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Sexual selection in a simultaneous hermaphrodite with hypodermic insemination: body size, allocation to sexual roles and paternity

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Theory predicts that variation in body size within a population of simultaneous hermaphrodites should affect sex allocation, leading to individual differences in mating strategies and increased investment in female function with size. Small animals with fewer resources should invest proportionally more of their resources in male function than large animals, resulting in sperm displacement and paternity patterns that are independent of body size. This study investigated the effect of body size on mating patterns, egg production (an indirect measure of sperm transfer) and paternity in *Alderia modesta*, a sperm-storing, hermaphroditic sea slug with hypodermic insemination. The relative sizes of two hermaphrodites affected the probability and duration of inseminations; smaller animals inseminated larger mates for longer than vice versa. Sperm transfer began at a smaller size and age than egg production, and estimates of both sperm transfer and egg production increased with body size. Paternity patterns varied widely; in this species, unpredictable sperm precedence patterns may be a consequence of hypodermic insemination and the lack of a well-defined sperm storage organ. Hypodermic injections across all sections of the body successfully transferred sperm and fertilized eggs. The function and consequences of hypodermic insemination are discussed.

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Although most investigations into the consequences of sexual selection have focused on dioecious animals, it may also be an important force shaping hermaphroditic traits (Charnov 1979; Arnold 1994). Sexual selection on traits related to mate attraction may be weaker in hermaphrodites (Greeff & Michiels 1999a), but because many simultaneous hermaphrodites mate multiply and store sperm, they are affected by forces similar to those leading to complicated mating strategies and sperm competition in animals with separate sexes (Charnov 1996; Michiels 1998). Unlike dioecious species, hermaphrodites have an additional mating strategy available to them in the face of sexual selection; they can adjust the ratio of resources invested in mating in the male role versus the female role, depending on current selection pressures and environmental conditions such as mating group size (Charnov 1982; Raimondi & Martin 1991; Trouvé et al. 1999). Sex allocation, or the trade-off between male and female investment, complicates sexually selected

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strategies, because any increased investment in one sexual role results in a decreased investment in the other.

There is a well-developed theoretical foundation for the effects of body size on mating strategies in dioecious species (Andersson 1994), hermaphroditic plants (Klinkhamer et al. 1997), sequentially hermaphroditic animals (Ghiselin 1969; Charnov 1982) and externally fertilizing simultaneous hermaphrodites (Petersen & Fischer 1996; St Mary 1997). Mating strategies of sperm-storing simultaneous hermaphrodites should also depend on body size, because an individual's size relative to that of a mate can have consequences for its sperm competition abilities, particularly when body size affects the resources (energy stores) available for gametes and the size of internal sperm storage organs (Charnov 1996). The presence of a storage organ that holds a limited amount of sperm can result in diminishing returns to increasing investment in sperm transfer (Charnov 1996; Parker 1998). A sperm donor with large body size and many resources may invest very little of its resources in sperm, and still be able to flush out the sperm of prior donors within the receptacle of a small recipient.

Recently, my colleagues and I developed a model to provide direct mathematical predictions for mating strategies in a population of promiscuous sperm-storing simultaneous hermaphrodites. This study expanded upon previous sex allocation models that did not allow for intrapopulation variation in strategies (Leonard 1990; Charnov 1996; Greeff & Michiels 1999b; Pen & Weissing 1999), by allowing for two body size classes (small and large), where size affects the resources available for each mating event but does not affect mating opportunities (Angeloni et al. 2002). In this theoretical population, individuals repeatedly mate and lay egg clutches whose paternity is determined by the relative composition of stored sperm. With diminishing returns to sperm transfer and under many population conditions, our model predicted that large animals should invest a reduced proportion of resources in sperm, and thus more in eggs, than should small animals (Angeloni et al. 2002). If investment can be flexibly adjusted depending on the current mate, more sperm should be transferred (and fewer eggs produced) when mating with a large animal than when mating with a small animal. Although small animals have reduced absolute resources, by investing a greater proportion of resources in sperm, they should achieve levels of sperm transfer and displacement similar to large animals. Thus, body size is not predicted to have a pronounced effect on the proportion of paternity gained in the eggs produced by a mate, similar to predictions for animals with separate sexes (Parker & Simmons 1994). Furthermore, high efficiency of displacing sperm in the population, or low cost of filling a mate's storage organ, should result in high last-mate paternity values and more energy invested in eggs for all sizes (Angeloni et al. 2002). Digestion of allosperm (sperm received from a mate) occurs in some hermaphrodites and may additionally complicate sex allocation by increasing investment in sperm (Greeff & Michiels 1999b; Greeff & Parker 2000), and random variation in paternity success should lead to increased investment in eggs (Greeff et al. 2001). To compare actual strategies with those predicted by these mathematical models, it is essential to measure mating strategies, sperm competition and paternity patterns in real hermaphrodites (Petersen 1991; Charnov 1996; Pen & Weissing 1999; Greeff et al. 2001).

By affecting sex allocation strategies, body size can have widespread consequences for a variety of behavioural traits: relative egg production and sperm transfer, mate choice and the time devoted to mating in each sexual role. The goal of this study was to investigate the effects of body size on these traits, and provide much-needed data on sperm transfer and paternity patterns in a hermaphroditic sea slug with hypodermic insemination, *Alderia modesta* (Opisthobranchia: Sacoglossa=Ascoglossa). Like other opisthobranchs, *A. modesta* does not self-fertilize and must mate to reproduce. It has an extendable penis with a sharp style to pierce the skin and inject sperm into spaces within the haemocoel of a mate (Fig. 1; Hand & Steinberg 1955). The genital system, particularly the ovotestis, occupies almost all of the entire body space (Evans 1953), but it is unknown how sperm travel to and fertilize eggs,

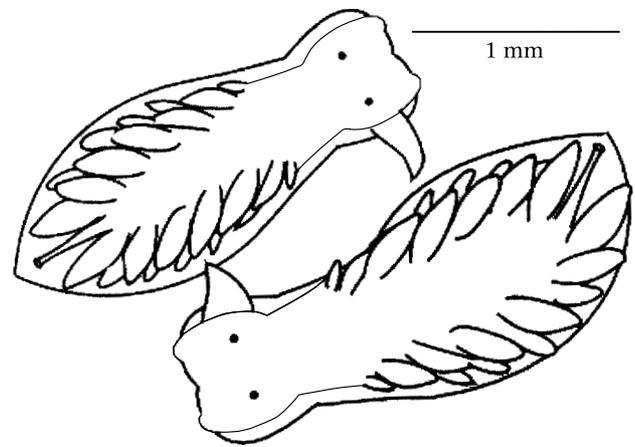


Figure 1. Dorsal view of two *A. modesta* about to inject each other. The flexible penis extends from the right side of the head before mating, and the penial style (approximately 50 μ long) pierces the skin of a mate.

or whether sperm can be digested (as in other opisthobranchs; Beeman 1970; Rivest 1984).

Hypodermic (or traumatic) insemination has evolved independently in a number of simultaneous hermaphrodites (e.g. sacoglossan and nudibranch sea slugs, flatworms, leeches), and several functions have been suggested. Hypodermic insemination may eliminate courtship time, allowing an individual to inseminate many mates when contact with conspecifics is brief (Trowbridge 1995). Hypodermic insemination may represent an escalation of sperm competition, allowing a sperm donor to gain more direct access to eggs and providing an advantage over other sperm donors, while the sperm recipient loses control over fertilization (Eberhard 1996; Michiels 1998; Michiels & Newman 1998). It may further allow individuals to transfer sperm without consent of a mate or without controlled reciprocal exchange of sperm (Michiels 1998; Michiels & Newman 1998). I investigated hypodermic insemination in light of these hypotheses by looking at the success rate of injections of varying duration and in different parts of the body, in terms of the amount of sperm transferred and the paternity gained. Do injections in certain body regions result in increased paternity by avoiding sperm competition or sperm digestion by the mate? Do rapid injections successfully transfer sperm while avoiding consent by the mate or reciprocal exchange of sperm? Does the entire body function as a large sperm storage organ, and if so, what are the consequences for sperm competition? To my knowledge, this is the first study to measure paternity and allocation to sexual roles in a hermaphrodite with hypodermic insemination.

METHODS

Study Animals and Their Maintenance in the Laboratory

Alderia modesta is found on high intertidal mats of the yellow-green alga *Vaucheria* spp. in temperate estuaries

along Atlantic and Pacific coasts (Engel et al. 1940; Hand & Steinberg 1955; Bleakney & Bailey 1967; Trowbridge 1993a, 2002). *Alderia modesta* is characterized by high levels of fecundity (up to 1000 eggs/day) and high population densities (up to thousands of animals per square metre; Seelemann 1967; Trowbridge 1993a, b; Krug 1998). Body size can vary greatly and depends on locality (Hand & Steinberg 1955; Bleakney 1988; Trowbridge 1993b). *Alderia modesta*'s life span is unknown in the field, but individuals can be maintained for at least several months in the laboratory and probably live less than 1 year, like other opisthobranchs (Thompson 1976); *A. modesta*'s life span has been estimated as 2–6 months in the northwest Atlantic (Clark 1975).

In southern California, populations of *A. modesta* show a reproductive polymorphism with some individuals producing large eggs that develop into lecithotrophic (nonfeeding) larvae and others producing many more small eggs that develop into planktotrophic (feeding) larvae (Krug 1998). Unlike planktotrophic larvae, which must feed several weeks before metamorphosis, lecithotrophic larvae metamorphose within a few days of hatching in the presence of *Vaucheria* spp., the adult food source, without feeding as larvae (Krug 1998, 2001). Thus, they were easy to raise in the laboratory and proved useful for rearing virgins. Virgins begin producing unfertilized egg clutches of a particular type before receiving sperm. Individuals producing lecithotrophic eggs were used as sperm recipients in paternity experiments because of the ease of rearing their offspring. Because eggs are formed before fertilization, larval type is maternally determined and not affected by paternity; when maintained under constant conditions in the laboratory, individuals do not change the developmental mode of their offspring depending on the current sperm donor (P. J. Krug, unpublished data). Thus, my use of animals producing lecithotrophic offspring in paternity experiments was unlikely to affect estimates of paternal reproductive success.

I collected *A. modesta* and portions of *V. longicaulis* mats from the mudflats of the Kendall-Frost Marine Preserve in north Mission Bay, San Diego, California, U.S.A. Animals were individually maintained at Scripps Institution of Oceanography at 16°C on a 12:12 h light:dark cycle in covered plastic culture dishes with 4 ml of fresh sea water. *Vaucheria longicaulis* patches were allowed to grow under constant light so that long filaments could be removed from the mud substrate and fed to *A. modesta*. Animals were transferred to clean water and *V. longicaulis* was added every other day.

I used total body length as a measure of size; body length is directly related to wet weight in this species (Trowbridge 1993b). Prior to being used in experiments, the length of each animal was measured to the nearest 0.1 mm through a dissecting microscope with an ocular micrometer. Because *A. modesta* stretches and contracts slightly while moving, I observed each animal for 1 min and recorded the maximum length during that interval.

Mating Patterns

After being maintained in isolation for 3 days, animals of varying lengths (0.8–4.7 mm) were paired for 30 min in 4-ml culture dishes and their behaviour was observed. I recorded movements, duration and location of contacts, mating characteristics (time of penis extension, penis injection, penis withdrawal and penis inversion) and the location of injection on the body of the mate (categorized three ways: left or right, anterior or posterior, and dorsal or ventral). I categorized mating events as 'reciprocal' if both animals were inseminated or 'unilateral' if only one individual injected its mate. I observed 122 pairs (244 individuals) in July–September 1997 and March–May 1998.

Egg Production and Sperm Transfer

To detect whether there is an early emphasis on male function in *A. modesta*, with sperm transfer initiating at smaller sizes than egg production, I indirectly recorded the onset of mating and sperm transfer in juveniles (≤ 5 days postmetamorphosis, < 1 mm in length and lacking full cerata or dorsal projections). I allowed groups of five lecithotrophic larvae to undergo metamorphosis and rearing in the same 4-ml culture dish for varying intervals (2, 4 and 5 days) before individual isolation. I then noted whether fertilized eggs were produced by isolated individuals to detect the storage of allosperm (sperm received from a mate) and, therefore, prior mating within juvenile groups. This allowed me to determine whether sperm production and transfer can occur at very small body sizes immediately following metamorphosis.

I measured the relation between body size and fecundity by counting the number of eggs produced by 90 animals within 2 days of collection from the field. The effect of isolation on their long-term egg production and sperm storage was measured over the next month by counting eggs deposited every 2 days. Partially fertilized egg masses are produced by animals with waning allosperm levels, and completely unfertilized egg masses are produced by virgins and also by animals that have depleted all of their allosperm stores. This allowed me to use the proportion of eggs within egg masses that were fertilized (began cleavage) as an indirect measure of allosperm stores and depletion rate. Directly measuring allosperm would be difficult in this species because it is stored throughout body tissues and is difficult to distinguish from autosperm (sperm produced by the individual itself; unpublished data). To verify that reduced fertilization rates over time in isolation represented depletion of allosperm stores, 10 individuals that had begun producing completely unfertilized egg masses were allowed to receive sperm again from one observed mating event. The 10 individuals were then returned to isolation and the fertilized proportions of their subsequent egg masses were counted to determine whether allosperm stores were replenished.

To estimate the effect of body size on relative levels of sperm transfer, 29 laboratory-reared virgins maintained in isolation were allowed to mate and receive sperm once

from a single field-collected individual and then returned to isolation (March–April 1999). I counted the eggs produced and the proportion fertilized every 2 days for each former virgin until all eggs produced were unfertilized. Variation in the number of fertilized eggs produced by former virgins (until egg masses were composed of >50% unfertilized eggs) and variation in the number of days until allosperm stores were depleted (when egg masses were composed of >50% unfertilized eggs) were used as relative measures of the amount of sperm transferred by sperm donors. I used both simple and multiple regression analysis to estimate the effect of sperm donor size, sperm recipient (former virgin) size, injection duration, injection location and recipient egg production rate on allosperm depletion rate and the number of eggs fertilized. Independent variables were removed from the multiple regression model by backward elimination if their associated P was greater than 0.15.

Paternity Experiments

Individuals producing only lecithotrophic eggs were used in paternity experiments because of the ease of rearing offspring through metamorphosis. Animals were collected from the field and allowed to lay at least one egg mass over a period of 4 days. Those producing only lecithotrophic eggs were genotyped for allozymes at the variable phosphoglucose isomerase (PGI) locus by polyacrylamide gel electrophoresis (Burton & Feldman 1981). Each individual was homogenized in 30 μ l of 15% (w/v) sucrose in 0.1 M Tris–borate–EDTA buffer (pH 8.9) with bromophenyl blue to colour the sample. Two μ l of each homogenate were run on a 1-mm-thick 6% acrylamide gel in 0.1 M Tris–borate–EDTA (pH 8.9) for 6.5 h at 120 volts, were stained for PGI (as in Harris & Hopkinson 1976) for 1 h and were scored. I saved egg masses produced by individuals that were scored with alleles different from the most common ‘medium’ allele. These egg masses were then reared to produce adults with known maternal alleles that could be used as sperm donors in mating experiments.

I used 13 laboratory-reared virgins, which each produced an unfertilized lecithotrophic egg mass, as sperm recipients in paternity experiments. Each virgin was allowed to mate and receive sperm from an animal with a known maternal PGI allele, lay an egg mass (43–152 eggs), then mate with another animal with a different maternal PGI allele. The second mating was usually within 2 (at most 6) days of the first mating, so that paternity values of the second sperm donor reflected sperm precedence or competition patterns, rather than loss or death of sperm from the first mating. Following the second mating, each former virgin was allowed to lay two egg masses (39–212 eggs).

I conducted seven additional paternity experiments using field-collected nonvirgins as sperm recipients. Each collected animal was allowed to lay a first lecithotrophic egg mass (62–189 eggs), receive sperm from a laboratory-reared animal with a known maternal PGI allele within 6 days of collection, and then lay two additional egg masses (56–168 eggs).

Following all experiments with virgin and nonvirgin recipients, 10–21 larvae were reared and genotyped from each of the first egg masses, and a sample of larvae (6–59; 11–71% of egg masses) were reared and genotyped from the two subsequent egg masses. Homogenates of the mother and putative fathers were run on the same gel with their respective offspring groups, allowing for paternity assignment of offspring. The proportion of offspring fathered by the last sperm donor, P_2 , was estimated in each egg mass as the proportion of genotyped offspring with the same paternal allele as the last sperm donor. In cases where the last sperm donor had only one allele that was different from the most common ‘medium’ allele, its frequency in the offspring was doubled to estimate total P_2 . Because paternity estimates were not significantly different between the two final egg masses, and between those experiments with virgins and nonvirgins, I pooled data from these groups for analysis. The effects of sperm donor and recipient body sizes, injection duration and injection location on P_2 were evaluated in both simple and multiple regression analyses. Independent variables were removed from the multiple regression model by backward elimination if their associated P was greater than 0.15.

All summary statistics of continuous variables below are reported as $\bar{X} \pm SE$, and statistical tests are two tailed. Power was calculated for tests with nonsignificant results at the given sample size and alpha of 0.05, and for a ‘medium’ effect level (following conventions of Cohen 1988).

RESULTS

Body Size and Mating Patterns

Fifty-nine of 122 pairings resulted in a mating event. An individual typically made contact with a potential mate and moved its oral lobes over the mate’s body before penis extension, penial style injection and sperm transfer, which lasted 337 ± 17 s. *Alderia modesta* mated size assortatively; animals more similar in length to each other were more likely to mate than those more different in length (logistic regression: $\chi^2_1=5.8$, $P<0.05$; Fig. 2). There was no tendency for injections to occur in any particular section of the body (dorsal versus ventral, left versus right, anterior versus posterior) (chi-square tests: all $\chi^2_1<0.8$, NS).

Reciprocal injections were more common (42 of 59 mating events; $\chi^2_1=10.6$, $P<0.01$) and lasted longer than unilateral injections (t test: $t_{99}=2.6$, $P<0.01$; reciprocal: 357 ± 19 s; unilateral: 242 ± 28 s). The probability of mating reciprocally rather than unilaterally depended on body size. Reciprocal insemination was more likely to occur with an increase in the size of the smaller member of a mating pair (logistic regression: $\chi^2_1=6.2$, $P<0.05$; Fig. 3), but did not depend on the size of the larger individual ($\chi^2_1=0.3$, NS) or the size difference between the two ($\chi^2_1=0.3$, NS; power=0.63).

Within pairs that mated reciprocally, there was a significant correlation between the injection times of the two mating animals ($r_{40}=0.60$, $P<0.0001$; Fig. 4) and a

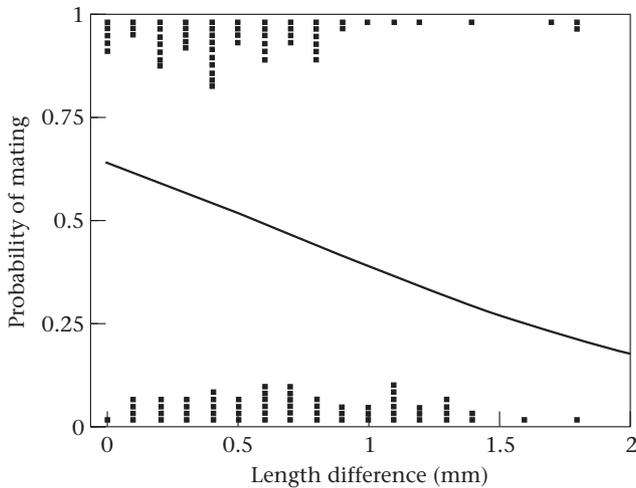


Figure 2. Mating was size assortative; the probability of mating decreased with an increase in the length difference between paired animals (logistic regression: $\chi^2_1=5.8$, $P<0.05$). Points at the top of the figure correspond to the size difference between pairs that mated; points at the bottom of the figure correspond to the size difference between pairs that did not mate.

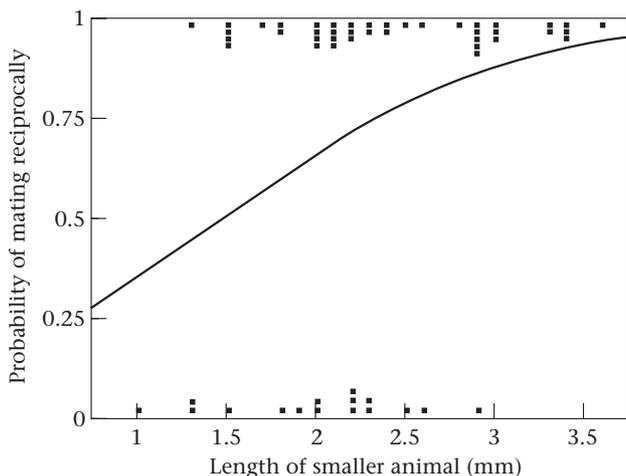


Figure 3. Among mating pairs, reciprocal insemination was more likely to occur than unilateral insemination with an increase in the body length of the smaller mate (logistic regression: $\chi^2_1=6.2$, $P<0.05$). Points at the top of the figure correspond to the size of the smaller individual in pairs that mated reciprocally; points at the bottom of the figure correspond to the size of the smaller individual in pairs that mated unilaterally.

size bias in injection times; the larger member of a pair was inseminated for longer than the smaller member of a pair (paired t test: $t_{41}=2.3$, $P<0.05$; mean difference=55 s).

In 11 of the 17 unilateral mating events, the smaller individual was the sperm donor; the larger individual donated sperm in six cases (binomial test: NS; power=0.42). The duration of unilateral inseminations did not differ between cases where the smaller or the larger animal donated sperm, although statistical power to detect such an effect was low (t test: $t_{15}=0.2$, NS; power=0.15).

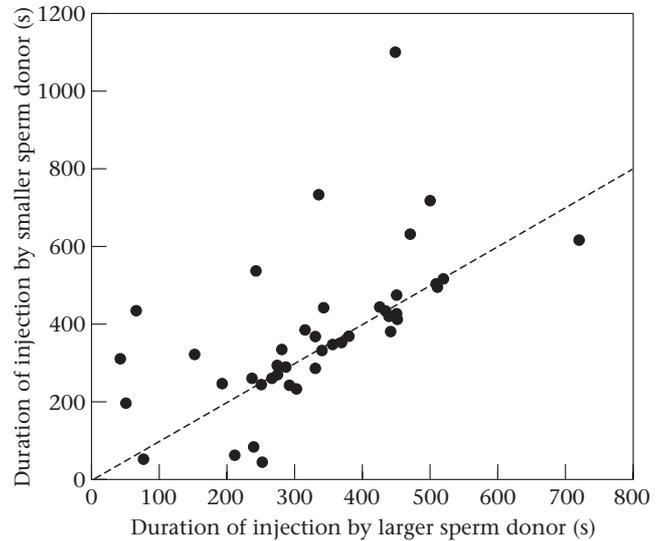


Figure 4. Among reciprocally mating pairs, injection times were positively correlated ($r_{40}=0.60$, $P<0.0001$). Dashed line represents equal durations of injections.

Effect of Body Size on Egg Production and Sperm Transfer

I detected the onset of sperm production and sperm transfer at smaller body sizes than egg production in juveniles. Fifty per cent of animals that had been exposed to same-age conspecifics for only the first 2 days post-metamorphosis went on to produce fertilized eggs, indicating mating, sperm transfer and sperm storage in juveniles less than 0.5 mm long ($N=14$). Seventy-five per cent of juveniles exposed to same-age conspecifics for the first 4 days postmetamorphosis (all <0.6 mm; $N=8$), and 91% exposed for only the first 5 days (all <0.7 mm, $N=22$) had received sperm and went on to produce fertilized eggs in isolation. The onset of egg production however occurred at larger sizes than did sperm transfer; the smallest animal that I observed producing eggs was 1.2 mm long and more than 10 days postmetamorphosis.

Egg production increased with body size (regression: $F_{1,86}=32.2$, $R^2=0.27$, $P<0.0001$; Fig. 5). Field-collected animals always had enough stored allosperm to fertilize all eggs produced within the first few days in isolation. They continued to produce egg masses with increasing proportions of unfertilized eggs as allosperm stores were depleted. Within the first week in isolation, the mean fertilized proportion of egg clutches dropped below 100%. After the first week in isolation, the mean fertilized proportion decreased by 5% per day and dropped below 50% between days 13 and 16 (Fig. 6). All animals that had ceased producing fertilized eggs and that were allowed to mate and receive sperm again began producing egg clutches that were 100% fertilized ($N=10$).

In 23 of 29 laboratory-reared virgins, a single injection by a sperm donor resulted in the fertilization of eggs. The fertilized proportion of each egg clutch produced by former virgins (sperm recipients) decreased over time from a mean of $99.3 \pm 0.5\%$ to below 50% after a mean of 16 days, reflecting the rate of allosperm depletion. A

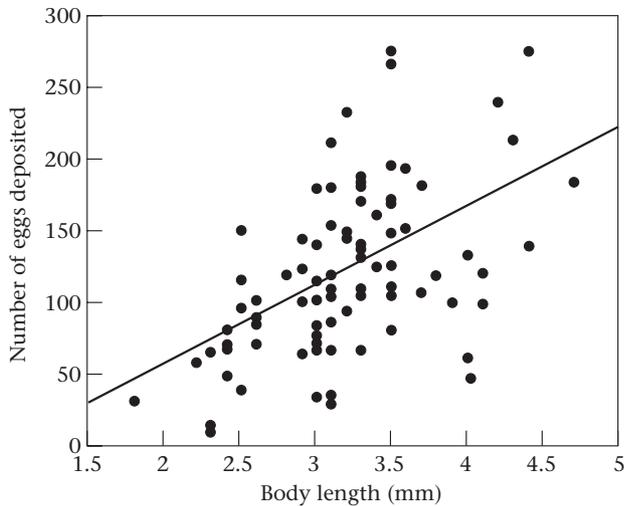


Figure 5. Fecundity (number of eggs produced within 2 days of collection from the field) increased with body length (regression: $F_{1,86}=32.2$, $R^2=0.27$, $P<0.0001$; $Y=-53.8+54.7X$).

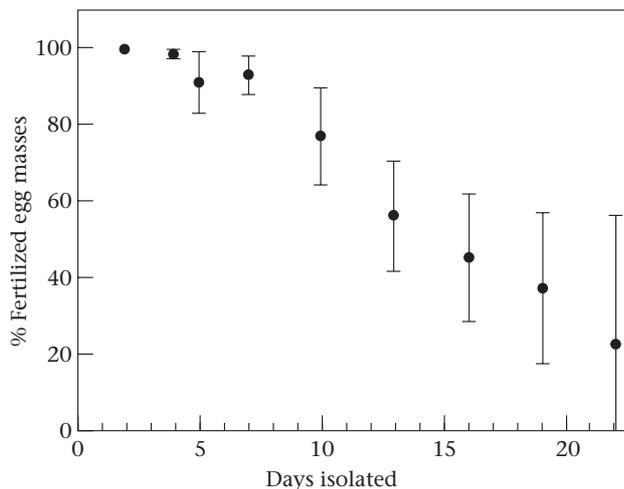


Figure 6. When maintained in isolation, field-collected animals produced egg masses with a decreasing proportion of fertilized eggs over time, indicating depletion of allosperm stores. Points correspond to mean proportion of eggs fertilized within egg masses; error bars span 95% confidence intervals.

mean number of 936 fertilized eggs were produced up to that point. However, those values ranged widely (range 5–24 days, 268–2164 fertilized eggs), and may have corresponded in part to variation in the amount of sperm received. Body length of the sperm recipient, injection time and injection site had no effect on the rate of allosperm depletion or the number of fertilized eggs produced. Sperm donor length and egg production rate, however, were significant predictors of allosperm depletion rate in multiple regression analyses. Sperm recipients produced mostly (>50%) fertilized egg masses for longer if they received sperm from a larger sperm donor ($F_{2,13}=7.17$, $P<0.05$) and if they produced fewer eggs per day ($F_{2,13}=10.48$, $P<0.01$; multiple regression: $N=16$, $R^2=0.53$). Sperm recipients produced more

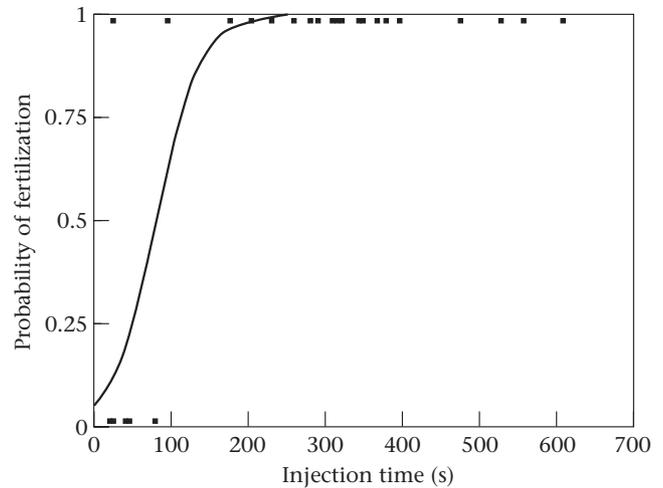


Figure 7. Very short injections were less likely than longer injections to result in the fertilization of eggs (logistic regression: $\chi^2_1=21.1$, $P<0.0001$). Points at the top of the figure correspond to the duration of injections that resulted in the fertilization of eggs; points at the bottom of the figure correspond to the duration of injections that did not fertilize eggs.

fertilized eggs (before egg clutches dropped below 50% fertilized) if they received sperm from a larger sperm donor ($F_{2,13}=12.43$, $P<0.01$) and if they produced more eggs per day ($F_{2,13}=22.89$, $P<0.001$; multiple regression: $N=16$, $R^2=0.78$). I found the same trends for the entire interval to 0% fertilized eggs or complete sperm depletion, but with a smaller sample size.

In six of 29 cases, a single injection of a laboratory-reared virgin by a field-collected animal did not lead to the production of fertilized eggs, but rather to the production of only unfertilized eggs. This was related to the duration of the injection; very short injections did not fertilize eggs (logistic regression: $\chi^2_1=21.1$, $P<0.0001$; Fig. 7). Mean injection time resulting in fertilization was 326 ± 29 s; mean injection time without fertilization was 42 ± 8 s.

Paternity

After receiving sperm from more than one mate, P_2 in the first two egg masses produced by sperm recipients ranged widely (0–0.88) with a mean of 0.40 ± 0.06 ; P_2 was almost significantly different from 0.50 (t test: $t_{19}=1.8$, $P=0.09$; power=0.58). I did not detect an effect on P_2 of any of the following variables: body sizes of sperm donor or sperm recipient, size difference between donors, duration of mating event, location of injection, time and number of eggs fertilized between the two mating events (in experiments with virgins) or status of recipient as a virgin or nonvirgin at the beginning of the study (for all tests, power <0.37). Injections into almost all sections of the body achieved paternity, including those directly into the extended penis of the mate and into cerata (dorsal projections). Only one injection location resulted in no paternity; in one case, sperm were injected near the anus and expelled out of it as they were transferred.

DISCUSSION

Body size affected the mating patterns of *A. modesta*, characterized by size-assortative, reciprocal, hypodermic inseminations. I found a high correlation between injection times of two mating animals, with the smaller individual investing more time injecting its larger mate than vice versa. Size-assortative, reciprocal mating, with a high correlation between injection times, indicates a form of conditional reciprocity, where animals are willing to be inseminated only if they can simultaneously inseminate a mate of similar egg production capacity (i.e. size; Michiels 1998). In mating trials, when an individual had completed sperm transfer, was uninterested in transferring sperm, or was unable to reciprocally inject a mate, it often appeared to struggle to break free of its mate and move away, sometimes dragging the mate by its penis. These behaviours suggest an unwillingness to receive sperm without simultaneously donating it. Because they live in high densities and can store sperm for long periods of time, *A. modesta* may be rarely allosperm-depleted, and thus only occasionally in need of receiving sperm. This kind of conditional reciprocity has been described by others as 'egg trading' or 'sperm trading' depending on which gamete is given conditionally (Fischer 1984, 1987; Leonard & Lukowiak 1984, 1991; Leonard 1991; Premoli & Sella 1995; Sella et al. 1997; Michiels 1998).

Pseudoceros bifurcus, a polyclad marine flatworm with hypodermic insemination, has a similar mating behaviour; individuals attempt to inject each other without being injected themselves, resulting in 'penis fencing' (Michiels & Newman 1998). However, in that species, unilateral insemination is more common, because the first animal to inject typically avoids being injected. In *A. modesta*, unilateral inseminations are less common and are often brief because the sperm recipient quickly pulls away from the sperm donor. Because very short injections are less likely to result in sperm transfer and fertilization, there may be selection in this species for two animals to settle into a longer, reciprocal mating event.

Although reciprocal inseminations are more common, *A. modesta* does occasionally mate unilaterally. I expected the probability of mating reciprocally to depend on the size difference between two mating animals. Sex allocation theory predicts that same-size animals should mate reciprocally, with pairs very different in size more likely to mate unilaterally (Angeloni et al. 2002). Instead, the probability of mating reciprocally increased with the absolute size of the smaller member of the mating pair.

The finding that the smaller member of a mating pair spent more time mating in the male role while the larger animal spent more time mating in the female role supports theoretical predictions for greater investment in male function by smaller animals (Angeloni et al. 2002). Differences in time invested to mating in each role may reflect underlying differences in sex allocation to reproductive structures and gamete production. Although mating events typically lasted only 5 min, the time and energy expended may represent a considerable investment in sperm, amplified by the break in feeding during a mating event; when not mating, *A. modesta* feeds

almost continuously and quite rapidly on strands of *Vaucheria* (Evans 1953).

Several studies on animal hermaphrodites have provided evidence for shifts in sex allocation with body size, using time spent mating in each sexual role, gamete production or volumes of reproductive organs as the currency for sex allocation (Otsuka et al. 1980; Petersen 1990; DeWitt 1996; Petersen & Fischer 1996; Yusa 1996; Angeloni & Bradbury 1999; Schärer et al. 2001). Several studies of gastropods have also found size-assortative mating similar to patterns in this study (Crozier 1917, 1918; Edwards 1968; Erlandsson & Johannesson 1994; Staub & Ribí 1995; Tomiyama 1996; Yusa 1996; Angeloni & Bradbury 1999; Michiels et al. 2001).

The ability to flexibly adjust mating patterns depending on the relative size of a current mate requires an ability to assess body size. *Alderia modesta* has poor vision, and probably uses chemical cues to find conspecifics. An individual can presumably then use both chemical and tactile cues to assess mate size before and during injections. Two animals typically push against each other and move their oral lobes over each other before mating. This may be a common method of size assessment in invertebrate hermaphrodites. Hermaphroditic flatworms have been shown to assess mate size using tactile and chemical cues (Vreys & Michiels 1997; Lüscher & Wedekind 2002).

Gamete production is another currency for measuring sex allocation and is predicted to depend on body size in sperm-storing hermaphrodites (Angeloni et al. 2002). I found an early emphasis on male function in *A. modesta*'s postmetamorphic life; sperm transfer occurs at smaller body sizes than does egg production, as in another opisthobranch, *Phostilla sibogae* (Todd et al. 1997). Juveniles transferred sperm to each other, and therefore mated in the roles of both sperm donor and sperm recipient, indicating an early onset for both male and female behaviours. However, very small sperm recipients stored allosperm without producing eggs until reaching a larger size, thereby delaying complete female function. This result suggests that individuals shift investment strategies with body size, or that oogenesis requires a greater investment of time and resources than does spermatogenesis.

Egg production increased with body size in *A. modesta*, confirming a previous finding by Krug (2001). Without knowing how body size affects sperm transfer, one cannot say whether or not this indicates a proportional shift towards greater investment in female function. I indirectly measured the amount of sperm transferred by sperm donors during mating events by monitoring the number of fertilized eggs produced by sperm recipients from that one mating event, and how quickly they depleted the sperm they received. This is a relative, not an absolute, measure of sperm transfer, because some sperm may be depleted by sperm mortality or digestion, and not used to fertilize eggs. Those animals that received sperm from a large donor produced more fertilized eggs and did not deplete allosperm as quickly as those that mated with a small donor. The most likely explanation for this finding is that larger sperm donors transferred sperm at

higher rates, because there was no effect of duration of insemination. Other possible explanations are that larger animals transferred seminal fluid with enhanced properties for postponing sperm death or preventing sperm digestion by the recipient, or that sperm recipients actively chose to digest or discard sperm from smaller sperm donors.

An effect of body size on sperm transfer rate may partially explain the finding that smaller animals injected larger mates for longer than vice versa in the observations of mating patterns. A smaller animal may need more time to transfer the same amount of sperm. Any differences in sperm transfer rates by animals of different sizes do not appear to affect the paternity achieved when there is sperm competition.

Because both egg production and sperm transfer rate probably increase with size, two additional questions must be answered to compare directly the relative effect of body size on egg versus sperm investment. (1) What are the relative energetic costs of producing eggs and sperm for this species? (2) What is the frequency of mating relative to egg production in the field? Obtaining this information in the future would allow for the calculation of a sex allocation ratio using gametes as the currency.

Although sex allocation theory predicts an effect of body size on the proportion of resources invested in male function, it does not predict a pronounced effect of body size on the actual proportion of sperm displaced within a mate, or the level of paternity achieved (Angeloni et al. 2002). I did not detect any effect of body size on paternity in *A. modesta*, which is consistent with theoretical predictions, but also possibly due to low sample sizes. The widely ranging and unpredictable P_2 values that I detected may be in part due to hypodermic insemination. Because *A. modesta* can inject sperm at any number of locations over the entire body of a mate, injections are unlikely to displace sperm directly from prior mating events, possibly contributing to unpredictable sperm precedence patterns. Although additional paternity data for hermaphrodites with hypodermic insemination are lacking (Baur 1998), Stutt & Siva-Jothy 2001 investigated paternity in a hypodermic inseminator with separate sexes, the bed bug *Cimex lectularius*, which showed significant levels of last-male sperm precedence ($P_2=0.68$). Perhaps P_2 values are more consistently high in that species because sperm transfer always occurs in the same location on the female's body.

Multiple factors probably contribute to variable sperm precedence, because it is also found in hermaphroditic gastropods that do not mate by hypodermic insemination. P_2 varies widely for the hermaphroditic land snail *Arianta arbustorum*, and there is a similar lack of effect of body size on paternity estimates, despite an increase in sperm transfer with donor body size (Baur 1994). Although sperm is not transferred by hypodermic insemination in that system, it is stored in multiple blind sacs, which could contribute to inconsistent sperm precedence patterns; sperm recipients may use blind sacs to sort and select sperm from particular sperm donors (Baur 1994). Body size does not affect the variable paternity patterns of the hermaphroditic sea slug *Aplysia californica*. In that

species, sperm is transferred through a genital opening to a seminal receptacle with a trend towards last-mate sperm precedence (Angeloni et al. 2003). Low P_2 estimates in the dart-shooting hermaphroditic snail, *Helix aspersa*, vary with the level of penetration of a love dart into the mate (Landolfa et al. 2001). A comprehensive look at paternity in other hermaphroditic animals could elucidate the relative importance of reproductive anatomy and behaviour on sperm displacement patterns.

Although there is currently no information on allosperm digestion in *A. modesta*, it does occur in another sea slug (Beeman 1970), and is probably common in other opisthobranchs and gastropods, including those with hypodermic insemination (Rivest 1984; Leonard 1991; Baur 1998). Allosperm digestion offers one possible explanation for the evolution of hypodermic insemination in *A. modesta*. Hypodermic injection may escalate sexual conflict by allowing the sperm donor to avoid the recipient's sperm digesting organs and directly fertilize eggs (Eberhard 1996; Michiels & Newman 1998). In its early evolutionary stages, hypodermic insemination may have provided a sperm competition advantage, along with an increased risk of transferring sperm to the wrong location for fertilization. I found hypodermic insemination to be an effective form of sperm transfer in *A. modesta*; injections over all sections of the body resulted in the fertilization of eggs with and without sperm competition. Although there may have been an advantage to localized injections in earlier stages of the evolution of hypodermic insemination, there is currently no apparent advantage for a sperm donor to inject in a particular location.

The ability to avoid precopulatory mating behaviours and mate quickly is another suggested advantage of hypodermic insemination (Trowbridge 1995). Rapid mating could allow for the insemination of a mate without consent or without reciprocal exchange of sperm in other species (Michiels & Newman 1998). However, this theory is unlikely to apply to *A. modesta*, because mating pairs showed precopulatory behaviours before insemination, which extended the duration of mating events. Very brief injections, which ended quickly because the sperm recipient was able to move away, did not usually result in the fertilization of eggs; to transfer sperm, mating pairs typically settled into longer reciprocal mating events. The duration of these mating events could easily match or exceed the times required for other forms of insemination, such as penis insertion through a vaginal opening. Further studies of fertilization success achieved by animals with hypodermic insemination would help to elucidate its function and potential role in sexual conflict.

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