Relative size influences gender role in the freshwater hermaphroditic snail, *Helisoma trivolvis*

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Simultaneous hermaphrodites have the unique challenge of allocating their available resources to egg and sperm production and behaviorally to a male and/or female mating role. Models that address the influence of body size on sex allocation predict that larger individuals should allocate proportionally more resources to female than male function and that this should translate into corresponding behavioral preferences during mating. We investigated the relationship between size and gender role in the hermaphroditic freshwater snail *Helisoma trivolvis*. We hypothesized that when 2 *H. trivolvis* mate, the larger would assume the female role and the smaller the male role. We also predicted that reciprocal mating would be more likely when partners were similar in size. We measured 180 snails, paired them, and observed their sex roles during copulation. The size difference between snails neither influenced the latency to copulation nor predicted whether mating was unilateral or reciprocal. In unilateral matings, the smaller snail acted as the male significantly more often than the larger snail. In order to test the hypothesis that increased activity of smaller snails influences gender role, we also measured movement rates in snails of various sizes but found no relationship between size and activity. These experiments indicate that in *H. trivolvis* body size does influence gender role in unilateral matings, and enable us to rule out activity as a direct determinant of male gender role. Whether snails mate reciprocally or not may depend on other factors such as previous mating history, time of isolation, or age. Key words: freshwater snails, gender role, *Helisoma trivolvis*, sex allocation, simultaneous hermaphrodite. [Behav Ecol]
levels of the hermaphroditic flatworm *Macrostomum lignano*, Vizoso and Scharer (2007) determined that well-fed worms allocated proportionately more resources to female but not male function.

Sex allocation has also been addressed less directly by measuring time spent mating in the male or female role during copulation. Large size has been associated behaviorally with a predominance of matings in the female role in the sea slugs, *Aplysia punctata* (Otsuka et al. 1980), *Aplysia kurodai* (Yusa 1996), and *Aplysia vaccaria* (Angeloni and Bradbury 1999). In the sea slug *Chelidonura sandrana*, individuals modify their male behavior dependent on the body size and previous experience of their partner (Anthes et al. 2006a). Individuals that mated with a larger partner spent longer times ejaculating than those with a smaller partner and copulated for a shorter time with a partner that had recently mated.

Pulmonate snails have model organisms for studies of reproductive allocation, gender roles, and gender conflict (Wethington and Dillon 1993, Dillon and Wethington 1994, Jordaens et al. 2007), and the size advantage model is generally supported by empirical data. In Physa species, when there is a size difference between potential mates, the smaller individual usually acts as male and the larger as the female (DeWitt 1994; DeWitt 1996; Ohbayashi-Hodoki et al. 2004). During the shell positioning phase of mating behavior in *Physa*, the potential sperm recipient—the “female”—may exhibit agonistic behaviors such as shell swinging, jerking, and genital biting, which usually interrupt copulation (DeWitt 1991). In field observations of *Physa*, mating occurred with no female rejective behaviors when the smaller snail played the role of male (DeWitt 1996). However, when a smaller snail was approached by a larger snail acting as male, rejective behavior was frequently observed. Wethington and Dillon (1996) hypothesize that these rejective behaviors are due to gender conflict—when snails attempt to assume the same role during copulation, in this case, the male role.

The above observations imply that these organisms have some way of assessing relative size. Although no mechanism has yet been identified, several hermaphroditic species have prolonged courtship behaviors that involve contact between the 2 individuals and may give tactile cues of size (e.g., the flatworm *Dugesia gonocephala*, Vreys and Michiels 1997; or the sea slug *Alderia modesta*, Angeloni 2003). Pulmonate snails crawl over one another before mating (Abdel-Malek 1952; Geraets and Joosse 1984; van Duivenboden and ter Maat 1988; DeWitt 1991; Trigwell et al. 1997) and may use this time of contact to assess relative size. It has also been suggested that because the snail eventually taking the male role is typically the more active of the 2 (van Duivenboden and ter Maat 1988; Trigwell et al. 1997), the speedier snail (assumed to be the smaller) ultimately becomes the male (Wethington and Dillon 1996).

However, not all evidence supports a link between body size and mating roles. In the opisthobranch *Bulla gouldiana*, gender roles were random with respect to relative size both in field samples and in laboratory pairings, and across samples smaller individuals were more likely to act as sperm recipients—contrary to the predictions of size-based sex allocation models (Chaine and Angeloni 2005). Similarly in *Ostrea olivaera*, another opisthobranch, when snails differed in size, larger animals initiated mating in the male role more often than did small animals, although frequent role switching occurred after the first copulation event (Gianguzza et al. 2004). In the land snail *Arianta arbustorum* (Baur 1992) and the pond snail *Lymnaea stagnalis* (Koene et al. 2007), matings were random with respect to body size both in spontaneous matings in large populations and when snails were placed in groups of 3 (one small, one medium, and one large). More empirical study is clearly needed to sort out the factors that may influence gender role and mating mode in hermaphrodites.

Here, we address the influence of body size on gender roles in *Helisoma trivolvis*, a freshwater pulmonate snail found mainly in North America in nutrient-rich eutrophic environments. *Helisoma trivolvis* are simultaneous hermaphrodites and are capable of reproducing by either outcrossing or less often by self-fertilization (Parapenae and Correa 1988). Mating has been described in this species by Abdel-Malek (1952): when 2 snails encounter one another, they crawl over each other’s shell until the foot of one (the “male” or potential sperm donor) adheres to the shell of the other (the “female” or potential sperm recipient). The preputium begins to evert—coming from the male opening on the right side of the neck; the organ moves along the body of the partner and eventually appears to attach to the right side of its body near the female opening or gonopore. Once the gonopore is located, intromission can occur, and sperm is transferred. This process can occur unilaterally, as described above, or reciprocally where each snail acts simultaneously as both male and female.

Anecdotal evidence suggests that body size affects gender roles in *Helisoma* as well. The positive relationship between body size and egg production has been recently demonstrated in *H. trivolvis* (Norton and Bronson 2006), and during mating, smaller *Helisoma* typically act as males (Murphy D, personal communication), but no quantitative data have been collected to date. We hypothesized that when 2 *H. trivolvis* of different size mate, the larger of the 2 would assume the female role and the smaller would adopt the role of male. We also predicted that reciprocal matings would be more likely when potential partners were similar in size than when size differences were greater and that latency to copulation would be greater in this case because copulation may be delayed by the necessity to negotiate mating roles prior to copulation (i.e., gender conflict). To test these claims, we paired snails of different sizes and observed their roles during copulation. To test the hypothesis that increased activity of smaller snails influences gender role, we measured rates of movement of snails of various sizes, predicting that movement rate would be inversely related to body size.

**METHODS**

**Body size and gender role**

Our study organisms were sampled from a laboratory population of approximately 100–200 *H. trivolvis* maintained in a 20-gallon aquarium under ambient temperature and light conditions and allowed to mate freely. This population originated from 30 snails sampled from another laboratory population (courtesy of A. Houde) and has been maintained in the laboratory since 2002. We isolated snails for each trial by placing them in individual plastic cups and fed them boiled romaine lettuce, replenishing the supply every 3–4 days when we changed water in the cups. We placed all snails in reversed light and dark conditions (14:10 light:dark with lights off at 01:00 PM), so that observations could be conveniently made in the dark—these snails are reproductively active at night. The snails remained isolated for at least 3 weeks (range = 21–37 days) before mating observations were made. We measured the shell diameter of each snail within 1–3 days of each mating experiment. Shell diameter (from shell opening across the apex) is highly repeatable (intraclass correlation for repeated measures on the same snail: $r = 0.993$, $n = 27$, $P < 0.001$) and highly correlated with body weight ($r = 0.947$, $n = 60$, $P < 0.001$), so all size measurements are reported here as diameter.

To assess mating role, we placed pairs of snails in 89.4-mm Petri dishes filled with conditioned tap water and observed...
them for copulation for 2–3 h during the dark phase (approximately 5 h after lights out). For identification, we marked one snail in each pair with a dot of nail polish. We observed pairs every 15 min using lights mounted on goggles and covered with translucent red plastic filters (Rosolux, no. 26 red, maximum transmission at 660 nm) in order to see the snails in the dark without disturbing them. These snails show little sensitivity to red light as measured by electoretinogram and optic nerve recordings (Patton and Kater 1972). We measured latency to copulation for each pair; copulation was determined to begin when the snail in the male role placed its preputium in the gonopore of the snail in the female role. When a pair of snails appeared to be in position, we moved them to a dissecting microscope for closer observation in order to confirm that copulation was occurring and to determine the gender role of each snail. Copulations were categorized as “small male” when the smaller individual acted as male (as evidenced by having its penis inserted into the partner’s gonopore), “large male” when the larger individual acted as male, or reciprocal (when penises of both individuals were simultaneously inserted into the partner’s gonopore).

For our first observations (Run 1), 60 snails were organized into large and small categories by placing them in order of shell diameter. Snails in the upper half of the size range were considered large, and those in the lower half were considered small. Pairings were made by placing the largest large snail with the largest small snail and so on, with the intention of creating pairs with a fairly consistent 2-mm size difference between individuals. Those snails that did not mate during the first 3-h period were returned to their cups, then measured, and paired again 10 days later. In this experiment, 17 pairs of snails mated, 12 did not. The entire experiment was replicated with new snails several weeks later (Run 2; n = 60); after the first observation period, unmated snails were isolated for an additional 7 days, placed in pairs again and measured, and finally the remaining unmated snails were tested 1 week later. In this experimental run, 10 pairs mated and 19 did not. One snail died before mating observations in each of runs 1 and 2, so the total number of pairs tested in each was 29.

For our final set of observations (Run 3), we organized another 60 snails into large and small categories using the method described above. This time, pairs were made by placing the largest large snail with the smallest small snail and moving inward so that the smallest large and the largest small snails were paired, with the intention of creating pairs with a variety of differences in size (ranging from less than 1 to 8 mm). We then observed pairs for copulation as described above. During the first observation period, 22 pairs of snails mated, so no snails were retested. As we calculated size differences in the first 2 runs, we realized that because of the rapid growth rate of the snails, and prolonged duration of the first experiments, the size differences of mated pairs in the first 2 runs were much more varied than we had intended and not significantly different than those in Run 3 (analysis of variance [ANOVA]; Fsex = 0.77, P = 0.467). A chi-square test for association (non-independence) to test whether the distribution of mating types (small male, large male, or reciprocal mating) varied among runs was nonsignificant (χ² = 8.99, degrees of freedom [df] = 4, P = 0.062), indicating no substantial effect of run on this outcome. We therefore combined all 3 runs for purposes of data analysis.

To test whether relative body size influenced gender role in unilateral matings, we performed a goodness-of-fit chi-square test, testing the null hypothesis that there would be equal numbers of matings in which the male snail was smaller or larger than its mate. To test whether size differences among snails influenced the type of mating (reciprocal vs. unilateral), we performed a binary logistic regression using absolute size difference as the predictor and the 2 mating types as the binary response. We predicted that unilateral mating would be more likely as size difference increased. Finally, to test whether latency to copulation was related to the size difference between partners, we performed a regression analysis of latency on size difference. We predicted that latency to copulation would decrease as size difference between partners increased.

### Rates of snail movement

To assess whether small snails might move more rapidly than large snails and thus be the first to encounter a potential mate and take the male role, we measured rates of movement in 32 snails with diameters ranging from 7.5 to 17.9 mm (x̄ = 11.54 ± 0.54 mm) by placing individual snails in the middle of a large Petri dish (150 × 15 mm) filled with water and placed on a 2 × 2 cm grid. We measured the latency to move (the time it took for each snail to cross the first gridline), as well as the number of lines crossed in three 5-min intervals.

Because snails may not have been motivated to move in these experiments, we repeated the experiments with a different group of snails, this time isolating 36 individuals (with shell diameters ranging from 5.9 to 19.3 mm, x̄ = 12.59 ± 0.55 mm) for 4 days without food before testing. Snails were placed in a square near the edge of the Petri dish, and a small piece of lettuce was placed at the opposite edge of the dish (if snails moved directly toward the lettuce, they would cross 7 gridlines to reach it). We then measured latency to move; the number of lines crossed at 5, 10, and 15 min; and time to contact with the lettuce.

Because the snail body sizes were not normally distributed (because they were deliberately chosen for a wide size range), we tested for differences between the 2 experiments with a non-parametric Kruskal–Wallis (K–W) test. There were no differences between these experiments in snail size (Kruskal–Wallis: H1,66 = 1.57, P = 0.210), the average latency to move (K–W: H1,66 = 0.59, P = 0.447), the number of lines crossed in any of the 5-min time periods (first 5 min, K–W: H1,66 = 0.86, P = 0.353; second 5 min, K–W: H1,66 = 0.39, P = 0.553; third 5 min, K–W: H1,66 = 0.02, P = 0.890), or total movement (K–W: H1,66 = 0.05, P = 0.829). We therefore combined data from the 2 experiments and used regression analysis to determine the relationship between size and movement.

### RESULTS

#### Body size and gender role

In 88 pairs of snails tested, we observed 49 matings and 39 instances where mating had not occurred within 2–3 h of pairing. There was no difference between mated and unmated pairs in the size difference between individuals (ANOVA: F1,86 = 0.48, P = 0.49). For those pairs that mated, the average size of small snails was 11.11 ± 0.18 mm and that of large snails was 13.36 ± 0.18 mm; the average size difference between individuals was 2.25 ± 0.20 mm with a range of 0.07–5.51 mm. Figure 1 shows the relationship between the size of the male (sperm donor) and female (sperm recipient) partners in unilateral matings. When mating was unilateral, situations in which the smaller snail acted as male (n = 28) occurred significantly more frequently than those in which the larger snail assumed the male role (n = 8) (χ² = 11.11, df = 1, P < 0.001).

Table 1 shows the distribution of copulation types in the 3 replicate experiments. In 13 of 49 cases (27%), mating was reciprocal. There was no relationship between absolute size difference and whether mating was reciprocal or unilateral (binary logistic regression; n = 49, P = 0.07, Figure 2). In fact, in the 13 reciprocal matings we observed, the average...
size difference between individuals was 2.88 mm, about 1 mm larger than the average size difference between unilaterally mating snails (\(\bar{X} = 2.03 \pm 0.22\) mm). Finally, there was no relationship between size difference and latency to copulation (Figure 3, \(R^2 = 0.03, P = 0.230\)). The latency to copulation was also similar among mating types (ANOVA: \(F_{2,46} = 0.79, P = 0.460\)).

**Rates of snail movement**

In the first experiment, the average time for a snail to begin moving and cross over a gridline was 131.5 \(\pm 40\) s, or just more than 2 min, and snails crossed an average of 12.4 \(\pm 1.2\) gridlines in 15 min. In the second experiment, when snails had been isolated and unfed prior to testing, the latency to move was 140.9 \(\pm 31.6\) s, and they crossed an average of 12.36 \(\pm 1.2\) gridlines. Fifteen of the 36 snails reached the lettuce in this time, and 7 of them began to feed. There was no relationship between size and latency to move (\(R^2 = 0.02, P = 0.194\)) or size and rate of movement as measured by the number of gridlines crossed in 15 min (\(R^2 = 0.02, P = 0.247\)). We did find that the number of grids crossed in the first 5 min was highly predictive of overall rate of movement (\(R^2 = 0.70, P < 0.001\)).

**DISCUSSION**

**Body size and gender role**

These data support our hypothesis that when 2 *H. trivolvis* of different size mate, the smaller snail would act preferentially as male and the larger would act as female. Our results are similar to those found in *Physa* species by DeWitt (1991, 1996) and Ohbayashi-Hodoki et al. (2004) and to those in a variety of other hermaphroditic species (Yusa 1996; Angeloni and Bradbury 1999; Scharer et al. 2001; Anthes et al. 2006a). This pattern is consistent with a size advantage model of sex allocation. Angeloni et al. (2002) predict a fitness advantage for both members of a mating pair when the smaller snail acts as male and the larger as female because fitness gains are expected to favor the male reproductive role at small sizes and the female role at larger sizes.

Why then do some pairs behave in ways that are unexpected? In 8 of the 49 matings, the larger snail acted as the male and the smaller as female. In these cases, factors other than body size may have influenced gender role. In both *Lymnaea* and *Physa* (van Duivenboden and ter Maat 1985; Wethington and Dillon 1996), isolation increases the likelihood that an individual

### Table 1

<table>
<thead>
<tr>
<th>Run</th>
<th>Small male</th>
<th>Large male</th>
<th>Reciprocal</th>
<th>Total mated</th>
<th>Total unmated</th>
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<td>13</td>
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<td>Total</td>
<td>28</td>
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<td>13</td>
<td>49</td>
<td>39</td>
<td>88</td>
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will behave as a male, presumably because of a buildup of sperm (Wethington and Dillon 1996). In *Lymnaea*, when individuals were isolated for 2 or 8 days and then paired, in all cases, the snails isolated for the longer time period acted as males during copulation (van Duivenboden and ter Maat 1985). Previous experience may also influence the gender role. In *Physa*, previously unmated snails were more likely to assume the male role than were snails that had mated recently (Wethington and Dillon 1996). In *Lymnaea*, if a snail acts as a male, then it is more likely to take a female role the next time it encounters a potential mate, but if it acts as a female, then its future role cannot be predicted (van Duivenboden and ter Maat 1985). Because snails in our experiments were isolated for at least 3 weeks before pairing, the effects of previous experience are expected to be less important, although sperm can be stored for more than 6 weeks in this species (Norton, unpublished observations).

When snails are of similar size, it is predicted that both would be willing to donate sperm, so reciprocal mating should be common (Angeloni et al. 2002). Gianguzza et al. (2004) observed both unilateral and reciprocal matings in the sea slug *Oxyyna olivacea* in pairs of similar size, but as predicted, they found more unilateral copulations when snails of different sizes were paired. We had also expected that reciprocal mating would be more likely to occur between snails of similar size, but this prediction was not borne out in our observations: there was no effect of average size discrepancy on mating type. In fact, the average difference in size between partners was slightly (but not significantly) greater in reciprocally mating pairs than in unilateral matings (Figure 2). Several factors may have led to reciprocity in some pairs with larger size differences. First of all, the snails in this experiment were isolated for almost 4 weeks, so both partners may have had depleted stores of allosperm (from another individual) and a buildup of autosperm (their own) and so were therefore willing to mate as both male and female (Wethington and Dillon 1996). Second, because the experiments were originally designed to create pairs with widely varying differences in size, in some pairs the size differences were quite large, as much as 5 mm. In fact, in 3 of the pairs that mated reciprocally, the large individual was more than 4 mm larger in diameter than the small individual, and the large snails in these cases were between 14 and 15 mm in size. Because it is unlikely that these snails will find even larger mates, they may be more likely to act as males as well as females (Ohbayashi-Hodoki et al. 2004). Alternatively, when the size difference between snails was so large, it was often difficult to tell if the snails were copulating, so often they were left for longer times before we removed them for closer observation, which may have allowed for reciprocity after an initial 1-way mating. This effect of experimental procedure may also have reduced the proportion of reciprocal matings between equally sized snails.

Angeloni (2003) also found that reciprocal mating in the sea slug *A. modesta* depended neither on the size difference between partners nor on the size of the larger individual. However, reciprocal mating was more likely with increasing absolute size of the smaller partner. She also found that in reciprocally mating pairs, the larger individual was inseminated for a longer time than the smaller partner. We saw no relationship between the size of the smaller or larger partner on mating type but were not able to determine the amount of sperm transferred by either partner. We did not quantify egg production in these snails after mating, but such a measure over time may give some insight into amounts of sperm transferred.

Finally we saw no relationship between the absolute size difference of members of a pair and the latency to copulation. Mating conflict (in this case, when 2 individuals of similar size prefer the same gender role) could potentially result in delaying copulation as potential partners sort out their roles. In our experiments, there was no evidence that mating conflict occurred (we saw no behavioral indications as reported by DeWitt 1991), and the latency to copulation did not differ among mating types. As Michiels (1998) points out, it is not easy to determine whether reciprocal copulations are the result of mutual willingness to mate in both gender roles or a way to settle gender conflict.

**Rates of snail movement**

The male role during courtship and mating is often described as and assumed to be more active than the female role (van Duivenboden and ter Maat 1988; DeWitt 1991; Wethington and Dillon 1996; Trigwell et al. 1997). Small snails may take on the male role because they move more quickly than large snails and thus are the first to encounter a new partner and move around it (Wethington AR, personal communication). We tested this hypothesis by measuring movement rates in snails of various sizes. The fact that there is no relationship between size and movement rate forces us to reject this hypothesis as a possible influence on gender role. The finding that initial movement rate (the first 5 min of testing) is highly predictive of movement over a longer time period (15 min) may be useful for other studies on movement.

Because rate of movement is not size related, and cannot explain how snails determine their size relative to another individual, we hypothesize that snails may assess relative size by moving over and touching their potential partners. This mechanism has been proposed to explain how several hermaphroditic invertebrates assess a potential partner (Vries and Michiels 1997; Angeloni 2003) and seems reasonable in this case because the snails spend most of their time together before mating crawling over and touching one another’s shells.

**CONCLUSION**

In conclusion, these experiments indicate that in *H. triozokis* body size does influence gender role in unilateral matings and enable us to rule out activity as a direct determinant of male gender role. However, contrary to the expectations of sex allocation theory, the type of mating (reciprocal or unilateral) was not related to body size differences. More flexible models may be needed to address this issue because whether snails mate reciprocally or not may depend on factors other than body size such as previous mating history, time of isolation, and perhaps even age. The mechanism of size assessment is unclear and needs more empirical study.

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