

High multiple paternity and low last-male sperm precedence in a hermaphroditic planarian flatworm: consequences for reciprocity patterns

N. PONGRATZ and N. K. MICHIELS

Institute for Animal Evolution and Ecology, University Muenster, Huefferstrasse 1, D-48149 Muenster, Germany

Abstract

It is difficult to predict a priori how mating success translates into fertilization success in simultaneous hermaphrodites with internal fertilization. Whereas insemination decisions will be determined by male interests, fertilization will depend on female interests, possibly leading to discrepancies between insemination and fertilization patterns. The planarian flatworm *Schmidtea polychroa*, a simultaneous hermaphrodite in which mating partners trade sperm was studied. Sperm can be stored for months yet individuals mate frequently. Using microsatellites, maternity and paternity data were obtained from 748 offspring produced in six groups of 10 individuals during four weeks. Adults produced young from four mates on average. Reciprocal fertilization between two mates was found in only 41 out of 110 registered mate combinations, which is clearly less than what is predicted from insemination patterns. Multiple paternity was high: > 80% of all cocoons had two to five fathers for only three to five offspring per cocoon. Because animals were collected from a natural population, 28% of all hatchlings were sired by unknown sperm donors in the field, despite a 10-day period of acclimatization and within-group mating. This percentage decreased only moderately throughout the experiment, showing that sperm can be stored and used for at least a month, despite frequent mating and sperm digestion. The immediate paternity a sperm donor could expect to obtain was only about 25%. Male reproductive success increased linearly with the number of female partners, providing support for Bateman's principle in hermaphrodites. Our results suggest that hermaphrodites do not trade fertilizations when trading sperm during insemination, lending support to the view that such conditional sperm exchange is driven by exchange of resources.

Keywords: genotyping, microsatellites, multiple paternity, sexual conflict, sexual selection, sperm competition

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Introduction

Mating with multiple partners has received much attention in studies of animal breeding systems (Reynolds 1996) and the use of polymorphic genetic markers has shown that it is widespread among both males and females (Birkhead & Møller 1998). Multiple mating by males can generally be explained by the direct benefits from increased likelihood of siring offspring. For females, however, many hypotheses have been formulated which

can be subdivided into immediate benefits (e.g. fertilization assurance) and long-term benefits (e.g. good genes) (Reynolds 1996; Jennions & Petrie 2000). But why do hermaphrodites mate multiply? To increase reproductive success via the male function, hermaphrodites may attempt to mate promiscuously like males do. Indeed, multiple mating and sperm competition appear to be common in hermaphrodites (Baur 1998; Michiels 1998, 1999) but evidence for multiple paternity is relatively rare and has been reported mainly from hermaphroditic gastropods (Mulvey & Vrijenhoek 1981; Leonard & Lukowiak 1991; Wethington & Dillon 1991; Baur 1994; Haase & Baur 1995; Baur 1998; Locher & Baur 2000) or

Correspondence: Professor Dr Nico Michiels. Fax: +44-251-83 24668; E-mail: michiels@uni-muenster.de

colonial hermaphrodites in which mates do not physically interact (Yund & McCartney 1994; McCartney 1997; Bishop *et al.* 2000). Using allozymes in pairs and triplets of three different strains of trematodes, Trouvé *et al.* (1996) showed that sperm exchange with more than one partner is possible. In a study of male allocation in relation to mating group size in a hermaphroditic tapeworm, Schärer & Wedekind (2001) showed increased sperm donation and sperm receipt with increasing group size, which suggests an adaptive response to the increased risk of sperm competition. Male allocation in the marine turbellarian *Macrostomum* also increases with mating group size, strongly suggesting sperm competition in larger groups (Schärer & Ladurner 2003).

An important issue in hermaphrodites is reciprocity of insemination and fertilization. Normally, one would expect hermaphrodites to attempt to inseminate as many mates as possible, in accordance with Bateman's principle (Charnov 1979), leading to 'unconditional reciprocity', i.e. reciprocal insemination that results from the unconditional willingness of both partners to donate sperm. Unconditional reciprocity implies that a sperm donor does not check whether the partner will reciprocate. Hence, donors will also give sperm to receptive partners that are not able to reciprocate. However, because the male investment in sperm can be considerable (Charnov 1996; Greeff & Michiels 1999; Pen & Weissing 1999), hermaphrodites may make sperm donation dependent on sperm receipt. Particularly when sperm represent a digestible resource and mates are plenty, sperm donors should prefer mates that reciprocate. This should lead to *conditional* reciprocal insemination or *sperm trading* (Greeff & Michiels 1999). In other words, by insisting on reciprocity, sperm donors can obtain a 'compensation' for their investment in the form of a digestible ejaculate. The open question here is, whether hermaphrodites also trade fertilizations: is the amount of paternity that one partner obtains related to what the other receives? Since it seems difficult for a sperm donor to affect its fertilization chances in internally fertilizing species (but see Rogers & Chase 2001), it is expected that sperm trading is primarily based on resource trading, not egg fertilization. Hence, we predict to see a discrepancy between insemination (male) and fertilization (female) patterns. Such a difference would also indicate differences in mating interests in the two sex functions of a hermaphrodite.

In the free-living, planarian flatworm *Schmidtea polychroa* it is possible to assign paternity to one of several candidate fathers using highly polymorphic microsatellites (Pongratz *et al.* 2001, 2002). An experiment was performed in which all juveniles hatching from all cocoons produced from six groups of 10 *S. polychroa* were analysed. Data were used to describe patterns of multiple paternity and long-term sperm storage or usage. This is the first time that such an analysis has been performed on planarians

and the first study of this scale in copulating, simultaneous hermaphrodites.

Mating and sperm transfer in Schmidtea polychroa

In *S. polychroa* sperm exchange occurs typically after having been *in copula* for ~30 min. Insemination is fast and happens virtually synchronously. After insemination, partners remain *in copula* for about another hour before separating. Copulations without insemination are relatively common (~35%). Unilateral inseminations are not uncommon (~27%), whereas reciprocal inseminations are most common (38%). Applying a binomial distribution, it can be shown that unilateral inseminations are significantly rarer than expected, the other types being more common, leading Michiels & Bakovski (2000) to conclude that sperm are traded. Further details are given by Peters *et al.* (1996), Michiels & Streng (1998) and Streng (1999). Sperm received in the *bursa copulatrix* migrate along the paired oviducts towards the sperm receptacles in the 'neck' region, adjacent to the ovaries. Although about 99.7% is resorbed within the reproductive tract soon after receipt (Sluys 1989; Vreys *et al.* 1997; Streng 1999), the remaining sperm may be stored and used for months. Individuals mate at a rate of once per 2 days under laboratory conditions (Peters *et al.* 1996). By comparing sexual and parthenogenetic forms Weinzierl *et al.* (1998, 1999) showed that investment in sperm in sexual forms is very high. Selfing does not occur in *S. polychroa* (personal observation). Another planarian in which sperm trading has been described is *Dugesia gonocephala*. Here, sperm are not just traded in the form of controlled exchange of whole ejaculates, but the actual volumes being exchanged are also balanced (Vreys & Michiels 1998).

Materials and methods

Experimental design

About 120 adult, sexual *Schmidtea polychroa* were collected in the river Sarca, about 3 km south of Arco (Italy). This population consists of sexual individuals of *S. polychroa*, of which parthenogenetic forms are common elsewhere (Beukeboom *et al.* 1996). Sampling took place during the reproductive season of *S. polychroa* and therefore individuals were assumed to have mated several times in the field. One hundred adult individuals were selected and randomly distributed among 10 groups of 10 individuals. Groups were kept at 18–20 °C in a 14 : 10 light : dark cycle in 1-L vials filled with water from a large, 600-L 'microcosm' containing algae, flatworms and snails, in which water was constantly circulated and filtered. The container was topped up with untreated tap water. Density in the field is variable and has not been properly

quantified. However, animals typically aggregate on the same stone in densities that can exceed those in the experiment (personal observation). Animals were fed minced beef liver and tanks were cleaned twice a week. To decrease the effect of allosperm received in the field and to allow individuals to accommodate to laboratory conditions, experimental observations and procedures were started only after 10 days (9 June 1998). Previous observations had shown that sperm digestion is pronounced (Streng 1999) and mating rates are high (Peters *et al.* 1996). It was therefore assumed that most 'foreign' sperm would be digested, flushed or displaced within a short time period. Previously mated individuals were deliberately chosen over virgins [young raised to adulthood or adults virginized by sectioning and regeneration as in Storhas *et al.* (2000)], because virgin individuals show different mating behaviour, as shown for isolated individuals by Michiels & Bakovski (2000). The retention of allosperm from mating partners from the field was, however, unexpectedly high (see Results). Although this made paternity analysis more complicated, it allowed us to quantify the gradual decrease of sperm from previous mating partners, yielding insights into sperm usage patterns that would not otherwise have been possible.

During the following 28 days, all freshly produced cocoons were collected once or twice a day, and kept separately. Cocoon size was measured using an image analysis system. One to two days after hatching the number of young per cocoon was counted. Young were flash-frozen and stored at -80°C . After 38 days in the laboratory, adults were separated, labelled and their body area was measured using an image analysis system. Tissue storage and DNA extraction followed the procedures described in Pongratz *et al.* (2001).

Multi-locus genotypes of four highly polymorphic microsatellite loci (SpATT12, SpATT16, SpATT18, SpATT20) were determined for all parents and offspring (Pongratz *et al.* 2001). There is no linkage disequilibrium between these loci, except between SpATT16 and SpATT18. Since SpATT16 was also found to include some null alleles (Pongratz *et al.* 2001), it was only used as additional evidence. PCR products were separated using an ABIPRISM 310 genetic analyser. Three groups were excluded from further processing because some adults died. Another group was excluded because it contained two individuals that shared seven of eight alleles. All results are based on the six remaining groups, A to F. In group B, one individual was found to be a triploid sperm-dependent parthenogen. It produced only two maternal offspring (parthenogenetically) and fathered no young — which would have been possible because parthenogens possess a functional male system (Weinzierl *et al.* 1998). This individual was ignored in further analyses ($n = 9$ for group B). A summary of the

genetic analyses of the adult individuals is included in the population study by Pongratz *et al.* (2002). They show that heterozygosities vary from 0.89 to 0.94 for our three key loci, with 16–22 alleles per locus.

Analysis of maternity and paternity

Since cocoons were collected from each group without knowing the cocoon-producer, both 'mother' (cocoon producer) and 'father' (sperm donor) had to be inferred from genotype data. While all potential mothers for a given cocoon had to be group members, the potential fathers could also be earlier sperm donors from matings in the field.

Maternity assignment was facilitated by the high frequency of half-sibs in cocoons as a result of multiple paternity. Additional cocoons could be assigned because an external sperm donor fathered all offspring. Maternity was formally assigned when all hatchling genotypes of a clutch indicated the same potential mother at all loci. This was possible in 91.5% of the cases. When two or more mothers were possible, information on adult body size, cocoon size and time of cocoon deposition was used. Cocoon size is strongly related to body size and shows little variance within individuals but high variance between individuals (mean squares within : between 0.028 : 0.137, $F_{52,159} = 4.9$, $P < 0.001$). Cocoon production is also regular and periodical, allowing exclusion of mothers on days on which another cocoon from that individual was identified. Combined, maternity could be assigned to a total of 97.9% of the cocoons ($n = 205$). From these, 797 hatchlings were obtained.

Paternity analyses were performed in several steps. First, paternity was excluded using presence or absence of paternal alleles as a criterion. If, for a given hatchling genotype, only one of the nine candidate sperm donors showed no mismatch at all four loci, paternity was assigned to this individual (92.7% of all hatchlings). When more than one candidate sperm donor showed no mismatch CERVUS 1.0 was used. This program applies likelihood tests and simulations, and assigns paternity to the individual with the highest LOD score (Marshall *et al.* 1998). Assignments were repeated for each of 29 hatchlings with unclear paternity within a brood. Paternities were assigned using a $> 80\%$ probability and only the three unlinked microsatellite loci (see above). Four young (0.6%) for whom paternity assignment remained ambiguous were removed from the data. When one or more allele mismatches between offspring and each potential father in the group were detected, the sperm donor was assumed to have been a previous mate in the field population. For all analyses, including those using CERVUS, it was assumed that no mutation in the microsatellite alleles had occurred. Paternity was determined from 748 offspring.

Fertilization pattern	Group						Total
	A	B	C	D	E	F	
No. of reproducing individuals	8	9	10	9	8	10	54
No. of young with known parentage*	85	155	120	159	82	147	748
Possible no. of mate combinations	28	36	45	36	28	45	218
Observed no. of mate combinations	12	16	21	24	16	21	110
with unilateral fertilization	8	10	17	12	10	12	69
with reciprocal fertilization	4	6	4	12	6	9	41
% reciprocal fertilization	33.3	37.5	19.1	50.0	37.5	42.9	37.3
Expected % reciprocal	68.8	68.1	66.6	68.1	68.8	66.6	67.8
<i>P</i> -values	0.008	0.009	< 0.001	0.057	0.007	0.021	< 0.001

*Including external paternity.

In the case of unilateral fertilization insemination may have been reciprocal. Only individuals that produced at least one offspring (paternally or maternally) were included. The expected frequency is taken from a simulation, which assumes random mating and uses the likelihood of non, unilateral or reciprocal insemination from Michiels & Bakovski (2000) (see Materials and methods). *P*-values are from one-sample χ^2 -tests in which observed and expected counts were compared.

Simulation of expected pattern of fertilization

To check whether the observed pattern of reciprocal or unilateral fertilization coincides with known reciprocal and unilateral insemination frequencies, expected values were generated in a simple simulation. First, 1000 'focal individuals' were assigned eight successive matings with mates picked randomly from a pool that equalled group size - 1, whereby the same mate could be picked more than once. Eight matings were chosen after testing a range of 4-15 because the resultant number of different partners with insemination in at least one direction closely approximated 4, the value obtained from paternity analysis. These eight matings were randomly assigned to 'none', 'unilateral', or 'reciprocal' insemination using the proportions 0.35, 0.27 and 0.38 for single mating events from Michiels & Bakovski (2000). Unilateral matings were further randomly subdivided into 'focal → mate' and 'mate → focal'. By aggregating data per focal individual, the average likelihood of reciprocity was obtained. Repeated mating between the same two individuals increased the likelihood of reciprocity and of successive unilateral matings in opposite directions, which can be seen as 'delayed reciprocity' (1.5% of all reciprocal matings).

Statistical analysis

Statistical analyses were performed using SPSS v.10.0. Means in text are \pm SD. The traits of two partners in a pair of hermaphrodites cannot be correlated using regular correlation coefficients. As an alternative, a one-way analysis of variance (ANOVA) was used, which also tests for similarity within pairs relative to between pairs (Sokal & Rohlf 1996; Vreys & Michiels 1997).

Table 1 Maximum and observed frequency of partner combinations that led to fertilization, split in unilateral and reciprocal fertilizations

Results

Fertilization patterns

Based on shared parentage, individuals had a minimum of 4.07 ± 1.72 mating partners from within their mating group in the course of 38 days (including 10 days acclimatization). Reciprocal paternity was found in 41 (37.3%) out of 110 realized partner combinations (Table 1). The observed counts diverged significantly from the expected values, indicating that reciprocal insemination does not imply reciprocal fertilization.

When looking at pairs that show reciprocal paternity, no trend was found to show that the number of young sired reciprocally was traded (Fig. 1): A one-way ANOVA was insignificant within each group ($P > 0.47$ for all groups except group C: $P = 0.074$), as well as for all groups combined (ANOVA $F = 0.74$, d.f. = 40,41, $P = 0.83$).

Multiple paternity within cocoons

In only 18.5% of all cocoons, a single sperm donor fathered all hatchlings (Fig. 2). In the remaining 81.5% at least two sperm donors could be distinguished. For the majority of cocoons, either two (40%) or three (35.6%) sperm donors were found. In one case, a different sperm donor was identified for each of five hatchlings in a single cocoon.

External sperm donors

Overall, 28.2% of the young produced in 4 weeks were sired by previous mating partners in the field (see Materials and methods section). The proportion of external sires decreased with time from almost 40% on day 9 to 0%

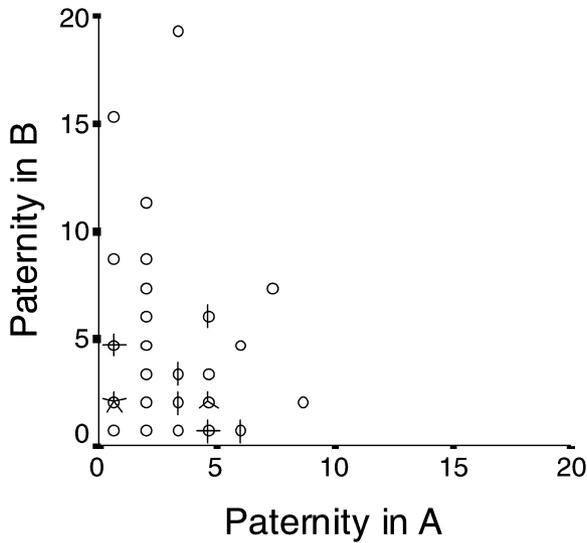


Fig. 1 Is paternity traded? Sunflower plot showing the relationship between numbers of shared offspring fathered reciprocally in pairs that exchanged fertilizations. Allocation to either axis is random since *a priori* allocation is not possible in pairs of hermaphrodites. Data from six replicate groups are combined. Petals of sunflowers indicate number of overlapping data points (≥ 2).

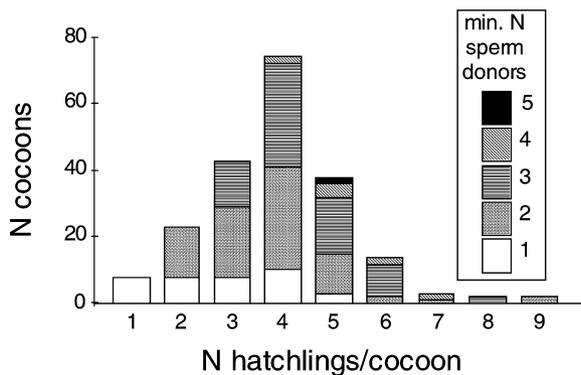


Fig. 2 Multiple paternity within cocoons. Overall, only 18.6% of cocoons contained hatchlings sired by only one sperm donor. All six replicates were combined.

at the end of the experiment (Fig. 3). The negative trend was not always significant, but this only indicates that the time window was still too short to allow for depletion of all external sperm.

Sperm precedence

Since it is not feasible to observe and assign all matings for 10×10 flatworms over a 4-week period, it is not known when or whether inseminations took place. To obtain an estimate of how many offspring an individual can expect to fertilize immediately after insemination, we looked for

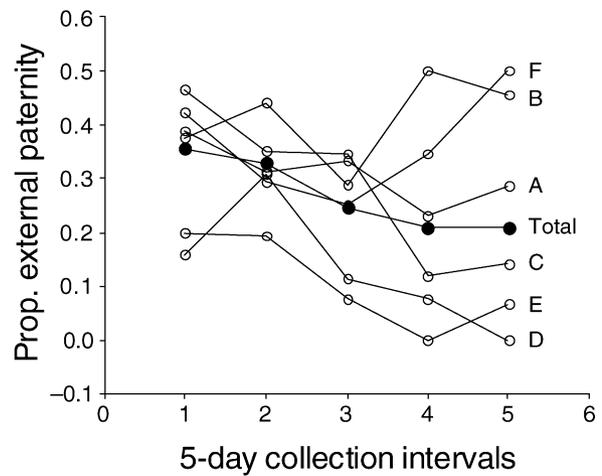


Fig. 3 The proportion of hatchlings sired by external sperm donors for five periods of cocoon collection. Values are shown separately for groups A to F as well as for the overall results (all groups combined). The apparent negative trend is significant for groups C, D, E and Total (Spearman Rank correlation analysis, $n = 5$, $P < 0.05$). A repeated measure analysis with the six experimental groups as subjects and period as within-subject factor, shows no significant effect (Friedman Test $\chi^2 = 6.4$, d.f. = 4, exact $P = 0.17$).

the first cocoon of each ‘mother’ in which a particular ‘father’ sired offspring. Assuming that this cocoon was the first produced after mating with that partner, the proportion of individuals sired in the cocoon is a measure of sperm precedence. Figure 4 shows that last-male sperm precedence (P_2) is low, but increasing from 25% (median) to +50% in later cocoons. This estimate is an overestimate as cocoons in which the most recent sperm donor sired no offspring are inevitably excluded. Spearman Rank correlations between P_2 and cocoon rank suggest that the positive trend is significant in at least three groups (group A, $P = 0.105$; group B, $P = 0.055$; group C, $P = 0.021$; group D, $P = 0.009$; group E, $P = 0.22$; group F, $P = 0.040$). This increase is a probably a consequence of repeated mating with the same partner over time.

Maternity and paternity as a function of sperm donation and receipt

How does the number of different sperm donors or sperm receivers affect the total number of offspring? For this analysis, only offspring were considered for which both parents were part of the mating group (external paternity excluded). Figure 5 and Table 2 show that paternity increased linearly with the number of mates. Unexpectedly, there was also a strong positive relationship between number of male partners and maternity in most groups (Fig. 5D, Table 2). The number of female partners did not affect the own female reproductive success (RS)

Group	N male partners		N female partners	
	MRS	FRS	MRS	FRS
A (n = 8)	0.159 (0.707)	0.658 (0.076)+	0.849 (0.008)**	0.086 (0.839)
B (n = 9)	0.172 (0.659)	0.015 (0.970)	0.967 (< 0.001)***	0.037 (0.925)
C (n = 10)	0.355 (0.314)	0.916 (< 0.001)***	0.922 (< 0.001)***	0.296 (0.406)
D (n = 9)	0.109 (0.780)	0.933 (< 0.001)***	0.780 (0.013)*	0.092 (0.814)
E (n = 8)	0.068 (0.872)	0.980 (< 0.001)***	0.903 (0.002)**	0.425 (0.294)
F (n = 10)	-0.194 (0.591)	0.840 (0.002)**	0.828 (0.003)**	-0.112 (0.759)

Table 2 Pearson correlations between the number or paternal or maternal offspring (male and female reproductive success, MRS and FRS) and the number of male or female partners in six replicated groups

A male partner is a sperm donor whose sperm was used to fertilize own eggs. A female partner is a sperm receiver who fertilized eggs with the received sperm. *P*-values are given between brackets. Significant values in bold. *n* is the number of individuals with at least one maternally or paternally produced offspring within each group.

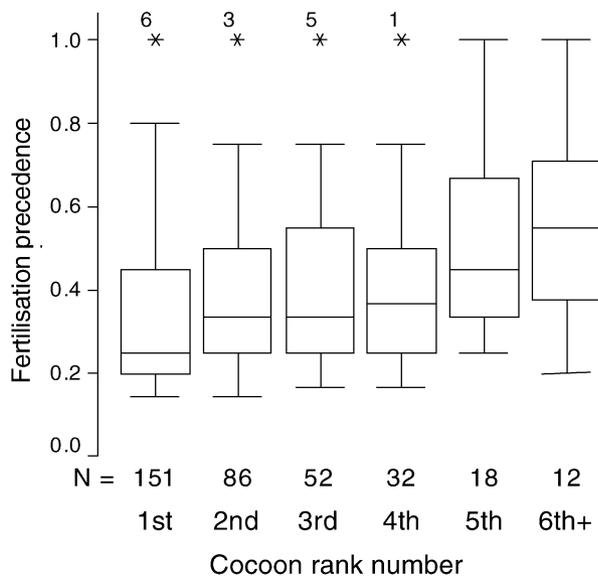


Fig. 4 Box-plot of relative paternity in successive cocoons produced by the same ‘mother’ individual, starting with the first cocoon(s) in which an individual fathered offspring. Data from six replicates combined. Boxes represents interquartile range, containing 50% of all values. Line across box indicates median. Whiskers show range, excluding outliers and extremes. Outliers (circles) are values between 1.5 and 3 box lengths from box edge. Extremes (asterisks) are cases with values more than three box lengths from the box edge. Numbers at the top indicate number of overlapping extremes.

(Fig. 5C), nor did the number of male partners affect the number of paternally sired offspring (Fig. 5B). This discrepancy once more illustrates the lack of trading of fertilizations mentioned above.

Discussion

Schmidtea polychroa reciprocity of paternity was absent during the investigated time window. High multiple paternity is the rule within and among cocoons, with low

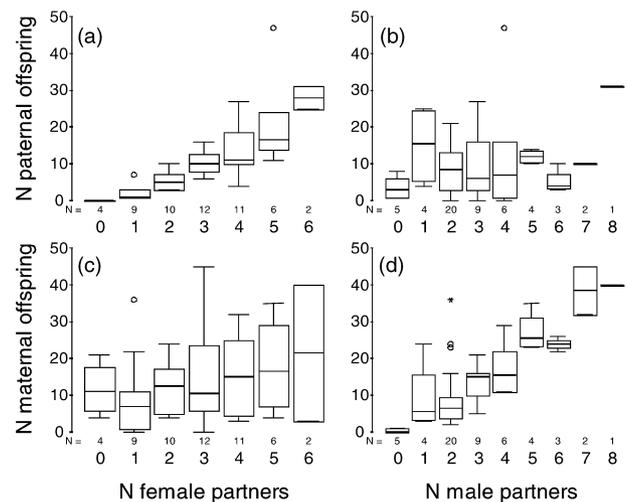


Fig. 5 Number of paternal (top) and maternal (bottom) offspring as a function of the minimum number of female (left) and male partners (right). The minimum number of partners is given by the parentage analysis. Matings that did not lead to shared parentage are therefore not included. All six replicates were combined. See Table 2 for statistics.

last-male sperm precedence. The data further show that male reproductive success increases with the number of female partners. The finding that female reproductive success increased with the number of male partners was unexpected. None of these effects were affected by variation in body size (Pongratz & Michiels in preparation). These findings are discussed below.

Reciprocal insemination vs. reciprocal paternity

Since it was not feasible to observe mating directly, the pattern of sperm exchange was estimated from a simulation based on data for single matings from Michiels & Bakovski (2000). By assuming eight matings per individual, our estimate of the likelihood of reciprocity was conservative. Peters *et al.* (1996) found a mating rate of

once every second day, which is much higher than the eight times in 38 days assumed here. Higher mating rates increase the difference between observed and expected counts. On the other hand, some pairs may have mated towards the end of the experimental time window, leaving not enough time to detect reciprocal fertilization. Nevertheless, the results indicate that even if reciprocity in fertilization could have been higher after a longer time, partners do vary in the way in which received sperm are used. The results therefore suggest that insemination does not automatically lead to paternity. When focusing on reciprocally fertilizing pairs only, there was also no relationship in the number of offspring sired by each partner.

Hence, although *S. polychroa* trades ejaculates (Michiels & Streng 1998; Michiels & Bakovski 2000) trading does not guarantee fertilization. It confirms the inherent uncertainty associated with the male function in many internally fertilizing hermaphrodites (Leonard & Lukowiak 1984; Leonard 1999). It also confirms that trading of fertilization cannot explain the evolution of conditional sperm donation or sperm trading, as already suggested by Greeff & Michiels (1999), who propose that conditionality arises from the trading of resources. In this discussion it is important to separate the function of insemination itself – which is to obtain paternity and can lead to unconditional reciprocity because both partners like to give sperm – and the evolution of conditional sperm exchange or sperm trading, which requires a commodity that is traded. This study suggests that this commodity is not fertilization of eggs.

Multiple mating

Individuals produced offspring from on average four different mating partners within 5 weeks. It is not possible to resolve whether certain pairs mated repeatedly and/or unsuccessfully within the course of the experiment. Michiels & Bakovski (2000) found that 48% of mates are not inseminated during a mating. Hence, actual mating rates are likely to be well above once a week, as already suggested by the simulations. It is not known whether the high mating rates and levels of multiple paternity shown here mirror those of natural populations of *S. polychroa* yet the diversity of external sperm donors suggests that it does: All individuals in this study had a minimum of one to four external partners (data not shown).

From the male perspective the advantages of multiple mating are obvious as it directly contributes to fertilization success (Bateman 1948). Our study confirms a strong relationship between the number of female partners and male RS (Fig. 5). Combined with previous data on sperm availability (Michiels & Streng 1998; Michiels & Bakovski 2000), these data strengthen the notion that obtaining paternity is a primary reason for mating in hermaphrodites (Charnov 1979).

The observation that female RS and the number of sperm donors was also strongly positively related is intriguing (Table 2). However, a statistical artefact may have caused this relationship: assuming a similar likelihood to mate with different partners for all individuals, receivers with high female fecundity will have more males among their offspring than low fecundity individuals. This is further strengthened by the fact that individuals that produce more cocoons, also mate more often (Peters *et al.* 1996), a possible consequence of differences in condition. The fact that individuals with high female fecundity did not have higher paternity in their partners (Fig. 5C) must be attributed to the fact that this effect is less direct and therefore more likely to be obscured by the fact that insemination does not imply fertilization.

Why do individuals insist on multiple sperm receipt? In *S. polychroa*, depletion of allosperm for fertilization of their own eggs appears unlikely as mating frequencies in the field and in the laboratory strongly exceed the rate required to maintain full fertility (Streng 1999). As mentioned above, Greeff & Michiels (1999) predict that individuals benefit from insisting on reciprocity as it represents a nutritional compensation for the investment made. Although this may explain multiple mating, it does not explain why multiple paternity is so high. In combination with the option of digesting received sperm, a subtle mechanism of sperm processing and storage in the female tract may be at work. This process may serve two purposes. On the one hand, multiple mating induces competition between sperm from successive sperm donors, assuring that maternal offspring inherit genes responsible for good sperm competition ability (Keller & Reeve 1995; Eberhard 1998) or even allow direct selection of the female function on sperm or mate quality (Eberhard 1996). On the other hand, the female function may actually benefit from high genetic diversity among offspring (Jennions & Petrie 2000). Since multiple paternity is high in cocoons, after fertilization and embryo development, there may be ecological reasons for high multiple paternity. Post-zygotic selection is, however, costly as young have to be raised before selection can run its course. In organisms where young compete for a common resource, the costs of postzygotic selection can be reduced (Lively & Johnson 1994). The so-called 'selective arena' model assures that maternal investment is allocated to the fittest juveniles only. Since several embryos compete for a common yolk mass in planarian cocoons, this model also applies to *S. polychroa* (Greeff & Michiels 1999; Storhas *et al.* 2000) and may help to explain the high levels of multiple paternity in this species.

Sperm storage

Because we used individuals that had mated in the field, a large proportion of the paternity had to be assigned

to unknown, 'external' fathers. After 10 days in the laboratory, external mates still sired 40% of all offspring. It took another 4 weeks to show a negative trend — despite *ad libitum* mating conditions. This is further support for the suggestion that the female genital system in general, and the sperm storage organs in particular, allow for some remarkable degree of control, which requires more attention in the future.

Conclusions

When given the opportunity, *S. polychroa* is a highly promiscuous species, with very high levels of multiple paternity, also within single cocoons, and low last-male precedence. Sperm trading does not guarantee fertilization. Male reproductive success is positively and linearly related to the number of mates. The same applies to female reproductive success and access to sperm donors, which may be the result of a statistical artefact.

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Norbert Pongratz is interested in fertilisation patterns, genetic diversity and phylogeography of flatworm populations. This work was part of his Ph.D. project. Nico Michiels is professor at the University of Münster. The main focus of his work is on the evolution of hermaphrodite mating systems, including as sexual conflict, mate choice, mate manipulation, sperm trading, sex allocation and the advantages and disadvantages of being a (simultaneous, non-selfing) hermaphrodite. Another research focus is on the evolution of sexuality versus asexuality. All projects integrate theory, field work, behaviour, histology, and life history measurements. Free-living flatworms are a key model organism.
