

Body size influences mating strategies in a simultaneously hermaphroditic sea slug, *Aplysia vaccaria*

L. ANGELONI and J. BRADBURY

Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA

Received 1 September 1998, accepted 17 December 1998

The relationship between body size and hermaphroditic mating strategies was studied by following a population of the sea slug, *Aplysia vaccaria*, for one year. Fourteen individuals were tagged with internal microchips, and all mating pairs were weighed. Tagged individuals mated repeatedly both as sperm recipients and sperm donors. There was a trend toward size-assortative mating and size-assortative spatial clustering, and animals acting as females were larger than the average mass for the population. These findings support prior studies showing an association between larger relative size and sperm recipient roles in hermaphroditic gastropods, and highlight the need for critical experiments to determine the mechanisms of role choice and mate choice.

KEY WORDS: sex allocation, sexual selection, body size, simultaneous hermaphrodite, *Aplysia*.

INTRODUCTION

Simultaneously hermaphroditic animals share with dioecious species the full complement of sexually selected strategies (MORGAN 1994), but can also have a spectrum of additional strategies involving sex allocation. Thus a hermaphrodite can adjust the amount of resource allocated to male vs female function depending on the costs and benefits associated with mating in each role. Differential sex allocation should be reflected in behavioral investments, such as relative time spent mating in each role. Body size has been an important factor for explaining mating strategies in sequential hermaphrodites (GHISELIN 1969), but has been left out of most models of sex allocation in simultaneously hermaphroditic animals (LEONARD 1990, CHARNOV 1996). In his recent model, CHARNOV (1996) predicted that sperm-storing simultaneous hermaphrodites should invest more in female function as sperm competition becomes more severe. However, he also noted that differences in body size would be expected to modulate sex allocation and other sexual strategies in ways which are not yet known. Although some evidence from studies of

invertebrate hermaphrodites supports the prediction that body size affects mating strategies (OTSUKA et al. 1980, DEWITT 1996, YUSA 1996, VREYS & MICHIELS 1997), others have not found such an effect (SWITZER-DUNLAP et al. 1984, LEONARD & LUKOWIAK 1985, ZAFERES et al. 1988, PENNINGS 1991, BAUR 1992, PETERS & MICHIELS 1996), leaving the relationship unclear.

In this paper, we study the relationship between sexual role and body size in a natural population of the sea slug, *Aplysia vaccaria* (Gastropoda Opisthobranchia). These animals have extremely high growth rates within an approximate 1-year life span, resulting in considerable variation in body size in natural populations (ANGELONI et al. 1999). The species usually exhibits unilateral sperm transfer, where one animal donates sperm to another but does not simultaneously receive sperm from that individual. A given individual can, however, donate and receive sperm simultaneously within a chain of three or more mating animals. Little else is described about the mating behavior of this species. Our study population exhibited abundant variation in body size and frequent mating, providing us with a unique opportunity to investigate the relationship between body size and sexual role in this species.

MATERIALS AND METHODS

We observed the study population from October 1995 to October 1996 at North Cardiff Beach, San Diego County, California (33°1'N, 117°17'W). We sampled the same 15 × 20 m rectangular portion of the rocky intertidal reef every 2-3 weeks during low tides (see ANGELONI et al. 1999 for a detailed description of the study site and methods). This site included eroded channels and pools which remained filled with water even during the lowest tides and harbored high densities of *A. vaccaria*. Each *A. vaccaria* within the study site was weighed and its location mapped. The proportion of total animals mating in a given census period ranged from 3 to 43% (ANGELONI et al. 1999). We classified the current mating status of each individual into four categories: (1) not mating; (2) mating as a sperm donor; (3) mating as a sperm recipient; (4) mating simultaneously as both a sperm donor and sperm recipient. Mating status was easily established by inserting a finger under the parapodia to determine whether or not adjacent animals were linked by an everted penis.

We tagged 19 *A. vaccaria* (12 in March, 7 in May 1995) with number-coded transponder tags which could be detected with a Trovan LID-500 Hand Held Reader (InfoPet Identification Systems, Inc.). We searched the study site for tagged animals on 4 consecutive days after the initial tagging and then again every 2-3 weeks. We noted the identity, mating status, mass and location of tagged animals when recaptured.

We wrote a Pascal program to test for non-random spatial distributions of body sizes using Monte Carlo randomization methods. Our program calculates the average body size difference between nearest neighbors for a given sample day. Then it randomly assigns nearest neighbors from the sample population and calculates the mean body size difference. This randomization process was repeated 5000 times (as suggested by ADAMS & ANTHONY 1996) to obtain a distribution of mean mass differences and to calculate the proportion of random means equal to or more extreme than the observed mean. This proportion is the *P*-value, directly providing the significance of the randomization test. The program additionally allowed us to exclude from the analysis animals that were more than a minimal distance away from any nearest neighbor. Based on a study of dispersion in this population (ANGELONI et al. 1999), we set this minimal distance at 0.3 m and assumed no interaction between individuals separated by more than 0.3 m.

We pooled data across all 20 sampling days to increase the sample size for detecting the effects of body size on mating patterns. Whereas previous studies of mating in hermaphroditic invertebrates were conducted over short time periods (e.g. CROZIER 1917, 1918; OTSUKA et al. 1980; BAUR 1992; PETERS & MICHIELS 1996; YUSA 1996), our study was conducted over

Overall there was a trend toward size-assortative mating ($r = 0.13$, $n = 190$, $P = 0.08$; Fig. 1). Mean body size difference between nearest neighbors was less than the predicted value for random assignment on 12 of 16 sample days (Wilcoxon signed rank test: $T = 17$, $n = 16$, $P < 0.02$). Nearest neighbors were significantly

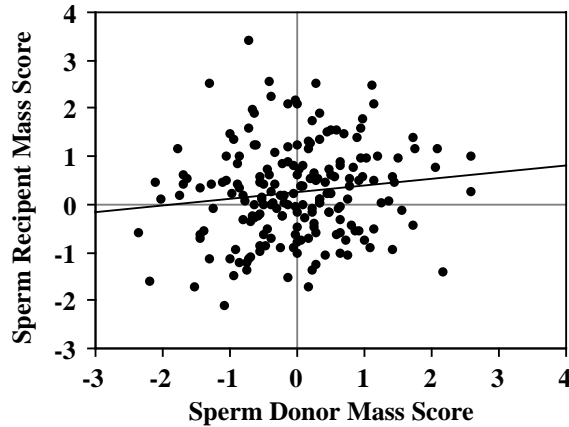


Fig. 1. — Regression of sperm recipient body mass on sperm donor mass for mating pairs of *Aplysia vaccaria* ($Y = 0.251 + 0.138 \cdot X$; $R^2 = 0.016$, $P = 0.08$). Mass score was calculated as standard deviations from the mean for that day to correct for growth in body size throughout the season.

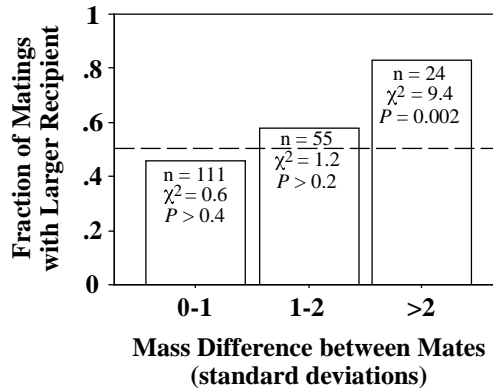


Fig. 2. — The fraction of 190 mating events in which the sperm recipient was larger than the sperm donor increased with increasing absolute size difference between mates (measured in standard deviation units around the mean mass for each census; G test: $G^2 = 12.5$, $df = 2$, $P = 0.002$). The same conclusion arises from a logistic regression of larger partner's sex on the absolute difference in pair members' body sizes ($\chi^2 = 11.35$, $df = 1, 188$, $P = 0.0008$). Dashed line in histogram shows 50% expectation if roles were assigned randomly. Although the two smaller size difference categories do not differ significantly from chance, the largest difference class shows a highly significant deviation (even with a Bonferroni correction) favoring large females (see χ^2 values and probabilities for each test on corresponding bar of histogram).

Table 2.

The number of 22 observed trios with each of the possible body size relationships between the three animals (terminal sperm donor, sperm donor and sperm recipient, and terminal sperm recipient); 1 = smallest of the three, 2 = mid-sized, 3 = largest of the three.

Donor	Both	Recipient	# trios
1	2	3	5
1	3	2	7
2	3	1	5
2	1	3	0
3	1	2	2
3	2	1	3

more similar in size than predicted by random dispersion on three sample days (invoking a Bonferroni correction and two-tailed tests: Oct. 24, $P < 0.001$; Dec. 7, $P < 0.001$; Mar. 15, $P < 0.002$).

Animals mating in pairs were larger than the average mass for the day they were measured (mean mass = 0.12 SD larger than the average of the population \pm 0.05 SE; one sample t-test: $t = 2.4$, $df = 379$, $P = 0.019$). This pattern was primarily due to the fact that sperm recipients were significantly larger than the population average (mean mass score = 0.25 ± 0.07 , $t = 3.5$, $df = 189$, $P = 0.0006$) and larger than their mates (mean difference = 0.252, $t = 2.9$, $df = 189$, $P = 0.004$). Those mating as sperm donors were not significantly different from the mean of the population (mean mass score = -0.02 ± 0.07 ; $t = -0.255$, $df = 189$, $P = 0.8$). The larger member of a mating pair was more likely to mate in the sperm recipient role as the difference between the body sizes of the mates increased (Fig. 2).

Animals mating in chains of three were significantly larger than the mean for the population (mean mass score = 0.32 ± 0.12 , $t = 2.6$, $df = 65$, $P = 0.01$). This is attributable to large individuals in the middle position, acting simultaneously as sperm recipients and sperm donors (mean mass score = 0.57 ± 0.21 , $t = 2.7$, $df = 21$, $P = 0.01$). Terminal sperm recipients and terminal sperm donors were not significantly different from average (recipient mean mass score = 0.34 ± 0.22 , $t = 1.5$, $df = 21$, $P = 0.1$; donor mean mass score = 0.05 ± 0.20 , $t = 0.3$, $df = 21$, $P = 0.8$). However, if animals in trios are ranked 1-3 for increasing relative body size, the terminal donor tends to be the smallest participant (mean rank 1.68), the middle donor/recipient the largest (mean rank 2.45), and the terminal recipient of intermediate size (mean rank 1.86) (Table 2). These rankings are significantly similar across the 22 trios (Kendall's coefficient of concordance, $\chi^2 = 7.18$, $P < 0.05$).

DISCUSSION

Our data on tagged animals show that individual *Aplysia vaccaria* mate in different roles (sperm donor, sperm recipient and both donor and recipient at the same time), on different occasions, and with different partners. They mate over a wide range of body sizes and therefore throughout a large portion of their lives. This data therefore adds to the growing body of evidence that *Aplysia* mates promiscuously. This is the first study on mating patterns in *A. vaccaria*, although sever-

al congeners have been shown to mate multiply in both sexual roles (e.g. LEDE-RHENDLER & TOBACH 1977, SUSSWEIN et al. 1984, SWITZER-DUNLAP et al. 1984, PEN-NINGS 1991, YUSA 1996).

The predominance of large sperm recipients can be explained by large individuals mating more frequently and/or for longer durations in the sperm recipient role. While we were unable to distinguish between these two possibilities with our sampling methods, YUSA (1996) found a similar result in the congener, *Aplysia kuro-dai*, that was due to both processes: larger animals mated as sperm recipients more often and for longer periods of time. Regardless of the mechanism, the outcome is the same; large animals spend proportionally more time mating as sperm recipients than small animals.

There are two alternative behavioral explanations for such a body size effect: (1) All individuals prefer to act in the female role, resulting in conflict between mates, and our finding is a reflection of dominance by the larger member of each pair. LEONARD & LUKOWIAK (1985, 1991) predicted that the female role should be preferred by hermaphrodites with organs for storing and/or digesting sperm because such structures allow for female control over fertilization. CHARNOV'S (1996) model also predicted greater resource allocations to female function in sperm-storing hermaphrodites. (2) Allocation to female function increases with body size because larger animals have an increased preference for mating in the female role or are more likely to be chosen as sperm recipients for their greater fecundity; egg production is consistently correlated with body size in gastropods (KANDEL & CAPO 1979, SWITZER-DUNLAP & HADFIELD 1979, TOMIYAMA & MIYASHITA 1992, ERLANDSSON & JOHANNESSON 1994, YUSA 1994). This is related to GHISELIN'S size advantage model for sequential hermaphrodites (GHISELIN 1969, DEWITT 1996) and is a likely outcome of extending CHARNOV'S (1996) model to include body size. Unlike the first explanation this one does not necessarily result in conflict between mates over role assignment. This explanation may involve a complicated decision process in which an individual's body size influences the mating role it prefers, which in turn affects the body size of the mate it chooses.

The finding that the larger member of a pair is more likely to take the recipient role as differences between partner body sizes increase (see Fig. 2) again suggests alternative explanations. It may be that the probability of mating as a sperm recipient increases with *absolute* body size. As body size increases, the size difference between mates also increases because the largest animals are likely to mate with animals closer to the mean. Hence the increasing bias toward large individuals mating as recipients when size differences are great could be an artifact of the effect of increasing absolute size. Alternatively, perhaps pairs use *relative* body size to determine mating roles with the larger preferentially being assigned the female role. The weaker role bias for pairs with smaller size differences may thus reflect an increasing difficulty of assessing relative size when differences are less than two standard deviations. Size assessment in these animals may not be very accurate. They have rudimentary eyes, no sound communication, and both touch and olfactory assessments could be challenging under the conditions in which mating occurs. Poor assessment abilities may also explain why we see a trend towards size-assortative mating, but no significant effects despite large sample sizes.

Both lab and field studies of other hermaphroditic gastropods have similarly shown that sperm recipients tend to be larger than sperm donors (OTSUKA et al. 1980, DEWITT 1996, YUSA 1996). It has been suggested that this is evidence of sexual selection on female function for large body size (YUSA 1996). In many dioecious

species heterogeneous body size is an outcome of sexual selection (e.g. sexual dimorphism or male polymorphism). However, in invertebrates such as opisthobranchs an early onset of reproduction and continued growth until the end of life generates mating populations with very heterogeneous size distributions independent of sexual selection. It remains possible that sexual selection has favored indeterminate growth or acts on growth rates in these animals and is thus affecting body size indirectly.

The size assortative aggregation patterns and the trend toward size assortative mating are likely related to each other. However, it is unclear which is the cause and which is the effect. Animals may seek out similarly-sized mates and remain adjacent to them for a period of time, resulting in nearest neighbors which are similar in size. Alternatively, they may aggregate size-assortatively for reasons not related to mating decisions, and may be subsequently more likely to mate with adjacent animals. For instance, local residency coupled with differential growth rates could explain a size assortative spatial pattern which could then lead to a size-assortative mating pattern. However, individuals have large enough home ranges relative to the distances between adjacent clusters of animals (ANGELONI et al. 1999) that these patterns are not explained by local residency.

Size-assortative mating appears to be common in gastropods (CROZIER 1917, 1918; EDWARDS 1968; ERLANDSSON & JOHANNESSON 1994; STAUB & RIBI 1995; TOMIYAMA 1996; YUSA 1996). It is usually argued that size-assortative mating should be observed in simultaneous hermaphrodites when fecundity, or female function, is related to body size and one of the following conditions is met: (1) pairs reciprocally inseminate each other, so that it is in the interest of both partners to mate with a large individual; or (2) large animals are competitively better at inseminating large recipients than small animals (RIDLEY 1983). The first condition is not met in this system; we rarely observed reciprocal inseminations and they are not common in the genus *Aplysia* (CAREFOOT 1987, PENNINGS 1991). Laboratory experiments are required to determine whether the second condition is true for this species.

The interplay between size-dependent role assignment and size-assortative mating is complicated and requires further modeling. Clearly, this is a system in which careful lab experiments may be required both to quantify size assessment abilities and to identify which party enforces the observed size dependencies in role assignment.

ACKNOWLEDGMENTS

For help in the field we thank Ian Billick, Alexis Chaine, Dahlia Chazan, Marc Dantzer, Catherine deRivera, Karen Fear, Paul Griffin, Darren Irwin, Laura Molles, Chris Nagy, Helen Neville, Jonathan Richmond, Amy Ritter, and Sukamol Srikwan. Kevin Crooks, Josh Kohn and Trevor Price provided valuable comments on the manuscript.

REFERENCES

- ADAMS D.C. & ANTHONY C.D. 1996. Using randomization techniques to analyse behavioural data. *Animal Behaviour* 51: 733-738.
- ANGELONI L., BRADBURY J. & CHAINE A. 1999. Growth, seasonality and dispersion of a population of *Aplysia vaccaria* Winkler, 1955. *The Veliger* 42: 1-9.

- BAUR B. 1992. Random mating by size in the simultaneously hermaphroditic land snail *Arianta arbustorum*: experiments and an explanation. *Animal Behaviour* 43: 511-518.
- CAREFOOT T.H. 1987. *Aplysia*: it's biology and ecology. *Annual Review of Oceanography and Marine Biology* 25: 167-284.
- CHARNOV E. 1996. Sperm competition and sex allocation in simultaneous hermaphrodites. *Evolutionary Ecology* 10: 457-462.
- CROZIER W.J. 1917. Evidence of assortative mating in a nudibranch. *Proceedings of the National Academy of Sciences of the USA* 3: 519-522.
- CROZIER W.J. 1918. Assortative mating in a nudibranch, *Chromodoris zebra* Heilprin. *Journal of Experimental Zoology* 47: 247-292.
- DEWITT T.J. 1996. Gender contests in a simultaneous hermaphrodite snail: a size advantage model for behaviour. *Animal Behaviour* 51: 345-351.
- EDWARDS D.C. 1968. Reproduction in *Olivella biplicata*. *The Veliger* 10: 297-304.
- ERLANDSSON J. & JOHANNESSON K. 1994. Sexual selection on female size in a marine snail, *Littorina littorea* (L.). *Journal of Experimental Marine Biology and Ecology* 181: 145-157.
- GHISELIN M.T. 1969. The evolution of hermaphroditism among animals. *The Quarterly Review of Biology* 44: 189-208.
- KANDEL P. & CAPO T.R. 1979. The packaging of ova in the egg cases of *Aplysia californica*. *The Veliger* 22: 194-198.
- LEDERHENDLER I.I. & TOBACH E. 1977. Reproductive roles in the simultaneous hermaphrodite *Aplysia dactylomela*. *Nature* 270: 238-239.
- LEONARD J.L. 1990. The hermaphrodite's dilemma. *Journal of Theoretical Biology* 147: 361-372.
- LEONARD J.L. & LUKOWIAK K. 1985. Courtship, copulation, and sperm trading in the sea slug, *Navanax inermis* (Opisthobranchia: Cephalaspidea). *Canadian Journal of Zoology* 63: 2719-2729.
- LEONARD J.L. & LUKOWIAK K. 1991. Sex and the simultaneous hermaphrodite: testing models of male-female conflict in a sea slug, *Navanax inermis* (Opisthobranchia). *Animal Behaviour* 41: 255-266.
- MORGAN M.T. 1994. Models of sexual selection in hermaphrodites, especially plants. *The American Naturalist* 144: S100-S125.
- OTSUKA C., ROUGER Y. & TOBACH E. 1980. A possible relationship between size and reproductive behavior in a population of *Aplysia punctata* Cuvier, 1803. *The Veliger* 23: 159-162.
- PENNINGS S.C. 1991. Reproductive behavior of *Aplysia californica* Cooper: diel patterns, sexual roles and mating aggregations. *Journal of Experimental Marine Biology and Ecology* 149: 249-266.
- PETERS A. & MICHIELS N.K. 1996. Do simultaneous hermaphrodites choose their mates? Effects of body size in a planarian flatworm. *Freshwater Biology* 36: 623-630.
- RIDLEY M. 1983. The explanation of organic diversity: the comparative method and adaptations for mating. *Oxford: Clarendon Press*.
- STAUB R. & RIBI G. 1995. Size-assortative mating in a natural population of *Viviparus ater* (Gastropoda: Prosobranchia) in Lake Zurich, Switzerland. *Journal of Molluscan Studies* 61: 237-247.
- SUSSWEIN A.J., GEV S., ACHITUV Y. & MARKOVICH S. 1984. Behavioral patterns of *Aplysia fasciata* along the Mediterranean coast of Israel. *Behavioral and Neural Biology* 41: 7-22.
- SWITZER-DUNLAP M. & HADFIELD M.G. 1979. Reproductive patterns of Hawaiian aplysiid gastropods, pp. 199-210. In: Stancyk S.E., Edit. *Reproductive ecology of marine invertebrates. Columbia: University of South Carolina Press*.
- SWITZER-DUNLAP M., MEYERS-SCHULTE K. & GARDNER E.A. 1984. The effect of size, age, and recent egg laying on copulatory choice of the hermaphroditic mollusc *Aplysia juliana*. *International Journal of Invertebrate Reproduction and Development* 7: 217-225.
- TOMIYAMA K. 1996. Mate-choice criteria in a protandrous simultaneously hermaphroditic land snail *Achatina fulica* (Ferrusac) (Stylommatophora: Achatinidae). *Journal of Molluscan Studies* 62: 101-111.
- TOMIYAMA K. & MIYASHITA K. 1992. Variation of egg clutches in the giant African snail, *Achatina fulica* (Ferrusac) (Stylommatophora: Achatinidae) in Ogasawara Islands. *Venus* 51: 293-301.

- VREYS C. & MICHIELS N.K. 1997. Flatworms flatten to size up each other. *Proceedings of the Royal Society of London (B)* 264: 1559-1564.
- YUSA Y. 1994. Size-related egg production in a simultaneous hermaphrodite, the sea hare *Aplysia kurodai* Baba (Mollusca: Opisthobranchia). *Publications of the Seto Marine Biological Laboratory* 36: 249-254.
- YUSA Y. 1996. The effects of body size on mating features in a field population of the hermaphroditic sea hare *Aplysia kurodai* Baba, 1937 (Gastropoda, Opisthobranchia). *Journal of Molluscan Studies* 62: 381-386.
- ZAFERES A., SKOLNICK A. & TOBACH E. 1988. Interindividual contact and copulation in *Aplysia dactylomela*. *Marine Behaviour and Physiology* 13: 221-238.