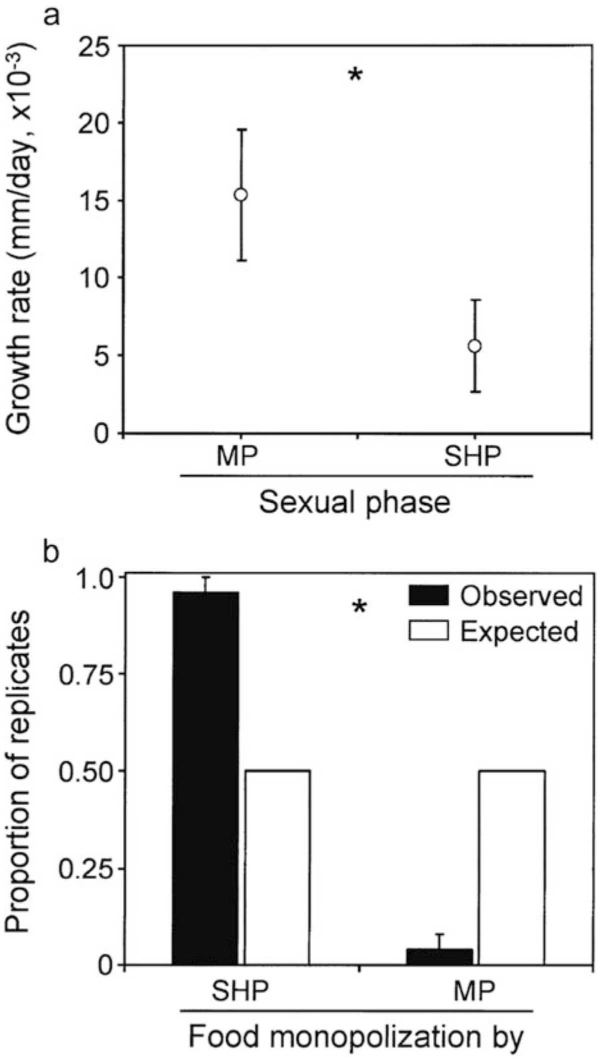

Fig. 10.13 Growth and resource holding power of the different sexual phases of *Lysmata wurdemanni*. (**a**) Growth rate (least squares mean ± SE) of male-phase individuals (MP) and simultaneous hermaphrodite phase individuals (SHP) reared alone in the laboratory. (**b**) Observed and expected proportion of the replicates in which MPs or SHPs retained a food item after 30 min of observation. Only those replicates in which agonistic interactions were observed were considered. The asterisk indicates significant differences between treatments. Bars in b are binomial standard error bars. Modified from Baeza (2006)

growth compared to other proxies (i.e., respiration rate, Baeza and Fernández 2002). Also, the growth rate of males was considered specific to the male function. Similarly, the growth rate of hermaphrodites was considered specific to the female function because energetic investment into the male function by small males, estimated as sperm mass, is less than 0.1% (Baeza 2007a, b).

Also, a second experiment demonstrated that large hermaphrodites were more successful in monopolizing food than small males (Fig. 10.13b). Overall, the experimental demonstration of sex-dependent growth rate and size-dependent resource holding power agree with predictions of the sex-dependent energetic cost model maintaining protandric simultaneous hermaphroditism in the genus *Lysmata*.

A second set of laboratory observations and experiments indicated that the time that hermaphrodites required for replenishing their sperm reservoirs after mating as males was much shorter (2 days) than the time required to brood two consecutive egg clutches (11 days). In caridean shrimps, including species belonging to the genus *Lysmata*, the inter-molt period (i.e., the time elapsed between molts) represents the time commitment to the female function per reproductive event. Hermaphroditic shrimps are known to spawn successively (i.e., consistently after every molt) (Bauer and Holt 1998). In turn, the time hermaphrodites require to fill their ejaculatory ducts (that serve as sperm reservoirs in shrimps) after they inseminated a "parturial" hermaphrodite represents the time commitment to the male sex function (Bauer and Holt 1998). Thus, the time commitment to the female was much greater than that to the male sexual function. Importantly, a second laboratory experiment demonstrated that small hermaphrodites experience heavier mortality due to fish predation than large hermaphrodites (Fig. 10.14). In general, experiments demonstrating sex-dependent reproductive time commitment and size-dependent mortality agree

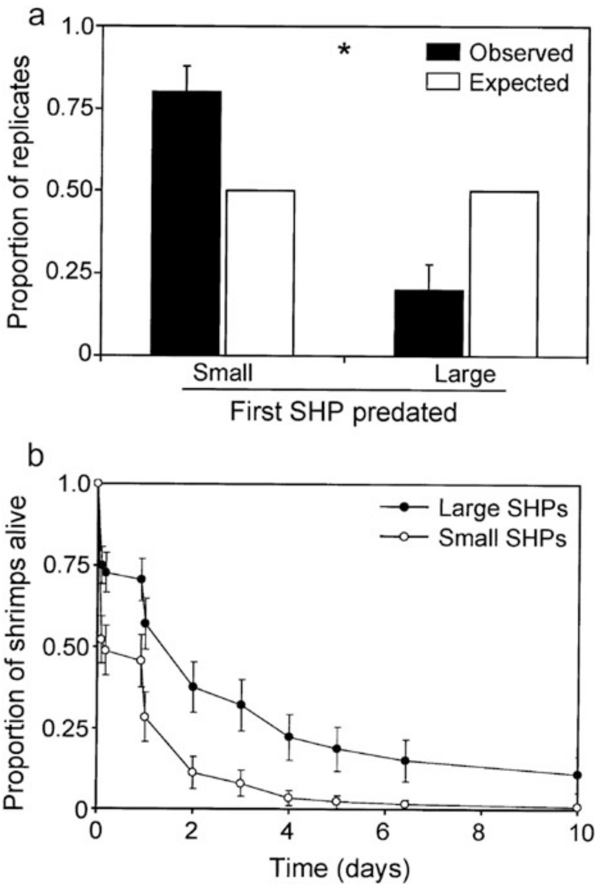

Fig. 10.14 Mortality due to predatory fishes of small and large simultaneous hermaphrodite phase (SHP) *Lysmata wurdemanni*. (**a**) Observed and expected proportion of the replicates in which either large or small SHPs were consumed first by fish. Bars represent binomial standard errors. (**b**) Proportion of small and large SHP shrimps staying alive over time (days). Points on curves were estimated for each treatment with Cox's maximum partial likelihood regression method. Modified from Baeza (2006)

Table 10.1 Models explaining the adaptive evolution of protandric simultaneous hermaphroditism

Model and predictions	Outcome
1. Sex-dependent energetic cost	
(a) Growth rate of MPs should be faster than that of SHPs	Supported
(b) Resource holding power of SHPs should be greater than that of MPs	Supported
2. Sex-dependent mortality rates	
(a) Fish-related predation of small SHPs should be greater than that of small MPs	Not supported
3. Sex-dependent time commitment	
(a) Time commitment of female reproduction should be longer than that of male reproduction	Supported
(b) Fish-related mortality of small SHPs should be greater than that of large SHPs	Supported

with predictions of the sex-dependent time commitment model maintaining protandric simultaneous hermaphroditism in the studied shrimp.

Lastly, during a third set of laboratory experiments, no evidence was found about sex-dependent mortality explaining the adaptive value of hermaphrodites given that, by contrast to what was predicted by the third model above, mortality due to predatory fishes suffered by hermaphrodites was not greater than that suffered by male.

Overall, Baeza (2006) found evidence that the mechanism postulated by two out of three models explains the sexual system of *L. wurdemanni* (i.e., the sex-dependent time commitments and sex-dependent energetic costs models) (Table 10.1). The above suggests that protandric simultaneous hermaphroditism in *L. wurdemanni* is maintained through the optimization of the sex allocation pattern of shrimps pertaining to different sizes/age classes. Baeza's (2006) approach also represents an example of the use of sex allocation theory in an attempt to understand mixed sex allocation patterns. A similar experimental approach remains to be conducted to understand the adaptive value of caridean shrimps exhibiting other sexual systems.

10.6 Discussion

10.6.1 Disparity in Sexual Systems and Ancestral Sexuality in the Infraorder Caridea

This study supports the notion that within monophyletic clades belonging to the species-rich and ecologically dissimilar order Decapoda, gender expression is the most diverse in caridean shrimps (infraorder Caridea). For instance, in the species-rich infraorders Brachyura (true crabs), Stenopodidea (boxer and glass-sponge shrimp), and Achelata (Panulira, spiny lobsters, and allies), gonochorism appears to be a fixed sexual system (Cobb and Phillips 1980; and references therein). Importantly, among true (brachyuran) crabs, the presence of intersex individuals has been reported in several species (LeBlanc 2007). However, these reports need confirmation and likely represent instances of endocrine disruption by chemical compounds, as reported before in other groups of invertebrates (see LeBlanc 2007; and references therein). In a few other monophyletic groups in which gonochorism is the predominant sexual system (e.g., the infraorder Anomura—hermit crabs, porcelain crabs, squat lobsters, and allies) as well as in other closely related shrimp clades (e.g., suborder Dendrobranchiata—penaeid shrimp and allies), only a few species exhibit sexual systems other than gonochorism. For instance, within the Dendrobranchiata, only a single species from the genus *Solenocera* has been reported to be strictly protandric (e.g., *S. membranacea*, Heergaard 1967; but see Chiba 2007). Similarly, in the suborder Axiidea (mud shrimps), only a single species belonging to the genus *Calocaris* appears to be hermaphroditic (i.e., *C. macandreae*, Buchanan 1963; Chiba 2007). Still, the disparity in terms of gender expression in the above groups does not rival that exhibited by the infraorder Caridea within the order Decapoda.

The condition of having separate sexes (gonochorism) is often assumed to be the most common sexual system in caridean shrimps. Subsequently, studies formally testing for gonochorism in this group are rather uncommon. Bauer (2000, 2002a, b, 2004) has suggested that the predominance of gonochorism as a sexual system in the Decapoda implies that the ancestral sexual system in the Caridea was gonochorism (Bauer 2000, 2002a, b, 2004). Importantly, the assumption of gonochorism being the ancestral condition and the most common sexual system in the Caridea needs to be considered with caution. For instance, detailed studies in species assumed to be gonochoric for a long time period have demonstrated them to be protandric (e.g., *Merguia rizhophorae*, Baeza 2010a, b). Conversely, other species believed to be strictly protandric (given reverse sexual size dimorphism) have been shown to be gonochoric after detail examination of their ecology, sexual dimorphism, and experimental testing of sex change (e.g., in the genus *Hippolyte*, Espinoza et al. 2008 and references therein). Perhaps more importantly, detailed examinations of the sexual system have been conducted only in a very few of the 3438 species formally described in the infraorder Caridea (De Grave and Fransen 2011). Certainly, many more studies examining the sexual system of caridean shrimp need to be conducted before any robust conclusion about the relative abundance of different gender expression patterns in this group can be reached. Such studies will also allow to develop a robust inference about the ancestral gender expression pattern in this species-rich clade. Studies on the sexual system in representatives of the Caridea ideally need to be coupled with data on the ecology of the species in order to improve our understanding about the conditions favoring different gender expression patterns, as is discussed below.

10.7 Origins of Protandric Simultaneous Hermaphroditism in the Infraorder Caridea

Transition pathways connecting the different sexual systems present in the Caridea have been proposed herein (Fig. 10.12). As pointed above, studies attempting to reveal the frequency of sexual system transitions within monophyletic groups represent a most relevant step to understand their adaptive value (see Weeks et al. 2009). Unfortunately, the information on the sexual system for the great majority of caridean shrimps is lacking (see above), and the internal phylogenetic relationships in the Caridea are far from settled (but see Bracken et al. 2009; Li et al. 2011; Baeza 2013). Such absence of information is constraining, at the moment, any exploration about transition probabilities among dissimilar sexual systems in this group that, in turn, will head lights on their adaptive value.

To the best of my knowledge, the question of transitions between sexual systems has been formally investigated within the infraorder Caridea only in the case of protandric simultaneous hermaphroditic shrimps in the families Lysmatidae + Barbouridae (Baeza 2013). The ancestral character-state reconstruction analyses conducted by Baeza (2013) did not provide strong support for Bauer's (2000) hypothesis about the evolution of protandric simultaneous hermaphroditism in this group. Restricted mobility of individuals belonging to an ancestral species due to their association with a host, and, hence, reduced probability of encountering mating partners, does not appear to have favored simultaneous hermaphroditism in caridean shrimps. Importantly, some authorities have pinpointed to evidence indicating that not a single but most often multiple causal factors affect the evolution of sexual systems in other monophyletic clades (Charlesworth 1999; Meagher 2007). Thus, "low abundance" by itself, as proposed by Bauer (2000), might not represent an evolutionary force strong enough to favor protandric simultaneous hermaphroditism in the families Lysmatidae + Barbouridae. Nonetheless, a combination of other factors acting in unison might favor the evolution of protandric simultaneous hermaphroditism in this group (Baeza 2013).

Considering the lifestyle (free-living) and socio-ecology (most likely group living or gregarious) inferred for the ancestral species of Lysmatidae + Barbouridae and recent studies on the behavioral biology of extant species in these families, Baeza (2013) proposed that two conditions acting together in the past, low male mating opportunities (which correlates with low density populations and/or with reduced mobility due to, e.g., high predation pressure) and brooding constraints, might have favored the transition to protandric simultaneous hermaphroditism in the environment occupied by the ancestral Lysmatidae + Barbouridae. Limited brood capacity in species that brood embryos, such as caridean shrimps, is expected to cause diminishing female fitness returns (sensu Charnov 1982) because sheltering space for offspring is gradually constrained as allocation to the female function increases. In this situation, reproductive effort that would otherwise be allocated to female reproduction should be allocated to male reproduction (Heath 1979). Few studies have explored brood limitation in marine invertebrates (Heath 1979; Strathmann et al. 1984; Baeza 2007a, b). Among them, that conducted on *Lysmata wurdemanni* demonstrated ~7.1% brood lost during embryo development due to brooding space limitations (Baeza 2007a, b). The above suggests that brooding constraints might indeed be important in driving diminishing fitness gains to the female function in shrimps from the families Lysmatidae and Barbouriidae. In turn, reduced sperm competition leading to a saturating male fitness gain curve (see below) might still occur in species that live in small groups or in aggregations, such as the ancestral Lysmatidae + Barbouriidae, if female-role individuals are monoandrous. Importantly, in the few species of *Lysmata* in which mating behavior has been studied in detail, hermaphroditic shrimps appear to be strictly monoandrous (Bauer and Holt 1998; Baeza 2007a, b). For instance, in the gregarious *L. wurdemanni*, female-role individuals copulate only once with a single other male-role shrimp seconds after molting and intensively use backward tail flipping (which rapidly propel shrimp away from conspecifics) to avoid male advances after insemination (Baeza 2007a, b, unpubl. data). Theoretically, the extent of saturation of the male fitness gain curve (sensu Charnov 1982) caused by a monoandrous behavior in group-living species is expected to be as strong as that caused by low abundance conditions in pair-living species (Charnov 1982; Baeza 2007a, b). The two conditions above decrease sperm competition (Michiels et al. 2009). Nevertheless, even if monoandry does not drive strong saturation of the male fitness gain curve (see below), monoandry acting together with brood constraints might represent conditions that are strong enough to favor protandric simultaneous hermaphroditism over gonochorism or other sexual systems in the Lysmatidae and allies. Overall, more studies on brood constraints and mating systems in shrimps are warranted to reveal those conditions favoring protandric simultaneous hermaphroditism in the Lysmatidae + Barbouriidae.

In general, clarification of the internal relationships within the Caridea together with natural history studies in the species belonging to this group is needed to continue improving our understanding about the adaptive value of sexual systems in shrimps. This is a challenging task, and I argue here to start with monophyletic clades exhibiting moderate or considerable diversity in sexual systems. Candidates include the family Thoridae (genus *Thor* and allies) and Rhynchocinetidae (genus *Cinetorhynchus* and *Rhynchocinetes*) as they represent monophyletic clades with extant disparity in sexual systems. For instance, the presence of gonochoric, strictly protandric, and partially protandric species in the genus *Thor* represents an outstanding opportunity for (1) the assessment of evolutionary transitions among the above sexual systems and (2) the exploration of those conditions favoring or constraining different sexual systems in the Caridea.

10.8 The Adaptive Value of Protandric Simultaneous Hermaphroditism

The discussion above about the transition between different sexual systems highlights another important question that needs to be further explored and that was implicit when formally testing for these transitions in the Lysmatidae + Barbouridae (see Bauer 2000; Baeza 2013): what are the environmental including social conditions that favor a particular sexual system over others? In other words, what environmental conditions facilitate transitions between sexual systems? To the best of my knowledge, this question has been formally investigated in the infraorder Caridea only in the case of protandric simultaneous hermaphroditism (Baeza 2006).

Baeza (2006) has shown that a combination of sex-dependent growth rate and size-dependent resource holding power together with sex-dependent reproductive time commitment and size-dependent mortality explains the maintenance of protandric simultaneous hermaphroditism (i.e., the existence of an early male phase in these simultaneous hermaphrodites) in the Lysmatidae + Barbouridae (see Baeza 2006). For instance, males grow faster than simultaneous hermaphrodites of the same size and age, an indication that the female function incurs higher energetic costs of reproduction than the male function (Baeza 2006). In turn, large individuals are more successful in monopolizing food than small individuals (Baeza 2006). Given that the growth rate of an individual decreases faster when reproducing as female than as male, small individuals with the greatest scope for growth that experience limitations on resource acquisition should reproduce only as males. In contrast, because large individuals have achieved most of their growth potential and have a greater resource holding power, these should allocate resources to both the male and female reproductive function (de Jong and Klinkhamer 1994; Baeza 2006, 2007a, b).

Importantly, the conditions above (i.e., size-dependent resource holding power, sex-dependent reproductive costs) appear to be quite common in several other species of marine invertebrates (including caridean shrimps) originally reported as strict simultaneous hermaphrodites. Thus, simultaneous hermaphroditism with an early male phase might be more common than originally noticed in nature (e.g., Crisp 1983; Premoli and Sella 1995; Tomiyama 1996; Todd et al. 1997; Manríquez and Castilla 2005). Future detailed studies examining the size-dependent sex allocation pattern of species initially reported as strict simultaneous hermaphrodites might confirm the notion that protandric simultaneous hermaphroditism is more common than originally reported in the literature.

10.9 Outlook

This study has reviewed the disparity in terms of sexual systems in the species-rich Caridea. Transition probabilities between sexual systems have been explored and suggested, and future studies on the natural history of and gender expression in the Caridea coupled with improvements in the understanding of the internal phylogenetic relationships in this clade are needed to understand gender expression evolution in this species-rich clade of marine invertebrates. Similarly, the selective forces favoring transitions between particular sexual systems or maintaining them once they have evolved have rarely been experimentally explored in this group (other than in protandric simultaneous hermaphrodites). The body of literature accumulated during the last decade in species exhibiting this peculiar sexual system has allowed to test sex allocation theory and to use this theoretical framework to improve our understanding about the conditions maintaining this rather unusual sex allocation pattern. I argue in favor of additional studies using (size-dependent) sex allocation theory as a framework to understand the evolution of sexuality in this species-rich clade of crustaceans. Lastly, phylogenetic studies in this clade are needed to reveal the number of times and ecological conditions that have favored different sexual systems in the infraorder Caridea.

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Chapter 11
Environmental and Genetic Sex Determining Mechanisms in Fishes

John Godwin and Reade Roberts

Abstract Teleost fishes are the most speciose group of vertebrate animals, and their study has been fundamental to understanding the mechanisms underlying sex determination, development, physiology, and evolution. Teleosts display extraordinary diversity in a variety of phenotypes, with sexual patterns and determination mechanisms being no exception. Sexual patterns range from familiar male–female gonochorism to various forms of hermaphroditism, including systems where individuals undergo functional sex change over their life cycle, or are capable of self-fertilization. There is also considerable variety in how gonochoristic species determine sex, with mechanisms using social, environmental, and genetic inputs to direct sexual development. Within genetic sex determination systems, there is additional diversity, with monogenic and polygenic systems found in various chromosomal contexts. Here, we review the above diversity and describe how it has revealed broad mechanistic and evolutionary insights into sexual phenotypes. We further discuss why fish display such amazing diversity in sex determination systems and how the necessary evolutionary transitions producing that diversity may have taken place.

11.1 Introduction

Teleost fishes are the most speciose group of vertebrate animals and show extraordinary diversity in morphology, habitats they occupy, behavior, and life history. This diversity extends to sex determination and differentiation where fishes exhibit an extraordinary variety of sexual patterns and sex determination mechanisms. Sexual patterns seen in fishes include the common and familiar pattern of gonochorism where individuals mature and function as either males or females. However, there are also unisexual species, species exhibiting alternate male mating phenotypes that may be either fixed or plastic in adulthood, functional sex change where individuals mature as one sex and then change to become the other sex at some point in

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adulthood, and simultaneous hermaphrodites that maintain functional ovarian and testicular tissue and can alternate in male and female spawning roles on a second-to-second basis (Fig. 11.1; Atz 1964; Reinboth 1980; Devlin and Nagahama 2002; de Mitcheson and Liu 2008; Godwin 2010; Lamm et al. 2015). Interestingly, there is also considerable diversity in the control of sex determination in gonochoristic species with social control of sex (Francis and Barlow 1993), temperature-dependent sex determination (TSD) in several groups (Luckenbach et al. 2009; Duffy et al. 2015), and genotypic sex determination (GSD). Even within gonochoristic species with GSD, there is a diversity of sex determination mechanisms across species and even within species (see *Polygenic Sex Determination*).

This chapter reviews the diversity of sexual patterns and sex-determining mechanisms in fishes. We describe the diversity of sexual patterns across fishes and then examine sex-determining mechanisms in more detail. As these sex-determining mechanisms can be either environmental or genetic, we discuss them separately. Lastly, we consider why fishes display such extraordinary diversity, whether there might be conserved features across the diversity of sex determination systems, and how transitions between different forms of sex determination might occur in fishes.

11.2 Sexual Patterns in Fishes

Fishes display the nearly full range of sexual patterns observed in vertebrates more generally (Fig. 11.1). This includes patterns that are not observed in other vertebrate animals in the form of functional sequential and simultaneous hermaphroditism as well as patterns that are not common in other vertebrate groups with unisexuality and fixed alternate mating phenotypes. This diversity, the availability of comparative taxa within the bony fishes (Osteichthyes) generally and often within lower taxonomic levels, and particularly the multiple independent evolutionary origins of some of the patterns make the bony fishes especially useful for exploring the evolution of sexuality more generally.

Most fish species are gonochoristic, meaning they mature as either a female or male and remain that sex throughout life. However, within gonochorists, we still observe interesting diversity in the form of discrete within-sex alternate mating phenotypes and temperature-dependent sex determination (discussed below in *Environmental Sex Determination*). Alternate mating phenotypes are seen in at least 140 species and 28 families of fishes (Taborsky 1998) and are an important part of the mating system in at least many species (see Gross 1996; Oliveira et al. 2005 for reviews). Well-studied examples at the behavioral and physiological levels include sunfishes [Centrarchidae (Gross 1982, 1991; Neff and Knapp 2009)], the plainfin midshipman [*Porichthys notatus*, Batrachoididae (Brantley et al. 1993)], and blennies [Blenniidae (Oliveira et al. 2001)]. These systems are characterized by large males that are typically aggressively dominant and exhibit strong courtship behavior and smaller males that instead employ “sneaking” or “streaking” tactics to

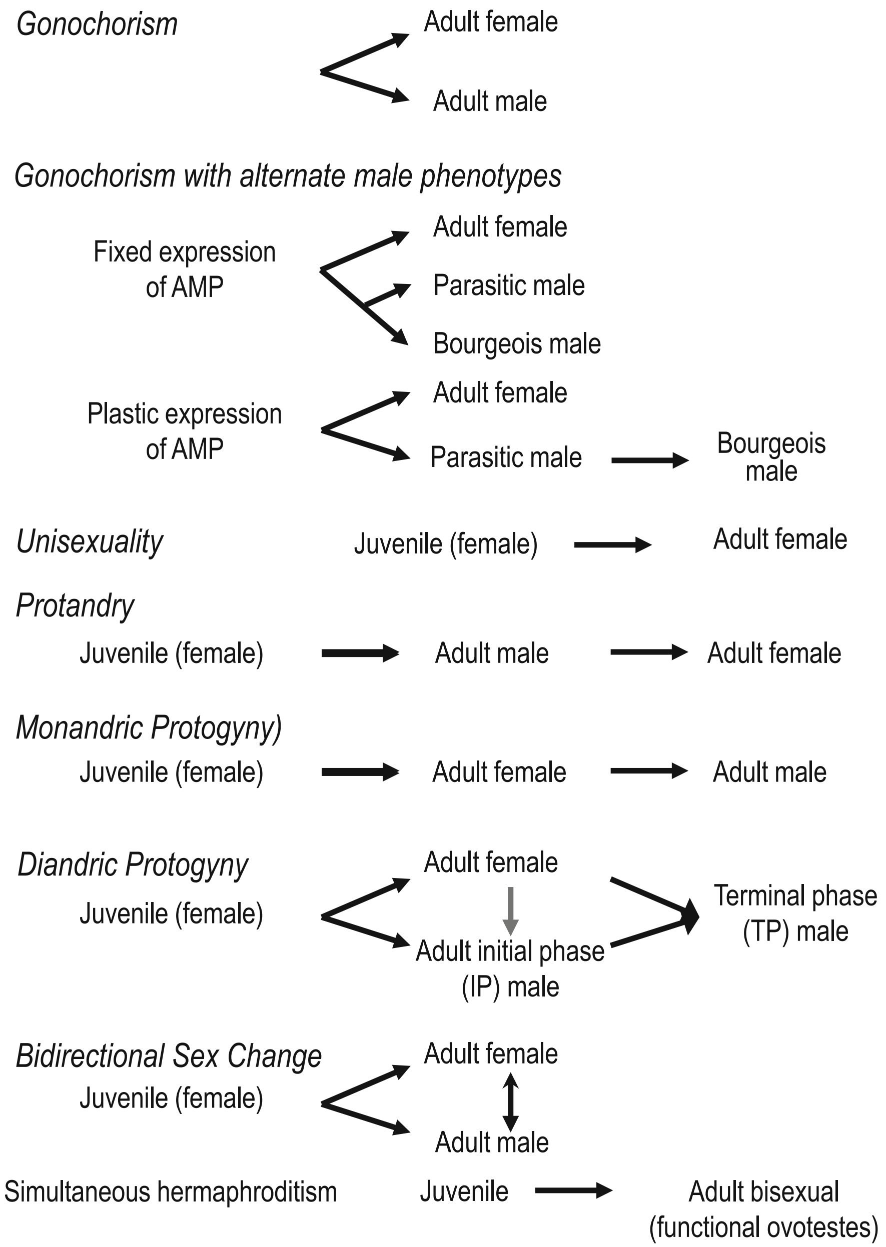

Fig. 11.1 Range of sexual patterns in fishes for both gonochoristic and hermaphroditic species. "AMP" refers to alternate male phenotypes

obtain fertilizations ["bourgeois" and "parasitic" males, respectively, in Taborsky's terminology (Taborsky 1998)].

Unisexuality in the form of gynogenesis occurs in five families of fishes (Poeciliidae, Atherinidae, Cyprinidae, Cobitidae, and Cyprinodontidae, see Avise 2015 for review) and is best studied in the Amazon mollies (*Poecilia formosa*), which was the first vertebrate discovered to reproduce asexually (Hubbs and Hubbs 1932). Females of this unisexual species reproduce through gynogenesis (Vrijenhoek 1994). Males make no genetic contribution, but mating with males from other *Poecilia* species is required to activate the diploid eggs.

Functional hermaphroditism was initially discussed for fishes by Atz (1964) and has now been described from at least 22 families of fishes (of ~450 total families, de Mitcheson and Liu 2008). Social control of this process was first reported in the early 1970s (Robertson 1972; Shapiro 1980; reviewed in Devlin and Nagahama 2002; Godwin 2010; Lamm et al. 2015). Hermaphroditism in fishes takes a variety of forms, and these are reviewed briefly here before we discuss mechanisms.

Functional sex change or sequential hermaphroditism is generally thought to be selectively advantageous when an individual can reproduce as one sex when small or young and as the other sex when larger or older. This is the "size-advantage model" proposed by Ghiselin (1969) (see also Warner et al. 1975 for a specific application of this model). The direction of sex change favored depends heavily on mating system and, in particular, whether large size favors males through increased ability to control resources important to females. This situation selects for female-to-male or "protogynous" sex change and is observed in well-studied examples from the wrasses, parrotfishes, and groupers studied mainly in coral reef habitats. Large body size confers an advantage on males in these species because mating success depends on controlling a general-purpose territory in which mating takes place (e.g., Robertson 1972; St. Mary 1994; Godwin 1995; Fricke and Holzberg 1974; Asoh 2003, 2005) or a temporary spawning territory during mating (the bluehead wrasse *Thalassoma bifasciatum*, groupers with spawning aggregations).

Species displaying protogynous sex change can exhibit a single path of development, termed "monandric protogyny," where juveniles mature into females that can later change to become males. Alternatively, other species exhibit "diandric protogyny" where juveniles mature into an initial-phase adult as either females or males that are often similar to females in external appearance and often behavior. Initial-phase females and males can change to become terminal-phase males. This typically involves changes in morphology and behavior for initial-phase males and morphological, behavioral, and gonadal sex change for females. There are rare examples of male-to-female sex change in these species when sex ratios become male biased (Kuwamura et al. 2011, 2014); illustrating this phenomenon can occur although it is likely to be an uncommon situation in nature.

Protandry or male-to-female sex change has been documented in seven families in four orders of fishes and is likely in three additional families in the Perciformes (de Mitcheson and Liu 2008). This pattern appears to be selectively advantageous with life histories like those in the well-studied sparid fishes (sea breams and porgies), where males do not control resources required by females and large body size may therefore not confer mating advantages. By contrast, female fecundity increases with body size and individuals realize a reproductive benefit by becoming female at larger sizes (Warner 1984). A different pattern is seen in anemonefishes of the genera *Amphiprion* and *Premnas*. These are damselfishes of the family Pomacentridae that are obligate symbionts of large tropical sea anemones that they aggressively defend as mated pairs (Godwin and Fautin 1992). Juvenile anemonefishes are immature females but develop spermatogenic tissue and function as mature males if they assume the male breeding role (Ross 1978; Hattori and Yanigisawa 1991; Godwin 1994a, b). Following disappearance of the large and dominant females from a pair, males undergo protandrous sex change to become mature and socially dominant females. It is interesting that some species of the damselfish genus *Dascyllus* also aggressively defend a cnidarian host yet instead show protogynous sex change and mating systems characterized by dominant males that control corals that smaller females rely on as a shelter resource (Godwin 1995). It may be that the fecundity of a dominant large female anemonefish exceeds what would be the combined fecundity of smaller individuals resident in an anemonefish group, making it selectively advantageous for this dominant individual to be female rather than male.

Bi-directional sex change involves individuals being able to undergo either female-to-male or male-to-female sex change, often repeatedly. This pattern has been confirmed in six families in the order Perciformes and is best studied in the gobies (Gobiidae; de Mitcheson and Liu 2008). Gobies tend to be small-bodied fishes that are bottom dwelling and weak swimmers. Working with the small coral dwelling *Gobiodon erythrospilus*, Hobbs et al. (2004) showed that individuals would adjust their sex in either a male or female direction depending on the sex of individuals they were placed on corals with, resulting in the development of heterosexual pairs. These authors argue that the limited mobility of these small fishes in a predator-rich coral reef environment selects for the ability to change sex in either direction in order to enable mating without potentially dangerous movement to find an opposite sex mating partner.

Lastly in terms of diversity of sexual patterns in fishes, species in nine families across three orders of fishes exhibit simultaneous hermaphroditism, characterized by expression of both mature testicular and ovarian tissue concurrently in the adult gonad. This sexual pattern is best documented in the aulopiform and serranid fishes within the Perciformes. As most aulopiforms, including those with strong evidence of simultaneous hermaphroditism, are found only in the deep sea, we know relatively little about their reproductive biology and almost nothing about their mating habits. By contrast, the serranids that exhibit simultaneous hermaphroditism are often relatively common on tropical and subtropical reefs and easily observed. Mating in one group of these serranids, the hamlets of the Caribbean (*Hypoplectrus* spp.*)*, involves a complex set of "egg trading" behaviors in which individuals switch between male and female spawning roles on a second-to-second basis during a brief spawning period around sunset (Fischer 1980, 1984). Serranids can also exhibit a complex pattern in which individuals mature as simultaneous hermaphrodites and then lose female function to become males with increases in body size (Hastings and Petersen 1986).

11.3 Environmental Sex Determination: Cues and Mechanisms

Sex determination can be influenced by a variety of environmental influences in fishes, but we focus on temperature and social interactions here as there is clear evidence these factors play important roles in nature.

11.3.1 Temperature-Dependent Sex Determination

Temperature-dependent sex determination (TSD) is well characterized in two groups of fishes, the Atheriniformes (mostly silversides) and the Pleuronectiformes (flatfishes). In a series of studies beginning in the 1980s, David Conover documented TSD in the Atlantic silverside (*Menidia menidia*, Conover and Fleisher 1986; Lagomarsino and Conover 1993; Duffy et al. 2015). This species shows both TSD and GSD with the relative proportions of individuals showing each pattern varying across a latitudinal gradient (Duffy et al. 2015). Specifically, populations in the southern part of the species range (e.g., South Carolina, USA) exhibit primarily TSD where cool temperatures produce predominantly females and warm temperatures produce predominantly males. By contrast, rearing temperature does not strongly affect sex determination for most populations in the northern parts of the species range, with sex ratios of approximately 50:50 resulting regardless of rearing temperature. Another well-studied atherinid fish that displays TSD is the pejerrey, *Odontesthes bonariensis.* As with silversides, cool temperatures of 13–19 °C during the critical period in development (1–5 weeks of age depending on temperature) of pejerrey induce female determination, while warmer temperatures produce progressively more males, peaking at 100% male determination at 29 °C (Strüssmann et al. 1996, 1997). Although both high and low temperatures can induce monosex development in the pejerrey, recent findings also indicate genotypic contributions to sex determination at intermediate temperatures attributable to the presence or absence of a gene termed Y-linked anti-Mullerian hormone (*amhy*, Yamamoto et al. 2014).

The other major and now well-studied example of TSD in fishes is from flounder of the order Pleuronectiformes. While TSD is known from several genera of flounders, the patterns and mechanisms are best characterized in the genus *Paralichthys* (Luckenbach et al. 2009; Mankiewicz et al. 2013). The Japanese and southern flounders, *Paralichthys olivaceus* and *Paralichthys lethostigma*, respectively, exhibit a sex determination response to temperature where both high and low temperatures produce male-biased sex ratios while midrange temperatures maximize female determination at approximately 50% (Yamamoto 1995, 1999). The temperatures that produce even sex ratios appear to be somewhat cooler in Japanese than southern flounder, which may be related to temperature differences in their wild ranges (Luckenbach et al. 2009). Interestingly, these species also exhibit a mixture of GSD and TSD as inheriting a Y chromosome from the male results in male sex

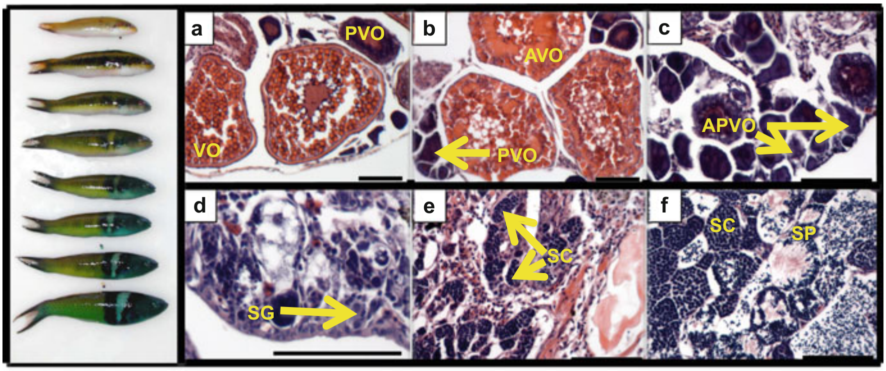

Fig. 11.2 Body color and gonadal sex change in the bluehead wrasse. Left panel depicts sequence of color changes from female to TP male (top to bottom) (image by J. Godwin). Right panels depict stages of gonadal sex change stages from ovary to functional testis (images by M. Lamm and J. Godwin). (**a**) Stage 1, mature ovary with vitellogenic (VO) and pre-vitellogenic oocytes (PVO); (**b**) stage 2, atretic vitellogenic oocytes (AVO); (**c**) stage 3, atretic previtellogenic oocytes (APVO); (**d**) stage 4, presumed spermatogonia (SG) and Leydig cells proliferate at this stage; (**e**) stage 5, spermatogenesis initiated and progresses with presence of spermatocysts (SC); (**f**) stage 6, mature, tailed sperm (SP). Gonadal stages follow classification by Nakamura et al. (1989). Scale bar = 50 μm. Figure redrawn from Lamm et al. (2015)

determination, while XX individuals have their sex determined by environmental cues. This is supported by very high proportions of females produced in matings between normal females and gynogenetically produced XX males (masculinized XX fish; Yamamoto 1999) (Fig. 11.2).

While temperature appears to be the critical cue affecting sex determination in flounder, others cues can also affect this process. Feeding cortisol to either southern or Japanese flounder effectively masculinizes sex ratios (Mankiewicz et al. 2013; Yamaguchi et al. 2010). Even tank color can affect sex ratios in developing southern flounder, with rearing in light blue tanks producing significantly more masculinized sex ratios than rearing in black or gray tanks (Mankiewicz et al. 2013). Interestingly, juvenile southern flounder in light blue tanks also showed significantly higher whole-body cortisol levels at a body size just prior to the onset of expression of gonadal aromatase, a key molecular marker of female sex determination (Fig. 11.3). We return to the potential central role of this glucocorticoid hormone below.

11.3.2 Social Determination of Sex

Social control of sex, or *behavioral sex determination* (BSD), is best characterized in tropical reef fishes and particularly in the wrasses (Labridae), damselfishes (Pomacentridae), and gobies (Gobiidae) but also with important studies focused on parrotfishes (Scaridae) and angelfishes (Pomacanthidae). Field studies

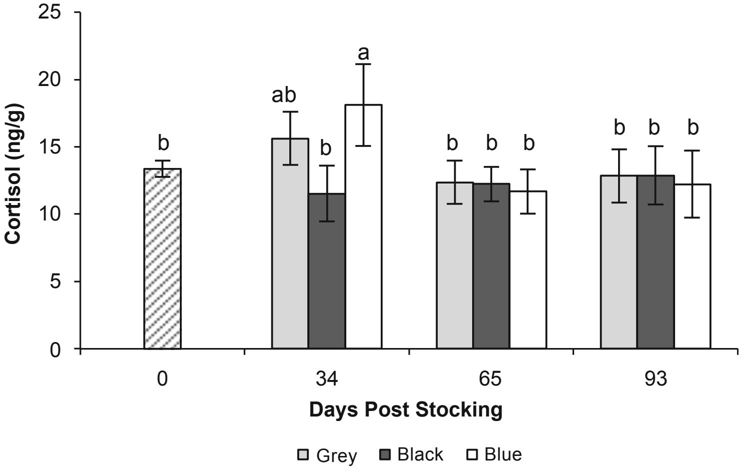

Fig. 11.3 Tank color effects on body cortisol concentrations in southern flounder. Thirty-four days post stocking was at approximately the putative period of sex determination. Values are ng/g body weight and significant differences are indicated by different letters (redrawn from Mankiewicz et al. 2013)

demonstrating social control of sex change have typically employed removal of dominant, secondary sex individuals and documented sex change in the next largest individuals present in a social group. The initial demonstration of socially controlled sex change by Robertson (1972) involved removal of dominant males from social groups of the Indo-Pacific cleaner wrasse *Labroides dimidiatus*. The largest females showed the beginnings of male-typical behavior within a few hours of male removal, and this behavioral transition was complete within a few days. Similarly in the bluehead wrasse (*T. bifasciatum*), removal of dominant terminal-phase (TP) males from small reefs led to the display of TP male-typical behaviors by large females beginning almost immediately and the fully display of TP male-typical spawning behaviors usually by the end of the first daily spawning period following removal of the dominant male(s) (Warner and Swearer 1991), a change that does not depend on gonads (Godwin et al. 1996). Removal of dominant females can also induce behavioral and gonadal sex change in subordinate males in anemonefish (Fricke and Fricke 1977; Fricke 1983; Godwin 1994a, b).

A variety of experiments under captive conditions have also shown behavioral and gonadal sex change mediated by the presence/absence of a dominant individual. Beginning in the 1980s, social control of sex change under captive conditions was demonstrated (Shapiro 1980). Ross and colleagues explored the key cues and conditions under which socially controlled sex change would occur in the protogynous Hawaiian saddleback wrasse, *Thalassoma duperrey* (Ross 1983). This study used floating wire-mesh cages and systematically varied the social exposure of female saddleback sex change candidates across 12 distinct treatments. These treatments included exposure to either larger and small conspecific individuals of the same sex (i.e., females), sexual phase (initial-phase males), or heterospecifics with the congeneric wrasse *Thalassoma ballieui*. These experiments demonstrated that socially controlled sex change in saddleback wrasses under these experimental conditions could be stimulated by the presence of smaller conspecifics, regardless of their sex, and inhibited by the presence of larger conspecifics. Exposure to a smaller individual of the congeneric *T. ballieui* did not stimulate sex change. The key sensory modality appeared to be visual as placing a barrier to only tactile contact between sex change candidates and smaller conspecifics did not prevent sex change, while a combined visual and tactile barrier did prevent sex change. Subsequent experiments with saddleback wrasses showed that while only single sex changes were observed in small groups of three females (by the largest females), sex change occurred in multiple large females in larger groups averaging seven females (Ross et al. 1990). These results were consistent with a "size-ratio threshold hypothesis." Working instead on small reefs with bluehead wrasses (*T. bifasciatum*), Warner and Swearer (1991) found that the number of large terminal-phase males present on a reef prior to TP male removal predicted the number of large females that would change sex following TP male removal. Experiments in Potter's angelfish, *Centropyge potteri*, in large experimental pens also support a key role for the relative exposure to larger and smaller conspecifics (Lutnesky 1994). Specifically, the results supported an *absolute encounter-rate threshold* hypothesis as large females were likely to change sex in experimental groups of 15 females and 1 male if held in a large experimental pen (102 m^2, 6/7 trials), but not if instead held in a much smaller experimental pen (12 m^2, 0/6 trials).

What are the key sensory modalities regulating sex change? The studies described above suggest visual cues and rates of encounter with dominant individuals are critical. Olfactory cues and particularly pheromonal signals are critical regulators of reproductive behavior and function in a variety of vertebrates including fishes (Stacey and Sorensen 2011; Stacey 2015). Evidence of pheromonal signals regulating social determination of sex is limited to a study in the Caribbean goby *Coryphopterus glaucofraenum*. Cole and Shapiro (1995) exposed solitary female *C. glaucofraenum* to water from empty aquaria or aquaria housing conspecific females, conspecific males, or females of another Caribbean goby species. Females exposed to waterborne cues, likely pheromonal, from aquaria holding conspecific females changed sex at significantly higher rates than females exposed to the other treatments. Interestingly, female bluehead wrasses undergoing sex change following removal of dominant TP males increase the display of a behavior termed "inspection." Inspections involve apparently close inspection of the cloacal area of initial-phase individuals, and the nares are close to this potential source of pheromonal signals (Godwin, pers. obs.). In many species of fishes, pheromonal signals can be detected at nanomolar concentrations and can dramatically activate the reproductive neuroendocrine axis (reviewed in Stacey and Sorensen 2011; Stacey 2015).

11.3.3 Physiological Regulation of Environmental Sex Determination

Are there fundamental mechanisms shared between different modes of ESD, and can elucidating these modes also potentially inform our understanding of transitions between ESD and the genotypic sex determination mechanisms? This section addresses physiological regulation of sex determination primarily in species displaying socially controlled sex change for BSD and species with TSD where it appears clear this plasticity is an important part of the life history. A full review of physiological regulation of ESD in fishes is beyond the scope of this chapter, but it has been reviewed recently (Devlin and Nagahama 2002; Ospina-Alvarez and Piferrer 2008; Luckenbach et al. 2009; Godwin 2010; Piferrer 2011; Lamm et al. 2015). The focus here is on what appears to be a critical role for estrogenic signaling in both TSD and BSD.

Sex determination can be manipulated in a large number of fish species by exposure to exogenous steroid hormones (Devlin and Nagahama 2002). Specifically with respect to estrogenic signaling, exposure to estrogens induces female development, while blocking endogenous estrogen production with inhibitors of the key rate-limiting enzyme for estrogen synthesis in the gonads, termed aromatase or *cyp19a1a*, instead induces male development (Devlin and Nagahama 2002). This critical role of estrogenic signaling in directing sexual development has been highlighted by several authors and has even been termed the "fish paradigm" with respect to the role of estrogen in brain sexual differentiation (Forlano et al. 2006; Le Page et al. 2010; Piferrer 2011).

A role for estrogenic signaling in socially controlled sex change is supported by differences in levels of estradiol 17β (E_2) across sexual phenotypes, changes in aromatase activity with sex change, and successful manipulations of sex change with both exogenous E_2 and aromatase inhibitors. Several studies have shown significantly higher circulating E2 levels in females of several species that decline with sex change including the saddleback wrasse (*T. duperrey*, Nakamura et al. 1989), the stoplight parrotfish (*Sparisoma viride*, Cardwell and Liley 1991a, b), and gobies (*Gobiodon histrio*, Kroon et al. 2003; *Lythrypnus dalli*, Lorenzi et al. 2008). Conversely, the protandrous anemonefish *Amphiprion melanopus* shows relatively low E_2 levels in males that rise with male-to-female sex change (Godwin and Thomas 1993). These patterns are consistent with decreases in both brain and gonadal aromatase activity at the onset of female-to-male sex change in the goby *Lythrypnus dalli* (Black et al. 2005, 2011) and decreases in brain aromatase mRNA with female-to-male sex change in bluehead wrasses (Liu et al. 2015).

Estrogen manipulations can effectively induce or prevent sex change in several species. Administering either an aromatase inhibitor (the nonsteroidal fadrozole) or the potent androgen 11-ketotestosterone induced female-to-male sex change in the three-spot wrasse *Halichoeres trimaculatus*, but administering estradiol simultaneously could prevent these effects (Higa et al. 2003). Similarly, Kroon and colleagues worked with field populations of the bi-directionally sex-changing goby

Gobiodon erythrospilus and showed that E_2 implants could induce male-to-female sex change, while implants of the aromatase inhibitor fadrozole could induce female-to-male sex change (Kroon et al. 2005). Consistent with an inhibitory effect of estrogenic signaling on sex change, bluehead wrasse females implanted with E_2 did not show behavioral sex change under permissive conditions (large TP males removed from social groups), while cholesterol-implanted control females did show the development of TP male-typical sexual and aggressive behavior (Marsh-Hunkin et al. 2013).

Temperature-dependent sex determination in fishes also appears critically dependent on estrogen signaling. These patterns are particularly well studied ecologically in the Atlantic silverside (*Menidia menidia*) and physiologically in another atherinid fish, the pejerrey (*Odontesthes bonariensis*), and several genera of flatfishes including particularly the paralichthid Japanese flounder *(Paralichthys olivaceus*) and southern flounder (*Paralichthys lethostigma*) (Duffy et al. 2015; Luckenbach et al. 2009; Montalvo et al. 2012). Paralichthid flounder display a complex sex determination system that includes elements of both GSD and TSD. The GSD component of this system appears to be XX/XY with offspring inheriting the Y developing as males (Yamamoto 1999; Kitano et al. 1999, 2000). Offspring that are XX instead show sex determination strongly influenced by temperature with midrange temperatures producing females, while both warmer and cooler temperatures produce predominantly males (Yamamoto 1999; Luckenbach et al. 2003). Several other flatfishes also show strong sex determination responses to temperature (reviewed in Luckenbach et al. 2009; Mankiewicz et al. 2013).

Temperature-dependent sex determination responses are strongly correlated with expression of the gonadal aromatase gene (*cyp19a1a*) in flounder. Juveniles developing at female-determining temperatures show elevated *cyp19a1a* expression relative to developing males, and this appears to be true in both laboratory and field populations (Kitano et al. 2000; Luckenbach et al. 2005). Elevated *cyp19a1a* expression is positively correlated with elevated expression of the forkhead box transcription factor L2 (*FoxL2*) that is associated with sex determination and differentiation responses in a variety of other vertebrates and is important in regulating aromatase expression (Mankiewicz et al. 2013). Conversely, Mullerian inhibiting substance (*MIS*) gene expression is low at female-determining temperatures and elevated at male-determining temperatures (Yoshinaga et al. 2004; Mankiewicz et al. 2013).

How are temperature and other environmental influences transduced into alterations in aromatase expression and estrogenic signaling? Very little is known about the critical early sensory mechanisms underlying these environmental influences, but accumulating evidence across different forms of ESD suggests glucocorticoid hormones associated with the endocrine stress axis are key regulators of gonadal aromatase expression and that glucocorticoid levels are responsive to both social and physical cues. Masculinizing temperatures are associated with elevated levels of cortisol in the pejerrey (Hattori et al. 2009) and Japanese flounder (Yamaguchi et al. 2010; Yamaguchi and Kitano 2012). Supporting a causal association between cortisol and male sex determination, administering exogenous cortisol masculinizes sex ratios in the pejerrey (Hattori et al. 2009), Japanese flounder (Yamaguchi et al. 2010), and southern flounder (Mankiewicz et al. 2013) and even in the gonochoristic medaka (*Oryzias latipes*, Kitano et al. 2012). This may be an indirect relationship with elevated temperatures being one example of a stressor that affects cortisol production. Consistent with this possibility, Mankiewicz and colleagues (2013) found that sex ratios were masculinized for southern flounder reared in light blue tanks, but not in darker tanks. This masculinization in light blue tanks was also associated with significantly elevated body levels of cortisol approximately coinciding with the developmental window for sex determination.

Elevated cortisol levels could masculinize offspring of species with TSD in two different ways. Cortisol can inhibit aromatase (*cyp19a1a*) expression and thereby reduce estrogen production (Yamaguchi et al. 2010). Interestingly, cortisol can also increase production of the masculinizing androgen 11-ketotestosterone (11-KT). Exposure of larval pejerrey to cortisol increased whole-body concentrations of 11-KT, expression of 11β-hydroxysteroid dehydrogenase (*11βHSD*, key synthetic enzyme in 11-KT production), and production of 11-KT in vitro by gonadal explants (Fernandino et al. 2012).

Cortisol has also been postulated as a physiological mediator in socially controlled sex change. Perry and Grober (2003) hypothesized that aggressive behavior from dominant TP males in bluehead wrasses stimulated estrogen synthesis in the gonad, reduced arginine vasotocin expression in the brain, and inhibited 11-KT synthesis by substrate competition at enzymes critical for cortisol synthesis (11-β-hydroxylase and 11β-hydroxysteroid dehydrogenase). This hypothesis was not supported by an experiment in the sandperch *Parapercis cylindrica* where cortisol implants did not prevent female-to-male sex change (Frisch et al. 2007). It remains possible that increases in cortisol may instead facilitate sex change and male sex determination as demonstrated in TSD species. Solomon-Lane et al. (2013) present a strong argument for this possibility based on a common role for glucocorticoid signaling in important life history transitions in vertebrates. They also present data showing sex-changing females in the goby *L. dalli* show increased whole-body cortisol in the first several days following assumption of social dominance with removal of males from social groups (Solomon-Lane et al. 2013). This can also be the case for protandrous sex change where the direction of change is male-to-female. The anemonefish *Amphiprion melanopus* shows significant increases in cortisol over the course of sex change in association with increases in aggressive behavior as sex-changing males become dominant (Godwin 1994a, b). These increases in plasma cortisol are also correlated with aggressive behavior on an individual level in *A. melanopus*.

11.4 Genetic Sex Determination in Fishes

As with environmental determination of sex, the genetic pathways controlling sex determination in fishes are diverse, encompassing the known variation in sex determination in other vertebrate taxa. The variation among fishes stands in particular contrast to uniform sex determination in birds and therian mammals, where almost all species of each class rely on the same sex determination allele, with rare exceptions (Tree of Sex Consortium 2014). The transitions between genetic sex determination systems among fish species are numerous and occur at varied evolutionary time scales, providing a comparative framework and rich context to study the evolution of novel sex determination systems. Additionally, genetic sex determination systems in fish can be found in a number of chromosomal contexts, from single-base differences on autosomes to degenerate sex chromosomes with emerging dosage compensation. Moreover, polygenic sex determination has been identified in an increasing number of fish species. In polygenic sex determination systems, multiple sex determination alleles interact within species and individuals, providing a natural model to explore how a novel sex determination allele might invade a population with an existing sex determination system.

11.5 Evolution of Genetic Switches Directing Sexual Development

Despite the increasing number of genetic sex determination loci mapped in vertebrates, only a handful of sex determination genes have been pinpointed. Fishes have been an excellent discovery resource, and except for the mammalian *Sry-related HMG box* (*SRY*) gene, all other vertebrate sex determination genes or pathways were first implicated in vertebrate sex determination in fishes (Fig. 11.4).

11.5.1 The Transcription Factors: sox3 and dmrt1

The first vertebrate sex determination gene identified and confirmed was *Sry* on the mammalian Y chromosome (Sinclair et al. 1990; Koopman et al. 1991). *SRY* is a highly diverged member of the *Sry-related HMG box* (*sox*) family of transcription factors, eventually shown to have evolved from the *sox3* gene (Katoh and Miyata 1999). Given the strong sequence divergence between *SRY* and *sox3*, the circumstances involving the initial evolutionary co-option of *sox3* as the mammalian sex determiner remain unknown; however, recent studies in the Indian medaka fish (*Oryzias dancena*) suggest some hypotheses. *Sox3* is responsible for sex determination in *O. dancena*, evolved via formation of a novel Y-specific *cis*-regulatory element that directs *sox3* expression in the gonad to drive testis development,

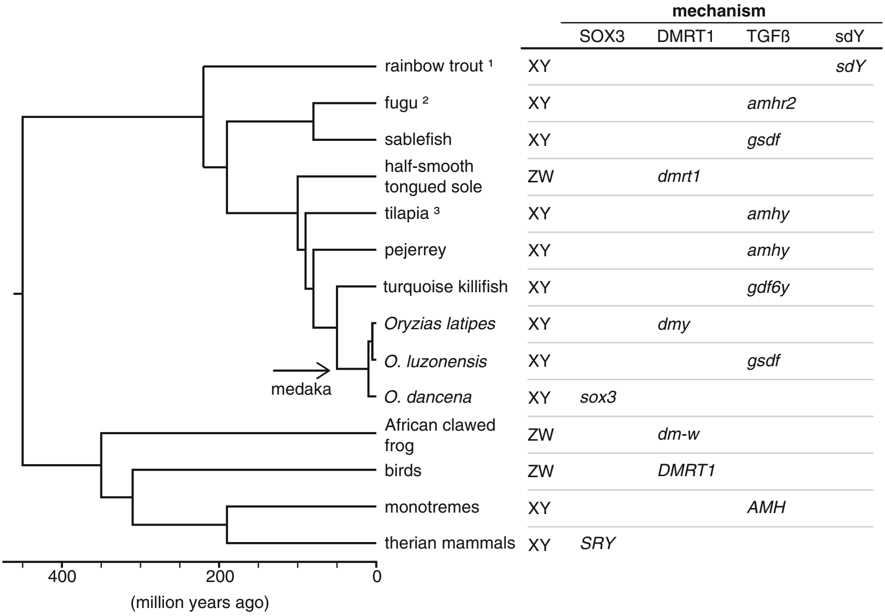

Fig. 11.4 Confirmed vertebrate master sex determination genes in phylogenetic context. To the right of the species tree XY versus ZW sex determination is indicated, and sex determination genes are organized by homology (sox3, dmrt1) or common pathway (TGFβ). [1]sdY was identified in rainbow trout but is the master sex determination gene in twelve salmonid species. [2]The same *amhr2* mutation is responsible for sex determination in *Takifugu* and two species of *Tetraodon*. [3] Tilapia (*Oreochromis niloticus*) has polygenic sex determination, and *amhy* is only the first sex determination gene identified; others exist at other loci (Figure by Emily C. Moore)

without evolution of *sox3* coding sequence (Takehana et al. 2014). In an independent event, *sox3* evolved similarly to become the sex determination gene in two other medaka species (*O. marmoratus* and *O. profundicola*; Myosho et al. 2015). Interestingly, downstream targets of *sox3* in *O. dancena* gonad include *gonadal soma-derived factor* (*gsdf*) (Takehana et al. 2014), discussed below for its role as a sex determination gene. These results suggest that at least in some contexts, turning on *sox3* expression in the gonad is sufficient to target key sexual development genes to drive sex determination.

The second vertebrate sex determination gene identified was *doublesex- and mab3-related transcription factor 1* (*dmrt1*), in the Japanese medaka fish (*O. latipes*) (Matsuda et al. 2002; Nanda et al. 2002). The Y-specific *dmrt1y* gene evolved via duplication of autosomal *dmrt1*, with the *dmrt1y* duplicate exhibiting an ontogenic shift in gene expression; *dmrt1* is only expressed in mature testes, while *dmrt1y* is expressed from very early larval stages into adulthood (Nanda et al. 2002). Interestingly, *dmrt1* has been strongly implicated as the sex determination gene in the ZW system in half-smooth tongue sole (*Cynoglossus semilaevis*) (Chen et al. 2014). *Dmrt1* in sole also exerts its influence by sexually dimorphic expression but under epigenetic control (methylation) in addition to possible evolution of *cis*-regulatory sequence (Chen et al. 2014). Moreover, temperature can shift methylation states at sole *dmrt1* resulting in sex reversal, providing a compelling mechanism for the coexistence of GSD and TSD within the species (Chen et al. 2014; Shao et al. 2014).

11.5.2 The Transforming Growth Factor-β Superfamily Genes: **amh, amhr2, gsdf, and gdf6y**

A compelling pattern is emerging as more than half of the fish sex determination genes that have been subsequently identified are members of the transforming growth factor-*β* pathway: *anti-Müllerian hormone* (*amh*), *anti-Müllerian hormone receptor type II* (*amhr2*), *gonadal soma-derived factor* (*gsdf*), and *growth differentiation factor 6* (*gdf6*).

In both Patagonian pejerrey (*Odontesthes hatcheri*) and Nile tilapia (*Oreochromis niloticus*), male sex determination alleles evolved through duplication of the *amh* gene (Hattori et al. 2012; Li et al. 2015). Similar to *dmrt1y* in the medaka *O. latipes*, the Y-specific *amhy* in pejerrey is expressed in the gonad much earlier than the ancestral form of the gene (Hattori et al. 2012). Interestingly, the amh receptor *amhr2* also acts as a primary sex determination gene, in three pufferfish species in the genus *Takifugu* (Kamiya et al. 2012). In *Takifugu*, a loss-of-function mutation in *amhr2* leads to female development when homozygous, while males are heterozygous for the ancestral and mutant forms (Kamiya et al. 2012). The evolution of *amh* and *amhr2* described above demonstrates that multiple components of a sex differentiation pathway can evolve as sex determiners (Fig. 11.5). Moreover, the signaling pathway can be turned on or off to produce a primary sex determination mechanism; where *amh* signaling increases via altered expression of ligand, male development results, and where it decreases via receptor mutation, female development is the outcome.

Two other transforming growth factor-*β* pathway ligands, *gsdf* and *gdf6*, have evolved as sex determination genes. Where *gsdf* acts as a male sex determiner in the medaka *O. luzonensis*, again a Y-allele evolved that is expressed earlier and at higher levels in XY individuals (Myosho et al. 2012). In *O. luzonensis*, the amino acid sequence of X and Y alleles of *gsdf* is identical, but upstream sequence is required for *gsdf*-driven male development, suggesting *cis*-regulatory evolution. Thus for three sex determination genes, *dmrt*, *amh*, and gsdf, sex determination alleles are expressed earlier than ancestral alleles (Nanda et al. 2002; Hattori et al. 2012; Myosho et al. 2012), suggesting a general evolutionary mechanism where novel sex determination genes can supersede existing sex determination mechanisms via genetic changes that allow them to trigger sexual differentiation at earlier developmental timepoints. Genetic mapping studies have also strongly implicated *gsdf* as the sex determination gene in species of the order Scorpaeniformes, specifically the

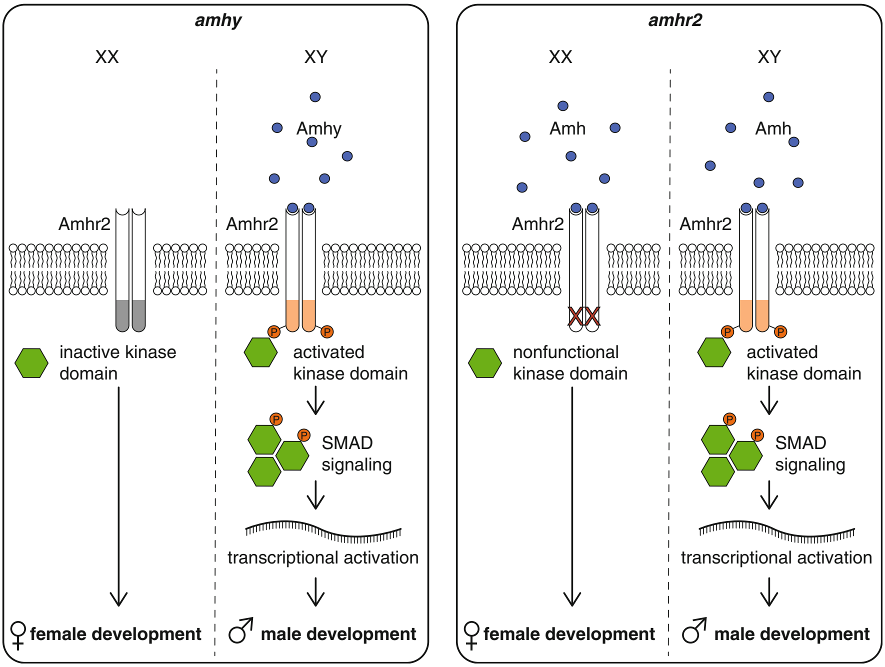

Fig. 11.5 Evolution of the anti-Müllerian hormone pathway for sex determination. Anti-Müllerian hormone pathway signaling is putatively activated by interaction of amh ligand dimers with amhr2 receptor dimers, along with other cofactors not shown. Dimerization induces kinase activity of the receptors, phosphorylating SMAD proteins and initiating a signaling cascade that can induce transcriptional activation to direct male development. Left panel, the ligand *amhy* became the master sex determination gene in Patagonian pejerrey and Nile tilapia, via evolution of a Y-specific duplication of *amh* with a novel expression pattern that activates the pathway in XY individuals. Right panel, the receptor *amhr2* became the master sex determination gene in multiple pufferfish species, via mutational loss of receptor kinase activity on what would become the X chromosome. The Y-allele of *amhr2* retains kinase function, allowing male sexual development only in XY individuals (Figure by Emily C. Moore)

sablefish (*Anoplopoma fimbria*) and two rockfish species of the genus *Sebastes* (Rondeau et al. 2013; Fowler and Buonaccorsi 2016). While developmental mechanisms for *gsdf* alleles are currently unknown in Scorpaeniformes, significant X-Y sequence divergence has been identified for the gene, including moderate insertions in the gene. Finally, *gdf6* has evolved as the male sex determination gene in the turquoise killifish *Nothobranchius furzeri* (Reichwald et al. 2015), with significant changes in both amino acid sequence and sexually dimorphic expression. Interestingly, the putative mechanism for gene expression differences of the Y-specific allele is loss of a regulatory microRNA binding site, via deletion of a portion of the 3' untranslated region of the gene (Reichwald et al. 2015).

11.5.3 Co-option of an Immune Gene: sdY

The sex determination genes listed above share modes of action and known roles in sexual development. By comparison, *sexually dimorphic on the Y chromosome* (*sdY*) is something of an oddball. First identified in rainbow trout (*Oncorhynchus mykiss*), *sdY* evolved via truncation of *interferon regulatory factor 9* (*irf9*) (Yano et al. 2012), which is part of a transcriptional complex that regulates immune response. Subsequent surveys have shown that *sdY* is responsible for sex determination in at least a dozen species of salmonids (Yano et al. 2013). While *sdY* is derived from the transcription factor *irf9*, it differs in fundamental ways from *SRY/sox3* and *dmrt* sex determination genes: *irf9* has no known role in sexual development, and the truncation leading to *sdY* involved loss of the DNA binding domain of *irf9*. Though the mode of action of *sdY* is unknown, its expression is sufficient to drive male sexual development (Yano et al. 2012).

11.6 Convergent Reuse of Sex Determination Genes

As sex determination genes are cataloged in fish and beyond, reuse of certain genes suggests repeated modification of a few pathways across broad taxa to direct sexual development (Fig. 11.4). Notably, the master sex determination genes identified in terrestrial vertebrates thus far are also all used for sex determination in some context in fishes. As mentioned above, the mammalian *SRY* evolved from *sox3*, which acts as a sex determiner in some medaka species (Myosho et al. 2015). Interestingly, circumstantial evidence also implicates *sox3* as a strong candidate for sex determination in some frog species (Uno et al. 2008; Furman and Evans 2016), suggesting its broad potential to determine sex across distantly related taxa. *Dmrt1* plays perhaps the most pervasive and ancient role in sex determination and development, with homologs acting in sexual development in nematodes and insects (Raymond et al. 1998). Outside of fish, *dmrt1* is also the master sex determination gene underlying the ZW sex determination systems in birds (Smith et al. 2009) and the African clawed frog (*Xenopus laevis*) (Yoshimoto et al. 2008), making it the only master sex determination gene definitively identified in three vertebrate classes. The transforming growth factor-β pathway can also be co-opted for sex determination outside of fish, demonstrated by the use of *AMH* as the putative master sex determination gene in the monotreme lineage (platypus) (Cortez et al. 2014). On the other hand, despite its key and conserved roles in sex development in many fishes including teleosts and the coelacanth (*Latimeria menadoensis*), *gsdf* appears to have been lost in tetrapods (Gautier et al. 2011; Forconi et al. 2013), demonstrating that it is dispensable for proper sexual development in other lineages.

The reuse of a handful of sex determination genes among divergent taxa hints at a highly conserved evolutionary toolkit for sexual development and/or evolutionary constraint on the types of genes that can evolve into master sex determination genes;

however, there are caveats to this line of thinking. First, the case of the salmonid *sdY* gene suggests caution in expecting only a few genes are capable of sex determination in fishes. Also, there is some inherent discovery bias in efforts to catalog sex determination genes in additional species, where mapping efforts are more likely to move to a quick resolution if a known master sex determination gene is present within a mapped genetic interval. That said, there are additional genes beyond those identified thus far that could predictably evolve into master sex determination genes but have not yet been identified as such. Developmental genetic experiments in medaka and other fish species suggest a signaling cascade including *sox3*, *gsdf*, and *dmrt1* that modulates *foxl2* and *cyp19a1a* to direct hormone synthesis and sexual development (Matsuda and Sakaizumi 2015; Herpin and Schartl 2011). *Foxl2* and *cyp19a1a* are described above for their role in environmental sex determination, and *cyp19a1a* is easily experimentally manipulated to produce sex reversal in fish (Baroiller et al. 2009; Kitano et al. 2000; Kwon et al. 2000). Genetic manipulation has identified other genes that when mutated produce complete, functional sex reversal, for example, two DNA repair genes in the Fanconi anemia gene family (*fancl* and *fancd1*) and the nuclear receptor *dosage-sensitive sex reversal on chromosome X* (*dax1*) (Rodriguez-Mar and Postlethwait 2011; Chen et al. 2016). Despite robust mapping efforts in a variety of fish species, these and other genes with similar attributes have not been implicated as genetic sex determiners, suggesting that some genes may be more likely than others to evolve as master sex determiners, perhaps due to constraints imposed by negative pleiotropic effects any gene evolution would produce.

11.7 The Evolution (or Not) of Fish Sex Chromosomes

Fish carry their diverse sex determination genes in equally diverse chromosomal contexts. Again, this variety stands in sharp contrast to birds and mammals, which have heteromorphic and highly degenerated W and Y sex determination chromosomes, respectively (Zhou et al. 2014; Lahn and Page 1999). Heteromorphic sex chromosomes were the first identified because they are readily apparent using standard karyotyping techniques, and well-accepted models have been developed to describe their evolution (Charlesworth and Charlesworth 2000). Briefly, when a novel sex determination allele arises on an autosome, it creates a new set of selective pressures on the chromosome, which in many cases will only be carried in one sex. Sexually antagonistic alleles (i.e., those that increase the fitness of one sex and decrease fitness in the other) arising in close proximity to the sex determination gene select for recombination suppression, which can be achieved by any chromosomal divergence, including inversions or other rearrangements, or accumulation of repetitive sequence (reviewed by Wright et al. 2016).

Extreme structural evolution of sex chromosomes is not inevitable in fishes, where only 10% of all species karyotyped show heteromorphic sex chromosomes, though this estimate includes species that may have nongenetic sex determination

(Devlin and Nagahama 2002). In fact, the pufferfish *Takifugu rubripes* and related species demonstrate that a sex determination allele can exist as a single-base variant with zero evidence of sex chromosome evolution or local recombination suppression, even after 6 million years of existence (Kamiya et al. 2012). However, most well-studied sex determination loci in fish show some degree of divergence, helping to empirically fill in predicted stages of sex chromosome evolution from early to late. For example, examination of the 10 million year old medaka (*O. latipes*) Y sex locus revealed a history of duplication, gene loss, and accumulation of repetitive sequence to produce a relatively small 258 kb non-recombining sex locus (Kondo et al. 2004, 2006). On the other hand, the at most 16 million-year-old Y chromosome in threespine stickleback (*Gasterosteus aculeatus*) exhibits broad divergence, including Mb-scale deletions, multiple evolutionary strata of X-Y divergence produced by pericentric inversions, and X-Y recombination restricted to a small 2 Mb pseudoautosomal region (Ross and Peichel 2008; Roesti et al. 2013; White et al. 2015); together these attributes recapitulate the evolutionary trajectory of the mammalian Y (Lahn and Page 1999). Sex chromosome divergence can also vary widely among sister species or even among populations within a species. Though they share a sex determination system, Y chromosomes among species of guppy (genus *Poecilia*) can vary quite drastically at the cytogenetic level (Nanda et al. 2014). Intriguingly, the degree of X-Y divergence in the killifish *N. furzeri* varies by population, with the span of recombination suppression and Y-specific variation ranging from 196 kb to 37 Mb around the sex determiner *gdf6y* (Reichwald et al. 2015). The various paths taken by the same sex determination locus in different lineages demonstrate that historical contingency plays a strong role in determining if, and how, sex chromosomes will evolve.

If sex chromosomes sufficiently diverge, gene content between them can vary, producing potentially detrimental impacts due to the two sexes having different gene dosages (e.g., two copies of X-linked genes in XX individuals and one copy in XY individuals). Dosage compensation describes strategies dealing with sex-related gene dosage issues and generally proceeds by mechanisms normalizing gene expression between sexes (reviewed in evolutionary context in Graves 2016). Dosage compensation is poorly understood in fishes. While it is largely unexpected in the many species with minimal sex chromosome divergence, dosage compensation has been explored in two fish species with heteromorphic sex chromosomes, with differing results. In the threespine stickleback, sex-linked dosage-sensitive genes appear to be maintained intact on both the X and Y under strong purifying selection, even as the rest of the Y chromosome diverges (White et al. 2015). Thus, no dosage compensation is required at the level of gene expression regulation. On the other hand, the ZW system in the half-smooth tongue sole displays strong dosage compensation in a 4 Mb region of the Z chromosome (Shao et al. 2014). In sole, normalization of male and female gene expression in the dosage-compensated region appears to involve DNA methylation. These two case studies make it clear that dosage compensation is possible but not inevitable during sex chromosome evolution in fishes.

In addition to the cumulative divergence of sex chromosomes described above, genetic sex determination factors can be found in a variety of interesting cytogenetic contexts. The *sdY* master sex determination gene conserved in numerous salmonids appears to act similarly to a mobile element, “jumping” around the genome such that it can be found in different locations in the genome among and even within species (Eisbrenner et al. 2014; Lubieniecki et al. 2015). In some species, the dominant sex determination chromosome resides on a fusion of two ancestral autosomes, while the paired recessive sex chromosomes remain unfused; for example, the blackspotted stickleback (*Gasterosteus wheatlandi*) has one Y and two X chromosomes, resulting in females having a diploid chromosome number of 42 and males a diploid number of 41 (Ross et al. 2009). In other species, “B” or supernumerary chromosomes appear to act as sex determination chromosomes; such chromosomes are nonessential, found in addition to the standard species karyotype, and may be present or absent among individuals (Camacho et al. 2000). Examples include various cichlid species from Lake Victoria (Yoshida et al. 2011) and Lake Malawi (Clark et al. 2016) where large B chromosomes act as female sex determiners, and polyploid gibel carp (*Carassius gibelio*), where supernumerary microchromosomes act in male sex determination (Li et al. 2016).

11.8 Polygenic Sex Determination Systems

In addition to the monogenic sex determination systems described above, polygenic sex determination systems have been identified in several fish species (Fig. 11.6). Polygenic sex determination occurs where multiple, independently segregating sex determination alleles or loci interact epistatically to determine sex within a species (Moore and Roberts 2013). Polygenic sex determination was first identified in platyfish (genus *Xiphophorus*), where three possible alleles segregate at a single locus to determine sex, in an XYW system (Kosswig 1964). Polygenic sex determination has been subsequently identified in a variety of fish species, notably in cichlids, where multiple studies have identified sex determination loci on different chromosomes. Pure cichlid species with demonstrated polygenic sex determination include the tilapia *Oreochromis aureus* with Chr1 XY and Chr3 ZW loci (Lee et al. 2004), species of the Lake Malawi genus *Metriaclima* with Chr5 ZW and Chr7 XY loci (Roberts et al. 2009; Ser et al. 2010), and the riverine species *Astatotilapia burtoni* with Chr5 XY, Chr13 XYW, and Chr18 XY loci (Roberts et al. 2016; Böhne et al. 2016), where the same chromosome numbering system is used for all species. Other examples of polygenic sex determination are accumulating in fish, including European sea bass (*Dicentrarchus labrax*), where at least four loci in the genome are associated with sex determination (Palaiokostas et al. 2015). The only polygenic sex determination system in fish where a sex determination gene has been identified is Tasmanian Atlantic salmon (*Salmo salar*), where the same *sdY* gene operates as an XY locus on three different chromosomes and linked mobile element-like sequences

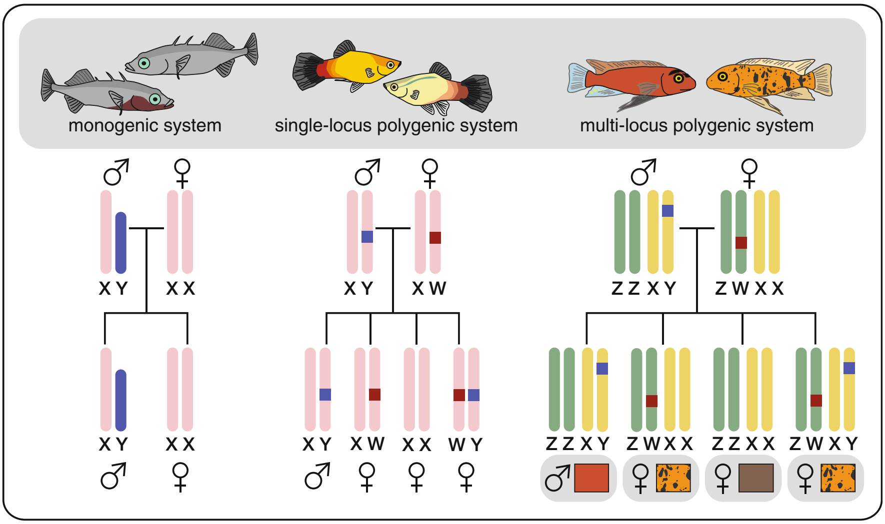

Fig. 11.6 Polygenic sex determination. Monogenic sex determination (left) is represented by the male heterogametic XX/XY system in threespine stickleback, where a degenerated Y chromosome inherited from the father determines male sex in offspring. Platyfish represent a single-locus polygenic XYW system (middle), where the inheritance of a Y allele by itself determines male development, but this effect is overridden by the presence of a W allele at the same locus; the system produces one genotypic male class and three genotypic female classes. African cichlid fish (e.g., the genus *Labeotropheus* here) represent a multi-locus polygenic system (right), where alleles at an XY locus on chromosome 7 (green) and a ZW locus on chromosome 5 (yellow) segregate independently. The W allele overrides the Y male determiner such that ZW/XY individuals are female, again resulting in one genotypic male class and three genotypic female classes for the cross shown. Additionally, a pigmentation allele tightly linked to the W locus produces a color polymorphism in female offspring, and aspects of male nuptial coloration have been mapped to the Y (represented by squares in offspring). Note that in both polygenic systems, other types of crosses with different outcomes are possible, since there are multiple genetic types of females (Figure by Emily C. Moore)

suggest a scenario where the *sdY* gene is able to "jump" around the genome (Eisbrenner et al. 2014; Lubieniecki et al. 2015).

Polygenic sex determination systems are expected to be unstable and evolve to simpler, monogenic systems, based on selection for the master sex determination gene producing the most robust sexual differentiation signal and/or highest reproductive fitness (Bull 1983). However, as species with polygenic sex determination are increasingly cataloged in fish and other taxa (Moore and Roberts 2013), it appears that polygenic sex determination may be an evolutionarily stable strategy in some cases. Whether or not polygenic sex determination systems are stable, they provide a model for evolutionary transitions from one genetic sex determination system to another, where presumably a novel sex determination gene would coexist and interact with an ancestral sex determination gene within a population.

11.9 Transitions Between Genetic Sex Determination Systems

Transitions in genetic sex determination systems are more commonplace in fish than other vertebrate taxa. Compared to the static sex chromosome systems of mammals (180 million years old) and birds (140 million years old), sex determination loci in fish are relatively young, with many on the order of 10 million years old or less, and closely related species having distinct sex determination systems (Fig. 11.4) (Cortez et al. 2014; Kikuchi and Hamaguchi 2013; Ross et al. 2009; Matsuda and Sakaizumi 2015). Importantly, polygenic sex determination systems in some fish species may represent evolutionary transitions in progress. Easy transitions in fish genetic sex determination systems are not surprising given insights from domestication and developmental genetic studies. One classic example, nearly a century old, involved the use of simple artificial selection over a few generations to drive the loss of a natural sex chromosome and arisal of a novel autosomal sex determiner in guppies (*Poecilia reticulate*) (Winge 1932). More recently, it was revealed that common research lines of the developmental model zebra fish (*Danio rerio*) have lost the species' naturally occurring sex chromosome (Wilson et al. 2014), apparently replaced with relatively complex polygenic sex determination systems during domestication (Liew and Orbán 2014).

While artificial selection can clearly produce losses and gains of genetic sex determiners in fish, the natural selective pressures driving evolutionary transitions among genetic sex determination systems are only beginning to be understood. How can a novel sex determination allele arise and outcompete a perfectly functional ancestral sex determination system, especially when such a fundamental fitness trait is involved? One hypothesis is that sexual conflict produced by a sexually antagonistic allele could drive the invasion of a linked novel sex determiner via positive selection pressures to ensure that the trait in conflict is only expressed in the appropriate sex (van Doorn and Kirkpatrick 2007). In other words, there should be selection pressure for alleles providing male-specific benefits to be Y- or Z-linked and those with female-specific benefits X- or W-linked, so that associated traits are expressed in the benefitting sex without impacting the fitness of the other. Rock-dwelling Lake Malawi cichlid fish with polygenic sex determination provides an empirical test of this hypothesis. Most males of these species have bright, gaudy nuptial coloration, and females generally have cryptic coloration, providing support for this hypothesis. An allele producing a cryptic color pattern that would disrupt male nuptial coloration is tightly linked to a relatively new W sex determination allele and thus may improve female camouflage without reducing sexual selection for males (Roberts et al. 2009). Interestingly, multiple aspects of bright male coloration are linked to the ancestral Y sex determination allele on another chromosome in these species (Albertson et al. 2014). Thus, the cryptic color morph is restricted to females, while bright coloration is expressed in males. It may be the case that the proper balance of selection pressures at these two sex determination loci maintains both in populations, allowing polygenic sex determination to be evolutionary stable rather than a transition in progress (Blaser et al. 2011). An additional factor that may drive genetic sex determination system turnover is negative selective pressures on an ancestral sex determination locus, due to accumulation of linked deleterious alleles (Blaser et al. 2013). While this hypothesis has not been directly tested in fish systems, it is notable that the oldest master sex determination gene documented in fish, *sdY* at approximately 60 million years of age (Yano et al. 2013), behaves as a transposable element that moves about the genome and thus could escape linkage to any linked deleterious alleles.

11.10 The Lability of Sex Determination in Fishes

Developmental genetic experiments clearly support lability of sex determination in fish, in the sense that straightforward experimental manipulations produce complete, functional sex reversal. For example, genetically engineered knockout of *gsdf* in medaka produces XY sex reversal (Imai et al. 2015), while overexpression of *gsdf* in tilapia produces XX sex reversal (Kaneko et al. 2015). Similarly, spontaneous mutational loss of *dmrt1* or its experimental overexpression in medaka produces XY and XX sex reversal, respectively (Matsuda et al. 2002, 2007; Otake et al. 2010; Masuyama et al. 2012). Also in medaka, a mutation that inactivates the kinase domain of *amhr2* produces complete sex reversal in males (Morinaga et al. 2007), in a parallel manner to the naturally evolved sex determination system in *Takifugu* (Kamiya et al. 2012), even though neither *amh* nor *amhr2* are known master sex determiners in medaka (Fig. 11.5). These and similar results in the literature suggest that several genes can readily evolve to have master sex determination gene attributes in fish via simple gain- or loss-of-function mutations. By contrast, genetic manipulation of master sex determination genes in the mouse produces incomplete sex reversal. An *Sry* transgene produces sterile XX males, *Dmrt1* knockout causes testis defects without feminization, and *Amh* knockout produces infertile pseudohermaphrodites (Koopman et al. 1991; Raymond et al. 2000; Behringer et al. 1994). Multiple factors likely contribute to the lack of lability of sex determination in mammals relative to fish, including extreme sex chromosome heteromorphy and accompanying dosage compensation, earlier developmental timing of gonad differentiation, and a higher degree of sexual dimorphism in the gonad.

What more general features of teleost biology could account for the extraordinary lability of sexual patterns in the group? Francis (1992) proposed that fishes, generally speaking, display a “polarity of development” between the brain and gonads that is fundamentally reversed relative to mammals. The gonads develop and begin producing gonadal steroid hormones quite early ontogenetically in mammals, with these hormones having important influences sexual differentiating the body and brain (Jost 1970). These are termed *organizational influences* and take place in mammals either prenatally or in the early postnatal period primarily (Phoenix et al. 1959; Arnold and Breedlove 1985; Wallen 2009) By contrast, the gonads develop relatively later in ontogeny for many fishes, often well after these animals are free-living juveniles functioning as essentially “miniature adults.” This provides at least the opportunity for these animals to integrate environmental information that may predict the fitness consequences of being either female or male into the sex determination “decision.”

The selective advantage of integrating environmental information into the sex determination process may then select for mechanisms of sexual determination and differentiation that are more labile than those found in tetrapods. One feature that appears to reduce constraints on sexual lability in teleosts is that the aquatic environment allows external fertilization, which is not an option for at least the amniote tetrapods (Warner 1978). External fertilization does not require the degree of specialization in gonadal ducts and associated structures that are observed with internal fertilization (e.g., mammals or birds), and this may, in turn, reduce morphological and genetic constraints on transitioning between reproductively successful morphs of the two gonadal sexes. Consistent with this hypothesis, there are no reports of sex change in internally fertilizing fishes although this could be confounded by the fact that internal fertilization is observed in freshwater fishes primarily and sex change appears very rare in freshwater fishes generally (Warner 1978).

Lastly, there may be a fundamental difference between fishes and tetrapods in the nature of sexual differentiation of the brain and behavior. Sexual lability can only be evolutionarily advantageous if the various aspects of the sexual phenotype ranging from gonadal function through morphological adaptations and behavior are effectively integrated to develop a reproductively successful phenotype. As noted above, steroid hormones have critical organizational influences on the brain and behavior in tetrapods (Phoenix et al. 1959 and related contributions in ensuing years). This may not be the case for fishes. The occurrence of sex change requires dramatic restructuring of the gonads and often morphological and behavioral changes as individual transitions from one sexual phenotype to another. The nature of these changes implies that gonadal steroid hormones are not having the sorts of early and permanent organizational influences documented for tetrapods. Comparisons of brain transcriptomes in zebrafish and bluehead wrasses do indicate there are relatively few differences in neural gene expression between adult females and males (Wong et al. 2014; Liu et al. 2015). The possibility that the brains of teleost fishes are not sexually organized by steroid hormones is also supported experimentally in the well-studied medaka (*Oryzias latipes*). Hiraki et al. (2012) documented striking differences in the expression estrogen and androgen receptors in the brains of male and female medaka. However, this group also showed experimentally that these differences were not influenced by genetic sex but were instead strongly and purely activationally influenced by current gonadal steroid hormone exposure. Purely activational responses of neural gene expression to gonadal steroid hormone environment may greatly facilitate integrating different components of the sexual phenotype to enable phenotypically plastic and reproductively successful life histories to evolve.

Very little is known about the extraordinarily rapid shifts between male-typical and female-typical behavior that are an important part of the mating sequence for simultaneous hermaphrodites like the Caribbean hamlets. This is a challenging problem to address at a physiological level but would likely be fascinating as it represents perhaps the best example of plasticity in sexual behavior in fishes.

11.11 Summary and Conclusion

Teleost fishes display the greatest diversity of sexual patterns of any vertebrate group. This is true both for documented genetic mechanisms of sex determination within gonochoristic species and environmental influences for species that show differing forms of ESD (TSD and BSD). This tremendous diversity is primarily found in the "triggers," whereas there does appear to be consistency in what may be the final common pathway for sex determination, which is whether or not estrogen synthesis is initiated and/or later inhibited in the developing gonad. A key goal in future studies should be elucidating the "transduction" mechanisms for activating (or deactivating) the aromatase transcription pathway. A second major goal should be to understand transitions between sex determination systems, where fish will provide a particularly strong comparative framework for study. Species that exhibit both GSD and ESD across populations or genotypes, recently diverged sister taxa with distinct sex determination systems, and species with polygenic sex determination should be particularly valuable models for these studies.

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Chapter 12
Causes and Consequences of Evolutionary Transitions in the Level of Phenotypic Plasticity of Reptilian Sex Determination

Nicole Valenzuela

Abstract Reptiles exhibit a remarkable variety of mechanisms by which the sexual fate of developing individuals is decided, ranging from systems of extreme phenotypic plasticity dependent on incubation temperature (TSD) to others of extreme canalization under the control of sex chromosomes (GSD). Our understanding of the extent of this diversity is growing, as is the insight about its molecular basis (genetic and epigenetic). However, further research is still overdue to fully decipher the causes and consequences of the independent and frequent transitions between TSD and GSD in reptile lineages. For instance, theoretically, it may be expected that TSD lineages suffer higher extinction because TSD may result in highly biased sex ratios that are detrimental for population dynamics, while sex chromosomes may promote higher speciation rates. Yet, support from empirical studies is conflicting and instead, some evidence points to longevity as an important mediator for the evolution of sex-determining mechanisms. While much effort has been devoted to test whether TSD or GSD is adaptive in various lineages, one of the most exciting areas of inquiry today relates to the increasing reports of intermediate systems of sex determination in reptiles (GSD + TSD = GSD susceptible to thermally induced sex reversal) as they shed critical light on the constraints (or lack thereof) for the evolution of sex determination in this group. The flourish of genomic approaches applied to the study of sex determination along with the refinement of phylogenetic comparative methods are also enabling the reconstruction of the enigmatic micro- and macroevolutionary history of reptilian sex determination.

12.1 Introduction

Sexually reproducing organisms with separate sexes across the tree of life vary wildly in the way they commit to the male or female developmental fate (Bachtrog et al. 2014; Bull 1983). These sex-determining mechanisms (SDMs) range from

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systems with a high degree of phenotypic plasticity to others with extreme developmental canalization, as occurs in vertebrates (Tree of Sex Consortium et al. 2014; Valenzuela and Lance 2004). Among amniotes, reptiles are particularly labile in the mechanisms they employ to determine sex. At one end of the plasticity continuum, we find reptilian systems that trigger sex-specific development according to the environmental temperature experienced during embryogenesis (temperature-dependent sex determination—TSD) in the absence of consistent genotypic differences between the sexes (thermal polyphenism), while in the other end, we find canalized systems where the trigger is primarily the individual's genotypic constitution (genotypic sex determination—GSD) such as occurs in chromosomal sex determination (Valenzuela et al. 2003, 2014; Sarre et al. 2004). Systems with intermediate levels of canalization/environmental plasticity lay between these two extremes. Reports of intermediate mechanisms in reptiles are increasing and include some well-documented cases where temperature overrides the genotypic signal from sex chromosomes in nature (Shine et al. 2002; Holleley et al. 2015; Radder et al. 2008), while other instances of potential co-occurrence of TSD and GSD have been refuted out empirically (Valenzuela et al. 2014; Mu et al. 2015). The causes and consequences of this remarkable diversity and the turnovers in SDM remain incompletely understood. Studies of sex determination encompass a variety of disciplines and levels of organization. They range from ecological research about the context where various SDMs are found and their effect on sex ratio production and population dynamics, to functional research about the molecular underpinnings of sexual development, to phylogenetic analysis of SDM prevalence and turnover, to theoretical studies about potential drivers of SDM evolution such as sex allocation, sexual selection, or genomic conflict. Here I review our current knowledge about transitions between TSD and GSD in reptiles, models about the relative advantages and disadvantages of each SDM, and whether they are supported by evidence from empirical tests or not.

12.2 Reptilian Sex Determination

A recent explosion of studies of sex determination facilitated by a combination of classic and modern molecular approaches reveals that TSD and GSD coexist within some vertebrate orders, families, and even species, as is the case in reptiles (Tree of Sex Consortium et al. 2014; Pokorná et al. 2011, 2014a, b; Ota et al. 1992; Gamble 2010; Gamble et al. 2014, 2015; Trifonov et al. 2011; Koubová et al. 2014; Schmid et al. 2014; Matsubara et al. 2013; Matsubara et al. 2014; Rovatsos et al. 2014a, b; Sulandari et al. 2014; Badenhorst et al. 2013; Montiel et al. 2017), yet some lineages are surprisingly conserved, such as mammals and birds, while the well-accepted conservation of snakes was recently debunked (Gamble et al. 2017) (Fig. 12.1). Further efforts to identify the SDM in additional reptiles are still warranted because the current taxonomic coverage of all studies combined (Tree of Sex Consortium et al. 2014) encompasses only a fraction of the existing reptiles, obscuring the

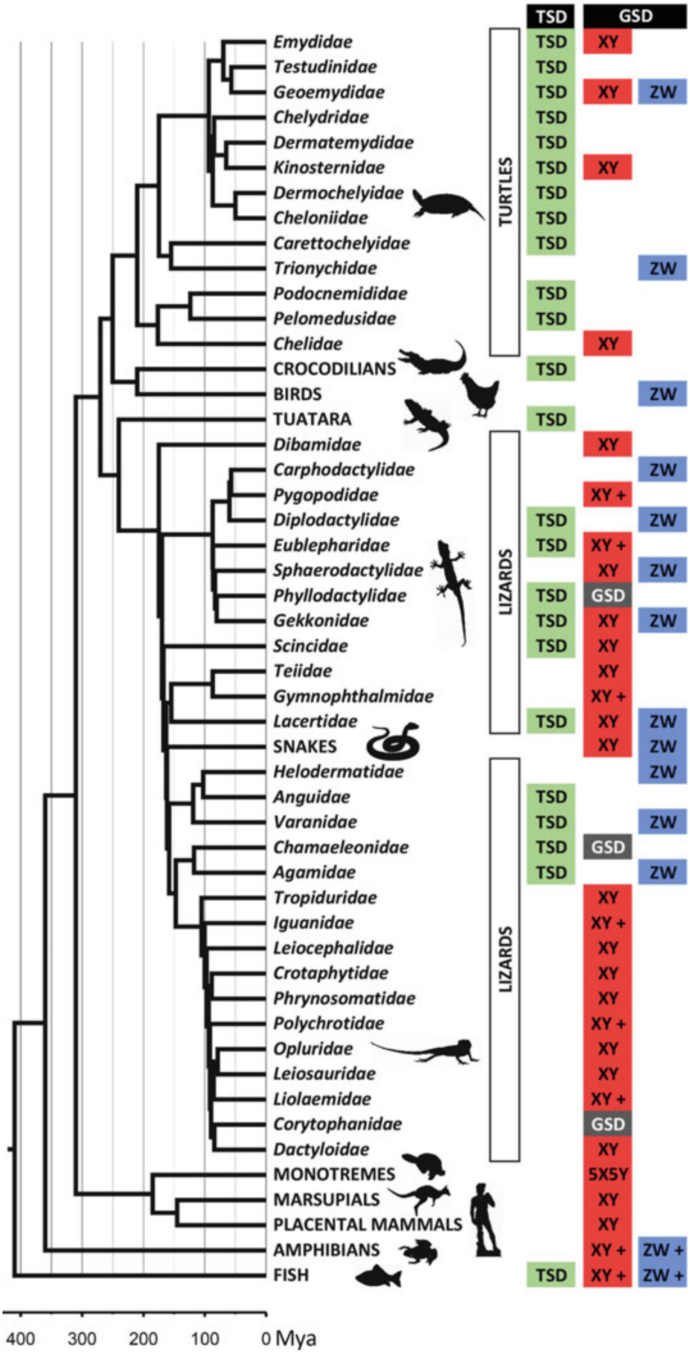

Fig. 12.1 Sex-determining mechanism in reptilian families and other vertebrates. Phylogeny and sex determination information are based on Gamble et al. (2017), Sabath et al. (2016), Chiari et al. (2012), and Jones et al. (2013)

evolutionary history of SDM and correlated traits in this group (Sabath et al. 2016). Interestingly, while TSD reaction norms in turtles commonly span temperatures that produce 100% males and 100% females (Ewert et al. 2004), this is more rarely the case in lizards (Harlow 2004) and crocodilians (Deeming 2004) where fewer species produce 100% males at any given temperature (but the nature of this constraint remains unknown). Several studies now indicate that TSD is the likely ancestral state in reptiles from which GSD evolved independently multiple times, with reversals occurring more often in squamates than in turtles (Sabath et al. 2016; Pokorná and Kratochvíl 2009; Valenzuela and Adams 2011), as detailed below (see section on SDM transitions in reptiles).

The molecular basis of sex determination in reptiles is also receiving considerable attention. Work in this area has concentrated in identifying reptilian homologs of genes known to be involved in sexual development in mammals and birds (Eggers et al. 2014; Smith 2010) and studying their expression patterns during reptilian gonadal development. More recent studies have interrogated reptilian genomes in an unbiased fashion using transcriptomics and illuminated the full composition of the gene network that regulates gonadal formation in turtles (Czerwinski et al. 2016; Radhakrishnan et al. 2017) and alligator (Yatsu et al. 2016). Because of the fascination that TSD has attracted since its discovery half a century ago (Charnier 1966), most of these molecular developmental studies have examined TSD reptiles (turtles, crocodilians, lizards) [e.g., (Czerwinski et al. 2016; Radhakrishnan et al. 2017; Yatsu et al. 2016; Bieser and Wibbels 2014; Rhen and Schroeder 2010; Barske and Capel 2010; Valenzuela et al. 2013; Schroeder et al. 2016; Shoemaker and Crews 2009; Ramsey and Crews 2009; Willingham et al. 2000; Paitz and Bowden 2013; Endo et al. 2008; Choudhary et al. 2000; Xin et al. 2014; Parsley et al. 2014; Inamdar et al. 2015; Parrott et al. 2014; Janes et al. 2013; Smith and Joss 1994; Smith et al. 1995), with the exception of *Apalone* softshell turtles, the only exclusively GSD reptile whose primary sexual development has been studied thus far in this vein (Valenzuela 2008a, b, 2010a; Valenzuela et al. 2006; Valenzuela and Shikano 2007)]. Combined, these efforts indicate that all the components of this regulatory gene network that have been investigated exist in reptiles (Fig. 12.2) with the notable exception of the *Sry* gene which is unique to eutherian mammals (Wallis et al. 2008). Despite the overall conservation in the composition of this network (Graves and Peichel 2010; Cutting et al. 2013), expression patterns of common elements differ within reptiles and among vertebrates (Valenzuela et al. 2013; Cutting et al. 2013). The elegant molecular architecture of sex determination/differentiation can be tipped off-balance by environmental insults as is the case of contaminants such as endocrine disruptors (EDCs) which can yield suboptimal phenotypes both in TSD and GSD reptiles (reviewed in Mizoguchi and Valenzuela 2016). Importantly, multiple elements in this network exhibit plastic responses to environmental temperature during gonadal formation in TSD taxa (differential transcription, translation, or protein activity by temperature) at various developmental stages (reviewed in Rhen and Schroeder 2010; Merchant-Larios et al. 2010). And some responses occur even before the canonical thermosensitive period, i.e., the time window when temperature exerts the

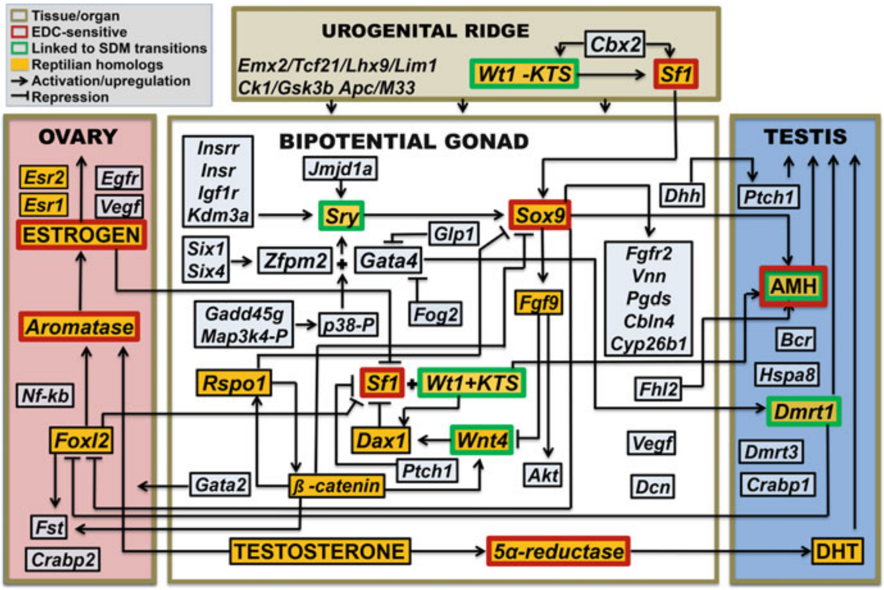

Fig. 12.2 Partial gene regulatory network of mammalian gonadal development, known reptilian homologs, and elements linked to repeated transitions in sex determination. Modified from Mizoguchi and Valenzuela (2016)

strongest influence on sex ratio production (Valenzuela et al. 2006, 2013; Valenzuela 2008a).

This plastic transcription in response to incubation temperature is not necessarily lost in its entirety during the evolutionary transition from TSD to GSD, as some network elements retain relic thermosensitive expression, such as *Wt1* and *Dax1* in *Apalone mutica* turtles (Valenzuela 2008a, b). Thus, TSD-to-GSD transitions require only that certain key downstream elements become insensitive to the differential signals from upstream elements that still respond to the environmental input in order for the plastic SDM to become canalized, as may be the case of *Sf1* in *A. mutica* (Valenzuela 2008a, b, 2010a; Valenzuela et al. 2006). Similar events may have taken place during other evolutionary SDM turnover in reptiles, but the lack of comparative molecular studies between additional TSD and GSD taxa in this group precludes generalizations at this point. Interestingly, however, some members of this network are linked to repeated transitions from TSD to GSD in reptiles and vertebrates (Fig. 12.2). For instance, the molecular evolution of a couple of amino acids of *Dmrt1* accompanies SDM turnover events in reptiles (Janes et al. 2014), and this gene is linked to the independently evolved sex chromosomes (GSD) of *Staurotypus triporcatus* turtles, *Gekko hokouensis* lizards, birds (where it is the sex-determining gene), platypus, plus some amphibians and fish (Graves and Peichel 2010; Brelsford et al. 2013; Montiel et al. 2016; Kawai et al. 2009; Kawagoshi et al. 2014; Grutzner et al. 2004). Additionally, *Wt1* is linked to the also independently evolved sex chromosomes of *Glyptemys insculpta* and *Siebenrockiella crassicollis* turtles

(Montiel et al. 2017; Montiel et al. 2016), which is intriguing because *Wt1* has been postulated as an upstream TSD candidate in the turtle developmental cascade that may play a key role in the early conversion of incubation temperature to sex-specific development, or it may be near the TSD factor that does so (Valenzuela 2008a). Moreover, the evolution of these turtle GSD systems involved chromosomal inversions that encompassed *Dmrt1* or *Wt1* (Montiel et al. 2017; Kawagoshi et al. 2009, 2012, 2014), a likely decisive event for the molecular evolution steps that might have led to GSD evolution and the subsequent divergence of these sex chromosomes themselves (Bachtrog et al. 2011; Charlesworth 2002). Importantly, because vestigial thermal sensitivity is sometimes retained in elements of this regulatory network by taxa with a derived GSD system (Valenzuela 2008a, b), it is plausible that transitions from GSD to TSD could also be facilitated via the co-option of some of those elements in certain lineages, but this remains an untested hypothesis.

12.3 SDM Transitions in Reptiles and What Drives Them

Earlier phylogenetic analyses found equivocal support for the ancestral SDM in reptiles when using family level (Organ and Janes 2008) or restricted sampling approaches (Janzen and Krenz 2004), but later species-level studies reconstruct TSD as the likely ancestral state in squamates (Sabath et al. 2016; Pokorná and Kratochvíl 2009), turtles (Sabath et al. 2016; Valenzuela and Adams 2011), and perhaps even amniotes (Pokorná and Kratochvíl 2016). Thus, GSD appears to have evolved multiple times in diverse reptilian lineages. Reversals back to TSD are more common in squamates, specifically in lizards (Sabath et al. 2016; Pokorná and Kratochvíl 2016), and only rare (if ever present) in turtles (Sabath et al. 2016; Valenzuela and Adams 2011; Literman et al. 2018). Consistently, the transition rates among SDMs varies in turtles and lizards, the two reptilian groups with labile sex determination, with GSD-to-TSD transitions being more prevalent than TSD-to-GSD transitions in lizards, whereas in turtles transitions are more rare and do not differ in direction (Sabath et al. 2016). Thus, turtles appear to retain their ancestral TSD state, whereas lizards have given up TSD for GSD fairly easily over evolutionary time.

But what are the ultimate drivers of these SDM transitions we observe in nature and that take place via the molecular changes described above, or their constraints? Are SDM turnover events random or adaptive responses to selective pressures? These questions have been the focus of extensive studies, both theoretical and empirical, and remain an area of active inquiry and debate, including in reptiles. It is clear that SDMs affect sex ratio production, and in doing so, they can alter effective population sizes and the rate of loss of genetic variation that are linked to extinction probabilities (Bachtrog et al. 2014; Valenzuela and Lance 2004; Girondot et al. 2004; Bessa-Gomes et al. 2004). Namely, because TSD produces sex ratios according to the environmental temperature experienced during development, the potential disadvantages of TSD are easily envisioned. Specifically, TSD populations could produce highly skewed sex ratios due to climatic variation which can cause population extinction and thus favor the evolution of compensating mechanisms to balance the sex ratios, including the evolution of a GSD system (Bull 1980). This matches the theoretical expectation that balanced sex ratios are the evolutionary stable strategy when the cost of producing males and females is the same (Fisher 1930). Also consistent with this idea, turtle lineages within which SDM turnover took place during >210 My of evolution split from their sister clades near peaks of global temperature that may have led to directionally biased sex ratios which were counterbalanced in some lineages by the evolution of GSD (Valenzuela and Adams 2011). The production of intersexes could be another potential detrimental by-product of TSD (Bull 1981), but intersexuality appears to be transient if present at all, at least in turtles (Pieau et al. 1998; Girondot et al. 1998), and simultaneous hermaphroditism is unknown in reptiles (Leonard 2013).

Life histories can play a significant role in triggering or impeding SDM transitions. For instance, the discrepancies in the rates of SDM transitions observed in turtles and lizards are linked to differences in longevity between these two groups (Sabath et al. 2016) which is expected because life span can render TSD and GSD adaptive, maladaptive, or neutral (Bull and Bulmer 1989; Valenzuela 2004; Freedberg and Debenport 2014; Schwanz and Proulx 2008). Namely, lizards abandon TSD and replace it with GSD more often than turtles likely because their generally shorter life span makes them vulnerable to highly skewed sex ratios produced by the vagaries of the environmental temperature (Sabath et al. 2016). And in fact, TSD turtles and lizards evolve toward greater life span compared to their GSD counterparts, an effect that is more pronounced in turtles than in lizards (Sabath et al. 2016). This also implies that given turtle longevity, TSD may be more of a neutral trait which is retained in this group because it works just as well as GSD (Bull 1980; Valenzuela 2004; Girondot and Pieau 1999). On the contrary, given the shorter life span of lizards, TSD in this group, when present, must be maintained by quite strong selective forces, or a transition to GSD would occur when TSD is detrimental. But how could TSD be adaptive at all?

Substantial effort has been devoted to study the potential adaptive advantage of environmental sex determination in general and of TSD in reptiles in particular, and how it could trigger the shift from GSD. One possibility is that biased sex ratios might be favored and TSD provides an adaptive plasticity mechanism to accomplish just that (Shaw and Mohler 1953). Various hypotheses have been proposed to explain when biased sex ratios might be adaptive. For instance, if species are structured into smaller demes, TSD may permit the overproduction of females that would bolster population growth (Bull and Charnov 1988). However, if populations are drastically small, TSD-induced sex ratio skews can push them beyond the limit where *Alee* effects causes their extinction instead of aiding population growth (Berec et al. 2001), in which case a shift to GSD would be favored. Instead, the *Charnov-Bull* model is the most robust of the theoretical models about how biased sex ratios induced by TSD may be adaptive, and it has withstood the test of time over four decades (Charnov and Bull 1977). This model predicts that when the environmental temperature experienced during development (or a correlated variable) confers males and females a lifetime differential fitness in a way that is unpredictable by the parents or the offspring, TSD is favored over GSD because its phenotypic plasticity affords the developing offspring the chance to differentiate into the sex that attains the highest fitness under particular environmental conditions.

The *Charnov-Bull* model does not apply to all cases ubiquitously or in the same manner. For instance, differential fitness may derive from sex-specific life histories, such as from sex-specific mortality (Burger and Zappalorti 1988), or if female quality depends on the site they are born to which they return to nest when adults (Reinhold 1998), an effect that is accentuated if males disperse more than females (Julliard 2000). But support for these alternatives is mixed. TSD was also proposed as a mechanism to induce sex-specific behavioral, morphological, or physiological effects (Tousignant and Crews 1995), or optimal growth by the larger sex in species with sexual size dimorphism because the fitness gain of the larger sex increases faster as a function of body size (via fecundity or mating success) than for the smaller sex (Ewert and Nelson 1991; Head et al. 1987). However, other factors such as temperature and resource availability post-sex determination are as important or more important in determining sexual size dimorphism in reptiles as is incubation temperature directly (Ceballos et al. 2014; Ceballos and Valenzuela 2011; Cox and Calsbeek 2009; Cox et al. 2006; Starostova et al. 2010). And further, sexual size dimorphism is independent of SDM in turtles (Ceballos et al. 2012). Nonetheless, cases where environmental temperature has an *indirect* effect on fecundity have provided the strongest evidence yet for the *Charnov-Bull* model in reptiles [*Amphibolurus muricatus* lizards (Warner and Shine 2008)], and fish [*Menidia menidia* (Conover and Heins 1987)]. In these two disparate short-lived vertebrates, the environmental temperature experienced by the developing offspring provides an indirect cue about the time left in the reproductive season before the arrival of the first winter when growth ceases and sexual maturation occurs. Individuals born earlier in the spring under colder temperatures develop as females and attain larger body sizes that afford them greater fecundity, whereas individuals born later under warmer temperatures develop into males whose fitness is not as impacted by body size. A recent theoretical model combines the sexual dimorphism hypothesis with life history effects and proposes that TSD is favored when males and females mature at different ages if temperature influences juvenile survival (albeit equally for both sexes), but when tested in turtles, this model was unsupported (Schwanz et al. 2016). It should be noted that TSD is not the only mechanism that permits sex ratio adjustments. Indeed, GSD species can alter sex ratios by a variety of means, including meiotic distorters, chromosome-specific fertilization, and sex-specific mortality, among others (Valenzuela et al. 2003; Uller and Badyaev 2009; Uller et al. 2006).

12.4 Constraints to SDM Transitions

The forces that maintain a given SDM constrain by definition the transition to an alternative SDM. For instance, as with the sexual dimorphism hypothesis, TSD could be favored if female nest site choice takes place in an egg-size-dependent manner in a species where larger eggs produce larger hatchings that attain higher fitness, provided that those choices also affect offspring sex (Roosenburg 1996). This explanation may not be widespread, and other maternal effects associated with egg size may be at work, such as steroid allocation (Radder et al. 2009) which is an important molecular regulator of sexual development (Fig. 12.2). Additionally, maternal control of offspring sex when coupled with offspring quality could lead to runaway sex ratio skews that would ultimately select for GSD evolution (Bull 1980). Alternatively, the inherent phenotypic plasticity of TSD may confer a preadaptation against climate change, particularly if genetic variation exists within and among populations for the thermal sensitivity in sex ratio production and for the molecular machinery regulating TSD sexual development (Ewert et al. 2004; Valenzuela et al. 2013; Kallimanis 2009; Escobedo-Galvan et al. 2011). This may also be facilitated by epigenetic modifications such as DNA methylation which is implicated in the molecular regulation of TSD (Navarro-Martin et al. 2011; Matsumoto et al. 2013) and has been linked to responses to environmental change (Angers et al. 2010). This scenario may also explain the lower extinction of TSD versus GSD families observed during the climate change of the Cretaceous/Paleogene transition (Escobedo-Galvan and Gonzalez-Salazar 2012; Silber et al. 2011). The reported inter- and intrapopulation variation in thermal sensitivity and the molecular regulation of sexual development (Holleley et al. 2015; Ewert et al. 2004; Valenzuela et al. 2013; Uller and Helantera 2011; Grossen et al. 2010) also speak against the idea that transitions from TSD to GSD have been prevented by phylogenetic inertia (Bull 1980). Other hypotheses postulate that TSD produces unisexual clutches as a sib-avoidance strategy to prevent inbreeding (Ewert and Nelson 1991; Burke 1993) or that the biased sex ratios observed in TSD taxa are favored by culturally inherited natal homing (Freedberg and Wade 2001). Constraints for the evolution of pure TSD systems and their implication have also been proposed (Uller and Helantera 2011; Valenzuela 2010b), but empirical tests in reptiles suggest that the TSD extreme of the SDM continuum may indeed exist (Valenzuela et al. 2014). Some of these and other hypotheses have been discussed in detail elsewhere (Valenzuela et al. 2014; Valenzuela 2004, 2010b; Uller and Helantera 2011; Beukeboom and Perrin 2014).

12.5 The Intermediate Steps of SDM Transitions

Evolutionary transitions between GSD and TSD imply that populations/species traverse intermediate states in their way from one extreme to the other of the SDM continuum (Valenzuela et al. 2003; Uller and Helantera 2011; Grossen et al. 2010; Beukeboom and Perrin 2014; Sarre et al. 2011). If “pure” GSD and TSD systems were the highest fitness peaks in the SDM optimality landscape and represent evolutionary stable states, then SDM turnover would require crossing fitness valleys where populations will suffer from lower fitness. Such scenario would make intermediate mechanisms unstable states and selection will favor the rapid transition to the alternative fitness peak, rendering these mixed SDMs transitory (Bull 1983; Valenzuela 2004; Uller and Helantera 2011) and probably rare in nature. This hypothesis is supported by the relative scarcity of intermediate SDM thus far reported.

However, alternative SDM mechanisms or certain properties of the molecular machinery that regulate sexual development might act as facilitators for SDM turnover by raising the fitness valley that species need to traverse from one to the other extreme. Or there may be neutral paths in the fitness landscape connecting various SDMs that permit readily transitions among them (Bull 1983). Indeed, an elegant recent model proposes that when sex determination is viewed as the result of a dosage-dependent process derived from the copy number of genes carried by sex chromosomes that is also susceptible to extreme temperatures in the viability range, transitions between GSD and TSD can occur easily (including transitions between male and female heterogamety and various TSD modes) as the system responds to frequency-dependent selection for sex ratio, provided that the sex chromosomes are not highly degenerate (such that YY or WW individuals are viable) (Quinn et al. 2011). This is consistent with quantitative models of gene-by-environment interactions that look at sex determination as a population- rather than an individual-level process, and provides a framework for SDM transitions when sex reversals are induced environmentally and sex ratio selection ensues (Grossen et al. 2010).

Other routes for transitions between male and female heterogamety are discussed elsewhere and range from drift models that lead to the fixation of sex chromosomes in small populations, to models of sexually antagonistic selection and chromosome fusion (Bachtrog et al. 2011; Beukeboom and Perrin 2014; Sarre et al. 2011; Pennell et al. 2015). Thus, perhaps some of these mixed SDM systems are more ancient and evolutionarily stable than originally thought rather than transitory steps between inevitable extremes in the SDM continuum. Ancestral SDM reconstruction (Sabath et al. 2016) combined with a dated squamate phylogeny (Pyron and Burbrink 2014) indicates that the mixed SDMs that are currently well documented in reptiles, in *Pogona vitticeps* (Holleley et al. 2015) and *Bassiana duperreyi* (Radder et al. 2008) lizards, are likely 25-50 My old and appear to be evolving still (Holleley et al. 2015; Radder et al. 2008). In contrast, the youngest turtle sex chromosome system known to date from *Glyptemys insculpta* (Montiel et al. 2017) and *Glyptemys muhlenbergii* (Literman et al. 2017) is ~20 My old (Montiel et al. 2017; Literman et al. 2017). Interestingly, *Bassiana duperreyi* also exhibits an egg-size-dependent allocation of yolk hormones which permits this lizard to balance sex ratios back to 50:50 adaptively when TSD causes a sex ratio skew in certain populations (Radder et al. 2009). In general however, the current scarcity of reports of mixed SDMs may be due simply to lack of proper sampling, because (a) a large proportion of species remain unexamined, (b) because sex chromosomes may be cryptic (Badenhorst et al. 2013; Ezaz et al. 2006), and (c) because search for mixed SDMs may not have been the target of many studies such and they may have been overlooked.

12.6 Consequences of SDM Transitions

The evolution of some traits is associated with transitions in SDM, but in some cases, it can be difficult to distinguish whether they are the cause or the consequences of SDM turnover. For instance, because of the effect of sex ratios on population dynamics, an association between SDM transitions and speciation or extinction is to be expected (Organ et al. 2009; Janzen 1994; Neuwald and Valenzuela 2011; Haldane 1922). But while the transition from TSD to GSD was proposed as a trigger of speciation in extinct lineages of marine reptiles (Organ et al. 2009), other studies found no association between diversification and SDM in Sauropsida (the clade of reptiles plus birds) (Organ and Janes 2008), nor on turtles, lizards, or squamates individually (Sabath et al. 2016). SDMs also correlate with adult sex ratio such that species with female heterogamety (ZZ/ZW) exhibit stronger male-bias than those with male heterogamety (XX/XY), a difference that can alter the demography of populations (Pipoly et al. 2015). On the other hand, SDM shifts in turtles correlate with an increase in chromosome reshuffling that alters diploid number but it is unclear if one occurs first and triggers the other (Valenzuela and Adams 2011). Transitions from TSD to GSD in reptiles involve the evolution of sex chromosomes (the most common GSD mechanism in animals), which in itself can have profound consequences for the evolution of sexual dimorphism, the onset of sexual dimorphism at earlier developmental stages, and its elaboration via the accumulation of sexually antagonistic genes in the heterogametic sex chromosome, compared to TSD taxa (Valenzuela 2010b; Rice 1984). Sexual dimorphism may also include sex-specific gene silencing, which may be affected by the sex chromosomal complement and not just the presence of the heteromorphic sex chromosome (Wijchers et al. 2010). Given the extent of the consequences of sex chromosome evolution (e.g., Bachtrog et al. 2011; Valenzuela 2010b; Pipoly et al. 2015; Rice 1984; Connallon and Jakubowski 2009), the question remains as to whether sexual dimorphism and sexual selection/conflict are less pronounced in species with strict TSD that lack sex chromosomes as appears to be the case for some reptiles (Valenzuela et al. 2014).

12.7 Conclusion

Reptiles hold a crucial key to deciphering the puzzle of the diversity of sex determination, the proximate mechanism that regulate sexual development, and the ultimate forces that drive its evolution. Comprehensive studies that integrate information across these levels, about the molecular architecture of sexual development and how it evolves under the particular ecological contexts of various lineages, will shed light on the causes and consequences of the evolution of sex determination. Despite significant efforts thus far, further research is needed to inventory the full extent of this diversity as our knowledge remains fragmentary, and new analytical methods require development to allow the reconstruction of its evolutionary history.

However, the rapid pace at which new technologies and phylogenetic methods are advancing is encouraging, and they should provide a comprehensive understanding of how and why nature leaves the control of such a fundamental process as is the production of males and females vulnerable to external inputs, and when it canalizes it away from environmental whims.

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