Sexual selection in earthworms: Mate choice, sperm competition, differential allocation and partner manipulation

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A B S T R A C T

Interpretations of hermaphroditism have been influenced by the long-held idea that organisms can be classified in a teleological series ranging from simple to complex, with humans placed at the top. Darwin considered that hermaphroditic animals, those which have both male and female sex organs, cannot be subject to sexual selection because the "union of sexes" makes them unable to perceive the attractiveness or rivalry of their partners, and moreover, because of their incapacity to exercise any kind of preference or choice. Until very recently, this view was generally accepted and justified on the grounds that the sensorial abilities of these animals are very limited and imperfect, basically because of their position at the lower level of the animal scale. In this paper we review new evidence that contradicts this vision. Indeed, recent studies suggest that earthworms are able to detect the degree of relatedness, the quality and mating status of their partners, and they are able to fine-tune control of transferred ejaculate volume and cocoon production. Overall, the picture that emerges is that earthworms are phenotypically very flexible, evaluation of partners is subject to intense selection, and sperm competition is important in shaping their mating behavior. Consequently, earthworms constitute an excellent model for studying sexual selection in simultaneous hermaphrodites. Interestingly, these results are more consistent with the latter observations of Darwin on earthworms than with his earlier conclusions on hermaphroditic animals. In his last book, written in 1881, Darwin affirmed, among other things, that despite being low in the scale of organization, earthworms possess some degree of intelligence. In the same book, Darwin postulated the role of earthworm behavior in soil formation, thus establishing the basis of modern soil ecology. Since earthworms exert considerable influence on the structure and function of terrestrial ecosystems, mating strategies that affect aggregations, dispersal or colonization may have important consequences on ecosystem functioning. Hence, earthworms are excellent models for integrating evolutionary and ecosystem ecology.

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1. Introduction

The essence of sexual selection, as defined by Darwin, is selection through competition for mates. Darwin first defined sexual selection in *On the Origin of Species*: "...what I call Sexual Selection. This depends not on a struggle for existence, but on a struggle between the males for the possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring." (Darwin, 1859, p 88). Fuller treatment of the theory came in *The Descent of Man*, in which he defined sexual selection as depending "on the advantage which certain individuals have over the same sex and species, in exclusive relation to reproduction" (Darwin, 1871, p. 256).

In chapter IX of the latter book Darwin argued that simultaneous hermaphrodites would not exhibit mate assessment or mate choice, primarily because sexual dimorphism cannot be expressed and because 'lower' invertebrates would lack the 'mental powers' to engage in 'mutual rivalry' (Darwin, 1871, p 321). He considered sexual selection to be restricted to higher animals and pointed out, for example, that "Annelids apparently stand too low in the scale, for the individuals of either sex to exert any choice in selecting a partner, or for the individuals of the same sex to struggle together in rivalry". Indeed, in many cases, hermaphroditism in invertebrates has been considered incompatible with sexual selection because of a lack of capacity for mate choice and/or intrinsically weaker selection for mate competition due to few mating opportunities (Charnov, 1979, 1987), limited opportunities for sex-specific trait expression (Morgan, 1994) and similar variance in reproductive success between sexes (Greeff and Michiels, 1999; reviewed in Arnqvist and Rowe, 2005). The frequent mating observed in many hermaphroditic animals may convey the impression of

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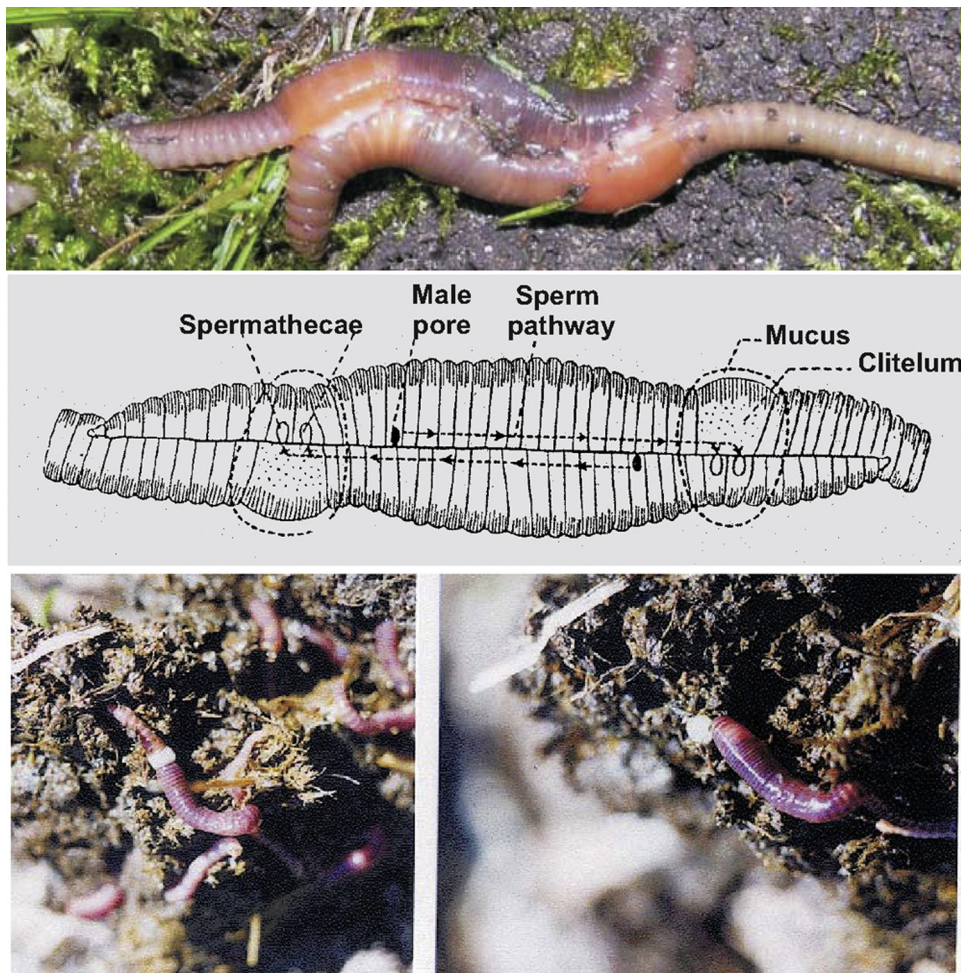
Fig. 1. Copulation (top panel) and cocoon formation (lower panels) in earthworms (Family Lumbricidae). A seminal groove, a depression in the outer body wall formed as a series of pits by the contraction of muscles, extends from the male pore to the clitellum (middle panel). These pits carry sperm as droplets from the male pore to the clitellar region, where it collects, and eventually enters the spermathecae of the partner earthworm with the aid of the *Tubercula pubertatis*. After copulation, earthworms separate and each clitellum produces a secretion that eventually hardens over its outer surface (lower panels). The earthworm then moves backwards, thus drawing the tube over its head; when the worm is completely free, the ends of the tube close to form the cocoon. The cocoons contains a nutritive albuminous fluid, produced by the clitellar gland cells, the ova, and the spermatozoa that were discharged into it as the tube passed over the spermathecal openings. Cocoons continue to be formed until all stored sperm has been used up. Photographs by J. Domínguez.

indiscriminate sexual interactions (Anthes, 2010). In contrast with this view, general theory predicts that sexual selection, i.e. selection acting on mating and fertilization success (Arnold, 1994; Arnqvist and Rowe, 2005; Clutton-Brock, 2007), and especially post-mating sexual selection (i.e., sperm competition and cryptic female choice) may also be an important selective force shaping mating behavior in hermaphroditic animals (Charnov, 1996; Michiels, 1998; Arnqvist and Rowe, 2005).

Earthworms represent the major animal biomass in most terrestrial temperate ecosystems (Lavelle and Spain, 2001; Bardgett, 2005). They are considered major ecosystem engineers because they significantly affect soil physical, chemical and biological properties, and play a key role in modifying soil structure and accelerating the decomposition of organic matter and nutrient turnover (Lee, 1985; Edwards and Bohlen, 1996). Belowground and aboveground components of ecosystems are strongly linked through a variety of both direct and indirect interactions that operate across different levels of ecological organization (Bardgett and Wardle, 2010). In this way, earthworms also exert important effects in shaping plant community composition and aboveground food webs (e.g. Eisenhauer et al., 2009, 2010).

According to Blakemore (2006), there are more than 6000 species of earthworms, although for the great majority of these only the name and morphology are known and nothing is known about their biology, life cycles or ecology. However some of these characteristics, such as reproductive systems and mating strategies, are very important drivers of population dynamics, aggregation, distribution and dispersion.

Earthworms are simultaneous hermaphrodites, and reproduction usually occurs through copulation and cross-fertilization, after which each of the mated individuals produces cocoons containing variable numbers of fertilized ova (Fig. 1). The resistant cocoons are usually deposited near the soil surface, except in dry weather when they are laid in deeper layers. Earthworms are iteroparous animals and display indeterminate growth, continuing to grow in size after completing their sexual development.

In comparison with other well studied simultaneous hermaphrodites, earthworms possess some characteristics that make them particularly suitable for studying post-copulatory sexual selection. Sperm are exchanged simultaneously and stored for a long time in spermathecae, where sperm from multiple donors is mixed until cocoon laying (Velando et al., 2008). Sperm digestion is widespread in hermaphrodites, which makes it difficult to distinguish sperm competition and mating investment (Michiels, 1998; Greeff and Michiels, 1999), although some earthworm species are unable to digest allosperm (Richards and Fleming,

1982). Moreover, earthworms spend a long time in copulation (Grove and Cowley, 1926), which provides a good opportunity for studying mating behavior.

2. Why are earthworms simultaneous hermaphrodites?

Simultaneous hermaphroditism is defined as the coexisting expression of male and female sex (i.e., sperm and egg production) in a single individual. It is favored whenever the overall reproductive success achieved by a hermaphrodite is greater than that of a pure male or a pure female (Charnov et al., 1976; Charnov, 1982).

Hermaphroditism is considered to be the ancestral state in animals, as shown by recent phylogenetic analysis (Iyer and Roughgarden, 2008), although many transitions between simultaneous hermaphroditism and gonochorism have occurred in Lophotrochozoa. The adaptive maintenance of simultaneous hermaphroditism has traditionally been associated with low population densities, and with sessile, slow-moving, and parasitic life styles. The occurrence of simultaneous hermaphroditism is classically attributed to the potential usefulness of self-fertilization in the absence of partners, and the benefit provided by the fact that every encounter with a conspecific individual is an encounter with a mating partner and allows outcrossing (Schärer, 2009; Anthes, 2010).

Although low population densities can explain hermaphroditism in the Oligochaeta, in many cases earthworm density is quite high, between 100 and 500 individuals/m^2, and even up to 2000 individuals/m^2 in temperate pastures and irrigated orchards (Lee, 1985; Dymond et al., 1997; Eisenhauer et al., 2007). Multiple matings are common in such dense populations, but more importantly, density and mating rates also fluctuate widely (varying frequently and unpredictably) (Edwards and Bohlen, 1996; Monroy et al., 2006), resulting in variations in the fitness of male and female functions.

Under a paradigm of adaptive evolution, animals would be expected to express a reproductive mode that maximizes fitness under the prevailing environmental and social conditions. While this type of adaptive flexibility is approached in broadcasting invertebrates with their simple reproductive structures, internal fertilization and complex reproductive morphologies in earthworms may restrict this flexibility. Such constraints may explain why earthworms maintain an apparently 'maladaptive' reproductive mode (Michiels et al., 2009; Anthes, 2010).

In this type of situation, when the environment is unpredictable on a time scale shorter than the generation time, phenotypic plasticity is expected to be favored (de Jong, 1995). Indeed, phenotypic plasticity in sex allocation may explain why hermaphroditism is maintained in large, dense fluctuating populations (Brauer et al., 2007). Here we present new evidence showing that earthworms are more flexible in the effort allocated to male and female reproduction than previously thought, thus explaining some bizarre behavior in these animals.

3. Evidence of sexual selection in earthworms

In the following sections, we summarize recent research on sexual selection in earthworms, showing that these organisms are excellent models for studying this topic in hermaphrodites. Since intra and intersexual selection may operate before, during or after copulation, we consider the recent findings on precopulatory mate assessment and choice, and on postcopulatory processes, i.e. sperm competition and cryptic female choice (differential allocation). We also show evidence that earthworms are able to manipulate partner decisions.

3.1. Mate assessment and choice

Some earthworm species undergo prolonged courtship with short and repeated touches between partners before mating and they spend a long time in copulation with constricted movements between partners, which provides ample opportunity for partner assessment (Michiels et al., 2001; Tato et al., 2006; Velando et al., 2006, 2008). Earthworms first need to be able to recognize and/or evaluate their partners in order to be phenotypically flexible according to mating situations. Field observations of the mating behavior of Lumbricus terrestris L. suggest the existence of a precopulatory behavior sequence, during which prospective partners visit each other's burrows (Nuutinen and Butt, 1997). The mechanisms used by earthworms to evaluate their partners are largely unknown, but probably involve tactile and chemical cues.

Earthworms possess a wide variety of specialized individual epidermal and subepidermal cells, free nerve endings in the epidermis, and some aggregations of cells within more complex structures. Although these cells are associated with the reception of tactile, positional, chemical stimuli all over their bodies, most are concentrated in the prostomium and anterior segments (Wallwork, 1983; Lee, 1985; see Fig. 2). The epithelium in the mouth region also accommodates groups of sensory cells that are associated with the detection of mucus secreted by other earthworms (Edwards and Lofty, 1972). These sensory organs include: (i) mechanoreceptors, which are probably free nerve endings in the epidermis; (ii) proprioceptors, which register deformations and stress in the body arising from the animal's own movement, its weight, or from external forces, most likely a type of multiciliate epidermal cell, whose cilia are bent over and lie horizontally beneath the cuticle; (iii) chemoreceptors, which probably include some multiciliate sensory cell, and the nuchal organs, which are pits or folded lobes on the body surface; and (iv) photoreceptors, single celled and widely distributed over the body surface, each containing an optic organelle composed of a transparent hyaline vacuole surrounded by a network of neurofibrils (Mill, 1978). Earthworms thus have a highly specialized recognition system that can potentially be used for mate evaluation.

In many invertebrates, partner size should be an important characteristic in mating patterns, because fecundity often increases with body size. In this case, and especially when the costs of insemination are not trivial, animals are expected to preferentially inseminate partners of equal or larger body size, because small partners will produce fewer eggs. In animals with indeterminate growth, such as earthworms, adult body size varies greatly, thus favoring size-dependent mate choice (DeWitt, 1996; Anthes, 2010). Egg production increases with body mass in earthworms (Domínguez et al., 1997) (as in many hermaphrodites), and larger partners should be preferred. As matings are reciprocal, a preference for inseminating larger individuals may ultimately result in pair formation between similar-sized individuals. This generates a positive correlation between the body size of mating individuals across the population, a pattern known as size-assortative mating.

Size-assortative mating has been observed in a natural population of the earthworm Eisenia fetida (Savigny) (Monroy et al., 2005) and such a non-random mating pattern probably reveals the existence of mate selection. In another study of mate choice, L. terrestris showed a preference for same-size partners under experimental conditions, although in the field, assortative mating was observed in some samples but not in others (Michiels et al., 2001). Differences between mating preferences and mating patterns may arise because this species lives in permanent burrows and mating choice is limited to the neighborhood (Michiels et al., 2001). Overall, these studies suggest that earthworms are able to evaluate
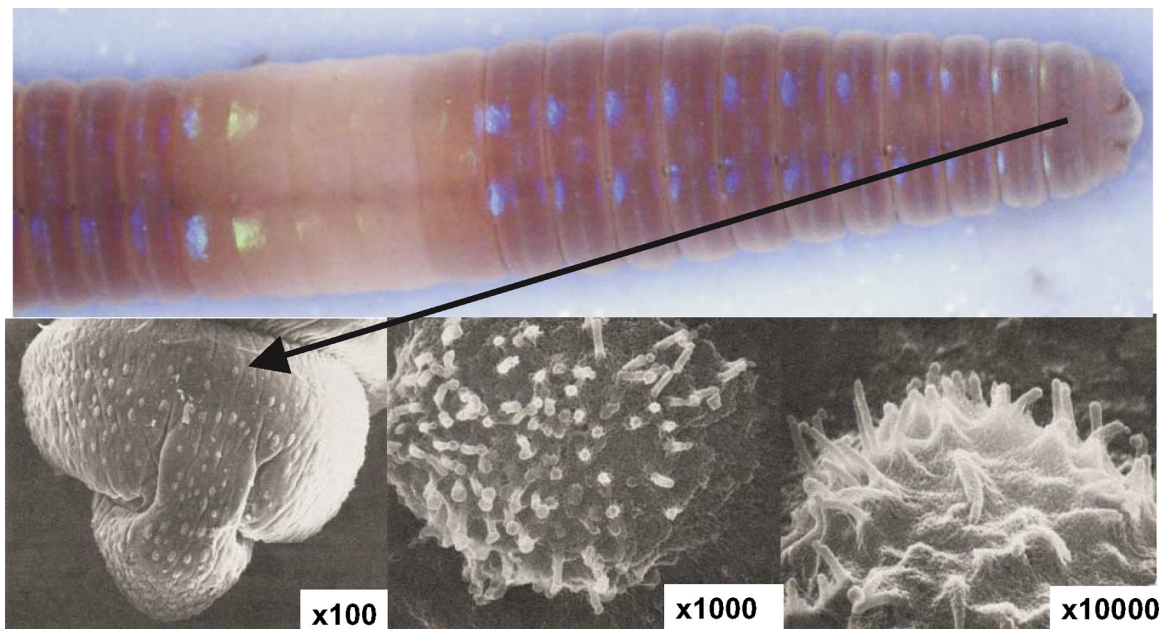
Fig. 2. Earthworm body surface (top panel) and scanning electron microscope photographs of external sensory buds on the *prostomium* and *peristomium* (lower left photograph; details of *prostomium* in middle and right, lower photographs). Photographs by J. Domínguez and J. Eiroa.

partner size, although mate choice may be constrained by the availability of preferred partners. On the other hand, assessment of a partner's body size may lead to prudent male mating effort, in which individuals donate larger ejaculates to more fecund partners (Wedell et al., 2002; Anthes, 2010). This effect was observed in *Eisenia andrei* Bouché, individuals of which approximately double their ejaculate size when inseminating a partner of twice their body mass (Velando et al., 2008; Fig. 3). Since growth in earthworms is indeterminate, body size may vary with age, but in the latter example all earthworms were raised from cocoons and were the same age. Future studies on the possible existence of an age-dependent mating system would clarify this question.
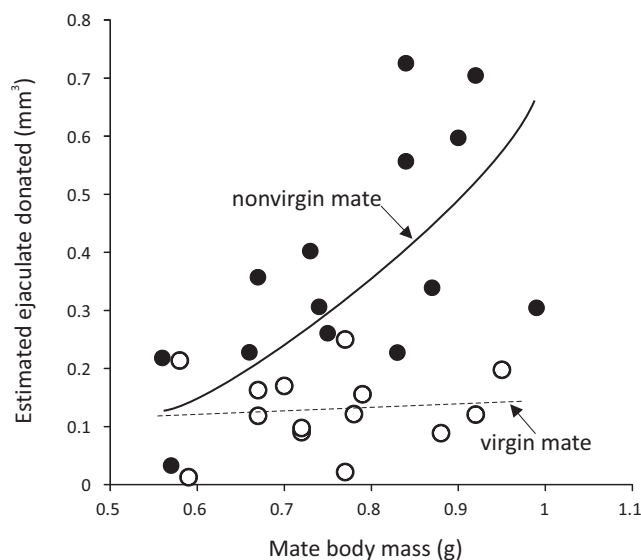
Fig. 3. Estimated volume of ejaculate (mm³) donated by the earthworm *E. andrei* after copulation with a virgin mate (white circles) and with a non-virgin mate (black circles), in relation to the mate body mass.
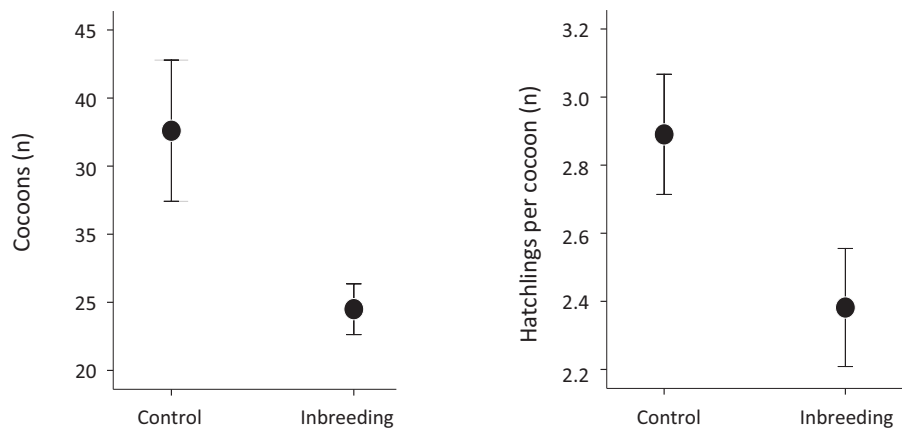*Source*: Adapted from Velando et al. (2008).

3.2. Sperm competition

Sperm competition may affect mating decisions, especially in those species in which multiple mating is very common (Wedell et al., 2002), such as some earthworm species (Monroy et al., 2006). Earthworms are capable of storing allosperm for prolonged periods, and the sperm from multiple donors probably compete for access to unfertilized eggs. Thus, mating history may be a relevant mate characteristic that will affect sperm allocation. Earthworms store sperm from previous matings in internal spermathecae, and therefore their mating history is not externally visible, making partner evaluation difficult. Despite this, a recent study revealed that earthworms are able to evaluate partner's mating history (Velando et al., 2008), suggesting a highly efficient recognition system in terms of mate evaluation.

Under sperm mixing from multiple donors, animals are strategically selected to adjust their male mating effort to the perceived intensity of sperm competition. Sperm competition risk models predict that the size of ejaculate should increase when ejaculates compete with the sperm from other males to fertilize the ova (Wedell et al., 2002; Engqvist and Reinhold, 2005). In this situation, fertilization success is probably similar to 'fair raffles' mixing, in which an increase in sperm transfer increases the probability of fecundation (Parker, 1998). Thus, in agreement with these models, *E. fetida* earthworms triplicate their ejaculates when mating with a non-virgin mate, i.e. after detecting a risk of sperm competition. Interestingly, such increases are greater when the worms are mated with larger (more fecund) partners, indicating that earthworms exert a flexible and fine-tuned control of ejaculate volume (Velando et al., 2008). This fine-tuned adjustment of ejaculate suggests that earthworms are prudent with their male resources and invest them in more successful mating encounters, as predicted in species with frequent mating encounters.

3.3. Differential allocation

After copulation, sperm recipients may bias paternity toward preferred individuals, a process known as "cryptic female choice" (Thornhill, 1983; Eberhard, 1996). In iteroparous animals,

Fig. 4. Number of cocoons (left panel) and number of hatchlings per cocoon (right panel) produced by the earthworm *E. andrei* in response to inbreeding situations. *Source*: Adapted from Velando et al. (2006).

reproductive investment in each mating encounter may be adjusted according to potential benefits, in a process known as "differential allocation" (Burley, 1988). In earthworms, the genetic similarity of the mating partner is a potentially important factor in biasing reproductive investment. Earthworms have low dispersal ability, so inbreeding may be common. Inbreeding typically leads to a significant reduction in fitness, and therefore it should be beneficial to avoid breeding with a genetically similar partner. Laboratory experiments have shown that the earthworm *E. andrei* adjusts its breeding effort according to the degree of mate relatedness. Individuals of this species were mated with their siblings and with non-siblings from the same population and it was found that inbreeding reduced cocoon production, indicating the existence of reproductive adjustment in earthworms according to the genetic divergence of their partners, i.e. female function in earthworms is also flexible (Velando et al., 2006; Fig. 4).

3.4. Sexual conflict: partner manipulation

The evolutionary interests of the sexual functions in hermaphrodites commonly diverge and generate a potential sexual conflict (Bedhomme et al., 2009). Sexual conflict has been well documented in dioecious animals (Arnqvist and Rowe, 2005), but also occurs in simultaneous hermaphrodites (Charnov, 1979; Michiels and Koene, 2006; Schärer and Janicke, 2009; Abbott, 2011). The coevolution of sexual antagonists may promote different mating strategies, in which sperm donors develop offensive traits (*male harm*) to increase their chances in sperm competition, while sperm receivers develop defensive traits to control who fathers their offspring (Arnqvist and Rowe, 2005). Clear evidence for male harm in hermaphrodites is still scarce, although traumatic sperm injection with damage to the female function is widespread among sea slugs, flatworms and leeches (Michiels and Newman, 1998; Arnqvist and Rowe, 2005; Anthes, 2010).

One important type of partner manipulation involves transfer of manipulative allohormones (Koene, 2005), i.e. bioactive substances that interfere with postcopulatory processes such as fertilization, re-mating, and resource allocation to female function (Michiels, 1998; Bedhomme et al., 2009; Schärer and Janicke, 2009). The transfer of allohormones during mating in some hermaphrodites (e.g., sea slugs, Anthes and Michiels, 2007; land slugs, Reise et al., 2007; land snails, Chase and Blanchard, 2006) suggests the important role that such mechanisms may play in hermaphrodite mating systems (Anthes, 2010). A well-known example is the dart-shooting behavior of the garden snail *Helix aspersa*, whereby partners shoot a calcareous dart into the partner's body prior to copulation. The bioactive compounds delivered in mucus surrounding the dart increase dart-shooters paternity (Chase and Blanchard, 2006).

In earthworms, possible allohormone transfer was studied in *L. terrestris*. During reciprocal copulation and sperm transfer, *L. terrestris* pierce 40–44 copulatory setae into the partner's body, causing damage, and also inject a substance from the setal glands (Grove, 1925; Koene et al., 2002). Experimental injections of this substance have shown that it increases sperm uptake and delays re-mating, both of which favor the sperm donor, but may be unfavorable to the sperm receiver (Koene et al., 2005). Thus, in some earthworm species donors are able to manipulate the behavior receivers by injecting allohormones. The way in which receivers defend paternity control should be explored in future studies.

4. Conclusions and future perspectives

Although there is an evident lack of knowledge about how sexual selection affects the reproductive behavior of earthworms, the picture that emerges from recent studies is that earthworms are phenotypically very flexible in terms of mating decisions, and that flexibility is modulated in both the male and female function. Whether or not this phenotypic plasticity in earthworm mating behavior entails phenotypic plasticity in sex allocation should be explored in the future. It has also been shown that partner evaluation of partners not only takes place, but is also subject to intense selection, and that sperm competition is important in shaping life history traits in earthworms. Earthworms therefore constitute an excellent model for studying sexual selection in simultaneous hermaphrodites.

Some practical aspects make earthworms particularly suitable for carrying out experimental evolutionary studies: individuals can be easily managed and reared; genetic markers are available for paternity analysis (Harper et al., 2006; Velavan et al., 2007; Novo et al., 2008), and earthworms can be marked individually (which enables e.g., the mating history of focal individuals to be traced) (Butt et al., 2009). There are also many types of life cycles among earthworm species within a slow–fast continuum. Species with a short generation time are especially suitable to perform experimental evolutionary studies and to disentangle genetic architecture of reproductive traits, and long-lived species for studying sexual selection in a life-history context. Earthworms also show high diversity in reproductive systems among species, e.g., in the number of testes and spermathecae (Adiyodi, 1988; Michiels, 1998), and are suitable for studying coevolution between male and female functions in comparative studies, as phylogeny will be resolved.

Interestingly, the findings outlined in this paper are more consistent with Darwin's latter observations on earthworms than with his earlier conclusions on hermaphroditic animals. In his last book, written in 1881, Darwin affirmed, among other things, that despite being low in the scale of organization, earthworms possessed some degree of intelligence. In the same book, Darwin (1881), Darwin postulated the role of earthworm behavior in soil formation, thus establishing the basis of modern soil ecology. Since earthworms exert considerable influence on the structure and function of terrestrial ecosystems, mating strategies that affect aggregation, dispersal or colonization may have important consequences on ecosystem functioning. Hence, earthworms are excellent models for integrating evolutionary and ecosystem ecology.

Acknowledgements

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