

Chapter 5

Reproduction of Earthworms: Sexual Selection and Parthenogenesis

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5.1 Introduction

Earthworms are generally considered to be cross-fertilization hermaphrodites (i.e., using reciprocal insemination, transferring, and receiving sperm in the same copulation). Although not all earthworms use this reproductive strategy, the best known species, *Lumbricus terrestris*, is a cross-fertilization hermaphrodite and this strategy seems to be the most widespread in earthworms. Nevertheless, cases of self-fertilization have been reported in earthworms; Domínguez et al. (2003) discussed that *Eisenia andrei* individuals bend themselves, allowing their spermathecal pores to contact the ventral zone of their clitellum. The sperm is then transported from the male pores to the spermathecae. This finding explains why 33% of isolated individuals in this study produced viable cocoons.

However, hermaphroditism is not the only reproductive mechanism and more parthenogenetic earthworms are being discovered all the time, most of which are polyploid. Parthenogenetic reproduction is very frequent in the family Lumbricidae, with more than 30 parthenogenetic species occurring in North America (Reynolds 1974). Parthenogenesis has also been reported in families such as Megascolecids, but has not been observed in other families, including Glossoscolecids.

“Asexual” reproduction by means of bipartition, stolonisation, budding, or similar processes has not been observed in earthworms and their ability to regenerate is limited. There are several reproductive models: discontinuous, semicontinuous, or continuous. In *Hormogaster elisae*, male and female gametogenesis are synchronized, beginning in autumn and ending in the summer. Male funnels are full of spermatozoa and the spermathecae contain spermatozoa throughout the year, but

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two peaks of reproduction have been observed, with the largest peak occurring in the spring and the second peak occurring in autumn (Garvín et al. 2003).

An excellent description of the earthworm reproductive system can be found in general zoology volumes and monographs such as Jamieson (2006), so it will be only succinctly described in the present chapter. Earthworms are usually hermaphrodites in which the testes and ovaries are accompanied by a series of organs with a male or female function. The female components typically include the ovaries (generally one pair in the 13th segment), ovisacs (in the 14th segment), oviducts, female pores (in the 14th segment), and spermathecae (of variable position and number). Male components typically include the testes and male funnels (in most cases, there are two pairs in the 10th and 11th segments and singularly a single pair in the 11th segment), seminal vesicles (of variable number, with 2–4 occurring in segments 9–12), deferent ducts, and male pores surrounded by atrial glands that are more or less developed. Other organs, such as testicular sacs (*Lumbricus* and *Octolasion*), accessory glands (prostates), or the thecal glands associated with the spermathecae, may also be present.

Some of the external reproductive organs, such as the clitellum, tubercula pubertatis, and sexual papillae, are developed at sexual maturity. The sexual papillae include modified genital chaetae and chaetal glands, which could be used to inject substances into the partner (see Sect. 8.2.2).

The union during copulation, which could last between 69 and 200 min in *L. terrestris*, is secured by tubercula and quetae. Copulation can occur at the surface in epigeic and anecic earthworms, which increases the depredation risk, and also occurs in deeper layers of the soil in the case of endogeic species. The more primitive type of copulation seems to be a simple juxtaposition of the male pores of one individual and the spermathecal pores of the other, with the direct transfer of spermatozoa. The presence of a penis has been observed in some cases, which in reality seems to be just an elevated papilla, as in the case of some *Pheretima* species.

In most of the species in the Lumbricidae family and in other families, the clitellum moves backwards and seminal grooves are developed from the male pores to the tubercula pubertatis. Spermatozoa flow through the seminal grooves to get into the partner's spermathecae pores. Details of sperm transfer are not well known with the exception of a few species such as *Pheretima sp.*, in which, according to Tembe and Dubash (1961), the sperm appears to be transferred sequentially, passing first to the anterior spermathecae and later to the posterior ones.

Bouché (1975) indicated that spermatophores have been observed in more than 20 species of lumbricids. Spermatophores are small capsules that adhere to the body wall and can be iridescent and full of spermatozoa. Their function is not clear. It has been suggested that the spermatophores may play a role in sperm transfer (Edwards and Bohlen 1996), thus avoiding sperm digestion in the spermathecae and fertilizing the ova during cocoon formation Michiels (1998). Nevertheless, Monroy et al. (2003) showed that spermatophores have no effect on the reproductive success of *Eisenia fetida* and were not able to demonstrate the specific function of these capsules.

Complex precopulatory behaviors have been described in partner selection in some species, including *L. terrestris*, in which individuals perform visits to their neighbors' burrows (Nuutinen and Butt 1997; Michiels et al. 2001, see Sect. 8.2.1). Development is direct in earthworms. Fertilization occurs within cocoons and one or more juveniles are produced for each cocoon.

The presence of parthenogenesis in earthworms was first observed many years ago, thanks to the contributions of authors such as Omodeo (1951), Casellato (1987), Jaenicke and Selander (1979) and Victorov (1997), among others.

Reynolds (1974) pointed out that in North America 35 species are amphimictic, 11 probably sexual, 4 facultative parthenogenetic, 1 possibly parthenogenetic, and 30 parthenogenetic. Casellato (1987) cited 25 parthenogenetic species or subspecies (12 of which had even ploidy numbers and 13 of which showed odd ploidy) and Victorov (1997) pointed out that in Russia, the number of polyploids almost equals the number of diploids, with a ratio of 46 polyploids: 52 diploids. He observed that polyploids (in cases of sympatry) tend to occupy the margins of the distribution areas. According to Edwards and Bohlen (1996), the association between parthenogenesis and high polyploidy in earthworms produces an unexpected level of heterozygosity, an advantageous condition that provides resistance to environmental stress.

5.2 Sexual Selection in Cross-Fertilization Earthworms

In simultaneous hermaphrodites, a trade-off between male and female sexual functions is expected because the two sexes share limited resources from the same individual. In addition, the strategy that maximizes fitness is different for the male and female functions. This has been explained previously by Bateman (1948), who showed that the higher the number of partners, the higher the fitness for the male function because it produces small sperm cells. Nevertheless, female function maximizes its fitness by seeking high quality mates because it produces large eggs and this function has to invest in cocoon production. As a consequence, there is a conflict between the sexes. Indeed, Porto et al. (2008) found a negative relationship between the present investment in male function and the future fertility of the female function in their research on *E. andrei*. Sexual selection is expected to occur because of female function as long as a sufficient number of mates are available.

5.2.1 Precopulatory Sexual Selection

Copulation is very costly and involves sperm and mucus production and long periods of time. Consequently, precopulatory selection is expected in environments where the density of earthworms is high.

One of the factors that could influence precopulatory sexual selection is the female fecundity of the partner, which may be related to body size. Large earthworms have not been found to produce more cocoons (Tato et al. 2006; Butt and Nuutinen 1998) but they do tend to produce heavier cocoons and larger offspring (Michiels et al. 2001). Size-assortative mating was indeed observed in the field for the epigeic *E. fetida* (Monroy et al. 2005) and for the endogeic *H. elisae* (Novo et al. in press), as well as in laboratory experiments for the anecic *L. terrestris* (Michiels et al. 2001). Earthworms selected similar-sized partners. Because every earthworm seeks a bigger partner, equilibrium is finally reached, resulting in partners with a similar weight, thus balancing the expectations of both mates on female and male functions. In the particular case of epigeic and anecic worms, which can copulate at the surface, this general tendency could be reinforced by a trade-off; worms can either select a bigger, more fecund partner or a smaller partner, which would decrease the risk of predation.

In ongoing laboratory experiments with *H. elisae*, we have observed that there is no such size selection in virgin individuals, although the bigger virgin individuals always managed to copulate so they seem to be more desirable.

Aside from size, reciprocation is sought from a potential partner. In simultaneous hermaphrodites, the primary purpose of mating is to fertilize the eggs of their partners, rather than to fertilize their own eggs. Therefore, the conflict of two earthworms copulating would be the amount of sperm that each of them is allowed to give (Michiels 1998).

Finally, the quality of the place where cocoons are deposited after copulation and the suitability of the burrow for offspring development (i.e., the moisture or litter content) could be important factors for precopulatory assessment. Ortiz-Ceballos and Fragoso (2006) studied parental care in *Pontoscolex corethrurus* and *Balanteodrilus pearsei*. They found that both species build up a chamber that they periodically clean and surround with fresh casts where a single cocoon is deposited. Grigoropoulou et al. (2008) observed that *L. terrestris* deposits the cocoons inside burrows, which may offer a protective location from the physical environment or may represent parental investment as they were also found to be coated with earthworm casts. These casts could be a means of maintaining the moisture content or protecting cocoons from predators.

The mechanism through which earthworms choose a mate, assess size, test reciprocity, or assess the burrow quality of their potential partners remains unknown, although there are some data on these factors. Chemical cues have been suggested in earthworms as a mechanism of finding and attracting the mate (Olive and Clark 1978; Edwards and Bohlen 1996).

Grove and Cowley (1926) suggested the existence of a courtship in *E. fetida* as they observed short and repeated touches between partners before mating. This type of contact, executed with the prostomium, was also observed by Nuutinen and Butt (1997) in *L. terrestris* and could last 90 min. The prostomium has been described as a sensory lobe with many chemoreceptors or sensory cells (Wallwork 1983).

During contact, the clitellum and associated structures could be indicators of female functionality and glandular margins of the male pores could be indicators

of male functionality. These structures could provide a means of evaluating the partner and assuring reciprocation. Reciprocation can also be assured by increasing the copulation time, which would prevent the partner from copulating with other earthworms. In addition, Nuutinen and Butt (1997) observed that *L. terrestris* visited the potential mate's burrow by inserting its anterior segments, but retaining the posterior segments in their own burrows, as a mechanism to evaluate the quality.

In case of the size assessment, it is also suggested that assortative mating could be due to a physical incompatibility of the copula among individuals of different sizes (Michiels et al. 2001), although this incompatibility would only result from large differences in size.

These selective forces depend on other factors, such as the density of earthworms or the distance of potential mates. Indeed, the low dispersal ability of these animals provides a restriction in the number of available mates. Earthworms have low migration rates, with observed natural dispersal rates of only 1.4–9 m year⁻¹ (Ligthart and Peek 1997; Hale et al. 2005) and are therefore expected to mate with partners living in their vicinity. In addition, in the case of the earthworms who copulate at the surface, a smaller distance to the partner would also minimize the risk of predation. There is evidence for this selective limitation produced by distance. Nuutinen and Butt (1997) investigated burrow visit patterns in *L. terrestris* and found that the nearer the burrow opening was, the more visits the worms made to assess the potential partner quality. In addition, Sahn et al. (2009) showed mate choice in the same species for its closest partner and Novo et al. (in press) found that *H. elisae* do not move long distances to find mating partners. Nevertheless, this low dispersal could cause inbreeding, which is generally accepted to be unadaptive and would reduce the fitness of the offspring. Partner selection has not been found to be dependent on relatedness (i.e., kin recognition), and Novo et al. (in press) did not find a correlation between mating probabilities and the level of heterozygosity in *H. elisae*. Regarding this, differential investment in offspring is thought to occur (Velando et al. 2006, see Sect. 8.2.2).

Finally, parasite concentrations may influence mate choice, since they can have a negative effect on earthworm growth as shown by Field and Michiels (2005) for the association between *Monocystis* and *L. terrestris*. In addition, earthworm skin color could be positively correlated with parasite concentration (Field et al. 2003), which could be a sign used to evaluate partners. Nevertheless, Sahn et al. (2009) failed in an attempt to show a relationship between parasite concentration and mate choice, and more studies are needed to assess this correlation.

5.2.2 Postcopulatory Sexual Selection

In spite of the precopulatory sexual selection, multiple mating is common in earthworms (Monroy et al. 2003; Sahn et al. 2009; Novo et al. in press) and all the allosperm received is stored and sometimes mixed (Novo et al. in press) in the

spermathecae. Therefore, postcopulatory sexual selection such as sperm competition (Parker 1970) or cryptic female choice (Thornhill 1983) could be expected.

The sperm remains viable in the spermathecae until fertilization. Butt and Nuutinen (1998) observed that *L. terrestris* was capable of successfully maintaining the received sperm up to 6 months. Meyer and Bowman (1994) reported that *E. fetida* continued cocoon production for up to 12 months after the earthworms were isolated from their partner, although these authors did not measure viability. Garvín et al. (2003) reported spermathecae full of spermatozoa during diapause in *H. elisae*. This would be advantageous for species with poor dispersal capacities or for species that occur in low densities that can copulate at any time of the year.

The maintenance of sperm for such a long time implies the existence of some kind of preservation mechanism. There is evidence suggesting that the spermathecal epithelium actively contributes to the successful maintenance of sperm by providing a favorable luminal environment (Grove 1925; Varuta and More 1972) or by producing nourishing substances (Vyas and Dev 1972; Jamieson 1992; Novo et al. (unpublished data))

A possible mechanism for postcopulatory sexual selection developed by the recipient is sperm digestion. Richards and Fleming (1982) observed spermatozoal phagocytosis by the spermathecae of the facultative parthenogenetic *Dendrobaena subrubicunda* and other lumbricids. This is likely related to the removal of aging or aberrant sperm during the months when cocoon production was minimal. Novo et al. (unpublished data) found sperm degeneration in the central area of spermathecae from *H. elisae* (Fig. 5.1a, b). These authors also observed sperm intrusions into the epithelium of spermathecae with high sperm contents, although these intrusions seemed to occur in areas where the sperm sought more nutrients rather than phagocytosis processes (Fig. 5.1c). Future ultrastructure studies will shed light on these mechanisms.

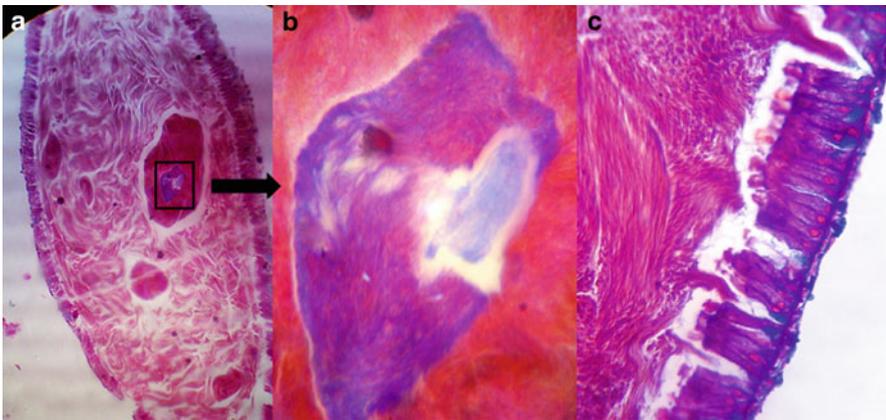


Fig. 5.1 Histological preparations of the spermathecae from *H. elisae*. Sperm degeneration (a and b in detail). Sperm intrusions in the epithelium of the spermathecae (c)

Another strategy for cryptic female choice could be the differential storage of the received allosperm within the spermathecae. The recipient can control the storage of sperm by increasing the complexity of these organs. Different species of earthworms have different numbers of spermathecae (Sims and Gerard 1999), although Novo et al. (in press) demonstrated using microsatellite markers that the four spermathecae from *H. elisae* contained sperm from the same individuals. Grove and Cowley (1926) observed that the transmission of sperm in *E. fetida* typically occurs on both sides of the individual, whereas in *L. terrestris* some individuals were found to have spermatophores on only one side of their body (Butt and Nuutinen 1998).

Moreover, some earthworms present different sperm loads within a single spermathecae. This has been observed in some hormogastrids (Qiu and Bouché 1998), and in *Megascolides australis*, in which spermatozeugmata (i.e., sperm in orientated bundles) were reported by Van Praagh (1995). In addition, the spermathecae may include one or more diverticula that arise from the duct (Butt and Nuutinen 1998).

Finally, the amount of sperm stored in each spermatheca could be controlled, and this occurs for *L. terrestris*, which predominantly store sperm in the two posterior spermathecae when there is no injection of all hormones (Koene et al. 2005, see later). Garvín et al. (2003) also observed that the second pair of spermathecae seems to be the main recipient of spermatozoa in *H. elisae*. However, Velando et al. (2008) showed that *E. andrei* distributes the sperm equally among the four spermathecae.

Cryptic female choice may also be achieved through differential investment in offspring. Velando et al. (2006) found that *E. andrei* adjusted the breeding effort to the degree of mate relatedness, showing that inbreeding and outbreeding cause a strong reduction of cocoon production, especially in genetic lines with high reproductive rates.

Sexual selection drives the evolution of strategies that increase the chances of fertilization for the donated sperm as a means of increasing paternity. Some of these strategies have been observed in earthworms. Velando et al. (2008) reported a behavior that could promote sperm competition in *E. andrei*, which can have a high degree of control over their own ejaculate volume after evaluating their partners. This species donated three times as much sperm as they did normally when mating with a nonvirgin mate. Moreover, such increases were greater when the worms mated with larger partners. Mariño et al. (2006) also showed a sperm trade in *E. andrei*, which adjusted the amount of sperm they release to the volume they receive from their mating partner during copulation. In addition, the total sperm volume they found in the spermathecae was correlated to the recipient's body mass, indicating that this adjustment is in accordance with the quality of the partner.

Koene et al. (2002) proposed that during mating, *L. terrestris* use their copulatory setae to pierce their partner's skin to inject an all hormone produced by the setal glands which manipulates the reproductive physiology of the partner and damages the body wall. The injection of this substance provokes a higher uptake of sperm, a more equal sperm distribution over the four spermathecae, and an increase the amount of time occurring before the next mating. The damage caused

by the injection itself could incur a considerable cost that inhibits another mating (Koene et al. 2005).

5.3 Parthenogenesis

5.3.1 Definition

Parthenogenesis is a very wide collective concept. Historically, classical authors addressed this concept on several occasions; although not defining the concept or providing an experimental approach, authors posed hints regarding the existence of this kind of reproduction. Although Bonnet provided experimental proof for this kind of reproduction in aphids in 1762, it was not until 1849 that Richard Owen coined the term. He defined parthenogenesis as “procreation without the immediate influence of a male”. As this general concept could include several typically asexual modes of reproduction such as fission or budding, several authors attempted to create new definitions for this term. A century later, Suomalainen (1950) defined it as “the development of the egg cell into a new individual without fertilization”. Later, Beatty (1957) defined it first as “the production of an embryo from a female gamete without the concurrence of a male gamete, and with or without eventual development into an adult”, but modified the definition in 1967 (Beatty 1967) by substituting “without any genetic contribution from a male gamete” for “concurrence of a male gamete”. In this way, Beatty extended the definition to include special types of parthenogenesis such as gynogenesis. Nevertheless, all of these definitions give rise to some terminological difficulties.

5.3.2 Types of Parthenogenesis in Earthworms

Several classifications have been used to define the different types of parthenogenetic mechanisms. To understand earthworm classification of parthenogenesis, it is worth mentioning the classifications proposed by Thomsen (1927); Ankel (1927); Suomalainen (1950) and White (1973); these are mainly based on the mode of reproduction, sex determination, and cytology.

The system of classification proposed by Thomsen (1927) and Ankel (1927) points out two main points: the zygoid–azygoid status of an individual and the maintenance of the zygoid chromosome number. It includes two main categories: generative or haploid parthenogenesis (in which chromosome reduction takes place in the eggs, and consequently the parthenogenetic offspring have an azygoid – haploid-number of chromosomes), and somatic parthenogenesis, in which parthenogenetic offspring have a zygoid–diploid or polyploid-chromosome number.

The difference between the two categories basically depends on the absence (apomixis) or presence (automixis) of chromosome conjugation and reduction. Both concepts are synonymous with White's concepts of ameiotic and meiotic parthenogenesis, respectively.

When considering sex determination, it is especially useful to use the classification of parthenogenesis proposed by Suomalainen et al. (1987): arrhenotoky, thelytoky and deuterotoky, or amphitoky (unfertilized eggs producing only male descendants, only females, or descendants of both sexes, respectively).

Parthenogenetic earthworms are generally automictic and thelytokous. Following the cytological studies of Muldal (1952); Omodeo (1951, 1952, among others) and Casellato and Rodighiero (1972), there is a premeiotic doubling of the chromosome number at the last oogonial divisions resulting in endomitosis, followed by the formation of chiasmatic bivalents and regular meiosis with the extrusion of two polar bodies. The genetic consequences of this cytological mechanism are similar to those of apomixis (i.e., the formation of clonal animals), as synapsis is restricted to sister chromosomes that are exact molecular copies of one another. The immediate genetic consequence of this mechanism is that heterozygosity is maintained. Following White (1973), all bivalents are structurally homozygous and multivalents are never formed. Consequently, this kind of reproduction is perfectly compatible with different degrees of polyploidy, especially in odd-numbered levels (Fig. 5.2).

Only one exception to the parthenogenetic mechanism described above has been found. *Dendrobaena octaedra* shows apomictic parthenogenesis: the chromosome number is not doubled in the oogonia, the chromosome number of the oocytes is unreduced, and there is only one equational maturation division (Suomalainen et al. 1987). For this species, Omodeo (1953) and later Gates (1972; as explained later in this chapter) described different parthenogenetic forms with a huge degree of morphological variation, which makes it very difficult to establish the evolutionary relationships among them. Omodeo (1953) suggested that "it could be the result of a breakdown of developmental canalisation in the absence of stabilizing selection", while White (1973) indicated that "it seems more likely that it indicates the coexistence of numerous biotypes differing significantly from one another genetically, even if not in their visible cytology".

Parthenogenesis is one of the main sources of morphological variability within reproductive structures of earthworms. This variability is related to the reduction in the investment in male structures: seminal vesicles, testes, spermathecae, genital setae, and prostates are reduced or even lacking; there is no sperm production (i.e., lack of iridescence in male funnels and spermathecae); and spermatophores are lacking (in some cases they are produced but are invariably empty). In *Octolasion tyrtaeum* (Muldal 1952; Jaenicke and Selander 1979), male structures are not reduced and pseudogamy is shown: individuals copulate to exchange spermatophores that are invariably empty. Thus, although spermatozooids are not necessary, this species needs a mechanical or chemical stimulus to trigger reproduction. Polymorphic degradation of reproductive structures is often observed in parthenogenetic organisms. In some species, such as *Eiseniella tetraedra* even hypergynous

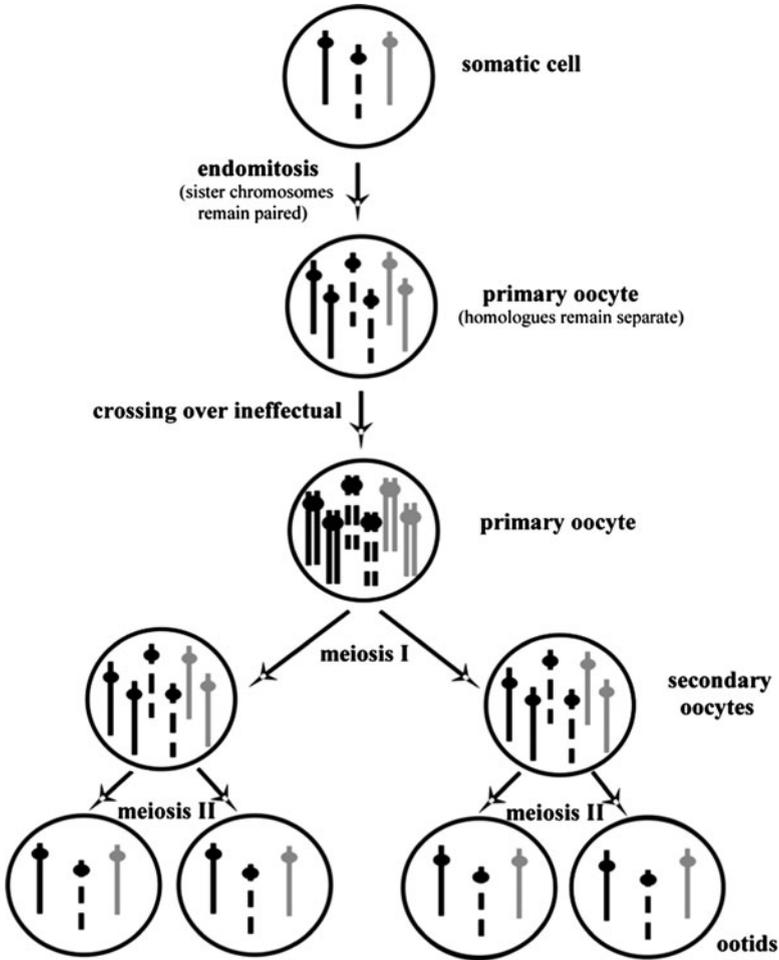


Fig. 5.2 Automitotic parthenogenesis: the genetic consequences of premeiotic restitution

individuals (with an extra pair of ovaries) can be found (Jaenicke and Selander 1979). However, in other parthenogenetic earthworms such as *Aporrectodea trapezoides*, both primary and secondary male sexual characters, such as perithecical papillae, tubercula pubertatis, spermathecae, swollen male porophores, and seminal vesicles, are retained. Recent studies show that pseudogamy is not observed in this species (Fernández et al. 2010). As discussed later, this seems to suggest very different origins of parthenogenesis in the different species.

Parthenogenesis is not homogeneously distributed in earthworms; it is only found in lumbricids and megascolecids. It is curious that it is not found (or not known to occur) in glossoscolecids or hormogastrids; this clearly shows that their life traits or evolutionary histories should be completely different and that somehow parthenogenesis and even polyploidy are not compatible or viable in this family.

5.3.3 *Parthenogenesis and Polyploidy*

Most part of the parthenogenetic earthworms are polyploids. Polyploidy ranges from tri- to dodecaploidy. From a cytogenetical point of view, automictic biotypes should be diploid (White 1973); nevertheless, in parthenogenetic lumbricids, polyploidy is the most common phenomenon. This is because, as explained later, the automictic mechanism in most lumbricids is premeiotic doubling, which leads to genetic consequences similar to an apomictic mechanism, leaving levels of heterozygosity unchanged from generation to generation (Suomalainen 1950). Because of premeiotic doubling, no multivalents are formed, so pairing only occurs between genetically identical sister chromosomes; this mechanism is compatible with odd-numbered polyploidy, as only bivalents are formed. This is the complicated chromosomal background that can give rise to different ploidy levels even within the same species. For example, in *Dendrobaena rubida*, diploid, triploid, tetraploid, hexaploid, and octoploid biotypes are known to occur, which clearly shows the extremely high liability of the genetic system. It has been proposed that automixis could be a step before apomixis (White 1973), which could mean that most lumbricids could be evolving toward an apomictic parthenogenesis. Polyploidy could be common in earthworms, as animals lacking the chromosomal determination of sex are particularly prone to this kind of reproduction, which is the main mechanism preventing the establishment of polyploidy in animals (White 1973). One of the main advantages of polyploidy in parthenogenetic species is the increase in genetic variability.

Since no study to date has elucidated the origin of parthenogenetic earthworms (as explained later in this chapter), it is not known if parthenogenetic earthworms may have arisen from hybridisation processes. These kinds of processes have been found to be very common mechanisms causing asexuality (only to the extent that parthenogenesis can be considered to be asexual reproduction) in animals and plants (Delmotte et al. 2003). Following this assumption, polyploidy (and particularly allopolyploidy) could provide important advantages, such as heterosis, to parthenogenetic species. This strong advantage could lead the parthenogenetic morphs to have more general purpose genotypes, allowing them to adapt to a wider range of environmental conditions than their sexual amphimictic ancestors (White 1973). There is much evidence that hybrid vigor could be responsible for the success of polyploids, but there is insufficient information to determine this with certainty.

5.3.4 *Genetic and Ecological Consequences of Cloning*

As stated by Hughes (1989), it is extremely difficult to define the advantages or disadvantages of parthenogenesis, as these depend on the situation; for some groups of animals, parthenogenesis is tremendously advantageous, while in others it is not.

Therefore, natural selection should control the pattern of occurrence in each group of animals.

Using molecular tools, very different degrees of genetic variability have been reported in different species. Both with allozyme electrophoresis and with mitochondrial gene sequencing, genetic variability was recorded as being high in *D. octaedra* (Haimi et al. 2007; Terhivuo and Saura 1996; Cameron et al. 2008) and *Aporrectodea rosea* (Terhivuo and Saura 1993; King et al. 2008), but low in *O. tyrtaeum* (Jaenicke et al. 1980; Heethoff et al. 2004) and *O. cyaneum* (Terhivuo and Saura 2003). In *A. trapezoides*, both mitochondrial and nuclear sequences resulted in an extremely high number of clones (Fernández et al. unpublished data.).

Judging from the number and distribution of parthenogenetic earthworms, one could expect that parthenogenesis is quite advantageous in this group. Parthenogenetic earthworms are widespread and very abundant, especially among peregrine species (Blakemore 1994) such as *A. rosea*, *A. trapezoides*, or *O. tyrtaeum*. Hughes (1989) pointed out the following advantages of parthenogenesis: both high levels of heterozygosity and exceptionally fit genomes, which are maintained and inherited by avoiding recombination and segregation; high reproductive rates, which could potentially be doubled by avoiding the production of males (i.e., no twofold cost in parthenogenetic reproduction); high colonizing abilities, since there is no need to mate; high values of reproductive potential, enabling clones to quickly replace losses; advanced polymorphism generated from selection at the level of the genome; and the delay or prevention of senescence as somatic replicas from undifferentiated somatic cells are generated. In reference to the last advantage, Hughes (1989) pointed out that several clones of oligochaetes did not show any signs of senescence after having been maintained for many generations.

5.3.5 *The Species Concept in Parthenogenetic Earthworms*

Parthenogenetic earthworms were wisely defined as “systematist’s nightmares” by Blakemore (1999). The biological species criterion cannot be applied to parthenogenetic earthworms, as each individual meets the criterion of being completely reproductively isolated not only from the parental species, but also from every sister clone. Several authors have attempted to resolve this problem, but an agreement has never been reached. Mayr (1963) suggested that the best solution would be to use a morphological criterion. Following this author (1963), a parthenogenetic species would be the one that “results in the combination of a single species of those asexual individuals that display no greater morphological difference from each other than from conspecific individuals or populations in related bisexual species”. He also proposed that clones can be combined into collective species when no essential morphological or biological differences have been observed. To complete this criterion, the author also argued that if a parthenogenetic line originated from an amphimictic species by an irreversible chromosomal event (such as polyploidy), it should be considered to be a separate and sibling species, although almost no

morphological differences could exist. This criterion has traditionally been used to define species in parthenogenetic lumbricids, though it can be difficult to apply as the degree of morphological variation is sometimes slight and the features defining parthenogenetic and even amphimictic species can overlap. This is a particularly big problem in complexes of very similar species containing both amphimictic and parthenogenetic species such as the “*Aporrectodea caliginosa* species” complex. In this context, other approaches, as discussed later, could be essential not only for properly defining parthenogenetic species, but also for determining the taxonomic status of each form in these species complexes.

Following Gates (1974), “the species is understood to include not only the interbreeding population, but also all recently evolved uniparental strains, clones, or morphs that clearly are affiliated with it”. This statement is useful when intermediate forms are found, but still does not solve the problem of how to resolve the status of parthenogenetic species with unknown (or extinct) amphimictic parental species. Another option would be to use the phylogenetic concept of species based on molecular markers, which would provide information about the genetic divergence between morphs or species. However, these tools are not so well developed in earthworms that they could provide a good idea as to the exact amount of divergence that should be used to differentiate between species. In addition, there is evidence of different degrees of divergence among closely related species in the different earthworms groups. The best way to define a parthenogenetic species (and amphimictic species, particularly when dealing with complex of species) is to use an integrative concept of species, using ecological, behavioral, morphological, and molecular data. A species should not be given a name if its biology is not well understood, but then, it is completely necessary to name the species. Parthenogenetic species are very common among the earthworms, and thus a solution needs to be found. The ideal study would be one using all of the available approaches to examine the same individuals so as not to incorporate any source of error or introduce any possible mistakes when identifying species. Making comparisons with previously published data is dangerous because different authors might have incorrectly identified species when dealing with parthenogenetic morphs or species from a complex, in which intermediate forms are typically found. The best means of eliminating this uncertainty is to deposit the individuals used in the experiments into a collection.

Gates (1974) categorized parthenogenetic morphs of *D. octaedra* using the presence or absence of different reproductive male structures. Gates (1974) defined morphs lacking spermathecae, male terminalia, testes, testis sacs, or seminal vesicles or those lacking several of these structures (e.g., athecal anarsenosomphic, with or without testes). He also included two categories of intermediate morphs with an incomplete or asymmetrical deletion of the above organs: hermaphroditic parthenogenetic morphs were defined as those that had reproductive organs in a juvenile state, while hermaphroditic morphs used biparental reproduction and were also parthenogenetic. Unfortunately, few studies have demonstrated the existence of these forms in every parthenogenetic species; the knowledge about the extension and degree of parthenogenetic morphs in parthenogenetic species is quite limited.

This is a problem both for clarifying the taxonomy of earthworms using this type of reproduction, and for understanding the origin of parthenogenesis in these species.

Gates (1974) and Blakemore (1999) suggested that parthenogenetic morphs should be given a name only when the parental amphimictic species can be determined. We totally agree with this statement. Nevertheless, as Blakemore suggested, the origin of the name, regardless of whether it was based on morphs or parthenogenetic forms, has no effect on the availability of a taxonomic name (ICZN 1999, Article 17.3). Moreover, Gates (1972) suggested that provision of names for all intermediate morphs of such species complexes was *ridiculous*.

Another limitation, as stated by Suomalainen et al. (1987), is that there are still very few examples of taxonomic diversification beyond the species level in parthenogenetic earthworms.

5.3.6 *The Origin of Parthenogenetic Forms*

Amphimictic ancestors of parthenogenetic forms are well known in many different animal groups, but this is not the case for Lumbricids. Hybridization has been proposed several times (e.g., Suomalainen et al. 1987) as a common origin of parthenogenetic animal species such as fishes, lizards, and salamanders. Among invertebrates, there are many examples of parthenogenetic forms originating from Hybridization in the literature. This is the case, for example, for parthenogenetic forms in delphacid leaf-hoppers or stick insects belonging to the genus *Acanthoxyla* which were described as having two haploid genomes, one of which came from an amphimictic parental species (Buckley et al. 2008). Suomalainen et al. (1987) also gave some examples among invertebrates in which parthenogenesis seems to have arisen through a single mutational event, or through multiple events. In these cases, parthenogenesis was a polyphyletic condition within a single species as, for example, in the psychid moth *Solenobia triquetrella*.

Little is known about the origin of parthenogenetic earthworms. Molecular biology will be very useful in shedding light on this topic. Several tools can be useful in reaching this goal. Traditionally, some studies using allozymes have been used to check genetic variability in parthenogenetic and sexually reproducing species that are related, such as *A. trapezoides* and *A. caliginosa* (Cobolli Sbordoni et al. 1987). However, the information obtained using this technique was not sufficient to evaluate hypotheses regarding the origin of parthenogenetic forms. An appropriate first approach would be to compare phylogenies using both mitochondrial and nuclear genes. To determine whether parthenogenetic species originated from hybridisation, alleles could be cloned in nuclear genes to check for the presence of different haploid genomes in diploid and, especially, polyploid parthenogenetic earthworms.

As stated earlier, there is a strong variation among parthenogenetic earthworms regarding the type of parthenogenesis that is observed; most of the species are automictic, but at least one is apomictic. Similarly, some species are pseudogamic

while others are not; some lack spermathecae while others have an extra pair of ovaries. The fact that parthenogenetic mechanisms are very labile in earthworms provides strong evidence that parthenogens could have originated in a number of different ways. Molecular biology will allow us to better understand why parthenogenetic earthworms have been so successful.

5.4 Conclusion

Reproduction models in earthworms are much more variable than it could seem *a priori*. Although direct cross-fertilization hermaphroditism may be seen as the most usual model, it is common to find different ones as self-fertilization or parthenogenesis. Even within the most widespread strategy, it is possible to find variations, such as presence of spermatophores.

During the last years, a great research effort has been made to shed light on some aspects of sexual selection, such as mate assessment, copulatory behavior, and sperm competition. Nevertheless, very interesting processes as origin and maintenance of parthenogenesis in earthworms are mainly unknown. Deeper research on both aspects would allow us to better understand the reproductive biology of these animals.

References

- Ankel WE (1927) Neuere Arbeiten zur Zytologie der natürlichen Parthenogenese der Tiere. *Z Indikt Abstamm Vererbungsl* 45:232–278
- Bateman AJ (1948) Intra-sexual selection in *Drosophila*. *J Hered* 2:349–368
- Beatty RA (1957) Parthenogenesis and polyploidy in mammalian development. Cambridge University Press, London, p 134
- Beatty RA (1967) Parthenogenesis in vertebrates. In: Metz CB, Monroy A (eds) *Fertilization*, vol I. Academic, New York, pp 413–440
- Blakemore RJ (1994) Earthworms of South East Queensland and their potential in brigalow soils. PhD Thesis, University of Queensland
- Blakemore RJ (1999) The diversity of exotic earthworms in Australia – a status report. In: Ponder W, Lunnay D (eds). Proceedings of “The other 99%” TRZS NSW, pp 182–187
- Bonnet C (1762) *Considerations sur les Corps Organisés*. Fayard, Tours, 1985:348
- Bouché MB (1975) La reproduction de *Spermophorodrilus albanianus* nov. Gen., nov. Spec. (Lumbricidae) explique-t-elle la fonction des spermatophores? *Zoologische Jahrbücher Abteilung für Systematik* 102:1–11
- Buckley TR, Attanayake D, Park D, Ravindran S, Jewell TR, Normark BB (2008) Investigating hybridization in the parthenogenetic New Zealand stick insect *Acanthoxyla* (Phasmatodea) using single-copy nuclear loci. *Mol Phylogenet Evol* 48:335–349
- Butt KR, Nuutinen V (1998) Reproduction of the earthworm *Lumbricus terrestris* Linné after the first mating. *Can J Zool* 76:104–109
- Cameron EK, Bayne EM, Coltman DW (2008) Genetic structure of invasive earthworms *Dendrobaena octaedra* in the boreal forest of Alberta: insights into introduction mechanisms. *Mol Ecol* 17:1189–1197

- Casellato S (1987) On polyploidy in Oligochaetes with particular reference to lumbricids. In: Bonvicini AM, Omodeo P (eds) On earthworms. Mucchi, Modena, pp 75–87
- Casellato S, Rodighiero R (1972) Karyology of Lumbricidae. 3rd contribution. *Caryologia* 25:513–524
- Cobolli Sbordoni M, De Matthaeis E, Omodeo P, Bidoli R, and Rodino E (1987) Allozyme variation and divergence between diploid and triploid populations of *Allolobophora caliginosa* (Lumbricidae: Oligochaeta). In: Bonvicini Pagliai AM, Omodeo P (eds) On earthworms, pp. 53–74. Selected Symposia and Monographs U.Z.I., vol 2. Mucchi, Modena
- Delmotte F, Sabater-Muñoz B, Prunier-Leterme N, Latorre A, Sunnucks P, Rispe C, Simon JC (2003) Phylogenetic evidence for hybrid origins of asexual lineages in an aphid species. *Evolution* 57:1291–1303
- Domínguez J, Velando A, Aira M, Monroy F (2003) Uniparental reproduction of *Eisenia fetida* and *E. andrei* (Oligochaeta: Lumbricidae): evidence of self insemination. *Pedobiologia* 47:530–534
- Edwards CA, Bohlen PJ (1996) Biology and ecology of earthworms, 3rd edn. Chapman and Hall, London, p 426
- Fernández R, Novo M, Gutiérrez M, Almodóvar A, Díaz Cosín D (2010) Life cycle and reproductive traits in the earthworm *Aporrectodea trapezoides* (Dugès, 1828) in laboratory cultures. *Pedobiologia* 53:295–299
- Field SG, Michiels NK (2005) Parasitism and growth in the earthworm *Lumbricus terrestris*: fitness costs of the gregarine parasite *Monocystis* sp. *Parasitology* 130:1–7
- Field SG, Schirp HJ, Michiels NK (2003) The influence of *Monocystis* sp infection on growth and mating behaviour of the earthworm *Lumbricus terrestris*. *Can J Zool* 81:1161–1167
- Garvín MH, Trigo D, Hernández P, Díaz Cosín DJ (2003) Gametogenesis and reproduction in *Hormogaster elisae* (Oligochaeta, Hormogastridae). *Inv Biol* 122:152–157
- Gates GE (1972) Contributions to North American earthworms. No. 3. IV. The trapezoides species group. *Bull Tall Timbers Res Stn* 12:146
- Gates GE (1974) Contributions to North American Earthworms. No. 10. Contributions to a revision of the Lumbricidae X: *Dendrobaena octaedra* (Savigny) 1826 with special reference to the importance of its parthenogenetic polymorphism for the importance for the classification of earthworms. *Bull Tall Timbers Res Stn* 15:57
- Grigoriopoulou N, Butt KR, Lowe CN (2008) Effects on adult *Lumbricus terrestris* on cocoons and hatchlings in Evans' boxes. *Pedobiologia* 51:343–349
- Grove AJ (1925) On the reproductive processes of the earthworm, *Lumbricus terrestris*. *Q J Microsc Sci* 69:245–290
- Grove AJ, Cowley LF (1926) On the reproductive processes of the brandling worm, *Eisenia fetida* (Sav.). *Q J Microsc Sci* 70:559–581
- Haimi J, Knott KE, Yliniemi I (2007) Does metal contamination affect clonal diversity of the parthenogenetic earthworm *Dendrobaena octaedra*? *Eur J Soil Boil* 43:268–272
- Hale CM, Frelich LE, Reich PB (2005) Exotic European earthworm invasion dynamics in northern hardwood forest of Minnesota, USA. *Ecol Appl* 15:848–860
- Heethoff M, Etzold K, Scheu S (2004) Mitochondrial COII sequences indicate that the parthenogenetic earthworm *Octolasion tyrtaeum* (Savigny 1826) constitutes of two lineages differing in body size and genotype. *Pedobiologia* 48:9–13
- Hughes RN (1989) A functional biology of clonal animals. Chapman and Hall, London, p 330
- ICZN (1999) International code of zoological nomenclature (4th edition). International Trust for Zoological Nomenclature, c/o Natural History Museum, London, p 306
- Jaenicke J, Selander RK (1979) Evolution and ecology of parthenogenesis in earthworms. *Am Zool* 19:729–737
- Jaenicke J, Parker ED, Selander RK (1980) Clonal niche structure in the parthenogenetic earthworm *Octolasion tyrtaeum*. *Am Nat* 116:196–205
- Jamieson BGM (1992) Oligochaeta. In: Harrison FW, Gardiner SL (eds) *Miscroscopic Anatomy of Invertebrates*, vol 7. Wiley-Liss, New York

- Jamieson BGM (2006) Non leech Clitellata. In: Rouse GW, Pleijel F (eds) Reproductive biology and phylogeny of Annelida. SP Science Publishers, USA, p 688
- King RA, Tibble AL, Symodson WOC (2008) Opening a can of worms: unprecedented sympatric cryptic diversity within British lumbricid earthworms. *Mol Ecol* 17:4684–4698
- Koene JM, Sundermann G, Michiels N (2002) On the function of body piercing during copulation in earthworms. *Invert Reprod Develop* 41:35–40
- Koene JM, Pfoertner T, Michiels NK (2005) Piercing the partner's skin influences sperm uptake in the earthworm *Lumbricus terrestris*. *Behav Ecol Sociobiol* 59:243–249
- Ligthart TN, Peek GJCW (1997) Evolution of earthworm burrow systems after inoculation of lumbricid earthworms in a pasture in the Netherlands. *Soil Biol Biochem* 29:453–462
- Mariño MF, Velando A, Domínguez J (2006) Do earthworms trade sperm? The 8th International Symposium on Earthworm Ecology Krakow Poland, 4–9 September 2006. Session 5 Behavioral and Evolutionary Biology. p 149
- Mayr E (1963) Animal species and evolution. Harvard University Press, Cambridge, p 797
- Meyer WJ, Bowman H (1994) Mating period and cocoon production in *Eisenia fetida*. The 5th International Symposium on Earthworm Ecology Columbus Ohio, 5–9 July 1994. p 128
- Michiels NK (1998) Mating conflicts and sperm competition in simultaneous hermaphrodites. In: Birkhead R, Moller AP (eds) Sperm competition and sexual selection. Academic, London, pp 219–254
- Michiels NK, Hohner A, Vormdran IC (2001) Precopulatory mate assessment in relation to body size in the earthworm *Lumbricus terrestris*: avoidance of dangerous liaisons? *Behav Ecol* 12:612–618
- Monroy F, Aira M, Velando A, Domínguez J (2003) Have spermatophores in *Eisenia fetida* (Oligochaeta, Lumbricidae) any reproductive role? *Pedobiologia* 47:526–529
- Monroy F, Aira M, Velando A (2005) Size-assortative mating in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae). *J Ethol* 23:69–70
- Muldal S (1952) The chromosomes of the earthworms I. The evolution of polyploidy. *Heredity* 6:55–76
- Novo M, Almodóvar A, Fernández R, Gutiérrez M, Díaz Cosín DJ (in press) Mate choice of an endogamic earthworm revealed by microsatellite markers. doi:10.1016/j.pedobi.2010.07.002
- Nuutinen V, Butt KR (1997) The mating behaviour of the earthworm *Lumbricus terrestris* (Oligochaeta: Lumbricidae). *J Zool Lond* 242:783–798
- Olive PJW, Clark RB (1978) Physiology of reproduction. In: Mill PJ (ed) Physiology of annelids. Academic, London, pp 271–368
- Omodeo P (1951) Corredo cromosomico e spermatogenesi aberrante in *Allolobophora caliginosa* trapezoides. *Boll Zool* 18:27–39
- Omodeo P (1952) Cariologia dei lumbricidae. *Caryologia* 4:173–178
- Omodeo P (1953) Specie e razze poliploidi nei lombrichi. *Convegno di Genetica, Ricerca Scientifica* 23, Suppl 43.9
- Ortiz-Ceballos A, Fragoso C (2006) Parental care of endogamic earthworm cocoons: is cleaning, construction, and cast surrounding of chambers related to hatching and survival of juvenile worms? The 8th International Symposium on Earthworm Ecology Krakow Poland, 4–9 September 2006. Session 5 Behavioral and Evolutionary Biology. p 150
- Owen R (1849) On parthenogenesis, or the successive production of procreating individuals from a single ovum. John van Voorst, London
- Parker GA (1970) Sperm competition and its evolutionary consequences in insects. *Biol Rev* 45:525–567
- Porto PG, Velando A, Domínguez J. (2008) Effects of mating frequency on sex allocation in the simultaneously hermaphroditic redworm (*Eisenia andrei*). The 15th International Colloquium on Soil Zoology Curitiba Brazil, 25–29 August 2008. Session 1 Soil Animal Biodiversity: the final frontier. S1T1P14
- Qiu JP, Bouché MB (1998) Contribution to the taxonomy of Hormogastridae (Annelida: Oligochaeta) with description of new species from Spain. *Doc Pedozool Integro* 4:164–177

- Reynolds JW (1974) Are oligochaetes really hermaphroditic amphimictic organisms? *Biologist* 56:90–99
- Richards KS, Fleming TP (1982) Spermatozoal phagocytosis by the spermathecae of *Dendrobaena subrubicunda* and other lumbricids (Oligochaeta, Annelida). *Int J Invert Rep* 5:233–241
- Sahm S, Velavan TP, Schulenburg H, Michiels NK (2009) Reconstruction of mating history – a retrospective analysis of *Lumbricus terrestris* mate choice criteria in natural populations. In: Velavan TP (ed) Population genetics of host–parasite interactions in *Lumbricus terrestris* and *Monocystis sp* (Apicomplexa: Gregarinaea). Phd Thesis, Tübingen University, Germany, pp 43–57
- Sims RW, Gerard BM (1999) Earthworms, notes for the identification of British species. In: Barnes RSK, Crothers JH (eds) Synopses of the British fauna (new series), no. 31 revised. Field Studies Council, Shrewsbury
- Suomalainen E (1950) Parthenogenesis in animals. *Adv Genet* 3:193–253
- Suomalainen E, Saura A, Lokki J (1987) Cytology and evolution in parthenogenesis. CRC, Boca Raton, p 216
- Tato A, Velando A, Dominguez J (2006) Influence of size and partner preference on the female body function of the earthworm *Eisenia andrei* (Oligochaeta, Lumbricidae). *Eur J Soil Biol* 42: S331–S333
- Tembe VB, Dubash PJ (1961) The earthworms: a review. *J Bombay Nat Hist Soc* 58:171–201
- Terhivuo J, Saura A (1993) Genic and morphological variation of the parthenogenetic earthworm *Aporrectodea rosea* in southern Finland (Oligochaeta, Lumbricidae). *Am Zool Fennici* 30:215–224
- Terhivuo J, Saura A (1996) Clone pool structure and morphometric variation in endogeic and epigeic North-European parthenogenetic earthworms (Oligochaeta: Lumbricidae). *Pedobiologia* 40:226–239
- Terhivuo J, Saura A (2003) Low clonal diversity and morphometrics in the parthenogenetic earthworm *Octolasion cyaneum*. *Pedobiologia* 47:434–439
- Thomsen M (1927) Studien über die Parthenogenese bei einigen Cocciden und Aleurodinen. *Z Zellforsch Mikrosk Anat* 5:1–10
- Thornhill R (1983) Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am Nat* 122:765–788
- Van Praagh BD (1995) Reproductive biology of *Megascolides australis* McCoy (Oligochaeta: Megascolecidae). *Aust J Zool* 43:489–507
- Varuta AT, More NK (1972) Cytochemical study of mucus and mucus secreting cells in spermathecae of the earthworms, *Pheretima elongata* (Perrier) and *Hoplochaetella powelli* (Michaelsen). *Ind Exp Biol* 10:239–241
- Velando A, Domínguez J, Ferreiro A (2006) Inbreeding and outbreeding reduces cocoon production in the earthworm *Eisenia andrei*. *Eur J Soil Biol* 42:S354–S357
- Velando A, Eiroa J, Dominguez J (2008) Brainless but not clueless: earthworms boost their ejaculates when they detect fecund non-virgin partners. *Proc R Soc B* 275:1067–1072
- Victorov AG (1997) Diversity of polyploid races in the family *Lumbricidae*. *Soil Biol Biochem* 29:217–221
- Vyas I, Dev B (1972) Histochemical localization of alkaline phosphatase in the spermathecae of the earthworm, *Barogaster annandalei* (Stephenson). *Acta Histochem* 42:344–350
- Wallwork JA (1983) Earthworm biology. Camelot, Southhamton
- White MJD (1973) Animal cytology and evolution. Cambridge University Press, London, p 961