Flatworms flatten to size up each other

C. VREYS¹* AND N. K. MICHIELS²

¹Zoology Research Group, Limburgs Universitair Centrum, B-3590 Diepenbeek, Belgium (cvreys@luc.ac.be)

²Max-Planck-Institut für Verhaltensphysiologie, PO Box 1564, D-82305 Seewiesen (Starnberg), Germany

SUMMARY

When copulations are costly, fertilization is reciprocal and fecundity is positively related to size, hermaphrodites are expected to favour large partners, leading to size-assortative mating. Size-related mate choice has, however, never been observed in hermaphrodites. In the flatworm *Dugesia gonocephala* copulations cost time and are reciprocal, and size is a positive predictor of female fecundity. Every copulation is preceded by a phase in which one partner glides on top of the other and both spread out and flatten, suggesting that partners assess each other's size. A total of 124 copulating pairs collected on four different dates, proved that mating is size-assorted in the field. In experiments with groups, more copulations took place between equally sized individuals than were expected when matings were random. In experiments with pairs, partners of different size exhibited twice as many mating attempts for the first copulation than did partners of the same size. We conclude that *D. gonocephala* employs a unique kind of pre-copulatory 'flattening' behaviour as a mechanism to signal as well as to assess relative size. This does not only confirm that hermaphrodites can mate assortatively when certain assumptions are met, it also proves that even lower invertebrates can show active mate choice.

1. INTRODUCTION

Darwin wrote that sexual selection is absent from true hermaphrodites because of the 'union of sexes' and because, as with all lower invertebrates, 'these animals have much too low mental powers to feel mutual rivalry or to appreciate each other's beauty or other attractions' (Darwin 1871). However, in species with this mode of gender expression male and female genitalia can be complex and diverse and mating behaviour is often elaborate (Hyman 1951; Eberhard 1985), suggesting that sexual selection is at work (Charnov 1979; Michiels 1997). Mating conflicts are known to have resulted in egg trading in external fertilizers like serranid fishes (Fischer 1984, 1987; Petersen 1995), and polychaetes (Sella 1985, 1988, 1990; Sella *et al.* 1997), whereas sperm are traded in internal fertilizers like the sea slug *Navanax inermis* (Leonard & Lukowiak 1984, 1985, 1991), and the planarian *Dugesia polychroa* (Michiels & Streng 1997). Sperm trading may offer one explanation for the often complex mating behaviour seen in e.g. terrestrial snails, which is assumed to assure the synchronous and reciprocal exchange of sperm (Lind 1976; Chung 1986; Adamo & Chase 1988; Reise 1995). What is unresolved, however, is whether and how hermaphrodites choose a mate among other hermaphrodites before even attempting to copulate. Note that this question differs from whether hermaphrodites prefer other hermaphrodites over conspecific males, as shown by Sella (1988) and Sella *et al.* (1997).

If we assume that the female function of a hermaphrodite rarely runs short of sperm (but see Levitan 1996), and that the benefits of receiving multiple ejaculates are limited, one could expect hermaphrodites to mate mainly in order to inseminate their partner rather than to receive allosperm (Charnov 1979). This view stems from gonochorists, where females usually limit male mating opportunities and, as a result, males rarely refuse to donate sperm when given an opportunity (Andersson 1994). In hermaphrodites, however, mating opportunities are not limited in this way because, since most members of the population may be willing to donate, all may readily copulate and accept sperm in order to have a chance to give sperm. Hence, a donor may not be limited by the number of mating partners, but by its own capacity to inseminate them. Because of sex allocation considerations, it may therefore be the sperm donor who becomes choosy about who to donate sperm to. Mate choice may therefore evolve when there is variation in the fecundity of the receiver, and when copulations are costly and the availability of alternative, receptive conspecifics is high.

In many hermaphrodites large individuals produce more eggs (Crozier 1918; Wolda 1963; Baur 1988; Baur & Raboud 1988; Yusa 1994) and copulations are costly (Lind 1976, 1988). As many species also occur at high densities, a preference for larger partners may evolve. Since sperm donation is usually reciprocal, small individuals may, however, be rejected by partners that are bigger than themselves, resulting in size-assortative mating (Ridley 1983). Size-assortative mating, however, has only been found in two cases. In

*Author for correspondence.

the first, however, it was due to the physical incompatibility of partners that differ significantly in size (Crozier 1918), whereas in the second it was a consequence of age-dependent mate choice (Tomiyama 1996). It has not been found in others (Baur 1992; Peters & Michiels 1996), nor has anyone to our knowledge described pre-copulatory behaviour in hermaphrodites that is suggestive of this kind of mate choice.

We investigated the pre-copulatory behaviour of the planarian *Dugesia gonocephala* Dugès 1830 (Platyhelminthes, Tricladida), a simultaneous hermaphrodite with reciprocal insemination and size-related female fecundity (Vreys & Michiels 1995). Selfing does not occur (Vreys *et al.* 1994). A unique kind of pre-copulatory behaviour, suggestive of 'partner sizing', precedes every copulation (Vreys *et al.* 1997a) (figure 1 *a,b,c*). When touched by a conspecific, receptive individuals spread and flatten in response. If the touching animal is receptive as well, it will begin to move its head sideways over the back of its partner (figure 1*a*). Subsequently, the touching individual may glide onto the back of its partner, resulting in a characteristic 'sandwich posture' in which one partner lies on top of the other (figure 1*b*). In this position, contact between the ventrally situated gonopores is not possible. If the sequence continues, both partners will flatten to more than double their (unflattened) surface area. Genital contact is established when the lower individual raises its caudal end (figure 1*c*). Copulation (figure 1*d*) lasts for 4.5 ± 2.2 h (range 0.2–10.6 h, $n = 111$). Penises are mutually inserted and spermatophores are predominantly transferred in both directions. Every copulation is preceded by this whole sequence, but most sequences are interrupted and end without copulation. Unreceptive individuals never flatten upon being touched, nor do they lie on top of each other. See Vreys *et al.* (1997a) for a detailed description.

To test whether the outcome of this behaviour indeed depends on the relative size of both partners and whether it results in size-assortative mating, three data sets were collected. First, we measured mating and non-mating animals collected in the field to look for size-assortative mating under natural conditions. Second, we determined the frequency of homogamic (size-assorted) and heterogamic (not size-assorted) matings in size-discrimination experiments in the laboratory. Finally, we looked at the outcome of mating attempts in homogamic and heterogamic pairs.

2. MATERIALS AND METHODS

(a) Size-assortative mating in the field

D. gonocephala gathers under stones in small streams throughout continental Europe. A total of 124 copulating pairs were collected from a sexual population of *D. gonocephala* in the Cottesserbeek near Cottessen, southeast Netherlands (50°7'N, 5°9'E) on 5 May ($n = 23$ pairs), 24 May ($n = 16$ pairs), 24 June ($n = 30$ pairs) 1995, and 5 June 1996 ($n = 55$ pairs). Stones were examined between 06:00 and 10:30 h. Pairs were removed using a soft paintbrush and immediately fixed in the field using a mixture of acetic acid and ethanol in

mean number of attempts until first copulation

Figure 1. Mating sequence of *Dugesia gonocephala*. (a) Receptive, touched individual flattens upon contact, whereas touching animal waves head sideways over partner's back. (b) Touching individual glides onto back of partner and both individuals flatten. (c) Lower individual raises posterior end and genital contact is established. (d) Copulation with simultaneous, reciprocal spermatophore transfer. The number of mating attempts until the first copulation was registered in 12 homogamic and seven heterogamic pairs. Percentages at each phase represent interruptions observed at this stage for homogamic (left) and heterogamic (right) pairs.

the ratio 1:3, respectively. In the laboratory they were treated with 5N HCl, Schiff's reagent and 45% acetic acid (Romeis 1968). Each individual was measured using an image analysis system (MicroScale TM/TC; Digithurst LTD) coupled to a video camera (Sony SSC-M370CE) with a macro lens. Due to their flat body shape (in live and fixed condition), body size can be conveniently expressed as dorsal surface area (mm²). Measurements of fixed individuals were converted to live size using a linear regression model (live size = $5.13 + 0.27 \times \text{size after fixation}$, $n = 37$, $r^2 = 0.94$, $p < 0.001$).

On 24 June 1995, we also collected all 192 non-mating animals from the 24 stones with copulating pairs and another 300 individuals from 19 randomly

examined stones without copulating pairs. Since the natural population (discontinuously) inhabits only about 200 m of the stream and is therefore not very big (but note that density may yield up to 300 animals m⁻²), non-mating animals were not killed but measured alive within three days after sampling using the set-up described above, and most were returned to the field on 28 June 1995. For the measurement, they were allowed to glide in a water-filled Petri-dish (diameter 11 cm), and for each individual the average of three separate measurements was used (6–7% variation among measurements). Since a considerable number of them were small, and presumably immature, 30 individuals between 8.8 and 26.0 mm² in length ($\bar{x} = 16.4 \pm 4.8$ mm²) were killed and checked for the presence of developed reproductive organs. Since the smallest adult measured 19.4 mm², all animals of length <19.4 mm² ($n = 193$) were considered immature and excluded from further analysis.

(b) Laboratory experiments

Live animals were collected in the Cottesserbeek and transported to the laboratory in plastic pots filled with stream water. Culture room temperature was maintained at $14 \pm 1^\circ\text{C}$ and the light set at 12L:12D (day starting at 07:00 h). Prior to the experiment, animals were acclimatized for one week in a group ($n = 100$ individuals) in a 48 l (80×60×10 cm) tank filled with filtered, aerated stream water. They were fed once with punctured, fresh *Chironomus* larvae, and once with frozen crayfish. Individuals were subsequently measured as outlined before and isolated for two days in 200 ml brown, plastic pots placed in a similar 48 l tank. A hole (diameter 34 mm) in opposite sides of each pot and covered with 0.25 mm² mesh ensured water exchange.

Observation chambers were made from transparent Perspex. A 2 mm lid was placed on top of a 4 mm thick bottom plate that contained one or more holes covered with 0.25 mm² mesh on the underside. Arena 1 contained one central chamber of 10×8×0.4 cm and was designed for group experiments. Arena 2 was subdivided into 12 chambers of 3×3×0.4 cm and was used for experiments with pairs. Arenas were suspended in a 24 l (60×40×10 cm) tank with the lid upward. The water of the observation tank was circulated through an additional 50 l buffer tank that was placed in a cooling chamber (14 °C). Two dim, white lamps (15 W) provided daylight (12L:12D, day starting at 07:00 am), whereas a red lamp used for dark-rooms illuminated the set-up during the night. Planarian sensitivity for red is negligible (Brown *et al.* 1968). Recordings were made using a sensitive, black-and-white video camera (Sony SSC-M370CE) with macro lens, coupled to a time-lapse video recorder (Mitsubishi HS-S 5600 E).

To allow interactions and choice based upon sequential encounters, two groups, each containing three small and three large individuals (50% size difference between dorsal surface of small and large individuals (1:1.5)), were monitored continuously for 17 d (from 16 July to 7 August and from 16 August to 2 September

1995, respectively). The number of homogamic and heterogamic copulations was recorded.

To examine pre-copulatory behaviour and the likelihood of matings when relative partner size is equal or different, six homogamic and six heterogamic pairs were video-recorded simultaneously on three different occasions (from 15 to 22 June, from 23 June to 5 July and from 6 to 16 July 1995, respectively). This resulted in a total of 18 homogamic (maximum size difference 1:1.1) and 18 heterogamic pairs (size difference 1:1.4–1:1.6). The relative size difference in heterogamic pairs was smaller than the maximum observed in the field (1:1.8, see §3). There was no overall absolute size difference between the two pair types (Mann–Whitney $U = 535$, $n = 18$, n.s.). In three homogamic and five heterogamic pairs one of the partners escaped or died in the course of the experiment. These pairs were excluded from the analyses. For the remaining pairs the number of pre-mating attempts until the first copulation was counted and it was noted for each attempt at which stage the mating sequence was terminated.

(c) Statistical analysis: how to correlate two hermaphrodites?

A product-moment correlation coefficient (r_p) cannot be used to correlate characteristics of two mating hermaphrodites since an *a priori* assignment of either partner to a specific axis is not possible. One solution is to calculate r_p after randomly assigning each partner to either axis. When repeated at least 100 times, the resulting averaged r_p can be used as a measure for the degree of association between two hermaphroditic partners (Baur 1992). However, this procedure provides value estimates and standard errors that will vary when the analysis is repeated. We propose therefore to calculate the intraclass correlation coefficient r_i (Donner 1986; Sokal & Rohlf 1995). First, a one-way ANOVA is calculated with pair-number as the classification factor. Second, r_i can be calculated as the proportion of the total variance explained by the variance between groups:

$$r_i = \frac{s_A^2}{s^2 + s_A^2},$$

$$\text{whereby } s_A^2 = \frac{MSB - MSW}{2} \text{ and } s^2 = MSW.$$

The values MSW (mean squares within) and MSB (mean squares between) are provided by the ANOVA table of most statistical packages. The p value is given by the probability of the F statistic. Note that r_i is a proportion that varies from 0 to 1. Although it is a measure of association within groups, it is not equivalent to the product-moment correlation coefficient.

All statistical tests were performed with Statistica (v.4.1) for Macintosh and SPSS (v.6.1.2) for Windows. The latter provides a t -test that corrects for unequal variances in the two groups that are compared (t_u in the results). This procedure reduces the degrees of freedom. Means are given with standard deviation.

Figure 2. Example of the relationship between the body sizes of two partners in a mating pair. Since axis-assignment is arbitrary for both partners, this graph is only a random selection of one of the many possible graphs that can be made and therefore statistical testing of this particular scatter plot is not meaningful (see §2). The sample shown is that of 24 June 1995 ($n = 30$ pairs). One pair showed a size difference of 1:1.8 (indicated with an arrow). This is the largest relative size difference seen in the field.

3. RESULTS

(a) Assortative mating in the field

In each of the four field samples, large individuals had large partners whereas small mated with small, demonstrating that mating is size-assortative (5 May 1995: $n = 23$ pairs, $r_1 = 0.40$, $p = 0.02$; 24 May 1995: $n = 16$ pairs, $r_1 = 0.67$, $p = 0.001$; 24 June 1995 (see figure 2): $n = 30$ pairs, $r_1 = 0.47$, $p = 0.003$; 5 June 1996: $n = 55$ pairs, $r_1 = 0.60$, $p < 0.001$). The largest size difference within a pair was $20.3 : 36.2 \text{ mm}^2$ (1:1.8) (figure 2). There was no difference in average body size when compared between stones (24 June sample, mating and non-mating animals combined; ANOVA, $F_{33,3} = 1.15$, n.s.); thus size-assortative mating did not result from different size-classes occurring on different stones. Compared to the average size of non-mating individuals ($n = 299$, $\bar{x} = 30.5 \pm 6.7 \text{ mm}^2$), mating animals ($n = 60$, $\bar{x} = 28.1 \pm 4.5 \text{ mm}^2$) were smaller ($t_u = 3.4$, d.f. = 117, $p = 0.001$) and also significantly less variable (Levene's $F_1 = 13.6$, $p < 0.001$). This statistical difference largely resulted from the fact that large individuals ($> 36.2 \text{ mm}^2$) were absent from the sample of mating pairs (figure 3). Large, non-mating individuals mated normally in the laboratory in further experiments.

(b) Matings in groups

When matings are random among three large and three small individuals, a ratio of 3:2 heterogamic:

Figure 3. Size distribution of mating ($n = 60$) and non-mating ($n = 299$) *Dugesia gonocephala* collected on 24 June 1995. Animals less than $< 19.4 \text{ mm}^2$ were excluded because they were defined to be immature. The x-axis labels indicate the midpoint of each 5 mm^2 interval.

homogamic (respectively) copulations is expected. We observed 1:3:2 small/large:small/small:large/large copulations in the first run and 3:2:2 small/large:small/small:large/large copulations in the second run. This results in 1:5 and 3:4 heterogamic:homogamic copulations in groups one and two, respectively, which is significantly different from the random expectation for group one (Binomial $p = 0.041$), but not for group two ($p = 0.29$), despite a similar trend ($p = 0.064$, probabilities combined (Sokal & Rohlf 1995)).

(c) Mating sequences in homogamic and heterogamic pairs

Twelve out of 15 homogamic pairs displayed pre-copulatory behaviour and subsequent copulation. Of the 13 heterogamic pairs, ten showed pre-copulatory behaviour, but only seven copulated (figure 1). The number of pairs that did copulate did not differ between the two pair types ($\chi^2 = 2.18$, d.f. = 1, n.s.). Heterogamic pairs showed, however, about twice as many mating attempts for the first copulation than did homogamic pairs (Mann-Whitney $U = 18$, $n_1 = 7$, $n_2 = 12$, $p = 0.037$, figure 1). Three heterogamic pairs which did not copulate, despite an average of 9.6 mating attempts, were excluded, thus the previous result is a conservative estimate. That most attempts were terminated in phases *a* and *b* (figure 1) further indicates that failures are not because of size differences in genitalia, because such effects can only occur in phase *c*.

4. DISCUSSION

As predicted for a simultaneous hermaphrodite with multiple mating opportunities, long matings and size-related female fecundity (Ridley 1983), mating is size-assortative in the hermaphroditic flatworm *Dugesia gonocephala*. All field samples showed a significant positive size-correlation between the partners of copulating pairs. Furthermore, mate-choice experiments in the laboratory showed that animals were able to discriminate between partners of different size and that equally sized individuals were more likely to mate. Although sample sizes were small due to the low

mating rate typical for this species, the observed effects differ strongly from those found in a similar experiment in the related *D. polychroa* where matings are more frequent (Peters *et al.* 1996) and size-assortative mating is absent (Peters & Michiels 1996).

Despite the fact that assortative mating by size is one of the most common mating patterns in natural populations of gonochorists (Ridley 1983), its underlying mechanism is often not well understood. Our experiments provide strong indications that size-assortative mating in *D. gonocephala* is the result of size assessment during courtship. Heterogamic pairs exhibited about twice as many mating attempts for the first copulation than did homogamic pairs. Most attempts were terminated before the gonopores were brought into juxtaposition, showing that problems in achieving genital contact were not the main cause of discontinued attempts. In fact, most attempts were terminated at a stage during which one partner glides on top of the other and first the lower, then both individuals, spread out and flatten (figure 1a and b, respectively). Planarians eyes lack a refractive apparatus which would enable them to form images (Hyman 1951; Smales & Blankespoor 1978). Therefore, intense body contact may be needed to estimate the size of a potential mate. The fact that partners separate after each unsuccessful attempt and that the time between two successive mating attempts can be very prolonged (Vreys *et al.* 1997a) will increase the likelihood of size-assortative mating when several receptive partners are available. Overall, our results strongly suggest that *D. gonocephala* is the first simultaneous hermaphrodite (and lower invertebrate) known to show true size-assortative mating on the basis of active size assessment.

It is clear that if hermaphrodites assess each other and make mating decisions accordingly, sexual selection is at work. The interesting difference with gonochorists is that the situation is symmetrical. In *D. gonocephala*, one behaviour (flattening) allows both mating partners to signal their own size and to assess the relative size of their partner. Cheating is impossible as a small animal cannot pretend to be big. The evolution of 'sizing' seems straightforward. As soon as a preference for larger partners has evolved (for the reasons suggested by Ridley (1983)), it is obvious that a partner that is being assessed and that responds by flattening is favoured by selection since it will appear bigger, therefore increasing its mating chances. Although one could regard flattening as a dishonest signal by the first individual that employed it, the traits 'flattening' and 'size-preference' will be linked immediately and spread through the population, making 'flattened body size' an honest signal.

Assuming that each individual prefers a partner that is equal or larger in size, large animals will have a lower mating rate because they reject most conspecifics. This may explain the surplus of unmated, large animals in the field population. Note that although this may negatively affect male reproductive success (RS), allo-sperm limitation in the female function is highly unlikely in *D. gonocephala* because of long-term sperm storage (Vreys *et al.* 1994). Moreover, large animals still have the benefit of having the highest female RS. All

this suggests that sexual selection will favour an average body size for a maximal male RS, whereas natural selection will favour large body size for a maximal female RS.

An unanswered question is why size-assortative mating is present in *D. gonocephala* while it is absent in other species that also meet the conditions put forward by Ridley (1983). Baur (1992) suggested that, in agreement with Parker's (1983) model for indiscriminate mate choice, time-constraints on activity and high searching costs explain the prevalence of random mating by size in the land snail *Arianta arbustorum*. In *D. polychroa*, mating activity is mainly limited to the night as well (Peters *et al.* 1996), but this is also true for *D. gonocephala* (Vreys *et al.* 1997a). Second, because planarians are known to aggregate under stones (Reynierse & Ellis 1967) and densities are often high, the cost of mate searching can be considered to be low for both *Dugesia* species. Furthermore, indiscriminate mating in *D. polychroa* was not caused by limited variance in mate quality: a 1.5-fold size difference between individuals does not affect the likelihood of copulation, despite the associated 40% lower cocoon production in the smallest partner (Peters & Michiels 1996). Hence, Parker's (1983) model, cannot explain the difference between the mating patterns of *D. polychroa* and *D. gonocephala*. More relevant differences may be those associated with the copulation itself. Copulations do not only last longer in *D. gonocephala* (12 min to 10.5 h versus 5 min to 2.5 h in *D. polychroa*), they also take place at a three times lower frequency (Peters *et al.* 1996; Vreys *et al.* 1997a), probably because *D. gonocephala* needs at least one day to prepare a new spermatophore (Vreys *et al.* 1997b), whereas *D. polychroa* transfers free sperm and can do so for maximally three times in one day (Peters *et al.* 1996). These differences may make mating in *D. gonocephala* more costly in terms of time investment and future mating opportunities.

We thank all the participants to the European Science Foundation workshop on the evolution of hermaphrodite mating systems in Bernried (October 1995), and Bruno Baur in particular, for their very stimulating discussions. We are grateful to Bruno Baur, Leo Beukeboom, Geert Molenbergs, Tim Sharbel, Rolf Weinzierl and Nikki Watson for their suggestions for improvement of the manuscript. C.V. was supported by a Research Assistantship of the Flemish Institute for Encouraging Scientific-Technological Research in Industry and by the Fund of Collective Fundamental Research (project G.0094.92).

REFERENCES

- Adamo, S. A. & Chase, R. 1988 Courtship and copulation in the terrestrial snail *Helix aspersa*. *Can. J. Zool.* **66**, 1446–1453.
- Andersson, M. 1994 *Sexual selection. Monographs in behavior and ecology*. Princeton University Press.
- Baur, B. 1988 Population regulation in the land snail *Arianta arbustorum*: density effects on adult size, clutch size and incidence of egg cannibalism. *Oecologia* **77**, 390–394.
- Baur, B. 1992 Random mating by size in the simultaneously hermaphroditic land snail *Arianta arbustorum*: experiments and an explanation. *Anim. Behav.* **43**, 511–518.
- Baur, B. & Raboud, C. 1988 Life history of the land snail *Arianta arbustorum* along an altitudinal gradient. *J. Anim. Ecol.* **57**, 71–87.

- Brown, H. M., Ito, H. & Ogden, T. E. 1968 Spectral sensitivity of the planarian ocellus. *J. Gen. Physiol.* **51**, 255–260.
- Charnov, E. L. 1979 Simultaneous hermaphroditism and sexual selection. *Proc. Natn. Acad. Sci. USA* **76**, 2480–2484.
- Chung, D. J. D. 1986 Stimulation of genital eversion in the land snail *Helix aspersa* by extracts of the glands of the dart apparatus. *J. Exp. Zool.* **238**, 129–139.
- Crozier, W. J. 1918 Assortative mating in a nudibranch, *Chromodoris zebra*. *J. Exp. Zool.* **27**, 247–292.
- Darwin, C. 1871 *The descent of man, and selection in relation to sex*. Photoreproduction 1981, Part II, p. 321. Princeton University Press.
- Donner, A. 1986 A review of inference procedures for the intraclass correlation coefficient in the one-way random effects model. *Int. Stat. Rev.* **54**, 67–82.
- Eberhard, W. G. 1985 *Sexual selection and animal genitalia*. Cambridge, MA: Harvard University Press.
- Fischer, E. A. 1984 Egg trading in the chalk bass, *Serranus tortugarum*, a simultaneous hermaphrodite. *Z. Tierpsychol.* **66**, 143–151.
- Fischer, E. A. 1987 Mating behavior in the black hamlet—gamete trading or egg trading. *Environ. Biol. Fish.* **18**, 143–148.
- Hyman, L. H. 1951 *The invertebrates. II. Platyhelminthes and Rhynchocoela*. New York: McGrawHill.
- Leonard, J. L. & Lukowiak, K. 1984 Male–female conflict in a simultaneous hermaphrodite resolved by sperm trading. *Am. Nat.* **124**, 282–286.
- Leonard, J. L. & Lukowiak, K. 1985 Courtship, copulation and sperm trading in the sea slug *Navanax inermis* (Opisthobranchia: Cephalaspidea). *Can. J. Zool.* **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). *Anim. Behav.* **41**, 255–266.
- Levitan, D. R. 1996 Effects of gamete traits on fertilisation in the sea and the evolution of sexual dimorphism. *Nature* **382**, 153–155.
- Lind, H. 1976 Causal and functional organization of the mating behaviour sequence in *Helix pomatia* (Pulmonata, Gastropoda). *Behaviour* **59**, 162–202.
- Lind, H. 1988 The behaviour of *Helix pomatia* L. (Gastropoda, Pulmonata) in a natural habitat. *Vidensk. Meddr. Dansk Naturh. Foren.* **147**, 67–92.
- Michiels, N. K. 1997 Mating conflicts and sperm competition in simultaneous hermaphrodites. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller). Academic Press. (In the press.)
- Michiels, N. K. & Streng, A. Reciprocity of insemination in a simultaneous hermaphrodite: is it conditional? *Behav. Ecol. Sociobiol.* (Submitted.)
- Parker, G. A. 1983 Mate quality and mating decisions. In *Mate choice* (ed. P. Bateson), pp. 141–164. Cambridge University Press.
- Peters, A. & Michiels, N. K. 1996 Do simultaneous hermaphrodites choose their mates? Effects of body size in a flatworm. *Freshwater Biol.* **36**, 623–630.
- Peters, A., Streng, A. & Michiels, N. K. 1996 Mating behaviour in a hermaphroditic flatworm with reciprocal insemination: do they assess their mates during copulation? *Ethology* **102**, 236–251.
- Petersen, C. W. 1995 Reproductive behavior, egg trading, and correlates of male mating success in the simultaneous hermaphrodite, *Serranus tabacarius*. *Environ. Biol. Fish.* **43**, 351–361.
- Reise, H. 1995 Mating behaviour of *Deroceras rodnae* Grossu & Lupu, 1965 and *D. praecox* Wiktor, 1966 (Pulmonata: Agriolimacidae). *J. Mollusc. Stud.* **61**, 325–330.
- Reynierse, J. H. & Ellis, R. R. 1967 Aggregation formation in three species of planaria: distance to nearest neighbour. *Nature* **214**, 895–896.
- Ridley, M. 1983 *The explanation of organic diversity*. Oxford: Clarendon Press.
- Romeis, B. 1968 *Mikroskopische Präparationstechnik*. München, Germany: Oldenburg Verlag.
- Sella, G. 1985 Reciprocal egg trading and brood care in a hermaphroditic polychaete worm. *Anim. Behav.* **33**, 938–944.
- Sella, G. 1988 Reciprocation, reproductive success, and safeguards against cheating in a hermaphroditic polychaete worm, *Ophryotrocha diadema* Akesson, 1976. *Biol. Bull.* **175**, 212–217.
- Sella, G. 1990 Sex allocation in the simultaneously hermaphroditic polychaete worm *Ophryotrocha diadema*. *Ecology* **71**, 27–32.
- Sella, G., Premoli, M. C. & Turri, F. 1997 Egg trading in the simultaneously hermaphroditic polychaete worm *Ophryotrocha gracilis* (Huth). *Behav. Ecol.* **8**, 83–86.
- Smales, L. R. & Blankespoor, H. D. 1978 The epidermis and sensory organs of *Dugesia tigrina* (Turbellaria: Tricladida). *Cell Tiss. Res.* **193**, 35–40.
- Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*. New York: W. H. Freeman & Co.
- Tomiyama, K. 1996 Mate-choice criteria in a protandrous simultaneously hermaphroditic land snail *Achatina fulica* (Férussac) (Stylommatophora: Achatinidae). *J. Mollusc. Stud.* **62**, 101–111.
- Vreys, C. & Michiels, N. K. 1995 The influence of body size on immediate reproductive success in *Dugesia gonocephala* (Tricladida, Paludicola). *Hydrobiologia* **305**, 113–117.
- Vreys, C., Michiels, N. K. & Schockaert, E. R. 1994 Evidence against self-fertilization in the hermaphroditic flatworm *Dugesia gonocephala* (Tricladida, Paludicola). *Neth. J. Zool.* **44**, 55–64.
- Vreys, C., Schockaert, E. R. & Michiels, N. K. 1997a Unusual pre-copulatory behaviour in the hermaphroditic planarian flatworm, *Dugesia gonocephala* (Tricladida, Paludicola). *Ethology* **103**, 208–221.
- Vreys, C., Schockaert, E. R. & Michiels, N. K. 1997b Formation, transfer and assimilation of the spermatophore of the hermaphroditic flatworm *Dugesia gonocephala* (Tricladida, Paludicola). *Can. J. Zool.* **75**, 1479–1486.
- Wolda, H. 1963 Natural populations of the polymorphic land snail *Cepea nemoralis* (L.). *Arch. Néerl. Zool.* **15**, 381–471.
- Yusa, Y. 1994 Size-related egg production in a simultaneous hermaphrodite, the sea hare *Aplysia kurodai* Baba (Mollusca: Opisthobranchia). *Publ. Seto Mar. Biol. Lab.* **36**, 249–254.

Received 1 July 1997; accepted 28 July 1997