Aspects of body size and mate choice in the simultaneously hermaphroditic pond snail *Lymnaea stagnalis*

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Abstract—Body size can be a good indicator of the quality of a potential mate in terms of fecundity. In many hermaphrodites, egg production is positively correlated with body size. Especially when donating sperm is costly, a preference for larger partners might be expected. Here we test this prediction for the great pond snail *Lymnaea stagnalis*. As expected, we find a clear effect of body size on egg production, and show that shell height can be used as a reliable predictor of body size. Additionally, behavioural observations reveal that these snails are not physically limited in mating with a much larger or smaller partner. Nonetheless, both in a choice experiment as well as in spontaneous copulations, we find no evidence of mate choice based on body size. These results contribute to a growing field of research which attempts to understand the evolution of the wide variation in the ways that hermaphroditic species respond to the size of potential mating partners.

Keywords: Basommatophora; fecundity; gastropod; hermaphrodite; sexual selection.

INTRODUCTION

Many animal species increase their reproductive fitness by choosing mates of better quality. Phenotypic variation in reproductive traits is fundamental for such choice and one important variable in many vertebrates and invertebrates is body size. Recent theoretical sex allocation models have emphasised the importance of body size for understanding hermaphroditic mating systems (Angeloni et al., 2002; Angeloni, 2003). Body size affects the timing of sex change in sequential hermaphrodites (Ghiselin, 1969), and evidence is accumulating that body size can affect mating strategies in simultaneously hermaphroditic animals (reviewed in

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Angeloni et al., 2002; Jordaens et al., 2007). In simultaneously hermaphroditic plants body size plays an important role. Such size effects occur because larger individuals usually have more resources to invest in reproduction, what is known as the budget effect (Cadet et al., 2004; Klinkhamer et al., 1997). With few exceptions, hermaphroditic plants with size-dependent sex allocation allocate more to their female function when larger (Klinkhamer et al., 1997), which typically results in a gradual shift in sex allocation with increasing size, i.e., growth (Cadet et al., 2004).

For simultaneously hermaphroditic animals body size reflects total resource level (Zonneveld, 1992; Ter Maat et al., in press), and thereby the available budget that can be divided over the male and female role (Schärer and Ladurner, 2003). Reproduction via the female function comprises egg production and allosperm storage (organ size) (Angeloni et al., 2002). There is ample evidence that female reproduction (eggs) increases with body size (DeWitt, 1954; Baur and Raboud, 1988; DeWitt, 1991; Vreys and Michiels, 1995; Peters and Michiels, 1996; Michiels and Bakovski, 2000; Schärer et al., 2001; Angeloni, 2003; Michiels et al., 2003; Gianguzzi et al., 2005). Such variance in mate quality, in this case fecundity, is a requirement for mate choice (Parker, 1983). Sexual selection can then favour a preference to inseminate larger partners because gains via the sperm donor’s male function are higher (DeWitt, 1996). Such evidence for mate choice based on size, i.e., a preference for inseminating a larger partner, has been reported in some species (Otsuka et al., 1980; DeWitt, 1996; Yusa, 1996; Lüscher and Wedekind, 2002; Ohbayashi-Hodoki et al., 2004), but not in others (Baur, 1992; Switzer-Dunlap et al., 1984; Peters and Michiels, 1996; Haase and Karlsson, 2004; Chaine and Angeloni, 2005).

Reasons for such species differences may lie in several constraining factors. For example, animals may mate with partners of the same size – size-assortative mating – because of a physical limitation that prevents a small individual from inseminating a larger one or vice versa (Crozier, 1917; Gianguzzi et al., 2004; but see Jordaens et al., 2005). However, the rejection of smaller partners by larger ones may also result in size-assortative mating patterns (Crozier, 1917; Tomayima, 1996; Yusa, 1996; Angeloni, 2003), and, in the case of conditional reciprocity where the exact matching of sperm quantity is a prerequisite, this can indirectly lead to matching sizes between pairs when sperm production correlates with size (Vreys and Michiels, 1998). Finally, animals may mate randomly with respect to size due to physiological or time constraints, e.g., water loss and predation risk (Baur, 1992). In such cases mate searching costs will be high, which will select against mate choice (Parker, 1983).

Without the above-mentioned constraining factors, donors may become choosy about who they will inseminate, especially when donating sperm is costly and the fecundity of partners varies (Chaine and Angeloni, 2005; Peters and Michiels, 1996). Since one important source of variation in fecundity is body size, a preference for larger partners is to be expected because a larger portion of the sperm will be used for fertilisation of eggs (or stored for later use, Angeloni et al., 2002). However,
Body size and mate choice in *Lymnaea* as highlighted by both Anthes et al. (2006) and Chaine and Angeloni (2005), there is wide variation in the way different species respond to partner size and how insemination takes place (i.e., unilateral, reciprocal, simultaneous, conditional). To summarise their suggestions in order to reach a full understanding of these patterns, additional data on single species are required and should focus on the following: whether i) there is a relation between fecundity and body size (Zonneveld, 1992); ii) there is a size-based gender role or size-dependent role preference (Van Duivenboden and Ter Maat, 1988); iii) and why role alternation takes place (Koene and Ter Maat, 2005); iv) size-assortative mating occurs; v) individuals respond differently to different partner sizes; vi) the male function is costly (De Visser et al., 1994); and vii) the female function is sperm limited (Cain, 1956). A number of these questions have already been addressed for the great pond snail *Lymnaea stagnalis* (see above references), while several others have yet to be investigated. In this study we aim at answering these remaining questions.

*Lymnaea stagnalis* is a simultaneous hermaphrodite that can self- and cross-fertilise (mixed mating system) with a preference for outcrossing and that experiences low inbreeding depression (Cain, 1956; Puurtinen, 2004). Despite a slight developmental protandry (Duncan, 1975), adult snails can mate in the male and female role (Van Duivenboden and Ter Maat, 1988). Within a copulation, one sexual role is performed and role alternation can occur afterwards (Koene and Ter Maat, 2005). These snails seem usually receptive as females (Van Duivenboden and Ter Maat, 1985), can donate and receive sperm relatively frequently (at least once per week, De Boer et al., 1997), and received sperm can be stored and used for approximately 3 months (Cain, 1956). Egg masses are produced at a relatively high rate (more than one mass per week, Ter Maat et al., 1982): combined with the above, this suggests that repeated mating can easily result in multiple paternity of the eggs within one egg mass. *Lymnaea stagnalis* is not always motivated to mate in the male role. Male sexual drive increases when individuals have not mated for several days. This occurs because, during sexual isolation, the size of the prostate gland which produces the seminal fluid increases (De Boer et al., 1997). This increase is detected by an evolutionary-conserved brain area via a small branch of the penial nerve (De Boer et al., 1997; Koene et al., 2000).

Zonneveld’s data (1992) indicate that larger animals produce more eggs. Moreover, the costs of copulating in the male role are high in *Lymnaea stagnalis* (De Visser et al., 1994). Hence this species has reasons to be choosy about the size of the mating partner. To investigate whether animals choose to inseminate larger individuals we made use of knowledge about the male motivational mechanism in this species; eagerness to mate in the male role increases with sexual isolation. Using isolated individuals we set up a choice experiment to investigate whether snails prefer larger partners. We also checked whether sexual roles are physically limited in pairs with extreme size differences, and, because isolation could interact with size preference, we measured spontaneously mating pairs.
MATERIALS AND METHODS

The great pond snail *Lymnaea stagnalis* is a pulmonate gastropod belonging to the Lymnaeidae. This species occurs commonly in European lakes, ponds and ditches, and can be easily collected in the field. Within field populations large differences in size can be detected (J.M. Koene, K. Montagne-Wajer and A. Ter Maat, unpubl. data). *Lymnaea stagnalis* can also be cultured in the laboratory under semi-natural conditions (Van Der Steen et al., 1969). For this study, mature specimens were obtained from our laboratory culture. They were housed individually in perforated polyethylene jars (460 ml) in the same experimental tank with continuous water exchange. The water was kept at 20°C and the light : dark cycle was 12 h : 12 h. Each snail was provided daily with one circular disk of lettuce with a surface area of 19.6 cm², which was completely consumed. All snail sizes were determined by measuring the shell height with callipers. Sizes of snails in spontaneous copulations were obtained by collecting and measuring copulating pairs, at a single time point, from each of the five culturing tanks that contained adults (N = 11 pairs per tank).

To observe egg production, 62 snails of four different size classes were used. Two groups of 16 snails with sizes of exactly 23 and 29 mm, respectively, and two groups of 15 snails with sizes of exactly 26 and 32 mm, respectively, were housed individually in perforated polyethylene boxes (460 ml) and kept as described above. During the following week we measured egg production by counting the number of eggs in each egg mass that was produced. For data analysis, we averaged the number of eggs per egg mass for individuals that produced more than one mass. In the largest size class (32 mm) two animals died during the course of the experiment. The remaining 60 animals laid a total of 83 egg masses; individual egg laying ranged between zero and three masses, and the average number of egg masses was 1.37 (± 0.64) per animal. The total body weight of the individuals was determined at the end of this 1-week period.

To test whether partners that differ greatly in size are able to copulate, we used 45 large (30.9 ± 0.8 mm) and 45 small (24.6 ± 0.8 mm) individuals that had been isolated for 1 week. We created three different kinds of pairs: two large individuals (L × L), a large and a small individual (L × S) and two small individuals (S × S). Because four large individuals died and one small individual escaped from its jar during the isolation period, we could form 12 L × L, 14 L × S, and 14 S × S pairs. We observed all the pairs for 7 h.

To test whether focal individuals have a preference for a large, medium (same size) or small partner we set up a choice experiment. We marked a different set of 30 focal animals with nail polish (27.2 ± 0.6 mm) and isolated them for 7 days before the start of the experiment. Their potential mates were kept in groups. Each group of either large (30.9 ± 0.7 mm), medium (26.9 ± 0.7 mm), or small (23.4 ± 0.7 mm) snails was divided into two perforated polyethylene boxes (5600 ml each). Thus, for each size class we had two boxes with 15 individuals each. Quartets were formed that consisted of the isolated (focal) individual and one grouped individual of each size class. Because one large individual died, we were able to form 29 quartets.
These quartets were observed for 4 h which allowed enough time for nearly all focal individuals to mate with one of the available partners.

RESULTS

Egg laying

The different size classes and the actual size at the end of the egg laying observations were very tightly correlated ($r = 0.982$, $N = 60$, $p < 0.0001$). The shell height increased on average by 0.42 mm (with upper and lower 95% confidence intervals of 0.55 and 0.28 mm, respectively). For clarity, we use the different size classes (not the actual sizes) for further egg laying analysis. The body weights of the animals from the different size classes were also clearly different from each other (ANOVA: $F_{3,56} = 199.187$, $p = 0.0001$; Post-hoc Student’s t, $p < 0.05$ for all comparisons; fig. 1A). The number of eggs produced per egg mass differed between the different size classes (ANOVA: $F_{3,52} = 9.529$, $p = 0.0001$). The post-hoc test (Student’s t) results are shown in figure 1B, where significant differences are indicated with different letters ($p < 0.05$). Individuals that laid no eggs were excluded from the latter comparison.

Spontaneous matings

Sizes of spontaneously copulating individuals ranged between 18 and 31 mm, and the smallest and largest animals were capable of copulating in the male and female role. Because the mean size in the tanks differed, we summarised the data by calculating the standard deviations from the mean (see Angeloni and Bradbury, 1999; Angeloni et al., 2003). We found no correlation between the size of the sperm donor and the sperm recipient ($r = -0.027$, $N = 55$, $p = 0.842$; fig. 2).

Copulation in pairs

32 (80%) of the 40 pairs copulated. Within the different types of mating pairs there was no significant difference in the likelihood of mating (Pearson $\chi^2 = 3.690$, $p = 0.158$; fig. 3). Because one $L \times S$ pair had not finished copulating at the end of the observation period, that pair’s value for insemination duration is missing. For the pairs with unequal sized snails, we made a distinction between large and small individuals performing the male role ($L \times S$ and $S \times L$, respectively; fig. 4). Comparison of insemination duration in the different pair types reveals a clear overall difference (ANOVA: $F_{3,27} = 6.262$, $p = 0.0023$). Post-hoc testing indicates that the $L \times L$ pairs and the $L \times S$ pairs inseminated longer that $S \times L$ and $S \times S$ pairs (Student’s t: $p < 0.05$; fig. 4). Hence, in the pairs in which the sperm donor was large insemination lasted longer.

Of the 32 pairs that copulated, 12 (37.5%) swapped roles after the first mating (fig. 3). Role alternations occurred at similar frequencies in the different pair types
(Pearson $\chi^2 = 2.855, p = 0.24$) and within all role alternating pairs there was no correlation between the first insemination duration and the second one ($r = 0.293, N = 12, p = 0.354$).

**Copulation in quartets**

In 24 (82.7%) of the 29 quartets copulation took place; in all cases it was the isolated animal that performed the male role. Isolated animals could choose to mate with a
partner that was larger, the same size (medium) or smaller than themselves. The isolated individuals took on average 48.4 min (upper CI = 66.5; lower CI = 30.4) before they started courting the partner they would eventually inseminate. During this initial time period they actively locomoted through their box and had brief encounters with all three potential partners. Only four circling events were observed in such encounters that did not lead to insemination. Thus, the isolated focals did not simply inseminate whoever they encountered first: nonetheless, they did not choose significantly more often one specific partner size (Pearson $\chi^2 = 3.25, p = 0.1969$). Insemination durations by the focal individual did not depend on the size of the partner (ANOVA: $F_{2,21} = 0.258, p = 0.7747$; post-hoc Student’s t: $p > 0.05$; fig. 5).

**DISCUSSION**

Within simultaneous hermaphrodites, different species vary widely in their response to the size of potential mating partners (Chaine and Angeloni, 2005; Anthes et al., 2006). As outlined in the introduction, for many species there is a lack of the information needed for a better understanding of the evolution of these patterns.
Figure 3. Contingency table showing the insemination pattern in the pairs. For each of the three different pair combinations of large (L) and small (S) animals the relative occurrences of no copulation (none), unilateral insemination and role alternation are shown. Hence, insemination is divided in unilateral and reciprocal (role alternation) events.

Figure 4. Insemination durations in pairs. Insemination durations (min) in the different pair types are shown. For the pairs with unequally sized snails, the graph shows insemination durations for large and small individuals performing the male role (L × S and S × L, respectively). The graph only comprises durations of first inseminations; those of second inseminations after role alternation are not included. The box plots indicate median, 25th and 75th quartile, and range. Significant differences are indicated with different letters.
Body size and mate choice in *Lymnaea stagnalis* to address some of the unresolved issues. We find a clear positive relationship between body size and fecundity (egg production). Our data also show that there is no physical limitation in copulations between partners with extreme size differences. Finally, we find no evidence for either size-assortative mating or a preference for insemination of a larger partner.

Our data on egg laying essentially confirm what was already indicated by a previous study (Zonneveld, 1992). The number of eggs per egg mass increases with the size of the animal. We used shell height as a measure of body size after having verified that this is indeed a very reliable measure to use in this species to predict body weight (see also Zonneveld and Kooijman, 1989). This tight relationship is essential for the plausibility of mate choice based on size. There are examples of size-assessment behaviours used by hermaphrodites, for example in flatworms (Vreys and Michiels, 1997) but not all species are necessarily able to assess reliably the mating partner’s size during courtship (Angeloni and Bradbury, 1999). What *Lymnaea stagnalis* can sense during courtship is the size of the partner’s shell. Assessment of the partner’s shell size could take place during the circling and positioning phases which occur after the male-acting animal has mounted the partner’s shell. The sperm donor crawls to the tip of the shell of the sperm recipient along the windings of the shell (circling) and then crawls downwards to reach the position for insemination (positioning). Evidently, the covered distance during these phases will be proportional to the size of the recipient. Such distance information could be used to assess the partner’s size and thereby, indirectly, its fecundity.

**Figure 5.** Insemination durations of the male acting individuals in the choice experiment (quartets). The graph shows insemination durations (min) in the three different choice options. The box plots indicate median, 25th and 75th quartile, and range. There are no significant differences.
Large differences in size may also physically limit mating, but we found no evidence that mating between partners of different size is restricted. Both large and small partners were perfectly able to mate in both sexual roles with a differently sized partner since we found no differences in the likelihood of insemination or role alternation between the different pair types. The average size difference of 6.8 mm lies within the range found in the field; size differences between the largest and smallest unparasitised adults found in the field range between 4 and 22 mm (J.M. Koene, K. Montagne-Wajer and A. Ter Maat, data unpubl.); parasitised individuals are often castrated (De Jong-Brink et al., 1999). Our observations of spontaneous matings show that animals of 18 mm are already able to mate as male as well as female. For this study we chose not to use animals smaller than 23 mm because their female function is not always fully mature (Koene and Ter Maat, 2004). In addition, we did not use animals larger than 32 mm because beyond that size survival goes down and egg laying levels off and subsequently decreases (Janse et al., 1989). As a result of these considerations, we did not look at the most extreme size differences. Nevertheless, our findings suggest that with their large and agile penis-carrying organ (preputium) these animals are able to inseminate smaller as well as larger partners, most likely also beyond the size differences used in this study.

The above shows that *Lymnaea stagnalis* experiences no physical limitation to inseminating much larger partners and that the latter are more fecund. Sexual selection theory predicts that a preference for larger, more fecund partners should be of particular importance when mating costs are high. De Visser et al. (1994) clearly showed that the male role is as expensive as the female role in *Lymnaea stagnalis*. Moreover, these animals can store, as well as digest, sperm. They can mate frequently and when sperm exchange happens this is unconditional (Koene and Ter Maat, 2005). Taken together, these conditions will select for sperm donors that are choosy about which partner they inseminate (Peters and Michiels, 1996). DeWitt (1996) showed that in *Physa*, another freshwater snail, the smaller individual of a pair usually donates sperm.

Despite all conditions seemingly being met for a strong preference to inseminate a larger partner, we did not find any evidence for it in our choice experiment with *Lymnaea stagnalis*. In the choice experiments we formed quartets in which only the focal animal was motivated to mate as a male due to a preceding sexual isolation period. The three potential mates had three different sizes and were not motivated to become male. Due to this set-up it is possible that we eliminated choosiness if an isolated animal simply picks the first individual it encounters to inseminate. However, individuals did have brief encounters with the three potential mates before initiating the courtship that led to insemination. During such encounters very few circling events were observed, indicating that the decision to mate with the encountered individual is not made during circling and/or positioning (when size assessment can take place) but at an earlier stage of the encounter. Moreover, if our experimental set-up did create overly eager males, we should still have detected a correlation in the spontaneous matings in the breeding tanks, but we found no
correlation whatsoever. These findings lead us to conclude that mating is random with respect to body size in this species. The lack of mate choice based on size suggests that there may be other constraints, such as physiology or time (Baur, 1992), that prevent choosiness based on body size.

We did find one effect of size on mating behaviour. Insemination duration was significantly longer when the sperm donor was large, i.e., both in L × L pairs and L × S pairs. It is, as yet, unclear whether larger animals simply take more time for sperm transfer, or whether they actually transfer more sperm to the recipient. The fact that we find the effect with large, as well as small, sperm recipients indicates that this is a property of the large sperm donor. Hence, the time invested in insemination per mating in Lymnaea stagnalis does not depend on partner size, as is the case for large individuals of Dugesia polychroa (Peters and Michiels, 1996). Angeloni (2003) reported that in the hypodermically impregnating sea slug Alderia modesta it is the smaller individuals that inseminate their larger mates for longer. In none of the abovementioned species has it been resolved what the insemination duration actually means in terms of sperm and/or seminal fluid transfer, and, therefore, this requires further investigation.

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