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Effects of body size on courtship role, mating frequency and sperm transfer in the land snail *Succinea putris*

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Sex role preferences in simultaneous hermaphrodites may vary with individual condition and partner quality across consecutive matings. Theoretical and empirical studies have highlighted an individual's body size and its relation with the partner's body size as potential factors that influence the preferred sex role. We studied effects of body size of focal individuals and partners on (1) mating frequency and mating interval, (2) courtship role and (3) number of sperm donated in successive copulations in the land snail *Succinea putris* where 'active' individuals mount the shell of their 'passive' partner before penises are intromitted reciprocally. We found body size-dependent differences in mating behaviour between small and large individuals: (1) smaller individuals of mating pairs were more likely to adopt the active role, (2) small individuals seemed to prefer inseminating larger partners, (3) a positive relation between body size and sperm number donated was found, (4) large individuals adjusted sperm number to their partner's body size, and (5) the smallest number of sperm was donated by large focal individuals to small partners. In addition, the number of sperm donated (1) increased with longer mating intervals and (2) decreased in later copulations indicating that the number of previous matings may affect male resource allocation. Our results support the view of models of sex role preferences, gender conflicts and solutions to gender conflicts that predict that the preferred sex role is variable within a species (e.g. size-dependent sex allocation models and the gender ratio hypothesis).

In simultaneous hermaphrodites, the sex role with the lower cost associated with mating (or the higher fitness gain per unit resource) is predicted to be preferred (Charnov 1979; Michiels 1998). When many individuals of a simultaneously hermaphroditic population share a preference for the same sex role, mating interests of potential mates will often be incompatible. For this reason, simultaneously hermaphroditic mating systems are prone to develop gender conflicts, that is, conflicts over the sex role during mating, the origin of which, and solutions to, is considered to shape the mating behaviour (Wethington & Dillon 1996; Baur 1998; Michiels 1998; Anthes et al. 2006a). Pinpointing the factors that determine sex role preferences therefore remains a key issue in understanding the behaviour of copulating simultaneous hermaphrodites (Anthes et al. 2006a).

A variety of models have been proposed to explain sex role preferences, gender conflicts and solutions to gender conflicts (reviewed in Anthes et al. 2006a). One school of thought proposes that sex role preferences depend on control of fertilization, that is, all members of a population invariably avoid the sex role with the highest likelihood of losing gametes during the process of fertilization (Leonard & Lukowiak 1984). For example, in hermaphrodites with internal fertilization, the male sex role has no direct control over paternity and so-called risk-averse models suggest that individuals should avoid copulating in the male role. Alternative models (e.g. Fischer 1988; Leonard 1991; Charnov 1996; Michiels et al. 2003) focus more on the benefits that each sexual function obtains through mating. Given long-term sperm storage in many hermaphrodites (Baur 1998), most of these models assume that 'female' fitness depends less on multiple mating than 'male' fitness does. This implies that hermaphrodite gender conflicts usually arise because mating partners prefer to be 'male' rather than 'female' (e.g. Charnov 1979; Fischer 1987; Michiels 1998; Greeff & Michiels 1999).

More recently, authors have suggested that the preferred sex role is variable within a species. In many simultaneously hermaphroditic species, there is considerable variation in sex allocation (reviewed in Schärer 2009) and sex allocation is thought to reflect mating behaviour in these organisms. Recently, this was empirically demonstrated in the free-living flatworm *Macrostomum lignano* where more male-biased individuals showed an increased mating rate (Janicke & Schärer 2009). Sex allocation

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models have therefore been applied to predict the factors influencing sex role preferences. In addition, Anthes et al. (2006a) showed that sex role preferences in hermaphrodites could be flexible and determined by the potential male and female fitness gain in each single mating interaction (i.e. the gender ratio hypothesis). Here, sex allocation is considered an a priori 'best guess' about future mating opportunities that sets the limits within which variation in sex role preferences can take place (Anthes et al. 2006a). With one and the same sex allocation, a hermaphrodite may prefer to donate sperm, for example, when mating with a virgin partner, but prefer to receive sperm, for example, after a period of isolation and allosperm depletion (Anthes et al. 2006a). Thus, sex role preferences may vary with individual condition and partner quality across consecutive matings.

Potential factors highlighted by both theoretical and empirical studies that may influence the preferred sex role of a hermaphrodite in a mating are (1) its body size (relative to that of its partner; Angeloni et al. 2002), (2) its sexual motivation and that of its partner (Halliday 1983; Ziv et al. 1989; Adamo & Chase 1990; Peters et al. 1996; De Boer et al. 1997; Westneat et al. 2000; McCarthy 2004; Koene & ter Maat 2005) and (3) its mating history and that of its partner (i.e. sperm competition Michiels et al. 2003; Anthes et al. 2006b). Indeed, under a wide range of individual and population conditions, size-dependent sex allocation models predict that smaller individuals with less resources for reproduction should invest a higher proportion of resources in sperm, and therefore a smaller proportion in eggs, than larger individuals with more resources for reproduction (Angeloni et al. 2002; Cadet et al. 2004; Vizoso & Schärer 2007). In addition, individuals of either size should invest more resources in sperm when copulating with a large partner than with a small partner (Angeloni et al. 2002) which was empirically demonstrated in the sea slug *Chelidonura sandrana* (Anthes et al. 2006b). Consequently, in species with unilateral penis intromission, smaller individuals should adopt the male sex role more often whereas larger individuals should adopt the female sex role more often (Angeloni et al. 2002). Some studies have provided strong support (Otsuka et al. 1980; Yusa 1996; Angeloni & Bradbury 1999; Angeloni 2003; McCarthy & Sih 2008) and others weak support (Pennings 1991; Angeloni et al. 2003) for this prediction. One study showed that small animals copulated in the female role (Gianguzza et al. 2004) while other studies did not find any effect of body size on sex role (e.g. Leonard & Lukowiak 1985; Baur 1992; Haase & Karlsson 2004; Chaine & Angeloni 2005; Koene et al. 2008). In addition, sexual isolation appears to trigger mechanisms causing changes in the motivational state of an individual and this may influence the mating behaviour and preferred sex role (Wethington & Dillon 1996; De Boer et al. 1997; McCarthy 2004; Koene & ter Maat 2005; Dillen et al. 2008; Facon et al. 2008). Male motivation seems to increase with increasing availability of autosperm and/or prostate fluids (De Boer et al. 1997; McCarthy 2004; Koene & ter Maat 2005; Facon et al. 2007) while female motivation seems to increase with decreasing amounts of allosperm stored (Wethington & Dillon 1996; Facon et al. 2008) or with increasing number or maturity of the eggs (McCarthy 2004). Finally, Michiels et al. (2003) and Anthes et al. (2006b) showed that sea slugs transfer less sperm when facing an increased likelihood of sperm competition (i.e. when mating with nonisolated individuals).

Davison & Mordan (2007) and Jordaens et al. (2009) reviewed the mating behaviour of hermaphroditic pulmonate gastropods (i.e. slugs and snails). One general outcome of these reviews was that in species with unilateral sperm transfer, the 'male' (sperm donor) mounts the shell of its partner that will act as the 'female' (sperm receiver e.g. Van Duivenboden & ter Maat 1988; Wethington & Dillon 1996). In contrast, snails with reciprocal sperm transfer adopt both sex roles at the same time (i.e. reciprocal mating; Davison & Mordan 2007; Jordaens et al. 2009). Nevertheless, in some species (of several genera) of land snails that mate reciprocally, one individual is more active during courtship and seemingly initiates copulation by mounting the shell of its, seemingly passive, partner (Jordaens et al. 2005; Dillen et al. 2009a). This is referred to as the 'courtship role' (also referred to as 'activity role', see Jordaens et al. 2005 and Dillen et al. 2008) with the shell mounter in the 'active' role and the lower individual in the 'passive' role (Jordaens et al. 2009). A case in point is the stylommatophoran land snail *Succinea putris* of which sexually mature individuals vary strongly in body size (i.e. shell height varies between 9 mm and 25 mm). Body size and sexual isolation affect the courtship role in this species (Jordaens et al. 2005; Dillen et al. 2008): smaller individuals mostly (98% of the matings) adopt the active role (Jordaens et al. 2005; Dillen et al. 2008) and sexually isolated individuals are more motivated to copulate, show a preference to adopt the active role, and transfer more sperm than nonisolated individuals (Dillen et al. 2008). In addition, the motivation to copulate after a period of sexual isolation of 2 weeks has been suggested to reflect a motivation to donate rather than receive sperm (Dillen et al. 2008). Because of the theoretical predictions of sex allocation theory, and previous observations in *S. putris* outlined above, we speculate that in reciprocally mating species where partners have a different courtship role, this difference in courtship role may be the result of the active and passive partners having different, body size- and condition-dependent, sex allocation strategies. As a result, the body size of an individual and that of its partner may predict the motivation to mate, the courtship role and the number of sperm that are transferred during copulation. In this study, we used *S. putris* to examine whether body size of focal individuals and of their partners had an effect on (1) the motivation to mate (i.e. mating frequency and mating interval), (2) the courtship role and (3) the number of sperm donated in successive matings.

METHODS

Study Species

The land snail *S. putris* (Pulmonata, Succineidae) is an outcrossing species (Dillen et al. 2009b) that possesses a sperm storage organ consisting of two spermathecal tubules in which allosperm is stored (spermatheca e.g. Bayne 1974) and a sperm-digesting organ (bursa copulatrix) in which excess sperm is digested and absorbed (Goméz 2001). Mating is by shell mounting with an active individual mounting the shell of a seemingly passive individual. Penis intromission is always, and sperm transfer mostly, reciprocal (Jordaens et al. 2005; Dillen et al. 2008, 2009a). During copulation, sperm is transferred by means of a pseudospermatophore. A high but variable number of sperm are transferred (up to $>6 \times 10^6$ sperm; Jordaens et al. 2005) which is far more than the amount that can be stored in the spermatheca and used to fertilize the eggs (Dillen et al. 2009a).

Experimental Set-up

Approximately 900 individuals of *S. putris* with a shell height of more than 9 mm were collected from a population in Wilrijk, Belgium, within the breeding period of the species (April–September; e.g. Rieper 1912; Hecker 1965). Individuals with a shell height of more than 9 mm contain sperm in their vesicula seminalis and display normal mating behaviour (L. Dillen, personal observation). Collected individuals were therefore considered to be reproductively mature. Snails were kept at 20 °C and on an 18:6 h light:dark cycle on moist paper, and were fed with lettuce and carrots that were provided ad libitum. After 3 days of isolation,