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Mating systems in the sea anemone genus *Epiactis*

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Abstract Four morphologically similar species in the sea anemone genus *Epiactis* exhibit overlapping distributions on the Pacific coast of North America; *E. prolifera*, *E. lisbethae*, *E. ritteri* and *E. fernaldi*. All brood their offspring up to the juvenile stage, but each has a different combination of internal versus external brooding and hermaphroditism versus gonochory (separate sexes). Specimens were collected from sites ranging from British Columbia to southern California between December 1988 and July 1992. Mating systems were inferred from genetic comparisons of mothers and offspring, histological analyses of sex expression and observations on brooding and spawning behavior. Allozyme and multilocus DNA fingerprint analyses of the gynodioecious hermaphrodite *E. prolifera* showed that offspring were all identical to their mothers, a result consistent with either asexual reproduction, self-fertilization or extreme biparental inbreeding. In the gonochore *E. lisbethae*, mothers and offspring were also electrophoretically identical, but variation in DNA fingerprints indicated cross-fertilization. Similar DNA fingerprint differences between mother and offspring in the gonochore *E. ritteri* implied that cross-fertilization also occurs in this species. No mother–offspring comparisons were performed on *E. fernaldi*, as this species was not observed brooding offspring during this study. Although incomplete, the results of this study increase our knowledge of the very unusual combination of reproductive modes in the genus *Epiactis*, and argue for further investigations of the evolution and genetic consequences of mating systems in these species.

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Introduction

Comparative studies of reproductive patterns are most effective when phylogenetic constraints and confounding effects of habitat are minimized, and are therefore best performed on closely related species with overlapping distributions. The sea anemone genus *Epiactis* Verrill, 1869 provides a particularly intriguing system for such studies. The four *Epiactis* species on the northeastern Pacific coast of North America all brood their young up to the juvenile stage, yet each has a different combination of internal versus external brooding and hermaphroditism versus gonochory (Fautin and Chia 1986). The species are: *E. prolifera* Verrill, 1869, an externally brooding, continually reproducing, gynodioecious hermaphrodite (first female, then hermaphroditic), ranging from Alaska to southern California (Dunn 1972, 1975a,b, 1977a,b); *E. lisbethae* Fautin and Chia, 1986, an externally brooding, seasonally reproducing gonochore known from British Columbia to Oregon; *E. ritteri* Torrey, 1902, an internally brooding gonochore ranging from Alaska to central California (Hand and Dunn 1974); and *E. fernaldi* Fautin and Chia, 1986, an internally brooding hermaphrodite known from Barkley Sound, British Columbia and San Juan Island, Washington.

Many details of reproductive mode (whether asexual or sexual; outcrossers or self-fertile, internally or externally fertilized) in these species have not been previously investigated. Only *Epiactis prolifera* had been subjected to genetic comparisons of parents and offspring. Electrophoretic studies by Bucklin et al. (1984) suggested that this species is at least facultatively self-fertile. Offspring of 22 homozygous adults were identical to the parent, while offspring of 3 heterozygous adults had phenotypic segregation ratios not different

from 1:2:1. Although these results imply self-fertilization, the sex of the adults was not determined, and self-fertilization clearly cannot explain offspring found in other studies on small, strictly female adults (Dunn 1975b).

Reproductive mode in the remaining three *Epiactis* species has not been described previously. Although all four species have seemingly functional gonads (Fautin and Chia 1986), this is not sufficient evidence that progeny are produced sexually. Two other actiniid anemones, *Actinia tenebrosa* and *A. equina* var. *mesembryanthemum*, also have apparently normal gonads (Larkman and Carter 1980; Ayre 1984), yet allozyme studies consistently showed brooded offspring to be identical to the adult (even for heterozygous loci), suggesting that offspring are produced ameiotically (Ottaway and Kirby 1975; Black and Johnson 1979; Carter and Thorp 1979; Gashout and Ormond 1979; Orr et al. 1982; Ayre 1984, 1988). Similarly, the scleractinian *Pocillopora damicornis* undergoes gametogenic cycles characteristic of externally fertilized corals (Harriott 1983a,b), yet brooded planula larvae appear to be produced ameiotically (Stoddart 1983), with no clear synchrony between oogenesis and planulation (Stoddart and Black 1985).

In this paper I describe mating systems in *Epiactis* species based on direct genetic comparisons (using allozymes and DNA fingerprinting) of adults and their brooded offspring. Alternatively, breeding systems can be inferred from the genetic structure of populations, but I consider this a weaker approach because genetic patterns can be produced by numerous factors not related to mating system (e.g. dispersal, selection, mutation, genetic drift, effective population size), and because the structural simplicity of cnidarian gonads is conducive to sexual lability, making it especially likely that a single species may reproduce in more than one way, thus blurring the genetic pattern observed at the population level. Indeed, both sexual and asexual reproduction occur in many cnidarian species (Chia

Table 1 *Epiactis* spp. Collection sites, habitats and dates. Sites are listed from north to south (*BC* British Columbia; *WA* Washington; *OR* Oregon; *CA* California) and include both intertidal (*i*) and subtidal (*s*) habitats. Site numbers are same as on Fig. 1 (*I* Island; *Pt* Point)

Species (Site No.), location	Habitat	Dates (mo/yr)
Epiactis prolifera		
(1) Blackfish I, BC	s	7/90
(2) Self Pt, Helby I, BC	s	7/90
(3) Execution Rock, Vancouver I, BC	i	7/90
(4) Neck Pt, Shaw I, WA	s	7/90, 6/91
(5) Brown I, San Juan I, WA	s	6/90, 6/91
(6) Eagle Pt, San Juan I, WA	i	6/90, 6/91
(7) Mar Vista, San Juan I, WA	i	6/90, 6/91
(10) Tatoosh I, WA	i	7/92[a]
(11) Cape Arago, OR	i	7/90, 5/91, 5/92
(12) Shell Beach, CA	i	2/90, 3/91
(13) Bodega Bay, CA	i	3–4/90, 5/91, 5/92
(14) Doran Rocks, CA	i	2/89
(15) Pigeon Point, CA	i	6/89, 10/89, 4/90, 10–12/91, 3–4/91, 8/91, 6/92
(16) Davenport, CA	i	3/89
(17) Big Creek, CA	i	5/90
(18) Ship Rock, Santa Catalina I, CA	s	3–5/89, 4/90
(19) Bird Rock, Santa Catalina I, CA	s	5/89, 4/90
(20) Blue Cavern Pt, Santa Catalina I, CA	s	5/89
Epiactis lisbethae		
(1) Blackfish I, BC	s	7/90
(2) Self Pt, Helby I, BC	s	7/90
(6) Eagle Pt, San Juan I, WA	i	6/91
(7) Mar Vista, San Juan I, WA	i	6/90, 6/91
(11) Cape Arago, OR	i	7/90, 5/91, 5/92
(15) Pigeon Pt, CA	i	5/90
Epiactis ritteri		
(3) Execution Rock, Vancouver I, BC	i	7/90
(10) Tatoosh I, WA	i	7/92[a]
(13) Bodega Bay, CA	i	12/88[b], 4/90, 5/91, 5/92, 7/92
Epiactis fernaldi		
(8) MacGinitie Cave, San Juan I, WA	i	7/90, 6/91, 11/91[c]
(9) South Beach, San Juan I, WA	i	7/90, 6/91

[a] Sample provided by R. Grosberg
[b] Sample provided by W. Clark
[c] Sample provided by D. McHugh

1976), and reproductive mode may be affected by environmental factors such as pollution level, as reported in the sea anemone *Cereus pedunculatus* (Rossi 1975). In *Actinia equina*, the results of direct and indirect methods do not agree; direct analyses showed brooded offspring to be genetically identical to the adult and thereby implied asexual reproduction, yet the extensive genetic variation within populations suggested that sexual reproduction also occurs (Orr et al. 1982). Population genetic structure has been assessed for North American *Epiactis* species (Edmands 1994), and will be described elsewhere in full (Edmands and Potts in preparation).

Both enzyme electrophoresis and multilocus DNA fingerprinting were used to compare adults and their brooded offspring because these techniques offer different advantages. The optimal test of a breeding system requires heterozygous mothers. A major advantage of enzyme electrophoresis is that heterozygotes can be distinguished from homozygotes. Multilocus DNA fingerprinting reveals substantially greater heterozygosity than enzyme electrophoresis due to extremely high mutation rates (Jeffreys et al. 1985a,b), and thus this technique has become popular for studies of parentage (Burke and Bruford 1987; Wetton et al. 1987; Burke 1989). However, multilocus DNA fingerprinting has the disadvantage that heterozygous loci cannot be unambiguously identified, because multiple loci are visualized on a single gel.

In addition to the genetic analyses, brooding adults were also examined histologically to determine sex expression. The presence of brooded offspring in males, females and immatures in *Actinia equina* (Chia and Rostron 1970) and *A. tenebrosa* (Ottaway 1979; Ayre 1984, 1988) is further evidence that brooded planulae may be produced asexually in these species. In *Epiactis prolifera* (a gynodioecious hermaphrodite), brooded young are found both on hermaphrodites and on females (Dunn 1975a,b), while in *E. lisbethae* (a gonochore) only females are believed to brood (Fautin and Chia 1986). *E. fernaldi* individuals are either nonsexual or hermaphroditic, and only hermaphrodites have been reported to brood (Fautin and Chia 1986). Finally, in *E. ritteri* (a gonochore), it is not known whether both sexes brood young.

Materials and methods

Collection and maintenance of specimens

All four *Epiactis* species occur intertidally on rock substrates, and two species (*E. prolifera* and *E. lisbethae*) also extend subtidally to ~ 10 m, where they are typically attached to eelgrass (*Zostera* sp.) or to *Cystoseira* spp. (Hand and Dunn 1974; Dunn 1977b; Fautin and Chia 1986). Samples were collected between December 1988 and July 1992 from sites ranging from British Columbia to southern California (Table 1; Fig. 1). At each site (defined as a single rocky outcrop), up to 30 individuals of each species present were sampled.

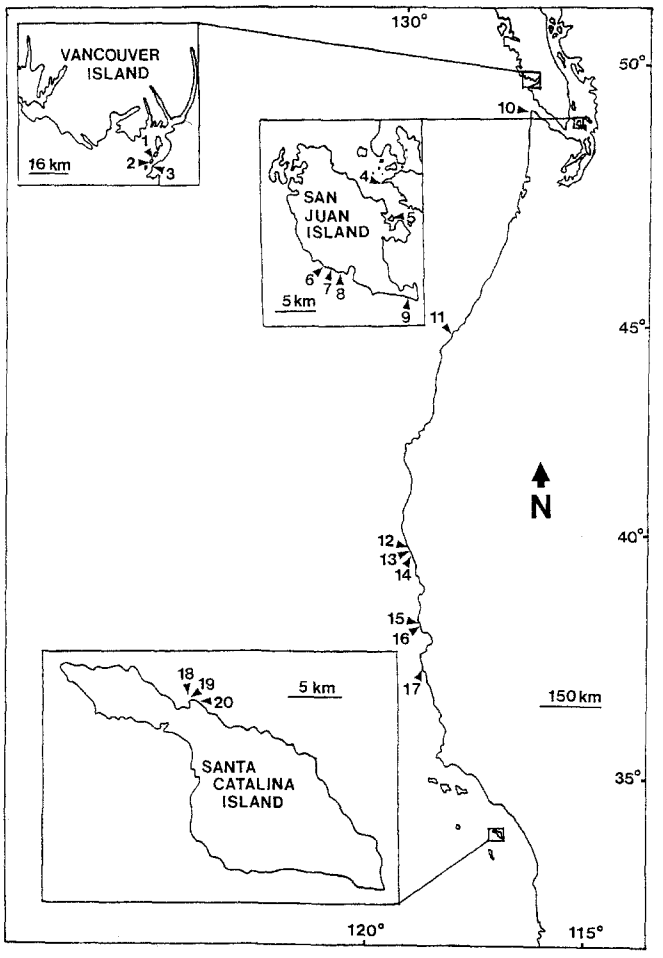

Fig. 1 Pacific coast of North America showing collecting sites of *Epiactis* spp. Numbers identify sites listed in Table 1

When possible, anemones were taken from at least 1 m apart to reduce chances of resampling the same clone. For the two *E. fernaldi* sites on San Juan Island, individuals were concentrated in too small an area to permit widely dispersed sampling, and therefore anemones were taken from a minimum of only 15 cm apart at these locations. Before collection, the pedal-disc diameter of most adults was measured to the nearest millimeter. Specimens were maintained alive in flowing sea-water tables at the Long Marine Laboratory, Santa Cruz, California, and were fed brine shrimp (frozen *Artemia* sp.). Conspecifics were maintained in the same sea-water table, allowing the potential for interbreeding. Throughout the course of this study, both these laboratory specimens and field populations were observed for information on reproductive mode. Records were kept on spawning and brooding behavior (requiring dissection of the internal brooders). Individuals used for genetic analyses were starved a minimum of 3 d before extraction or freezing (-70 °C). Before freezing, offspring were separated from their mothers with a metal spatula.

Histology

For determination of sex expression, anemones were examined histologically following standard methods described in Humason (1967) and Dunn (1975b) to determine the presence/absence of spermatagonia and eggs. Individuals used for histological analyses, which included most of the adults used for the DNA fingerprint

assays, were preserved within a few days of collection, except for one *Epiactis ritteri* which was analyzed after it released young in captivity. Anemones were cut longitudinally into quarters, dehydrated in an ascending ethanol series, cleared with xylene and embedded in paraffin. Blocks were sectioned at 6 μm, concentrating on the lower half of the anemone, where gonads have been found previously (Dunn 1975b). Sections were stained with Erhlich's hematoxylin (Humason 1967) and Eosin Y.

Starch gel electrophoresis

For the electrophoretic study, *Epiactis prolifera* were collected from Pigeon Point and Ship Rock, California and *E. lisbethae* from Cape Arago, Oregon (Table 1; Fig. 1). Methods followed standard procedures outlined in Ayala et al. (1973), Tracey et al. (1975) and Harris and Hopkinson (1976). All individuals analyzed electrophoretically were frozen (-70 °C) at least overnight. For adults and large offspring (>3 mm in basal disc diameter), a small portion (~ 0.1 cm^3) was sliced from the base of the anemone and sonicated in an equal volume of extraction buffer. For small brooded offspring (<3 mm in basal disc diameter), each individual was squashed onto a seawater-dampened filter-paper wick between two glass slides (following Bucklin et al. 1984). Gels were made of 10.5% starch (Sigma). Twenty loci (Table 2) were resolved using three buffer systems (TC1, Ward and Beardmore 1977; TC2, Shaw and Prasad 1970; and LiOH, Shaw and Prasad 1970) and 16 enzyme stains. Stain recipes were essentially those in Murphy et al. (1990), except for CAT and EST-D which were minor modifications of recipes in Aebersold et al. (1987).

A total of 146 adult *Epiactis prolifera*, including 45 brooders, were surveyed initially at up to 20 loci to determine which enzymes were most variable. The brooders were then analyzed along with their offspring (average of 4.3 offspring per adult) for the 4 to 5 enzymes judged most likely to differ between mother and progeny. Analyses were limited to 4 to 5 enzymes because most offspring were only large enough to run one gel. In a later study, eight brooding *E. lisbethae* with particularly unusual genotypes based on population studies (Edmands 1994) were analyzed along with their offspring (average of 5.1 offspring each) for 4 to 5 enzymes. All subsequent paternity analyses were done with DNA fingerprinting which revealed greater genotypic variation.

DNA fingerprinting

For this study, brooding *Epiactis prolifera* were collected from Pigeon Point, California and brooding *E. lisbethae* from Cape Arago, Oregon (Table 1; Fig. 1). DNA isolation methods followed Coffroth et al. (1992), with the following modifications: tissue (either fresh or frozen in liquid nitrogen) was added to *preheated* buffer, two phenol:chloroform:isoamyl alcohol (25:24:1) extractions preceded the chloroform:isoamyl alcohol (24:1) extraction, and there was no RNase step. DNA (~ 10 μg) was digested with an excess of restriction endonuclease. Initial screening of four enzymes (*Bam*HI, *Eco*RI, *Hae*III, and *Hinf*I) determined that *Hae*III gave the clearest fingerprint bands and provided the greatest fragment polymorphism; therefore only *Hae*III was used in all subsequent analyses. Samples were then electrophoresed on a 1X TBE 1% agarose gel. Fifteen-lane (25 × 15 cm) gels were run at 19 mA for 24 h, while 24-lane (27 × 20 cm) gels were run at 19 mA for 36 h with recirculating buffer. The gel was transferred to nylon membrane (BRL "Photogene") for a minimum of 24 h using standard capillary transfer (Maniatis et al. 1982).

Two probes were used: whole-bacteriophage M13mp8 (Vassart et al. 1987) labeled by random priming with Boehringer Manheim's "Genius" kit and Jeffreys' 33.6 probe (Jeffreys et al. 1985a, b; Jeffreys and Morton 1987) covalently linked to alkaline phosphatase (Molecular Biosystems, San Diego). Probing techniques were based on the protocol provided in Tropix' "Southern Light" kit. Multiple exposures were typically made of each gel to maximize the number of scoreable bands. Autoradiographs were scored visually. Only samples run on the same gel were compared, and restriction fragments were scored as shared between two individuals if the bands differed by <1 mm in alignment. Bands were included in the analysis only if the corresponding gel segment was scoreable in all lanes (i.e. a band present in the parent was ignored if the analogous area was too light or blurry in one or more of the offspring lanes). Genetic variation within families was estimated by bandsharing similarity. Similarity (S) between each pair of individuals was calculated as

Table 2 Enzyme/buffer combinations for starch gel electrophoresis [*E.C.No.* Enzyme Commission identification number (Murphy et al. 1990; Shaklee et al. 1990)]

Enzyme	(Abbreviation)	No. of loci scored	E.C. No.	Buffer
Catalase	(CAT)	1	1.11.1.6	LiOH
Esterase	(EST)	1	3.1.1.–	LiOH
Esterase-D	(ESTD)	2	3.1.–.–	TC2
Glucose dehydrogenase	(GDH)	1	1.1.1.47	LiOH
Glucose-6-phosphate dehydrogenase	(G6PDH)	1	1.1.1.49	TC1
Glucose-6-phosphate isomerase	(GPI)	2	5.3.1.9	LiOH
Glutamic-oxaloacetic transaminase	(GOT)	1	2.6.1.1	TC1
Hexokinase	(HK)	1	2.7.1.1	TC1
Isocitrate dehydrogenase	(IDH)	1	1.1.1.42	TC1
Malate dehydrogenase	(MDH)	2	1.1.1.37	TC1
Malic enzyme (NAD^+)	(ME)	1	1.1.1.38	TC1
Peptidase (leucine tyrosine substrate)	(PEP)	2	3.4.11/13.–	TC1
6-phosphogluconate dehydrogenase	(PGDH)	1	1.1.1.44	TC2
Phosphoglucomutase	(PGM)	1	5.4.2.2	TC1
Superoxide dismutase	(SOD)	1	1.15.1.1	TC2
Xanthine dehydrogenase	(XDH)	1	1.1.1.204	TC2

$2n_{xy}/(n_x + n_y)$, where n_{xy} is the number of bands shared between Lanes x and y, and n_x and n_y are the total number of bands in Lanes x and y (Lynch 1990). The complement of the mean value $(1 - \bar{S})$ is the average percent difference (APD).

Results

General observations

Field and laboratory observations of brooding and spawning are summarized in Table 3. Captive anemones were maintained at ambient ocean temperature in flowing sea-water tables. *Epiactis prolifera* and *E. ritteri* were observed in the field in all months of the year, while field observations on *E. lisbethae* and *E. fernaldi* were restricted primarily to June through August, except for three annual trips to Cape Arago, Oregon in May (1990–1992), planned specifically to collect *E. lisbethae* while it was brooding offspring. Two captive *E. prolifera* (an externally brooding gynodioecious hermaphrodite) spawned 20 to 50 pink or orange spheres 300 to 500 μm in diameter in the spring (April 1990 and June 1991). These spheres (either eggs or zygotes) were expelled onto the oral disc and subsequently moved down the side of the column where they became attached. Within 2 wk these offspring were visible as polyps, and within $\simeq 4$ mo they began leaving the adult and crawling onto the surrounding substrate. *E. prolifera* brooded offspring throughout the year, both in the field and in captivity. Within a brood ($\bar{x} = 11$ per adult in natural populations), offspring often differed substantially in size and occasionally they also differed in color.

Three captive *Epiactis lisbethae* (an externally brooding gonochore) spawned spheres on up to three occasions: all three in March 1990; two in February 1991; and two in January 1992. Although two of the original individuals were still alive in 1993, no spawning was observed. *E. lisbethae* offspring remained attached to the parent for up to 6 mo, attaining a size of up to 13 mm. This considerably exceeds the size of the largest brooded juvenile (6.7 mm) found in Dunn's studies (1972, 1975a, b) on *E. prolifera*. In the field, *E. lisbethae* brooded offspring from May (when observations began) through July, and progeny were always of approximately the same size. On collecting trips to Cape Arago, Oregon, in mid-May of three consecutive years, *E. lisbethae* juveniles had reached moderate size by this time in 1990 and 1991; however, in 1992 only very large offspring were present, and many adults had prominent scars from recently departed young. It appears that spawning occurred earlier that year, as it did in the laboratory, possibly due to increased water temperature during an El Niño event (Philander 1992).

Epiactis ritteri (an internally brooding gonochore) was never observed spawning gametes, and none were found brooding offspring in the field. One individual

Table 3 *Epiactis* spp. Brooding of offspring and spawning of eggs or zygotes in field (*F*) and laboratory (*L*). Sample sizes (number of adults) in parentheses

Species	Adult size (mm, $x \pm$ SD)	Brooding offspring			Spawning spheres (eggs or zygotes)			
		Proportion	Months	Offspring/Adult	Months	No. of adults	No. of spheres	Sphere diam
E. prolifera	18 ± 7 (280)	F: 149/280	F: Jan–Dec (149) L: Jan–Dec (37)	F: 11 ± 6 (149) L: 13 ± 5 (5)	L: Apr, Jun	2	20–50	300–500 μm
E. lisbethae	28 ± 11 (48)	F: 8/48	F: May–Jul (8) L: Jan–Sep (6)	F: 4 ± 2 (8) L: 60 ± 15 (6)	L: Jan–Mar	2–3	> 100	~ 400 μm
E. ritteri	20 ± 7 (58)	F: 0/58	L: Mar[a] (1)	L: 3 ± 0 (1)				
E. fernaldi	17 ± 6 (19)	F: 0/19						

[a] Release of internally brooded offspring

which had been maintained in a sea-water table for >1 yr did release offspring in the spring. Despite the rarity of reproduction, this species survived well under laboratory conditions, with several individuals living for >3 yr.

Epiactis fernaldi (an internally brooding hermaphrodite) was never observed brooding offspring or spawning gametes in either the field or the laboratory. This species did poorly under laboratory conditions; specimens collected on three separate occasions never survived longer than 10 mo.

Histological analyses

As there are essentially no anatomical reproductive structures in these anemones, individuals studied histologically were scored as female if they contained only eggs, male if they contain only sperm packets, hermaphrodite if both eggs and sperm were present, and nonsexual if no gametes were present. Results are given in Table 4.

In *Epiactis prolifera*, no males or nonsexual individuals were found. Both females and hermaphrodites were bearing young when collected, and all adults analyzed (collected at various times of the year) contained gametes. In *E. lisbethae*, brooding individuals were either female or nonsexual. Non-brooders identified as *E. lisbethae* included individuals in all reproductive categories. In *E. ritteri*, the only individual seen with progeny during the course of this study was female. Non-brooders were either nonsexual, male or female. No hermaphrodites were seen. *E. fernaldi* were never found brooding and no males were detected. All non-brooders were either nonsexual, hermaphrodite or female.

Table 4 *Epiactis* spp. Sex expression determined by histology

Species, reproductive condition	No. of nonsexuals	No. of females	No. of males	No. of hermaphrodites	Σ
E. prolifera					
brooding	0	4	0	7	11
not brooding	0	2	0	1	3
E. lisbethae					
brooding	2	8	0	0	10
not brooding	3	3	2	1	9
E. ritteri					
brooding	0	1	0	0	1
not brooding	3	3	1	0	7
E. fernaldi					
brooding	0	0	0	0	0
not brooding	8	3	0	2	13
Σ	16	24	3	11	54

Electrophoretic analyses

In preliminary electrophoretic analyses of paternity in *Epiactis prolifera* and *E. lisbethae*, all brooding mothers were homozygous for the 4 to 5 enzymes surveyed, although not all individuals were fixed for the same alleles. Frequencies of parent and offspring phenotypes for those loci found to be polymorphic within each species are shown in Table 5. In all cases, brooded offspring were identical to their mothers at all loci, even for rare alleles such as the "fast" allele at GPI-1 in *E. prolifera* and the "slow" allele at PEP-1 in *E. lisbethae*. Table 6 shows allele frequencies and observed heterozygosity in the populations from which these families came, and demonstrates that alternative homozygotes were frequently found within the same population (e.g. the CAT locus in Cape Arago *E. lisbethae*, the EST locus in Bird Rock *E. prolifera*). A more complete study of population structure using 20 allozyme loci (Edmands 1994; Edmands and Potts in preparation)

Table 5 *Epiactis* spp. Phenotypes of single adults and their brooded offspring for up to five polymorphic enzyme loci, with alleles coded by electrophoretic mobility (*S* slow; *M* medium; *F* fast). *E. prolifera* were collected from Pigeon Point, California, except for six families (*) collected from Bird Rock, California. All *E. lisbethae* families were collected from Cape Arago, Oregon

Species, parent phenotype	No. of families	No. of offspring	No. of offspring identical to parent
E. prolifera			
MDH-1(SS)MDH-2(SS)	6	32	32
CAT(SS)GPI-1(SS)GPI-2(MM)MDH-1(SS)MDH-2(SS)	9	33	33
CAT(SS)MDH-1(SS)MDH-2(SS)	10	33	33
GPI-1(SS)GPI-2(MM)	7	32	32
GPI-1(FF)GPI-2(FF)	1	2	2
MDH-1(SS)MDH-2(SS)GPI-1(SS)GPI-2(MM)	4	18	18
CAT(SS)EST(FF)GPI-1(FF)GPI-2(FF)	2	6	6
CAT(SS)EST(FF)GPI-1(SS)GPI-2(MM)*	6	37	37
E. lisbethae			
CAT(SS)GPI-1(SS)GPI-2(MM)PEP-1(FF)PEP-2(FF)	4	21	21
CAT(SS)GPI-1(SS)GPI-2(SS)PEP-1(FF)PEP-2(FF)	2	11	11
CAT(SS)GPI-1(SS)GPI-2(MM)PEP-1(SS)PEP-2(SS)	2	9	9

Table 6 *Epiactis* spp. Numbers of adult individuals sampled (*n*), allele frequencies (*S* slow; *M* medium; *F* fast) and observed heterozygote frequencies (H_o) for 8 polymorphic allozyme loci in two populations of *E. prolifera* and one population of *E. lisbethae*

Locus Allele, H_o	*E. prolifera*		*E. lisbethae*
	Pigeon Point	Bird Rock	Cape Arago
CAT			
(*n*)	(30)	(23)	(17)
S	1.00	1.00	0.88
F	0.00	0.00	0.12
H_o	0.00	0.00	0.00
EST			
(*n*)	(30)	(22)	(16)
S	0.00	0.09	0.00
F	1.00	0.91	1.00
H_o	0.00	0.00	0.00
GPI-1			
(*n*)	(30)	(23)	(17)
S	0.90	1.00	1.00
F	0.10	0.00	0.00
H_o	0.00	0.00	0.00
GPI-2			
(*n*)	(30)	(23)	(17)
S	0.02	0.00	0.18
M	0.88	1.00	0.82
F	0.10	0.00	0.00
H_o	0.03	0.00	0.00
MDH-1			
(*n*)	(30)	(23)	(16)
S	0.87	1.00	1.00
F	0.13	0.00	0.00
H_o	0.00	0.00	0.00
MDH-2			
(*n*)	(30)	(23)	(16)
S	0.87	1.00	1.00
F	0.13	0.00	0.00
H_o	0.00	0.00	0.00
PEP-1			
(*n*)	(30)	(23)	(16)
S	0.00	0.00	0.13
F	1.00	1.00	0.87
H_o	0.00	0.00	0.00
PEP-2			
(*n*)	(30)	(23)	(16)
S	0.00	0.00	0.13
F	1.00	1.00	0.87
H_o	0.00	0.00	0.00

revealed departures from random mating within populations of both *E. prolifera* (9 populations, standardised genetic variance within populations, $\bar{F}_{IS} = 0.94$) and *E. lisbethae* (3 populations, $\bar{F}_{IS} = 0.96$). Because of this evidence for population substructure, these allozyme data cannot be used to estimate outcrossing rates, as the effects of biparental inbreeding cannot be distinguished from the effects of self-fertilization. Because allozyme heterozygosity was low and no brooding heterozygotes were found in this initial study, all subsequent progeny assays were done with DNA fingerprinting.

DNA fingerprinting

In the DNA fingerprinting analyses (Table 7), all 12 *Epiactis prolifera* mothers had identical offspring (Fig. 2), whether probed with M13 or Jeffreys' 33.6. In *E. lisbethae*, 4 out of 6 families had slight variation in their banding patterns (Fig. 3), with an average percent difference (APD) of 0.07 for the M13 probe and 0.17 for the 33.6 probe. The only *E. ritteri* family assessed had moderate variation, with an APD of 0.48.

Discussion

Sex expression and timing of reproduction

Patterns of sex expression and brooding periodicity in these four *Epiactis* species were generally consistent with previous descriptions. As reported by Dunn (1972, 1975b), both female and hermaphroditic *E. prolifera* brooded offspring throughout the year, and brooded young were often of substantially different sizes (Edmands personal observations), suggesting that individuals of this species reproduce continuously. The possibility remains that individuals identified as female were actually hermaphroditic; in these anemones, as with other simultaneous hermaphrodites (McGrath and O'Foighil 1986), the proportion of gametes allocated to sperm is very small; therefore the presence of sperm cannot be ruled out definitively without sectioning and examining every gonad in its entirety.

In *Epiactis lisbethae*, females and apparently non-sexual individuals were both brooding young. This differs from results for previous studies in which only females were reported to brood offspring (Fautin and Chia 1986), although gonads may have regressed by the time the anemones were examined histologically. Also, one non-brooding individual identified as *E. lisbethae* was hermaphroditic, which is in conflict with previous evidence that this species is gonochoric (Fautin and Chia 1986). Whether this individual was a reproductive anomaly or was actually a mis-identified *E. prolifera* is not known, as the specimen was not examined genetically.

Histological examination of the internal brooder *Epiactis ritteri* supports previous observations (based on 5 individuals) that this species is gonochoric (Hand and Dunn 1974). None of the *E. ritteri* collected in the field were brooding, and only one female (which had been maintained for > 1 yr in the laboratory) released offspring in March. Unpublished notes of R. Fernald on field-collected anemones he identified as *Cnidopus ritteri* (now *E. ritteri*, Fautin and Chia 1986) and maintained in the laboratory for up to several years, report that one or more individuals spawned sperm in February and that juveniles were released in December, February and April. Knowledge of the mode and timing of

Table 7 *Epiactis* spp. Results of DNA fingerprinting of single adults and their brooded offspring. All DNA samples were digested with *Hae*III and analyzed using either M13 or Jeffreys' 33.6 probe. Sexes determined histologically as female (*F*), male (*M*), hermaphrodite (*H*) or nonsexual (*N*) (*Ind* individual; *APD* average percent difference amongst family members)

Species, probe	Ind	Sex	No. of bands scored	No. of progeny	Proportion of progeny identical to adult	APD
E. prolifera ($n = 12$)						
M13	A	H	12	4	1.00	0
M13	B	H	16	7	1.00	0
M13	C	F	23	3	1.00	0
M13	D	?	27	8	1.00	0
M13	E	H	22	9	1.00	0
M13	F	F	20	4	1.00	0
M13	G	?	25	2	1.00	0
M13	H	H	26	5	1.00	0
M13	I	H	12	5	1.00	0
M13	J	F	12	4	1.00	0
M13	K	H	14	4	1.00	0
M13	L	?	14	5	1.00	0
33.6	G	?	19	2	1.00	0
33.6	H	H	19	5	1.00	0
E. lisbethae ($n = 6$)						
M13	A	N	23	2	0.00	0.11
M13	B	F	14	4	0.75	0.03
M13	C	F	15	4	0.50	0.23
M13	D	N	16	4	1.00	0
M13	E	F	19	3	1.00	0
33.6	A	N	26	2	0.00	0.19
33.6	F	F	22	5	0.40	0.15
E. ritteri ($n = 1$)						
33.6	A	F	20	3	0.00	0.48

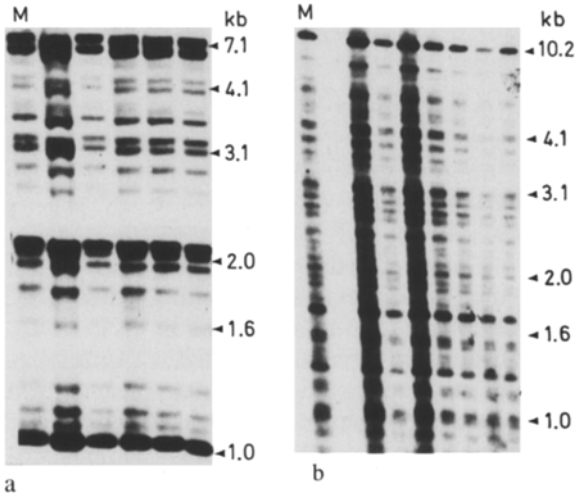

Fig. 2 *Epiactis prolifera.* Mother–offspring comparisons of DNA fingerprints in Family H (**a**) and Family D (**b**). Genomic DNA was digested with *Hae*III and hybridized with Jeffreys' 33.6 probe. In both families, mothers (*M*) were identical to their offspring (unlabeled)

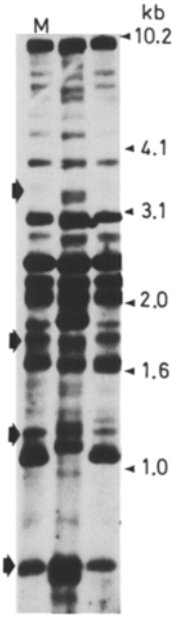

Fig. 3 *Epiactis lisbethae.* Mother–offspring comparison of DNA fingerprints in Family A. Genomic DNA was digested with *Hae*III and hybridized with Jeffreys' 33.6 probe. Mother is designated as *M* and offspring are unlabeled. Both offspring differed from parent, with variation scored in regions indicated by arrows on left

reproduction in *E. ritteri* is limited to these anecdotal accounts.

Epiactis fernaldi examined in this study were either nonsexual, female, or hermaphroditic. Fautin and Chia (1986) examined 15 individuals and found 12 hermaphrodites, 1 female and 2 nonsexual individuals. As the female was smaller than the average hermaphrodite, they concluded that *E. fernaldi* is hermaphroditic, with female gonads developing slightly before the male gonads. Fautin and Chia found *E. fernaldi* brooding offspring in late summer in the field on San Juan

Island, but none of the *E. fernaldi* collected in the present study (also from San Juan Island) during June, July and November were brooding, and none reproduced in the laboratory. However, *E. fernaldi* did poorly under laboratory conditions, with no individuals surviving longer than 10 mo. In contrast, individuals of the other three *Epiactis* species collected from the San Juan Islands and further north lived well in the laboratory, with some individuals surviving > 3 yr, and two of the three species reproducing on multiple occasions.

Reproductive mode

In *Epiactis prolifera*, both the allozyme and DNA fingerprint data were identical for each parent and its offspring, providing no means of distinguishing amongst different modes of reproduction. The lack of genetic variation within families together with extreme heterozygote deficiencies within populations is most similar to the pattern expected from selfing, and indeed self-fertilization has been shown to occur in this species (Bucklin et al. 1984). However, self-fertilization cannot explain the origin of young on the four adults in this study found to be strictly female (Table 4). These offspring must be the result of either asexual reproduction (e.g. somatic budding from diploid tissue or ameiotic parthenogenesis) or biparental inbreeding. Brooded offspring are known to be produced asexually in other sea anemones (Black and Johnson 1979; Orr et al. 1982) and in corals (Stoddart 1983). While the extremely low heterozygosity in *E. prolifera* (observed heterozygote frequencies, $\bar{H}_o = 0.003$; Edmands 1994) is generally unexpected for asexual species (Shick and Lamb 1977; Hoffmann 1986), this result is certainly possible in small populations with low mutation rates and weak selection for heterozygotes. The possibility of cross-fertilization with genetically similar neighbors also cannot be ruled out, given the evidence for substantial population substructure.

In *Epiactis lisbethae*, allozyme patterns also did not differ between parents and offspring, but DNA fingerprints differed in 4 out of 6 families. This species is gonochoric, so this variation is evidence of outcrossing, not self-fertilization. The possibility also exists that some or all the externally brooded juveniles may be "adopted", as has been suggested for internally brooded planulae in *Actinia equina* (Chia and Rostron 1970). While the limited number of variable bands within families and the evidence for population subdivision preclude a test of this hypothesis with the available data, cross-fertilization is a much simpler explanation for the slight genetic differences between parents and offspring. Because DNA fingerprint variation within families is small (APD = 0.17 for the 33.6 probe) compared to variation within populations (APD = 0.30 for the 33.6 probe; Edmands 1994), this suggests that mating occurs mainly with closely related neighbors. This genetic pattern thus resembles that found in highly subdivided plant populations, where even self-incompatible species show significant heterozygote deficits due to very limited pollen flow (Levin 1978; Ennos and Clegg 1982).

Only one individual of *Epiactis ritteri* was found to be brooding during the course of this study. Since fingerprint results for this family show differences between parent and offspring (APD = 0.48), with no two individuals showing identical band profiles, this species appears to be, at the least, a facultative outcrosser. Because this species appears to have separate sexes and because there were fingerprint bands in the offspring that were not present in the parent, this variation cannot be due to self-fertilization.

No brooding *Epiactis fernaldi* were found in this study. These anemones are known only from two sites on San Juan Island, and possibly from remote areas of southeast Alaska (L. Francis personal communication). The rarity of these anemones rules out further destructive sampling, and their mode of reproduction remains unknown. Because of the very low levels of within-population variation ($\bar{H}_o = 0$ for 20 allozyme loci, APD = 0.04 for minisatellite loci; Edmands 1994), parent–offspring comparisons with the available markers may, in any case, have been inconclusive.

This study confirms the importance of direct genetic comparisons of parents and offspring for a complete description of mating systems. As was the case in the controversy over reproduction in *Actinia* anemones, the results of parent–offspring comparisons in these *Epiactis* anemones could not have been predicted from data on population structure or sex expression. Despite evidence of extreme deviation from random mating within populations, *E. lisbethae* appears to be highly cross-fertile. Similarly, despite the regular occurrence of both functional females and hermaphrodites in *E. prolifera*, genetic data provided no direct evidence of cross-fertilization. This makes adaptive explanations for the very unusual strategy of sequential gynodioecy in *E. prolifera* difficult, as obligate outcrossing in females is central to hypotheses concerning the evolution of gynodioecy in *E. prolifera* (Dunn 1975a) as well as in plants (Lewis and Crowe 1955). A more thorough description of the reproductive mode in *E. prolifera* might be revealed through a more comprehensive allozyme study focusing on heterozygotes, or through higher-resolution genetic techniques such as single-locus minisatellites or microsatellites, where heterozygotes can be identified. The reproductive mode in all four *Epiactis* species might also be further elucidated by studies of sperm ultrastructure, as sperm morphology has been shown to correlate well with internal versus external fertilization (Franzen 1970; Harrison 1985). The unique combination of reproductive modes in these *Epiactis* species clearly presents a challenge to our

understanding of the evolution and genetic consequences of reproductive patterns, and promises to be fertile ground for future work.

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References

Aebersold PB, Winans GA, Teel DJ, Milner GB, Utter FM (1987) Manual for starch gel electrophoresis: a method for the detection of genetic variation. NOAA natn mar Fish Serv tech Rep US Dep Commerce 61: 1–9

Ayala FJ, Hedgecock D, Zumwalt GS, Valentine JW (1973) Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. Evolution, Lawrence, Kansas 27: 177–191

Ayre DJ (1984) Effects of environment and population density on the sea anemone *Actinia tenebrosa*. Aust J mar Freshwat Res 35: 735–746

Ayre DJ (1988) Evidence for genetic determination of sex in *Actinia tenebrosa*. J exp mar Biol Ecol 116: 23–34

Black R, Johnson MS (1979) Asexual viviparity and population genetics of *Actinia tenebrosa*. Mar Biol 53: 27–31

Bucklin A, Hedgecock D, Hand C (1984) Genetic evidence of self-fertilization in the sea anemone *Epiactis prolifera*. Mar Biol 84: 175–182

Burke T (1989) DNA fingerprinting and other methods for the study of mating success. Trends Ecol Evol 4: 139–144

Burke T, Bruford MW (1987) DNA fingerprinting in birds. Nature, Lond 327: 149–152

Carter MA, Thorp CH (1979) The reproduction of *Actinia equina* var. *mesembryanthemum*. J mar biol Ass UK 59: 989–1001

Chia F-S (1976) Sea anemone reproduction: patterns and adaptive radiation. In: Mackie GO (ed) Coelenterate ecology and behavior. Plenum Press, New York, pp 261–270

Chia F-S, Rostron MA (1970) Some aspects of the reproductive biology of *Actinia equina* (Cnidaria: Anthozoa). J mar biol Ass UK 50: 253–264

Coffroth MA, Lasker HR, Diamond ME, Bruenn JA, Bermingham E (1992) DNA fingerprints of a gorgonian coral: a method for detecting clonal structure in vegetative species. Mar Biol 114: 317–325

Dunn DF (1972) The natural history of the sea anemone *Epiactis prolifera* Verrill, 1869, with special reference to its reproductive biology, Ph.D. dissertation. University of California, Berkeley

Dunn DF (1975a) Gynodioecy in an animal. Nature, Lond 253: 528–529

Dunn DF (1975b) Reproduction of the externally brooding sea anemone *Epiactis prolifera* Verrill, 1869. Biol Bull mar biol Lab, Woods Hole 148: 199–218

Dunn DF (1977a) Dynamics of external brooding in the sea anemone *Epiactis prolifera*. Mar Biol 39: 41–49

Dunn DF (1977b) Variability of *Epiactis prolifera* (Coelenterata: Actiniaria) in the intertidal zone near Bodega Bay, California, J nat Hist 11: 457–463

Edmands S (1994) Genetic and evolutionary consequences of various reproductive strategies in the sea anemone genus *Epiactis*. Ph.D. dissertation. University of California, Santa Cruz

Ennos RA, Clegg MT (1982) Effects of population substructuring on estimates of outcrossing rate in plant populations. Heredity, Lond 48: 283–292

Fautin DG, Chia F-S (1986) Revision of the sea anemone genus *Epiactis* (Coelenterata: Actiniaria) on the Pacific coast of North America, with descriptions of two new brooding species. Can J Zool 64: 1665–1674

Franzen A (1970) Phylogenetic aspects of the morphology of spermatazoa and spermiogenesis. In: Bacetti B (ed) Comparative spermatology. Academic Press, New York, pp 29–46

Gashout SE, Ormond RFG (1979) Evidence for parthenogenetic reproduction in the sea anemone *Actinia equina* L. J mar biol Ass UK 59: 975–987

Hand C, Dunn DF (1974) Redescription and range extension of the sea anemone *Cnidopus ritteri* (Torrey) (Coelenterata: Actiniaria). Wasmann J Biol 32: 187–194

Harriot VJ (1983a) Reproductive ecology of four scleractinian species at Lizard Island, Great Barrier Reef. Coral Reefs 2: 9–18

Harriot VJ (1983b) Reproductive seasonality, settlement and post-settlement mortality of *Pocillopora damicornis* (Linnaeus), at Lizard Island, Great Barrier Reef. Coral Reefs 2: 151–157

Harris H, Hopkinson DA (1976) Handbook of electrophoresis in human genetics. American Elsevier, New York

Harrison PL (1985) Sexual characteristics of scleractinian corals: systematics and evolutionary implications. Proc 5th int coral Reef Congr 4: 337–342 [Gabrié C et al. (eds) Antenne Museum–EPHE, Moorea, French Polynesia]

Hoffmann RJ (1986) Variation in contributions of asexual reproduction to the genetic structure of populations of the sea anemone *Metridium senile*. Evolution, Lawrence, Kansas 42: 357–365

Humason GL (1967) Animal tissue techniques. W.H. Freeman, San Francisco

Jeffreys AJ, Morton DB (1987) DNA fingerprints of dogs and cats. Anim Genet 18: 1–15

Jeffreys AJ, Wilson V, Thein SL (1985a) Hypervariable 'minisatellite' regions in human DNA. Nature, Lond 314: 67–73

Jeffreys AJ, Wilson V, Thein SL (1985b) Individual-specific 'fingerprints' of human DNA. Nature, Lond 316: 76–79

Larkman AV, Carter MA (1980) The spermatozoon of *Actinia equina* L. var. *mesembryanthemum*. J mar biol Ass UK 60: 193–204

Levin DA (1978) Genetic variation in annual *Phlox*: self compatible versus self incompatible species. Evolution, Lawrence, Kansas 32: 245–263

Lewis D, Crowe LK (1955) The genetics and evolution of gynodioecy. Evolution, Lawrence, Kansas 10: 115–125

Lynch M (1990) The similarity index and DNA fingerprinting. Molec Biol Evolut 7: 478–484

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor, New York

McGrath D, O'Foighil D (1986) Population dynamics and reproduction of hermaphroditic *Lasaea rubra* (Montagu), (Bivalvia: Galeommatacea). Ophelia 25: 209–219

Murphy RW, Sites JW Jr, Buth DG, Haufler CH (1990) In: Hillis DM, Moritz C (eds) Molecular systematics, Sinauer, Sunderland, Mass, pp 45–126

Orr J, Thorpe JP, Carter MA (1982) Biochemical genetic confirmation of the asexual reproduction of brooded offspring in the sea anemone *Actinia equina*. Mar Ecol Prog Ser 7: 227–229

Ottaway JR (1979) Population ecology of the intertidal anemone *Actinia tenebrosa*. II. Geographical distribution, synonomy, reproductive cycle and fecundity. Aust J Zool 27: 273–290

Ottaway JR, Kirby GC (1975) Genetic relationships between brooding and brooded *Actinia tenebrosa*. Nature, Lond 255: 221–223

Philander SG (1992) El Niño. Oceanus 35(2): 56–61

Rossi L (1975) Sexual races in *Cereus pedunculatus* (Boad.) Pubbl Staz zool Napoli (Suppl): 462–470

Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature for protein-coding loci in fish. Trans Am Fish Soc 119: 2–15

Shaw CR, Prasad R (1970) Starch gel electrophoresis of enzymes: a compilation of recipes. Biochem Genet 4: 297–320

Shick JM, Lamb AN (1977) Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. Biol Bull mar biol Lab, Woods Hole 153: 604–617

Stoddart JA (1983) Asexual production of planulae in the coral *Pocillopora damicornia*. Mar Biol 76: 279–284

Stoddart JA, Black R (1985) Cycles of gametogenesis and planulation in the coral *Pocillopora damicornis*. Mar Ecol Prog Ser 23: 153–164

Tracey ML, Nelson K, Hedgecock D, Shleser RA, Pressick ML (1975) Biochemical genetics of lobsters: genetic variation and the structure of the American lobster (*Homarus americanus*) population. J Fish Res Bd Can 32: 2091–2101

Vassart G, Georges M, Monsieur R, Brocas H, Lequarre AS, Cristophe D (1987) A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science, NY 239: 683–684

Ward RD, Beardmore JA (1977) Protein variation in the plaice (*Pleuronectes platissa*). Genet Res 30: 45–62

Wetton JH, Carter RE, Parkin DT, Walters D (1987) Demographic study of a wild house sparrow population by DNA fingerprinting. Nature, Lond 327: 147–149