

Altruistic sperm donation in a sperm-dependent parthenogenetic hermaphrodite is stabilized by reciprocal sperm exchange

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Abstract

Sperm-dependent parthenogenetic animals require sperm from an ‘altruistic’ mate in order to trigger embryogenesis, but without genetic contribution from the sperm donor. The stability of sperm donation is puzzling, particularly in populations that consist of sperm-dependent parthenogenetic hermaphrodites. Here, selection should favour total reduction of the male function, which would lead to population extinction. We investigated mating behaviour in a population of the sperm-dependent parthenogenetic, hermaphroditic flatworm *Schmidtea polychroa*. In this species, parthenogens possess a fully functional male reproductive system. We predicted that individuals should trade sperm and, hence, only donate when receiving sperm from their partner. Results from 54 pairs indicate that conditional reciprocity is common, possibly even more so than in previously studied sexual conspecifics. In contrast to sexuals, however, parthenogens do not increase allocation to sperm with body size. Taken together, the data indicate that in order to receive sperm and ensure full maternal fertility, parthenogenetic *S. polychroa* must be able to produce and donate a minimum of self-sperm. As such, sperm trading contributes to the stability of apparently ‘altruistic’ sperm donation. This study is also the first convincing example of sperm trading in internally fertilizing hermaphrodites with a strong preference for the female role.

Key words: sexual conflict, altruism, sperm-dependent parthenogenesis, hermaphroditism, sperm trading planarian, Plathelminthes, *Schmidtea polychroa*

INTRODUCTION

A peculiar form of asexual reproduction is sperm-dependent parthenogenesis, also called gynogenesis or pseudogamy (reviewed by Beukeboom & Vrijenhoek, 1998). Sperm-dependent parthenogens require sperm from a sperm donor (male or hermaphrodite) in order to initiate the parthenogenetic development of their eggs. After gamete fusion the sperm's chromosomes degenerate within the egg's cytoplasm or are expelled from the zygote (but see Schartl *et al.*, 1995; Beukeboom *et al.*, 1996b, 1998). Mating systems like these are potentially unstable, since they depend on the altruistic donation of sperm by an individual who cannot gain paternity in return. In gonochoric systems (i.e. species with separate sexes), males can benefit from mating with parthenogenetic females because this can increase their attractiveness for sexual females (Schlupp, Larler & Ryan, 1994). However, the benefits of sperm donation are less clear in non-selfing

sperm-dependent hermaphrodites, living in purely parthenogenetic populations. In such systems, sperm donation appears always maladaptive and selection will favour a strong reduction in male allocation (Weinzierl *et al.*, 1998).

The freshwater planarian flatworm *Schmidtea (Dugesia) polychroa* consists of two main biotypes: diploid sexuals and polyploid parthenogens. Both forms share three important characteristics: (1) they are outcrossing, simultaneous hermaphrodites with internal fertilisation; (2) they require sperm from a partner to produce fertile eggs; (3) they produce haploid, fertile sperm, which is very unusual in the case of polyploid parthenogens. Parthenogens are sperm-dependent. Since selfing does not exist in this species, they require sperm from a conspecific to initiate egg development. All examined populations of *S. polychroa* in central and Western Europe are exclusively parthenogenetic (Beukeboom *et al.*, 1996a). In these populations sperm donors cannot gain fitness through the male function, so altruistic sperm donation should be selected against. Parthenogens indeed produce fewer sperm (Weinzierl *et al.*, 1998) and more cocoons (Weinzierl, Schmidt & Michiels, 1999) than sexual conspecifics. As predicted

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by Greeff & Michiels (1999) sperm investment is very high in sexuals, presumably as a consequence of sperm competition and sperm digestion, which are both known to be strong (N. Pongratz & A. Streng pers. comm.). Sexual individuals can compensate for this cost by insisting on reciprocal insemination and 'trade sperm'. We define sperm trading in a broad sense as any form of conditional reciprocity in insemination. This can take the form of conditional, reciprocal exchange of whole ejaculates and/or balanced exchange of equal amounts of sperm. Sperm trading is well documented in sexual planarians (Michiels & Streng, 1998; Vreys & Michiels, 1998; Michiels & Bakovski, 2000). By trading sperm, individuals receive a digestible ejaculate from the partner as a compensation for their own investment (Greeff & Michiels, 1999). This view differs from that of Leonard (1999) who proposes that it is the uncertainty of fertilization characteristic for the male function that explains the evolution of sperm trading in [sexual] hermaphrodites with internal fertilization. Although this seems unlikely for an outcrossing population in which paternity is as important as maternity (Greeff & Michiels, 1999), Leonard's theory does apply to sperm-dependent, parthenogenetic hermaphrodites. Here, an extreme asymmetry exists in the potential gain via either sex function and individuals can be expected to strongly prefer the female rather than the male sexual role.

Here, we address the question whether sperm-dependent parthenogens solve their sexual conflict by exchanging sperm conditionally. In order to test this, we investigated sperm exchange in *S. polychroa* from an exclusively sperm-dependent parthenogenetic population. For the reasons given above we expected them to be reluctant to donate sperm. Yet, sperm receipt is required for maternal fertility. A solution to this dilemma would be to conditionally exchange sperm. Here, we test whether sperm exchange in sperm-dependent parthenogens is conditional. We also included estimates of sperm production. The methods used in the present study are similar to those used in Michiels & Bakovski (2000) for sexual *S. polychroa* and we shall compare both studies in the Discussion.

METHODS

Schmidtea (Dugesia) polychroa is a true simultaneous hermaphrodite, in which matings and cocoon production completely overlap, with no obvious regular pattern. Cocoon production can be up to 1 per day and is up to 40% higher in parthenogens relative to sexuals. Under favourable conditions (high prey availability and temperature of 10–20 °C) individuals are sexually active all year round. In the field, the main reproductive season is from early spring to mid-summer, but cocoons can be found through much of the year.

Individuals were collected from a purely parthenogenetic lake population of *S. polychroa* (Ammersee, Herrsching, SSW of Munich) (Beukeboom *et al.*, 1996a). All animals were collected from 30 × 10 m² of shallow

water in the bay of Herrsching. This population has been studied for over 6 years and is known to consist of triploid parthenogens (Beukeboom *et al.*, 1996a; unpubl. data).

Immediately after collection, 105 random pairs were formed and placed in plastic weighing boats with approx. 50 ml of lake water at room temperature. The diurnal cycle was simulated with darkness from 22:00 to 06:00. Pairs were observed continuously from 15 July 1998, 18:00 until 18 July 1998, 16:00. Copulatory activity was checked every 15 min, which is sufficient to register all but the shortest, ineffective copulations (Peters, Streng & Michiels, 1996; Michiels & Streng, 1998). Pairs that had finished copulation were killed in a drop of 5 N HCl, and fixed in ethanol:acetic acid 3:1. Pairs that did not mate were fixed at the end of the observation period. By sacrificing individuals after mating, we obtained only 1 data point per pair. From previous observations, we know that individuals do not mate repeatedly during the same night, but remate only a few days later (e.g. Peters *et al.*, 1996). Since they forage actively in the meantime, the same 2 individuals will only meet again by chance. The outcome of a single mating can therefore be seen as the final outcome of a mating interaction between any 2 individuals.

Amounts of sperm in different parts of the genital system were determined using techniques described and discussed in detail by Michiels & Streng (1998). In brief, fixed animals were stained with Schiff's reagent and cleared. Drawings of the 2 sperm filled sperm ducts were made under a binocular microscope using a *camera lucida*. If an individual donated sperm, the resulting 'sperm clump' in the bursa of its partner was drawn as well. The area of each drawing was measured using an image analysis system. In order to compare the amount of available self-sperm between all animals, we summed the surface of the 2 sperm ducts and that of the donated sperm clump (if any) in each individual. We used this as a measure of the total amount of self-sperm available before copulation. Previous attempts to transform the surface of the coiled sperm ducts and the spherical sperm clump in the bursa into comparable estimates of sperm mass showed that, whatever 3-D model is used, the correlation between volume and surface estimates is always very close (pers. obs.). It is nevertheless important to stress that we use an estimate of sperm availability. Dissection of the bursa and sperm ducts is, unfortunately, not possible in fresh specimens and sperm are coagulated in Feulgen-treated specimens.

Some individuals fell apart during processing, leaving 92 pairs for further analysis. Body surface area was digitally measured after fixation. Individuals with areas below 9 mm² never copulated and were probably sexually immature. Yet, out of 43 individuals smaller than 9 mm², 14 had sperm, and all possessed a male copulatory organ. In order to avoid having to select 'immature' and 'mature' individuals on unclear grounds, we only analysed data for pairs that mated within the observation period ($n = 54$) and refrained from making explicit or implicit comparisons between mating and non-mating individuals. Body size of mating individuals was

Table 1. Patterns of sperm transfer as observed in parthenogenetic *Schmidtea polychroa* (present study, left part of the table) and comparison with data from sexual *S. polychroa* (right part, data from Michiels & Bakovski, 2000). Expected values were calculated using a binomial distribution

Type of sperm transfer	Parthenogenetic forms (this study)			Binomial	Sexual forms (Michiels & Bakovski)		
	Obs.		Exp.		Obs.		Exp.
No exchange (NN)	10	>	3.63	q^2	37	>	25
Unilateral (YN or NY)	8	<	20.74	$2pq$	29	<	53
Bilateral (YY)	36	>	29.63	p^2	40	>	28

The proportions p and q are given by the numbers of individuals that did (Y) or did not (N) donate sperm (only shown for parthenogens): Y:N = 80:28 = 0.7407:0.2593. Observed frequencies differed strongly from expected values (Chi-square test: $\chi^2 = 20.4$, d.f. = 1 because expected values were calculated from the data; $P < 0.001$). See Michiels & Bakovski (2000) for sexuals.

$\bar{X} \pm SD = 13.80 \pm 2.72 \text{ mm}^2$ ($n = 108$). Size analysis confirmed that pairs were assembled randomly with regard to size.

Statistical Analysis

Statistical analyses were performed using SPSS v. 10.0. The mating experiments yielded frequency data on the 3 possible outcomes of a mating: no, unilateral or reciprocal sperm exchange. In order to test whether the observed data are indicative of conditional reciprocity, we calculated expected frequencies in the following way. The null-hypothesis is that an individual's decision to donate sperm (Y) or not (N) is independent of sperm donation by the partner. If this is true, the 3 possible outcomes of matings (NN, NY and YY) should be binomially distributed for a given probability of N and Y. The latter 2 values are the overall number of individuals that did (Y) or did not (N) donate sperm in a given experiment. The expected relative frequencies are then given by $p(N)^2$, $2p(N)q(Y)$ and $q(Y)^2$ and can be used to calculate the expected number of pairs of each type when sperm donation is independent of the partner, and solely depends on own characteristics. Traits of 2 partners in a pair cannot be correlated in hermaphrodites because an *a priori* allocation to the X- or Y-axis is not possible. As an alternative, we used a 1-way analysis of variance, which is the appropriate solution to this problem (Vreys & Michiels, 1997). Box-plots (Fig. 2) represent the median \pm 1 quartile (box) and range (lines), excluding outliers and extremes. Outliers are cases with values between 1.5 and 3 box lengths from the upper or lower edge of the box (indicated as circles). Extremes are cases with values more than 3 box lengths from the upper or lower edge of the box (indicated as *). Means in text are \pm standard deviation.

RESULTS

Sperm exchange

Out of 54 pairs that mated in the course of the observation period, 18% did not exchange sperm, 15% exchanged sperm unidirectionally and the remaining 67% showed

reciprocal insemination (Table 1). A comparison with expected frequencies shows that a symmetrical outcome (no or bilateral exchange) was much more common than expected by chance, whereas unilateral exchange was rare. This is indicative of conditional reciprocity. The three types of matings did not differ in duration (Fig. 1). Note that, due to the fact that observations were collected in 15 min intervals rather than continuously, very short matings may have been overlooked. The pattern of sperm exchange was related to the amount of self-sperm available before the mating (Fig. 2). Individuals with fewer sperm were more likely to copulate without donating sperm. Reciprocity was particularly likely for individuals that had more sperm.

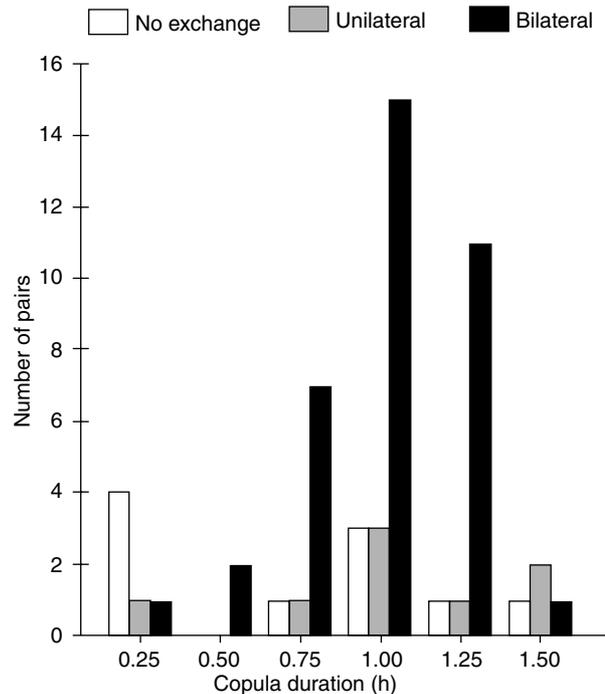


Fig. 1. Copulation duration in pairs of parthenogenetic *Schmidtea polychroa*, separated by the outcome of each mating (see legend). There was no difference in average copulation duration between the three groups (Kruskal–Wallis $\chi^2 = 2.70$, d.f. = 2, $P = 0.26$).

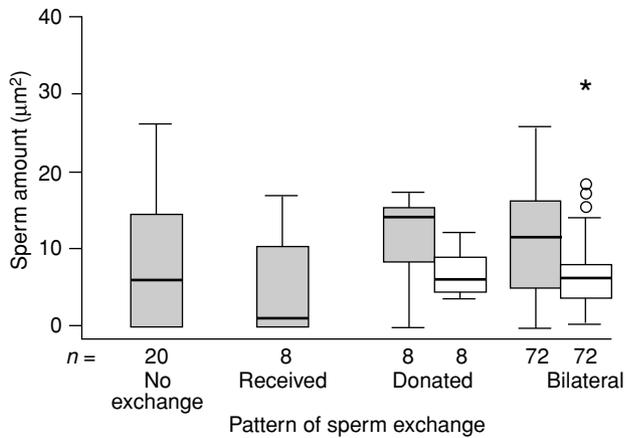


Fig. 2. Box-plot showing estimates of sperm amounts in mating individuals, split for self sperm amounts in the sperm ducts (grey) and the sperm clump donated to the partner (white), subdivided according to the pattern of sperm exchange. A comparison of total self-sperm available (*sum of ducts and clump*) shows significant effects between groups (one-way ANOVA $F = 8.88$, d.f. 3, 104, $P < 0.001$). Post-hoc comparisons (Ryan, Einot, Gabriel and Welsch F -ratio statistic) show that the first two groups differ from each of the last two.

Sperm availability

Surprisingly, there was no relationship between the availability of self-sperm and body size ($r = 0.048$, $n = 108$, $P = 0.62$), but the size of the donated sperm clump increased strongly with the amount available (adjusted $r^2 = 0.69$, $F = 168.6$, d.f. = 1, 77, $P < 0.001$). One extreme value (see definition in Methods) donated an exceptionally large amount of sperm (0.0195 mm^2 ; sample average $0.0043 \pm 0.0030 \text{ mm}^2$), and was ignored for this analysis. This had no effect on the conclusion ($P < 0.001$ in both cases). Approximately 32% of the available sperm was donated to the partner. Within reciprocating pairs, the amounts of sperm that were exchanged between partners were not correlated (one-way ANOVA $F = 1.47$, d.f. 35, 36, $P = 0.13$). Mating parthenogens had roughly 1/40 the amount of self-sperm of that recorded for mating sexuals (Michiels & Bakovski, 2000).

DISCUSSION

Our results indicate that parthenogenetic *S. polychroa* increase the likelihood of receiving sperm by giving sperm. This implies that they need to produce self-sperm in order to obtain maternity in the form of fertile parthenogenetic eggs. Many individuals had only very little if any sperm, but mated nevertheless. Our results confirm data obtained by Streng (1999), who described the process of ejaculation in sexuals and parthenogens in detail. An alternative explanation for the result found here would be that sperm donation depends on the relatedness between mating partners. In the case of parthenogenetic

populations, genetically identical individuals would benefit if they would be able to recognise each other. However, in a previous study on sexual forms, we did not find avoidance effects in genetically identical pairs relative to unrelated pairs (Peters & Michiels, 1996). Another explanation could be a generally higher level of sperm availability in sperm donors. A relationship between sperm availability and the likelihood of donating sperm has been shown before (Michiels & Streng, 1998). However, this does not explain why sperm donation by one partner depends on that of the other, as suggested by the binomial distribution (Table 1).

Survival of parthenogenetic populations

The results can be compared with those of Michiels & Bakovski (2000), who did an identical experiment using sexual individuals population of *S. polychroa* from northern Italy (Table 1). Such a comparison, however, needs to be done with care, because geographical locations and dates differ (May in Michiels & Bakovski, 2000, July in this study). A striking difference is that sexuals produce more sperm with increasing body size. This effect had already been shown by Michiels & Streng (1998) and was therefore not mentioned by Michiels & Bakovski (2000). Yet, they also found an increase in sperm production with body size ($r = 0.26$, $n = 294$, $P < 0.001$). Sexuals invest heavily in sperm because of the importance of paternity, which on average represents 50% of the lifetime reproductive success of an outcrossing hermaphrodite. In addition, sperm investment is increased by sperm competition and sperm digestion. Hence, in sexuals, conditional reciprocity can be seen as a mechanism that is maintained by the importance of male fitness, the accordingly high investment in sperm and the opportunity to digest sperm, as explained in detail by Greeff & Michiels (1999). Sperm limitation leading to female infertility is very unlikely in sexual *S. polychroa* (Michiels & Bakovski, 2000).

The situation is different in purely parthenogenetic populations. Here, paternity is absent or very weak, as in the planarian *Polycelis nigra* where paternal leakage occasionally occurs (Beukeboom *et al.*, 1996b). Moreover, sperm competition or sperm digestion are irrelevant to the sperm donor. Hence, investment in sperm is accordingly low (Weinzierl *et al.*, 1998; this study). The fact that parthenogens did not show an increase in sperm production with size suggests that they produce just enough sperm in order to be able to trade sperm with a partner. In addition, Storhas, Weinzierl & Michiels (2000) showed that sperm from parthenogenetic partners does not ensure full fertility, suggesting that in purely parthenogenetic populations, sperm limitation may play an important role for the female function. This may explain the high rate at which parthenogens mated in this study, presumably in an attempt to collect enough sperm to ensure maximal maternal reproductive success. Hence, in order to prevent donating sperm to a cheater who accepts

sperm, but refuses to reciprocate, conditional reciprocity is expected. This study suggests that conditional exchange is even more explicit in parthenogens than in sexuals (Michiels & Bakovski, 2000). This is supported by the observation that the duration of copulations with unilateral transfer is as long as that of bilateral inseminations. In sexuals, unilateral inseminations are significantly shorter than reciprocal inseminations (Michiels & Streng, 1998). This suggests that parthenogens do not give up a non-reciprocating partner as easily as sexuals do, once again suggesting that sperm receipt and sperm limitation may be more important in parthenogenetic *S. polychroa* than in their sexual conspecifics. The need for giving self-sperm to facilitate allosperm receipt may stabilize sperm-dependent parthenogenetic populations, in addition to other mechanisms proposed by Weinzierl *et al.* (1998).

Male vs female preferences

In *S. polychroa* sexual and parthenogenetic forms have now been found to trade sperm, but for different reasons. The mating interests of a parthenogen are female, resulting in conditional reciprocity for reasons similar to those proposed by the gamete trading model (Leonard & Lukowiak, 1984; Leonard, 1999). However, this model was not designed for asexual or parthenogenetic systems, but for sexual hermaphrodites for which data are inconclusive (Leonard & Lukowiak, 1991). Leonard's hypothesis may apply more generally to systems in which the expected gain from the male and female function is systematically different and this study may be the first true example of such a case. In sexual hermaphrodites sperm trading is better explained by assuming a male biased mating interest (Greeff & Michiels, 1999). A male mating interest in sexuals is suggested by 'female' avoidance behaviour (Rudolph 1979*b*; Rudolph & Bailey, 1985), explicit male attempts to inseminate unilaterally (Michiels & Newman, 1998), high male allocation (Rudolph 1979*a*; Michiels 1998; Weinzierl *et al.*, 1998), mating behaviour associated with the availability of self-sperm rather than lack of allosperm (Michiels & Streng, 1998; Vreys & Michiels, 1998; Michiels & Bakovski, 2000), male manipulation of the female function of the partner (Koene & Chase, 1998) and male-initiated copulation (Rudolph 1979*b*; DeWitt, 1996; Wethington & Dillon, 1996). A complete overview of the diversity of gastropod mating behaviour is given by Tompa *et al.* (1984) and Baur (1998). See Michiels (1998) for a review of other hermaphroditic animals.

CONCLUSION

We conclude that conditional reciprocity in parthenogenetic *S. polychroa* is strong, as expected. Costs are kept low by not increasing sperm production with size. These results explain at least partly the apparent stability and success of this mode of reproduction in large parts of

Europe. By insisting on reciprocity, they cause a trade-off between sperm production and female fertility, which favours the maintenance of a minimal male function.

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REFERENCES

- Baur, B. (1998). Sperm competition in molluscs. In *Sperm competition and sexual selection*: 255–305. Birkhead, T. R. & Møller, A. P. (Eds). New York: Academic Press.
- Beukeboom, L. W. & Vrijenhoek, R. C. (1998). Evolutionary genetics and ecology of sperm-dependent parthenogenesis. *J. Evol. Biol.* **11**: 755–782.
- Beukeboom, L. W., Seif, M., Mettenmeyer, T., Plowman, A. B. & Michiels, N. K. (1996*b*). Paternal inheritance of B chromosomes in a parthenogenetic hermaphrodite. *Heredity* **77**: 646–654.
- Beukeboom, L. W., Seif, M., Plowman, A. B., de Ridder, F. & Michiels, N. K. (1998). Phenotypic fitness effects of B chromosomes in the pseudogamous parthenogenetic planarian *Polycelis nigra*. *Heredity* **80**: 594–603.
- Beukeboom, L. W., Weinzierl, R. P., Reed, K. M. & Michiels, N. K. (1996*a*). Distribution and origin of chromosomal races in the freshwater planarian *Dugesia polychroa* (Turbellaria: Tricladida). *Hereditas* **124**: 7–15.
- DeWitt, T. J. (1996). Gender contests in a simultaneous hermaphrodite snail – a size-advantage model for behaviour. *Anim. Behav.* **51**: 345–351.
- Greeff, J. M. & Michiels, N. K. (1999). Sperm digestion and reciprocal sperm transfer can drive hermaphrodite sex allocation to equality. *Am. Nat.* **153**: 421–430.
- Koene, J. M. & Chase, R. (1998). Changes in the reproductive system of the snail *Helix aspersa* caused by mucus from the love dart. *J. Exp. Biol.* **201**: 2313–2319.
- Leonard, J. L. (1999). Modern portfolio theory and the prudent hermaphrodite. *Inv. Reprod. Dev.* **36**: 129–135.
- 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. (1991). Sex and the simultaneous hermaphrodite: testing models of male–female conflict in a sea slug, *Navanax inermis* (Opisthobranchia). *Anim. Behav.* **41**: 255–266.
- Michiels, N. K. (1998). Mating conflicts and sperm competition in simultaneous hermaphrodites. In *Sperm competition and sexual selection*: 219–254. Birkhead, T. R. & Møller, A. P. (Eds), New York, London: Academic Press.
- Michiels, N. K. & Bakovski, B. (2000). Sperm trading in a hermaphroditic flatworm: reluctant fathers and sexy mothers. *Anim. Behav.* **59**: 319–325.

- Michiels, N. K. & Newman, L. J. (1998). Sex and violence in hermaphrodites. *Nature* **391**: 647.
- Michiels, N. K. & Streng, A. (1998). Sperm exchange in a simultaneous hermaphrodite. *Behav. Ecol. Sociobiol.* **42**: 171–178.
- 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.
- Peters, A. & Michiels, N. K. (1996). Evidence for a lack of inbreeding avoidance by selective mating in a simultaneous hermaphrodite. *Invert. Biol.* **115**: 99–103.
- Rudolph, P. H. (1979a). The strategy of copulation of *Stagnalis elodes* (Say) (Basommatophora: Lymnaeidae). *Malacologia* **18**: 381–389.
- Rudolph, P. H. (1979b). An analysis of copulation in *Bulinus (Physopsis) globosus* (Gastropoda: Planorbidae). *Malacologia* **19**: 147–155.
- Rudolph, P. H. & Bailey, J. B. (1985). Copulation as females and use of allosperm in the freshwater snail genus *Bulinus* (Gastropoda: Planorbidae). *J. mollusc. Stud.* **51**: 267–275.
- Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J. T., Schmid, M. & Parzefall, J. (1995). Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* **373**: 68–71.
- Schlupp, I., Larler, C. & Ryan, M. J. (1994). Benefit to male sailfin mollies of mating with heterospecific females. *Science* **263**: 273–274.
- Storhas, M., Weinzierl, R. P. & Michiels, N. K. (2000). Paternal sex in parthenogenetic planarians: a tool to investigate the accumulation of deleterious mutations. *J. Evol. Biol.* **13**: 1–8.
- Streng, A. (1999). *Sperm exchange in simultaneous hermaphroditic freshwater flatworms* Doctoral Thesis. Ludwig Maximilians Universitaet, Munich.
- Tompa, A. S., Verdonk, N. H. & van den Biggelaar, J. A. M. (1984). *The Mollusca, 7. Reproduction*. Orlando, Florida: Academic Press.
- Vreys, C. & Michiels, N. K. (1997). Flatworms flatten to size up each other. *Proc. R. Soc. Lond. B* **264**: 1559–1564.
- Vreys, C. & Michiels, N. K. (1998). Sperm trading by volume in an internally fertilising hermaphrodite with mutual insemination. *Anim. Behav.* **56**: 777–785.
- Weinzierl, R. P., Berthold, K., Beukeboom, L. W. & Michiels, N. K. (1998). Reduced male allocation in a parthenogenetic hermaphrodite (*Dugesia polychroa*, Tricladida, Platyhelminthes). *Evolution* **52**: 109–115.
- Weinzierl, R. P., Schmidt, P. & Michiels, N. K. (1999). High fecundity and low fertility in parthenogenetic planarians. *Invert. Biol.* **118**: 87–94.
- Wethington, A. R. & Dillon, R. T. (1996). Gender choice and gender conflict in a non-reciprocally mating simultaneous hermaphrodite, the freshwater snail, *Physa*. *Anim. Behav.* **51**: 1107–1118.