

Chapter 13

Genomic Perspectives on the Long-Term Absence of Sexual Reproduction in Animals

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Abstract Sexual reproduction, the exchange and recombination of genetic material between different individuals, is commonly viewed as one of the most important sources of genomic diversity in animals. This genomic diversity is subject to natural selection and, consequently, the fittest genomes relative to the environment survive and persist. According to this vision, the absence of sexual reproduction in animals is believed to inexorably lead to an evolutionary dead end as asexual animals become unable to adapt to changing environmental conditions. Yet, several animal lineages suspected to have been reproducing exclusively asexually for millions of years actually survived environmental changes and are not necessarily restricted to specialized ecological niches. The sources of genomic variations that have contributed to the evolutionary success and persistence of these lineages is currently unknown. Here we will review and discuss these known cases of long-term survival of asexually reproducing animal lineages with a focus on recent genomic findings.

13.1 Introduction

The most common reproductive mode throughout the animal tree of life is sexual reproduction, in which meiosis and fertilization occur sequentially. It is also the most widespread both in terms of species and phyla. One reason commonly cited for its evolutionary success is the series of advantages associated with sexual

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reproduction. These advantages comprise higher genome plasticity through mixing and recombination of different haplotypes expected to provide better adaptability (Weismann 1886; Burt 2000), as well as the possibility to overcome invasion of deleterious mutations within genomes (Muller 1932; Muller 1964). Therefore, it is believed that sexually reproducing species are more likely to survive environmental changes and persist over long evolutionary time periods, whereas species reproducing exclusively asexually cannot persist in the long term and represent evolutionary dead ends. Indeed, the absence of recombination and mixing reduces possibilities for an overall genetic variability and there is an ongoing accumulation of deleterious mutations. Most examples of strictly asexually reproducing animals belong to short emerging branches dispersed among clades of sexually reproducing taxa in the animal tree of life (Butlin 2002). Additionally, these recently emerging asexual lineages generally possess only part of the genotypic diversity and occupy a more restricted ecological niche than their sexual ancestors (Doncaster et al. 2000; Janko et al. 2008). This dominance of sexual animals supports the view that sexual reproduction confers advantages over asexuality. In apparent contradiction with this view, a few animal lineages are suspected to have survived in the absence of sexual reproduction for several millions of years. How they survived environmental changes and persisted in the long term in the absence of known mechanisms to generate genetic diversity remains unexplained. In this chapter, we define as ancient asexuals, animal lineages that have been surviving in the absence of sexual reproduction for at least 10 million years. To our knowledge, four such animal lineages have been reported so far and are considered as putative ancient asexuals (Fig. 13.1, Table 13.1): two groups of arthropods (darwinulid ostracods and oribatid mites), one nematode lineage (the tropical root-knot nematodes), and the notorious bdelloid rotifers considered by Maynard Smith as being “something of an evolutionary scandal” (Maynard Smith 1986). The whole genome of the root-knot nematode *Meloidogyne incognita* has recently been sequenced and annotated (Abad et al. 2008). This nematode reproduces without meiosis and without sex and the availability of the genome sequence of its sexual sister species *Meloidogyne hapla* (Opperman et al. 2008) will allow a detailed examination of the genomic consequences of the loss of sex. In parallel, the genome sequence of the bdelloid rotifer *Adineta vaga*, an ancient asexual, is currently underway, and it will be interesting to compare it with the genome of a monogonont rotifer (a closely related group that reproduces both sexually and asexually) in order to identify the genomic signatures of a complete loss of sexuality. Comparison of these two genomic models (nematodes and rotifers) that have survived for a long time in the absence of sexual reproduction will allow determining whether some genomic singularities are shared between different lineages of asexually reproducing animals. To our knowledge, no genome sequence data is currently available for the two arthropod examples (oribatid mites and darwinulid ostracods), but their genomes will be worth exploring in the future to fill the current lack of model and large-scale data for animal species considered as ancient asexuals.

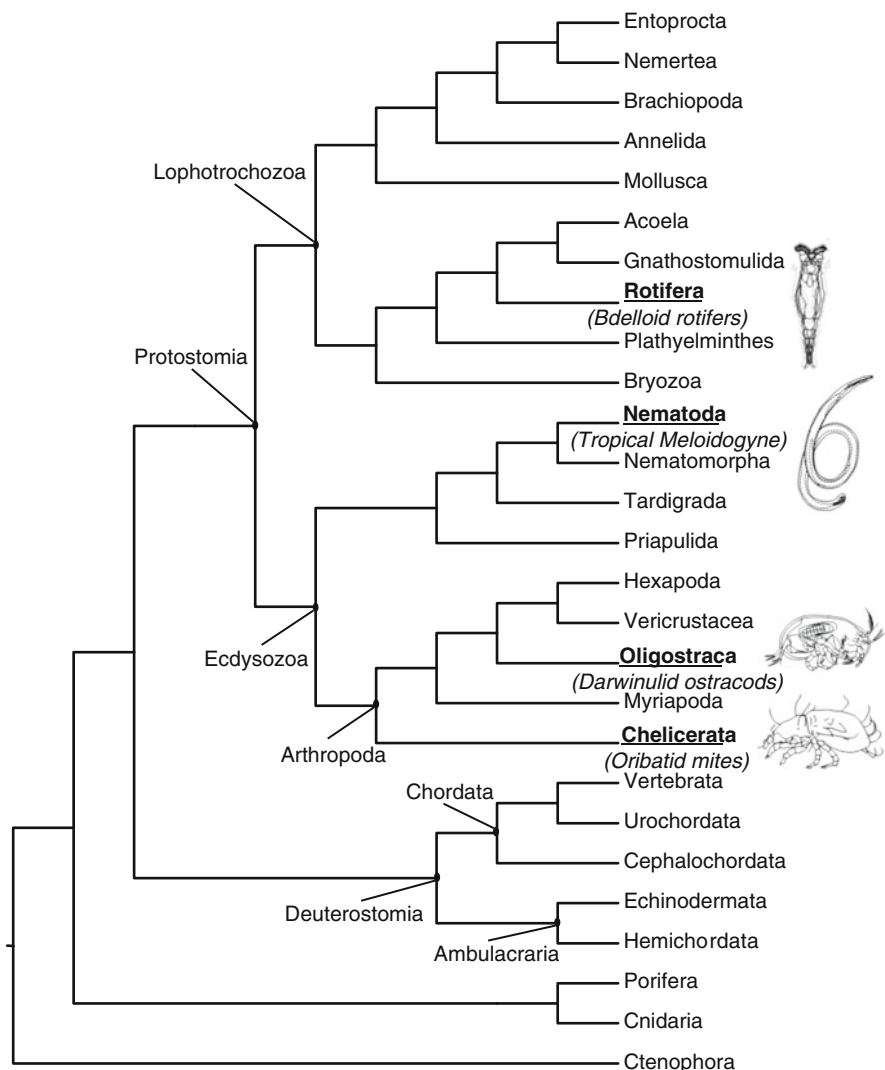






Fig. 13.1 Phylogenetic position of ancient asexuals in the animal tree of life. This schematic representation of the phylogeny of animal species is based on a phylogenomic analysis performed on 71 animal taxa based on 150 different genes (Dunn et al. 2008). The phylogenetic position of the different groups within the Arthropoda lineage is based on a focused phylogeny performed on 75 arthropod species and covering 62 genes (Regier et al. 2010). The main animal divisions are indicated at corresponding nodes. Clades that contain the four animal lineages considered as ancient asexuals in the present chapter are *underlined* and in *bold*

Table 13.1 Characteristics of four known lineages estimated to have survived in absence of sexual reproduction for more than 10 million years

				
Characteristics	Darwinulid ostracods	Oribatid mites	Root-knot nematodes	Bdelloid rotifers
Number of living species	35	10 000	80	460
Habitat	Aquatic	Soil, trees, aquatic	Soil, parasite of plant roots	Freshwater, semi-terrestrial (mosses, lichens)
Phylogenetic group	Arthropoda	Arthropoda	Nematoda	Rotifera
Asexual species				
Model species	<i>D. stevensoni</i> <i>V. cornelia</i>	<i>P. peltifer</i> <i>M. nasalis</i>	<i>M. incognita</i> <i>M. arenaria</i> <i>M. javanica</i>	<i>A. vaga</i> <i>P. roseolata</i>
Parthenogenesis	Mitotic or meiotic?	Meiotic with inverted meiosis sequence	Mitotic => female clones	Mitotic => female clones
Males	Virtually absent, only 3 <i>V. cornelia</i> live males (functional ?)	Very rare and not functional.	Rare, not functional.	No
Genomic singularities associated with ancient asexual status				
Presence of highly-diverged gene copies	No	No	Yes	Yes
Polyploidy/Hybridization	Not described	Not described	Yes Hybridization or two ancient parental haplotypes	Yes WGD or Hybridization => degenerate tetraploid
Homogenization mechanism, low divergence level	Yes, efficient DNA repair?	Yes, efficient DNA repair?	Not described	Yes, gene conversion?
Ancientness of asexuality: Datation source				
Age of asexuality	~25/200 million years	~100/200 million years	~17/43/80 million years	~35/40 million years
Fossil	Yes	No	No	Yes
Emergence of obligate asexual reproduction	Single?	Multiple	Multiple	Single?
Nucleotide divergence	Low at the nuclear level.	High divergence observed in the COI mitochondrial gene	Low at the mitochondrial level, high at the nuclear level	High divergence observed in the HSP82 region
Genomic discrepancies with prediction for ancient asexual status				
Presence of retrotransposons	Yes	?	Yes	Yes
Sexual/Amphimictic relative species				
Facultative / cyclical parthenogenesis	None	Yes and cases of reversion towards sexuality	Clade II Meloidogyne	monogonont rotifers
Obligate sexuals	None	-	<i>M. microtyla</i> <i>M. carolinensis</i> <i>M. macrotyla</i>	-

13.2 An Overview of Putative Ancient Asexual Animals

As mentioned in the introduction, a number of animal lineages appear to contradict the common view that species unable to reproduce sexually represent evolutionary dead ends. Each of these lineages is considered to have survived in the absence of sexual reproduction for more than 10 million years (Neiman et al. 2009). Here, we present the four known such lineages and the associated evidence supporting their long-term abandon of sexual reproduction (Fig. 13.1, Table 13.1).

Frequently, obligate asexuality in animals is inferred from the absence of males, their extreme rarity, or their sterility in extant populations, whereas the ancientness

of asexuality is usually assessed by examining the fossil record, by comparing the divergence level between nuclear or mitochondrial genes in asexual and sexual lineages or by estimating the genetic divergence within individuals (the so-called Meselson effect). In this last case, high allelic sequence divergence observed within asexual individuals (Birky 1996) is interpreted as long-term evolution in the absence of sexual recombination because of the independent accumulation of allelic mutations. However, it has been shown that ameiotic recombination such as gene conversion occurs in asexual species and may reduce heterozygosity (Omilian et al. 2006): consequently, the absence of Meselson effect cannot be considered as a proof of sexual reproduction.

Evolutionary theory also predicts that ancient asexuals should contain few or no functional retrotransposons because sex, despite facilitating the spread of these elements within populations, also limits their intragenomic proliferation. In asexuals, the uncontrolled multiplication of deleterious retrotransposons could predictably lead to their extinction (Arkhipova and Meselson 2005b). Another evolutionary expectation in species that have abandoned meiosis and fertilization a long time ago is that genes involved specifically in those processes should have accumulated a high number of deleterious mutations and become nonfunctional. Although methods such as “meiosis detection toolkit” have been proposed to assess ancientness of asexuality (Schurko and Logsdon 2008), it can still be argued that these genes might have been co-opted for other processes unrelated to meiosis and sexual reproduction.

13.2.1 *Darwinulid Ostracods*

Darwinulid ostracods are small, bivalve crustaceans for which a rich fossil record is available. The darwinulids are exclusively non-marine brooders and only 35 living species have been described (Martens et al. 1998). Researchers generally agree that no traces of male darwinulid ostracods have been found in the fossil record since 65–100 million years ago; and although putative males have been reported for the species *Darwinula stevensoni* for periods comprised between 200 and 100 million years, they actually turned out to be females according to a more detailed analysis (Martens et al. 2003). Based on this long-term absence of observed males, darwinulid ostracods appear to have been surviving without sexual reproduction for at least 200 million years. However, three males of the darwinulid species *Vestalenula cornelia* have recently been found and described and it is not clear whether they represent nonfunctional male relicts or whether they actually participate in rare sexual reproduction (Smith et al. 2006). To date, no copulation in *V. cornelia* has been observed and sperm could not be found in the three males nor in sympatric females (Schön et al. 2009). At the molecular level, analysis of three nuclear regions of a darwinulid species revealed no Meselson effect. Indeed, the level of nucleotide divergence within and between individuals was low compared to a related fully sexual lineage (Schön and Martens 2003). Whether this is due to rare

cases of sexual reproduction, to gene conversion, or to a particularly efficient DNA repair mechanism remains to be elucidated. The genome of a darwinulid species was screened for the presence of RT-encoding non-LTR retrotransposons (LINEs) and two novel families were characterized, one being apparently functional, but their role and position have not yet been determined (Schön and Arkhipova 2006).

Until now, the only evidence for the ancient asexual status of darwinulid ostracods is the absence or extreme rarity of males. Schön et al. (2009) suggested that the “model-species” *D. stevensoni* is the most likely candidate to be a true ancient asexual. Indeed, no recent or fossil male has been found since at least 25 million years and this species appears to feature genetic mechanisms that homogenize their genome and maintain their general-purpose genotype (Schön et al. 2009). Such a generalized genotype appears to allow survival in a wide range of ecological conditions (Van Doninck et al. 2002; Van Doninck et al. 2003), as further detailed in Sect. 13.3.1.

13.2.2 Oribatid Mites

Oribatid mites are a species-rich group with around 10,000 currently described species inhabiting soils, trees, and aquatic habitats. They are small arthropods belonging to the Acari, and parthenogenesis, a mode of asexual reproduction, is supposed to have emerged multiple times independently in this clade. Although asexuality is the most frequent reproductive mode in the oribatid phylum, reversion toward sexuality has been reported to occur in some species of this group (Domes et al. 2007). The exact mode of asexual reproduction is not clear yet but a recent review suggests terminal fusion automixis with holokinetic chromosomes and inverted meiosis sequence (Heethoff et al. 2009). In oribatid mites, the hypothesis of an obligate asexual reproduction is not supported by the absence of males but rather by their rarity and sterility. Also supporting this idea is the apparent absence of cyclical parthenogenesis in these species (Palmer and Norton 1991), and the balanced sex ratio observed in sexual lineages (Heethoff et al. 2007). Concerning the ancientness of asexuality, divergence levels in the mitochondrial COI gene between and within clades of *Platynothrus peltifer* suggest that asexual reproduction is at least 100 million years old for this species (Heethoff et al. 2007). Another fully asexual species in this group, *Mucronothrus nasalis*, is thought to be 200 million years old (Hammer and Wallwork 1979). Similarly to darwinulid ostracods, no Meselson effect has been identified in oribatid mites and analyses of nuclear regions also suggest homogenization mechanisms in the absence of sexual recombination (Schaefer et al. 2006). This low divergence at the nuclear level contrasts with the high divergence level observed in the mitochondrial gene COI, used as an indication of ancientness. The presence of active retrotransposons within their genome has not yet been screened.

13.2.3 *Root-Knot Nematodes*

Root-knot nematodes (*Meloidogyne* genus) comprise ca. 80 described species, dwell in soil, and parasitize plant roots. Phylogenetic analyses have shown that in this lineage, asexual reproduction through obligate mitotic parthenogenesis has emerged at least two times independently (Holterman et al. 2009). These lineages have thus not only abandoned sexual reproduction but also meiotic division. The *Meloidogyne* clade I contains tropical root-knot nematodes (e.g., *M. incognita*, *M. arenaria*, *M. javanica*) that are considered as ancient mitotic parthenogenetic species (De Ley et al. 2002; Castagnone-Sereno 2006; Holterman et al. 2009). Clade II, its most closely related clade (Holterman et al. 2009) is essentially composed of facultative meiotic parthenogenetic species like *M. hapla* but also comprise two species described as obligate sexuals (*M. microtyla* and *M. spartinae*). Clade III, which holds an outgroup position relative to clades I–II, contains species that have all been described as facultative meiotic parthenogens (e.g., *M. chitwoodii*, *M. fallax*), except *M. oryzae* that is considered as a mitotic parthenogenetic species (Holterman et al. 2009). The common assumption that tropical *Meloidogyne* species (*M. incognita*, *M. arenaria*, *M. javanica*) are obligate parthenogens is not based on the absence of males. Indeed, males are observed in these tropical root-knot nematodes but they are rare and are assumed not to contribute genetically to the offspring (Castagnone-Sereno 2006). Furthermore, meiosis has never been observed in these nematodes and offspring results from mitotic division from the female germline, thus giving rise to clones (Van der Beek et al. 1998). High frequency of polyploidy, aneuploidy, and variable chromosome number within one species have all been reported in strict parthenogenetic *Meloidogyne* (Sasser and Carter 1985; Castagnone-Sereno 2006). Such observations are commonly viewed as indicative of frequent asexual reproduction although not necessarily of obligate asexuality.

On the basis of a comparative analysis of enzymatic profiles in the genus *Meloidogyne*, Esbenschade and Triantaphyllou (1987) estimated the last common ancestor of tropical mitotic parthenogenetic (strictly asexual) and of facultative meiotic parthenogenetic (able to reproduce sexually) nematodes to be ca. 43 million years old. Based on the phylogenetic tree presented by these authors, the age of the last common ancestor of mitotic species is estimated to be ca. 17 million years old. Another analysis, based on the comparison of the level of divergence of the mtDNA between mitotic and meiotic RKN, suggests that the last common ancestor of these two lineages may be as old as 80 million years (Hugall et al. 1997). In both analyses, the authors acknowledge that these divergence times could be overestimated due to an AT-rich composition or other biases. Anyhow, taking these potential biases into account, the last common ancestor of the obligate asexual root-knot nematodes is unlikely to be more recent than several millions of years.

A recent analysis offers a different interpretation of the evidences for long-term asexual reproduction in *Meloidogyne*. In a phylogenetic analysis of various nuclear genes, Lunt (2008) confirms the occurrence of large divergence in allelic sequences in tropical *Meloidogyne*, as expected in an asexually reproducing species due to

Meselson effect, and shows that the alleles do not cluster according to recognized morphological species in phylogenies. However, due to high similarity at the mtDNA level between different tropical apomictic (strict parthenogen) species, the author interprets the observed high allelic sequence divergence as the result of past interspecific hybridizations rather than as Meselson effect.

13.2.4 *Bdelloid Rotifers*

Bdelloid rotifers are common microinvertebrates inhabiting freshwater environments and semiterrestrial habitats such as mosses, lichens, and temporary pools. Bdelloidea, in which more than 460 morphospecies have been described, is the only class of the phylum Rotifera composed entirely of obligate parthenogenetic species (Segers 2007; Segers 2008). Despite much observation since the eighteenth century, neither males nor vestigial male structures have ever been observed in bdelloid rotifers. However, males have been clearly identified in monogonont rotifers, the sister class reproducing by cyclical parthenogenesis (Velázquez-Rojas et al. 2002; Leasi et al. 2010). The absence of males and the fact that single females can be reared in laboratories to produce “female” clones have led to the hypothesis that bdelloid rotifers are indeed asexual. Hsu (1956a, b) studied bdelloid oogenesis and demonstrated that oocytes are produced without any chromosome pairing or reduction in chromosome number and that after two mitotic divisions, one egg and two polar bodies are produced. These cytological results indicate the absence of meiosis and hence a reproduction by obligate mitotic parthenogenesis in bdelloid rotifers.

The presence of bdelloid fossils in old amber dated 35–40 million years (Waggoner and Poinar 1993) indicates that bdelloid rotifers originated more than 40 million years ago. Another signature of the ancient asexual status of bdelloid rotifers is that, unlike monogonont rotifers and other tested eukaryotic animals, bdelloids seem to lack high-copy number retrotransposons within their genome (Arkhipova and Meselson 2000). Commonly, those elements will propagate within the genome and if specific meiotic mechanisms are absent to control their proliferation; their unchecked invasion will lead to the extinction of the lineage (Arkhipova and Meselson 2000). Therefore, asexuals can only persist if vertically transmitted deleterious elements are maintained at a low level or are absent within their genome, a situation observed in bdelloids.

High levels of allelic divergence in the *hsp82* region were first reported by Mark Welch and Meselson (2000), suggesting that Meselson effect, an accumulation of mutations between former alleles that may lead to functional divergence, did occur in bdelloid rotifers. However, more recent studies of both the *hsp82* and histone regions of bdelloid genomes have demonstrated that they are in fact degenerate tetraploids, resulting either from an ancient whole genome duplication (autotetraploidization) or an interspecies hybridization (allotetraploidization) (Mark Welch et al. 2008; Hur et al. 2009; Van Doninck et al. 2009). Consequently, their genome

is structured as two colinear pairs of genomic regions corresponding to two ancient lineages (A and B). The two lineages A and B have only few genes in common and these genes present a high level of nucleotide divergence. Within each lineage, the divergence between copies is low although a few gene copies diverge by as much as 20% (Ks value), indicating that over time, in the absence of recombination or homogenization mechanisms, synonymous divergence accumulates (Mark Welch et al. 2009).

13.3 The Challenges of Long-Term Asexuality

13.3.1 *Adaptability Without Sex*

One argument to explain why sexual reproduction is the most widely represented reproductive mode in animals is that it allows better adaptation (Weismann 1886; Burt 2000). By allowing mixis between arrangements of alleles (haplotypes), sexual reproduction produces at each generation new combination of alleles that can provide a selective advantage. An allele may turn out to be advantageous only when expressed together with other alleles in a combined effect. In asexual species, such a combined advantageous effect is substantially less likely to occur as emergence of a new mutation is restricted to one individual and its offspring and has no chance to mix with mutations that occurred independently in other individuals. Similarly, in diploid species, if a mutation provides an advantage only when it is present in the homozygote state, this mutation has to occur twice and independently in the two former alleles of an asexual lineage (Kirkpatrick and Jenkins 1989), unless gene conversion using the “advantageous” gene as template occurs (Mandegar and Otto 2007). Another aspect is that an advantageous mutation cannot spread easily in populations of asexual species. Indeed, assuming that such a mutation has occurred in one individual, it can only be transferred to its own offspring, but to be spread in the population, it has to be by competitive replacement of the offspring of other individuals (that may bear other mutations that would have been beneficial in different conditions). Again fixation of an advantageous mutation is supposed to be much longer and difficult in asexual populations than in sexual ones, according to the Fisher-Muller accelerated evolution theory (Fisher 1930). Intuitively, we may postulate that sexual lineages possess a better adaptation potential to environmental or ecological changes than asexuals. Furthermore, if the asexual lineage emerged from an individual genotype of the source sexual lineage, it probably possesses only a reduced frozen subset of the whole pool of genetic diversity present in the source population (Vrijenhoek and Parker 2009). In such a case, asexuals must occupy more restricted ecological niches than their sexual relatives. However, if the asexual lineage possesses a more versatile genotype than the source sexual lineage(s), as a result of hybridization for example, the asexuals may present a broader niche than their sexual relatives. In all cases, in the

absence of sexual recombination, parthenogenetic species lack an important mechanism of genotypic plasticity. Thus, while they may have an adaptive advantage in a relatively stable environment due to a frozen efficient genotype and a reproductive efficiency since males are not produced, they appear clearly disadvantaged in cases of multiple environmental changes as it is expected for lineages that have been surviving for long evolutionary periods. As counterintuitive as it may appear, the four asexual animal lineages discussed here exhibit a wide geographical distribution.

In darwinulid ostracods (all asexuals), species that are ubiquitous and cosmopolitan, like *Darwinula stevensoni*, seem to contain a general-purpose genotype (GPG), i.e., a genotype providing a broad environmental tolerance (Van Doninck et al. 2002, 2003). Unlike sexuals, for which selection acts over individual genes, in asexuals such as darwinulids the unit of selection appears to be the complete genome. As a consequence, natural selection over time can favor clones with a wide tolerance (see Vrijenhoek and Parker 2009) and once such a GPG clone evolved, it can be maintained because the absence of recombination will avoid breaking up those well-adapted genotypes. How such a GPG evolved in the darwinulid ostracods is not known but the cosmopolitan species clearly have a wide tolerance of variations in abiotic factors whereas endemic darwinulids exhibit a narrow tolerance (Van Doninck et al. 2003).

In oribatid mites, an analysis of the ecological distribution according to the reproductive mode has been performed to test several predicates of differences between sexual and asexual lineages (Cianciolo and Norton 2006). No evidence for difference in ecological niche breadth between sexual and asexual lineages could be found. Another postulate commonly held is that the frequency of asexual lineages should be negatively correlated to the biological diversity in an ecological niche. The same analysis showed no negative correlation between these two features. Thus, no significant difference could be found in ecological pattern or niche breadth between sexual and asexual lineages of oribatid mites.

In root-knot nematodes, apomictic (asexual, clade I) species have a broader host spectrum as well as a wider geographical and ecological distribution than their amphimictic (sexual, clade II) relatives (Triantaphyllou 1985; Castagnone-Sereno 2006). This observation is in total contradiction with the postulated better adaptability of sexual species. In the particular case of plant parasites, this apparent evolutionary success of asexual lineages may be related to their competitive advantage due to the “twofold cost of sex” in a relatively stable and uniform environment as recently proposed for agricultural pests (Hoffmann et al. 2008). However, this argument only holds partially in root-knot nematodes as amphimictic competitors of apomictic species are able to perform facultative meiotic parthenogenesis, and are thus not completely subject to the twofold cost of sex. Furthermore, if these tropical root-knot nematodes have actually been surviving for millions of years without sex, they predated the development of agriculture and must have survived in competition with sexual relatives in unstable environments. Hence, other mechanisms of currently unknown nature may provide these obligate parthenogenetic nematodes with a competitive advantage.

Bdelloid rotifers are common micro-invertebrates inhabiting freshwater environments but also temporary habitats that dry out frequently. They are able to colonize such environments because they are both asexual and desiccation resistant. Asexuality allows individual bdelloids to colonize empty patches or to reestablish a population after experiencing severe bottlenecks. Desiccation resistance enables bdelloids to inhabit environments that are prone to desiccation and to easily disperse as dried propagules (Ricci 1998). Indeed, many bdelloid species are cosmopolitan, exhibiting a worldwide distribution (Fontaneto et al. 2008) and reaching a surprisingly high level of diversity at local scale (Fontaneto et al. 2006). Moreover, a recent study by Wilson and Sherman (2010) showed that bdelloids can eliminate a lethal fungal parasite by drying out completely for a prolonged period and escape by wind dispersal. Therefore, the combination of asexuality and desiccation seems to allow bdelloids to thrive in unstable environments and to compete with biotic factors. Finally, research by Fontaneto et al. (2007) demonstrated that bdelloids have been able to diversify into distinct evolutionary entities, successfully adapted to specific niches, in the absence of sexual reproduction. Thus, evolution and speciation appear to have proceeded unimpeded by this group's lack of sexuality.

From the four examples discussed in this section, there is presently no evidence that asexually reproducing animal species present a narrower geographical distribution or a more restricted (specialized) ecological niche than their sexual relatives (if any). In contrast, some asexuals even present a wider distribution than their sexual relatives. Specific mechanisms of yet unknown nature or peculiar genomic structures observed in these organisms may be related to their adaptability despite the lack of sexual recombination.

13.3.2 Rates of Evolution and Clonal Decay

According to Muller's ratchet theory (Muller 1964), strictly asexual lineages should undergo "clonal decay" and disappear within a few thousand years. Therefore, the persistence of some lineages for much longer periods of time without sex contradicts this model and we expect those lineages to have a low rate of mutation accumulation (maybe due to a particularly efficient DNA repair system). Supporting this hypothesis, darwinulid ostracods (Schön et al. 1998) and oribatid mites (Schaefer et al. 2006) have been shown to undergo slower rates of molecular evolution than their sexual relatives. Thus, at least two out of the four examples of ancient asexuals presented here exhibit relatively slower rates of evolution, but, is that true for the other examples?

In bdelloid rotifers, the rate of accumulation of potentially deleterious mutations appears to be higher than in their closest sexual relatives the monogonont rotifers (Barraclough et al. 2007). Other works comparing bdelloids and monogononts showed slightly higher rates of non-synonymous substitutions and slightly lower rates of synonymous substitutions in bdelloids as compared with monogononts

(Mark Welch and Meselson 2001). However, it should be noted that in the case of bdelloid rotifers, this apparently high rate of mutation accumulation is probably related to the tetraploid genome structure (made of two colinear pairs of chromosomes with a high level of divergence between pairs). Within one colinear pair, lower levels of divergence have been shown, including tracts of near identity that may result from homogenization events such as gene conversion (Mark Welch et al. 2009). These homogenizing events are suspected to occur during rounds of desiccation and recovery that involve DNA double stand break repair.

In the root-knot nematode *M. incognita*, no precise evaluation of the rate of mutation accumulation has been conducted so far to our knowledge. However, an analysis of the internal transcribed spacers (ITS) of nuclear ribosomal genes showed an extremely high heterogeneity of sequences within apomictic (obligate asexuals) *Meloidogyne* species whereas the heterogeneity was virtually absent in sexually reproducing *Meloidogyne* (Hugall et al. 1999). Nevertheless, as for bdelloid rotifers, this apparent high divergence at the genetic level has to be put in parallel with a peculiar genomic structure. Indeed, in *M. incognita*, most of the genome is present as two highly diverged copies (~8% divergence at the nucleotide level) that may represent former allelic regions or the result of an interspecies hybridization. This feature, not observed in the close relative facultative sexual species *Meloidogyne hapla*, is discussed further in Sect. 13.4.1. Concerning possible mechanisms of homogenization such as gene conversion, none have been revealed so far in apomictic *Meloidogyne*.

Overall, no clear tendency regarding the rate of mutation accumulation appears to emerge in the considered ancient asexual animal lineages. Half of the examples show higher rates of mutation accumulation than their sexual relatives, though it is certainly related to peculiar genomic structures, while the other half exhibit lower rates. There is no clear evidence for a positive or negative correlation between asexual reproduction and the rate of accumulation of potentially deleterious mutation when considering these examples. Assuming that all examples truly represent ancient strict asexuals, we cannot argue in that case that sexual reproduction provides an evolutionary advantage in terms of resistance to the accumulation of potentially deleterious mutations.

13.4 Genomic Consequences of Long-Term Asexuality

No whole-genome sequence for an obligate asexually reproducing animal was available until recently, with the publication in 2008 of the genome of the tropical root-knot nematode *Meloidogyne incognita* (Abad et al. 2008). Interestingly, the genome of a facultative sexually reproducing relative was published a few months later the same year (Opperman et al. 2008) and comparison of these two genomes will allow identifying genomic marks of long-term asexual reproduction. Genome sequence data is also emerging in bdelloid rotifers as the genome of *Adineta vaga*, a long-term obligate asexual, is currently being sequenced and assembled. We will

thus focus here on the genomic singularities that emerged from the analysis of the genome sequence of *M. incognita* and from the preliminary assembly of the genome of *A. vaga*.

13.4.1 Insights from the Genome of *M. incognita*

The *M. incognita* genome, sequenced using a whole-genome shotgun strategy and assembled with Arachne (Jaffe et al. 2003) yielded 2,817 supercontigs. The size of the assembly, totaling 86 Mb, is almost twice the size (between 47 and 51 Mb) that had been estimated experimentally using flow cytometry approach (Leroy et al. 2003). Interestingly, an all-against-all comparison of supercontigs revealed that the genome of *M. incognita* is mainly composed of pairs of homologous yet divergent copies. The average divergence level at the nucleotide level observed between two homologous pairs is ca. 8% (Abad et al. 2008). For comparison, the average level of nucleotide divergence between individuals within an animal species is usually below 2% and higher levels of dissimilarity is considered as an indication of speciation (Birky et al. 2005). Highly divergent pairs in the genome of *M. incognita* cannot be interpreted as a mixture of individuals from different lineages or populations since the sequenced material results from repeated infections from the clonal offspring of a single female. Thus, this high divergence level can be considered to occur within one individual. The highly divergent pairs can represent former allelic regions or they can be the result of hybridization between two sexual progenitors from distinct but closely related species (Triantaphyllou 1985; Castagnone-Sereno 2006; Lunt 2008). In both cases, long-term absence of sexual recombination may have allowed mutations to accumulate independently and persist to reach the currently observed high divergence level as proposed under the “Meselson effect” model (Mark Welch et al. 2004). These features, associated to the relatively high frequency of observed polysomy and aneuploidy, are compatible with a strictly mitotic parthenogenetic reproductive mode. The peculiar genomic structure observed in *M. incognita*, composed of pairs of highly diverged regions, is not found in its facultative sexual relative *M. hapla*. Indeed, this species, able to do meiosis, harbors a small genome (54 Mb, the smallest so far for an animal) totally conform to the predicted size based on flow cytometry experiments and no trace of pairs of diverged regions has been found (Opperman et al. 2008; Bird et al. 2009). To evaluate whether the peculiar genome structure observed in *M. incognita* had consequences at the protein level, predicted proteins were grouped in cluster of at least 95% identical sequences, using the program CD-HIT (Li and Godzik 2006). The results of this clustering showed that more than 69% of protein sequences were more than 5% divergent to any other. This indicates that the observed 8% average divergence at the nucleotide level between pairs of similar genomic regions include non-synonymous substitutions that may be associated to functional divergence between gene copies.

In the case of root-knot nematodes, it appears that one remarkable consequence of the long-term absence of sexual reproduction and meiotic recombination is a

genome constituted of a juxtaposition of pairs of homologous but divergent copies that might represent former paternal and maternal haplotypes. These divergent copies include genes that encode proteins divergent enough to potentially support subfunctionalization or neofunctionalization events. It will be necessary to check whether similar peculiar genomic structures are observed in other obligate asexual root-knot nematodes as well as in other species that have abandoned sexual reproduction a long time ago in order to find out whether this might represent a general genomic signature of long-term asexual reproduction.

The only other feature that emerged as an idiosyncrasy in the genome of *M. incognita* as compared to those of *M. hapla* and other nematodes was the proportion of the genome covered by repetitive elements, including transposable elements. More than 36% of the *M. incognita* genome is covered by such elements (Abad et al. 2008). This is substantially higher than for other nematodes, as only 17% were reported in *M. hapla* (Opperman et al. 2008); 16.5% and 22%, respectively, reported for *C. elegans* and *C. briggsae* (Stein et al. 2003), 17% in *P. pacificus* (Dieterich et al. 2008), and between 12% and 15% in *B. malayi* (Ghedini et al. 2007). Whether some of these transposable elements are active and potentially play a role in the plasticity of the genome of *M. incognita*, as previously suggested (Castagnone-Sereno 2006), remains to be determined.

13.4.2 Emerging Results from the *Adineta vaga* Genome Project

Initial investigations of parts of the genome of bdelloid rotifers appeared to match what was expected for ancient obligate asexuals: high intraindividual divergence (ca. 15% at nucleotide level) between what was believed to be ancient allelic sequences (Mark Welch and Meselson 2000), and an apparent lack of retrotransposons (Arkhipova and Meselson 2000). Later on, however, the picture started to change completely: the highly divergent gene copies co-occurring in *Philodina roseola* were found to be actually ohnologs (Mark Welch et al. 2008), i.e., paralogs resulting from complete genome duplication (Wolfe 2000), whereas the level of divergence between ancient alleles was markedly lower (ca. 3%) and not very different from the range observed in sexually reproducing species such as *Ciona savignyi* (Small et al. 2007). Subsequent observations in *Adineta vaga* (another bdelloid species) confirmed this result (Hur et al. 2009). Recently, a wide diversity of transposable elements was found in *Adineta vaga* near chromosome ends (Arkhipova and Meselson 2005a), most of them inactivated or decaying but some still apparently active. Another unexpected finding at the telomeric regions of *Adineta vaga* was the discovery of abundant horizontally transferred genes (Gladyshev et al. 2008). Similarly, a high number of genes acquired via horizontal transfer was found in the genome of the root-knot nematode *M. incognita* but also in its facultative sexual relative *Meloidogyne hapla* as well as in many other plant-parasitic nematodes, including obligate sexuals (Danchin et al. 2010).

Therefore, this feature may not be indicative of the absence of sexual reproduction and it would be interesting to check whether similar abundance of genes acquired by lateral transfer is found in sexual relatives of bdelloid rotifers such as monogononts.

Since all these results were obtained from the analysis of a few selected genomic fragments (fosmids), an international consortium decided to sequence the complete genome of *Adineta vaga* in order to check the generality of these observations and conduct more in-depth analyses. Sequencing was performed using mostly paired-end pyrosequencing (Margulies et al. 2005), but the assembly proved challenging due to the medium-range heterozygosity of this genome, a problem also encountered in other whole-genome shotgun sequencing projects such as the ascidians *Ciona savignyi* and *Ciona intestinalis* (Vinson et al. 2005; Kim et al. 2007; Small et al. 2007). While very divergent sequences assemble separately and very similar ones are fused during assembly process, intermediate-level heterozygosity result in incomplete fusion that makes a reference sequence particularly difficult to produce.

Although the assembly and annotation of the complete genome sequence of *Adineta vaga* is still in progress, the first preliminary results from this project seem to confirm the absence of the Meselson effect in this species. Indeed, the average divergence level between former allelic regions within a colinear pair is around 3% over the whole genome of *Adineta vaga*. This figure appears surprisingly low for an organism whose last genomic homogenization through meiosis is supposed to have occurred several millions years ago. In comparison, the average divergence level between homologous regions that might represent former alleles in *Meloidogyne incognita* reaches 8%. Either *Adineta* does actually perform meiosis, albeit rarely (and a search for meiosis-related genes in the complete genome sequence will be required to bring a definitive answer to this question), or there must be some other mechanism acting to homogenize ancient alleles and prevent their divergence. The alternation of desiccation and rehydration phases in the life cycle of bdelloid rotifers (Gilbert 1974) may provide such a mechanism. As shown by experiments on *Deinococcus radiodurans* (Mattimore and Battista 1996), desiccation usually results in DNA double-strand breaks. In eukaryotes, double-strand breaks are repaired through heteroduplex formation (Resnick 1976), which, in turn, often leads to gene conversion (Bishop et al. 1987), i.e., the copying of one region of a chromosome over the homologous region of another chromosome, thus resulting in sequence homogenization. Moreover, such repair mechanism only works if two homologous regions are not too divergent. Hence, bdelloid rotifer that would have accumulated a large amount of divergence between homologs would probably not survive desiccation, as their damaged DNA could not be repaired. At the present time, however, the only experimental evidence for the occurrence of gene conversions in bdelloid rotifer comes from the isolation and sequencing of *hsp70*- and histone-containing fosmids in *A. vaga* and *P. roseola* that revealed several tracks of sequence identity or near-identity between ancient alleles (Hur et al. 2009). Therefore, this result will have to be confirmed and quantified at the genome

scale to find out whether gene conversion really plays a role in limiting the divergence between homologs in bdelloid rotifers.

13.5 Concluding Remarks

Strict asexuality and its ancientness in animal species both remain difficult to establish. The four animal lineages we have described in the present chapter represent, to our knowledge, the most plausible ancient asexual candidates. However, for none of these lineages, ancient asexuality can be stated in an absolutely incontestable manner. The evidences used to indicate asexuality rely on the absence of current observation of sexual-specific features such as males, meiosis or fertilization, while support from the fossil record or divergence level between individuals are used to state age of asexuality. The extensive study of the genomes of presumed long-term asexuals, showing, e.g., that key genes involved in sexual reproduction or meiosis are absent, may represent the most solid evidence in the near future. Considering that the lineages presented here are most probably ancient asexuals, several peculiarities and features can be sorted out. Recent genomic data for bdelloid rotifers and root-knot nematodes suggest that a peculiar genomic structure in which at least part of the genes are present in divergent copies may support functional divergence and provide a genetic pool for adaptation. These singular genomic structures may represent partial alternatives to sexual reproduction as a source of genomic plasticity necessary for adaptation in changing environment. On the other hand, in darwinulid ostracods, it has been proposed that a more or less fixed general-purpose genotype has allowed these species to survive in a variety of environments.

Another constraint linked to the absence of sexual recombination is that deleterious mutations are not eliminated as rapidly and tend to accumulate if no alternative elimination mechanism exists. In oribatid mites and in darwinulid ostracods, it has been observed that asexual lineages present lower rates of accumulation of mutations than their sexual relatives, possibly due to particularly efficient DNA repair mechanisms. In bdelloid rotifers, a homogenization mechanism, possibly via gene conversion during DNA repair after desiccation, has been proposed to maintain a low level of divergence between gene copies within a colinear pair while allowing high divergence between copies in different pairs. Overall, it appears that ancient asexuals may have evolved substitutes to sexual reproduction that would allow their genomes to adapt to environmental changes while maintaining a low level of potentially deleterious mutations. With the first genome for an animal reproducing strictly without sex recently available and the forthcoming release of another such genome, we are currently at the dawn of the genomic era for asexual animals. Moreover, comparative analysis with genomes of close sexual relatives will probably shed light on new features in the genomes of asexual species that might represent signatures of the long-term absence of sexual reproduction.

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