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Gender choice and gender conflict in a non-reciprocally mating simultaneous hermaphrodite, the freshwater snail, *Physa*

AMY R. WETHINGTON & ROBERT T. DILLON, JR
Department of Biology, College of Charleston, U.S.A.

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Abstract. Like freshwater pulmonate snails in general, mature *Physa heterostropha pomilia* (Say) are hermaphroditic but cannot reciprocally inseminate each other simultaneously. One member of a pair assumes the male role and the other the female, after which swapping may occur. The tendency for a pair of prospective copulants to elect the same sexual role was defined as gender conflict, and distinguished from sexual conflict, the more widely noted tendency for females to reject low-quality males. Experiments assessed whether previous reproductive history affected the likelihood of mating and the sexual role assumed and evaluated the strength of gender and sexual conflicts. Snails were selected from isofemale lines fixed for albinism at two complementary loci. Ten pairings were made involving all combinations of four categories of reproductive history: previously selfing virgin, not previously selfing virgin, previously selfing mated, and not previously selfing mated. Encounter times, evasive behaviours, and polarity of mating were recorded. Results suggested that the probability of initiating copulation as male in any encounter increased with autosperm store, and that snails mounted as female may decrease the overall probability of mating by rejective behaviour when their reserves of allosperm are high. There was no evidence that snails ever use any mechanism to increase their likelihood of being mounted as female. Evasive behaviours observed in encounters between virgin snails were best attributed to male–male gender conflict, but such behaviour patterns seemed to derive from sexual conflict when virgin snails encountered previously mated snails. Male–male gender conflict did not, however, detectably lengthen contact times, lead to bouts of sperm trading or ultimately lower mating success.

The tendency for males to be eager to mate, and for females to be more discriminating, has been a familiar observation since the days of Darwin. Bateman (1948) attributed this phenomenon to the intensity of sexual selection acting on males. Although his attention was primarily directed toward dioecious animals, Bateman recognized that sexual selection might also occur in simultaneously hermaphroditic plants. The theory, as generalized by Charnov (1979), predicts conflict between simultaneous hermaphrodites vying to mate in the male role by virtue of the disparity in the energetic costs of eggs and sperm. The phenomenon of egg trading in such diverse groups as hermaphroditic sea basses (Axelrod & Hamilton 1981; Fischer & Peterson 1987) and polychaete worms (Sella 1985, 1988) has been viewed as a mechanism to resolve this conflict.

Leonard & Lukowiak (1984, 1985, 1991) suggested that sperm may be the currency of trade in the hermaphroditic sea slug *Navanax*. Leonard's (1990, 1991) game-theoretical model, the Hermaphrodite's Dilemma, predicted that mating in populations of hermaphrodites will in general be reciprocal, with mechanisms evolving to prevent cheating by specialization on the preferred sexual role. Based on the Prisoner's Dilemma game (Rapoport & Chammah 1965), Leonard's model accords each player two options: to cooperate (offer to mate in both roles) or to defect (insist on preferred role).

We suggest, however, that in some circumstances at least one member of a pair of simultaneous hermaphrodites may elect a third option,

Correspondence: R. T. Dillon, Jr, Department of Biology, College of Charleston, Charleston, SC 29424, U.S.A. (email: DILLONR@COFC.EDU). A. R. Wethington is now at the Department of Biology, Indiana University, Jordan Hall 142, Box 42, Bloomington, Indiana 47405, U.S.A.

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		Hermaphrodite 2		
		Resist game	Offer to play both roles	Insist on playing preferred role
Hermaphrodite 1	Resist game	No mating		
	Offer to play both roles	Sexual conflict	Swap No conflict	
	Insist on playing preferred role	Sexual conflict	Unilateral mating No conflict	Gender conflict

Figure 1. Extension of Leonard's (1990) Prisoner's Dilemma model, where a game of mating is played between two non-reciprocally mating simultaneous hermaphrodites. The margins are expanded to include a third option, resistance to mating in any capacity. The body of the figure classifies the conflict arising, if any.

not to play the game. Such an animal may show no tendency to initiate copulation as a male (because of low stores of its own sperm, for example) and, by analogy to the dioecious situation elaborated by Bateman, may resist being mounted as a female. The (male–female) conflict arising when a player meets a non-player seems distinct from the conflict between two players (Fig. 1). At issue is whether any mating may take place at all, not the ultimate genders assumed. Because player–non-player conflict seems more similar to that arising in dioecious organisms, we suggest applying the older term, sexual conflict (in its male–female sense). We further suggest a new term, gender conflict, to describe that situation where two non-reciprocally mating simultaneous hermaphrodites (players) vie to assume the same sexual role with each other. Such gender conflict might theoretically be male–male, female–female, or both. It should not be confused with sexual conflict in the male–male sense, as when stags vie for a harem. The purpose of this study was to distinguish between (male–female) sexual conflict and gender conflict in the basommatophoran pulmonate snail, *Physa heterostropha* (Say), a common inhabitant of North American fresh waters, and to estimate the extent to which either sort of conflict may affect the overall probability of mating between two individuals.

Laboratory lines of *P. heterostropha* attain male functional maturity at a mean age of 5.7 weeks under our culture conditions, and become hermaphroditic with the onset of female maturity at a mean age of 7.4 weeks (Wethington & Dillon 1993). Reared in isolation, the first onset of the production of viable offspring by self-fertilization occurs at mean age of 22 weeks. This delay in reproduction, together with a high rate of autosterility and lowered fecundity, reduces the lifetime fitness of self-fertilizing snails substantially below that of out-crossers (unpublished data), which seems to be generally true of basommatophoran pulmonates (DeWitt 1954a; DeWitt & Sloan 1959; van Duivenboden 1983; Jarne & Delay 1990; Jarne et al. 1991). Previously selfing snails generally mate immediately when furnished with a partner, and switch from fertilization by endogenous sperm (autosperm) to fertilization by exogenous sperm (allosperm) within 24 h (Paraense 1955; Richards 1973; Rollinson et al. 1989; Wethington & Dillon 1993).

A number of generalities emerge from the many thorough descriptions of mating behaviour in freshwater pulmonates (DeWitt 1954b; Barraud 1957; Rudolph 1979a, b; Smith 1981; van Duivenboden & ter Maat 1988; DeWitt 1991). When two snails meet, one mounts the other's shell and positions itself near its partner's aperture facing in the same direction. The top snail then everts its penis, containing a milky white fluid, inserts it into the recipient's gonopore, and intromission occurs. The two snails may then either disengage, mate again in the same role, or (in some cases) exchange sexual roles. Basommatophoran pulmonates cannot simultaneously mate in a reciprocal fashion. Estimates of storage capacities for allosperm are generally 50–100 days, sufficient to fertilize several thousand eggs (Rudolph 1983; Monteiro et al. 1984; Rollinson & Wright 1984; Rudolph & Bailey 1985;

Vianey-Liaud et al. 1991). Copulatory plugs seem to be deposited by *Lymnaea stagnalis* (van Duivenboden & ter Maat 1988), *Stagnicola elodes* (Rudolph 1979a), and *Bulinus globosus* (Rudolph 1979b), although such plugs may be temporary. Evidence of multiple insemination in pulmonates has been offered by Mulvey & Vrijenhoek (1981), Rollinson et al. (1989), and Wethington & Dillon (1991).

To distinguish gender conflict from sexual conflict, it must be established that a pair of hermaphrodites, in their struggle prior to mating, have both elected the same gender. van Duivenboden & ter Maat (1985), studying gender choice and mating probability in *L. stagnalis*, suggested that the masculinity of a pulmonate snail (its probability of mating as a male) increases with time since previous mating, as its autosperm store presumably increases. They related the probability that a mating occurred between two snails only to the masculinity of the more masculine individual; snails seemed to be equally receptive as females at all times. We termed this hypothesis of male behaviour 'autosperm build-up' and female behaviour 'complete receptivity'.

The snails observed by van Duivenboden & ter Maat (1985) were reared in mass culture, and were not isolated for more than 12 days. It is not clear that stored autosperm levels (and hence the probability of initiating copulation as a male) in isolated snails should continue to rise asymptotically, even after the onset of self-fertilization. Increased energy allocation to the female function in selfing organisms, with a concomitant drop in pollen or sperm production, is widely reported (reviews by Goldman & Wilson 1986; Barker 1992). Thus another hypothesis is that the probability of a snail seeking to copulate as male decreases with the onset of self-fertilization, after extended isolation. The trigger might be physiological or behavioural; the snails may be aware of other individuals in proximity. We termed this alternative to the autosperm build-up hypothesis of male behaviour 'autosperm re-allocation'.

The hypothesis of complete female receptivity, as proposed by van Duivenboden and ter Maat, does not predict sexual conflict of the sort widely observed throughout the animal kingdom (Parker 1979). Behaviour interpretable as mate rejection in pulmonate snails mounted as female has been often reported, however (Barraud 1957; Rudolph 1979b; van Duivenboden & ter Maat 1988; DeWitt 1991). Thus we offer 'rejective' as a second hypothesis for female modification of male behaviour, suggesting that the phenomenon should be most observable in snails recently inseminated, with large reservoirs of allosperm. Under this rejective hypothesis of female receptivity, snails mounted as females may lower their ultimate likelihood of receiving sperm by evasive manoeuvring, shell shaking, and other behaviour patterns, especially if the prospective suitor is of low quality.

Snails are not predicted to attempt to elicit insemination under any of the hypotheses of mating thus far considered (male build-up, male re-allocation, completely receptive female and rejective female). Because snails reared in isolation suffer delays in reproduction, however, and self-fertilized offspring suffer inbreeding depression, a reasonable hypothesis is that such virgin snails might seek a female role by receptive behaviour, display or pheromone release. We term this third hypothesis of female behaviour 'inductive'. Mucus trail following has been documented in a variety of gastropods, including *Physa* (Cook 1992; Karowe et al. 1993). Although such behaviour has usually been attributed to feeding or homing, stimulatory cues might be included in the mucus of individuals especially receptive to mating.

The inductive female hypothesis and the rejective female hypothesis are potentially complementary. Female receptivity in any encounter may be a function of allosperm stores (or time since copulation as a female), such that female behaviour ranges from active encouragement of all prospective suitors when allosperm stores are depleted to extreme mate discrimination when allosperm stores are high. Thus we offer a fourth hypothesis of female mating behaviour, 'inductive/rejective', which is a combination of the two hypotheses just described.

Here we report the results of mating experiments designed to distinguish between two hypotheses of mating probability in the male capacity and between four hypotheses of female mating behaviour, using our laboratory lines of *Physa*. We present data on the frequency of successful unilateral mating and swapping, as well as behavioural observations from encounters between individual snails of four different reproductive histories. After establishing hypotheses for gender choice and mating probability in both capacities, we then examined the evidence for sexual conflict and gender conflict in non-reciprocally mating simultaneous hermaphrodites.

METHODS

Experimental Animals

We performed mating experiments using two of the albino isofemale lines of *Physa heterostropha pomilia* described by Dillon & Wethington (1992, 1994). Line 29 is homozygous recessive at the *alb1* locus, and line 7 is fixed for albinism at the complementary locus *alb2*. Out-cross progeny are immediately distinguishable by their pigmentation; progeny produced by self-fertilization are albinos. Juvenile snails from these two lines were isolated at about two weeks post-hatch, well in advance of maturation, and cultured in 220 ml plastic cups using methods previously described (Wethington & Dillon 1993). Ultimately it was necessary to rear over 100 snails from each of the two lines in several cohorts.

We designated four categories of snails. Previously selfing virgin (PSV) snails were isolated individuals that had demonstrated the ability to produce viable (albino) offspring by self-fertilization. Not previously selfing virgin (NPSV) snails were isolated individuals that were mature in both male and female function, but not yet producing offspring by self-fertilization. Because we have been unable to identify clear size or age thresholds for maturation in *Physa*, we isolated a large number of even-aged juveniles from each line and carefully monitored their egg production. We measured the first snail laying viable eggs by self-fertilization, and assumed that any larger individual of the same line was a reproductively mature NPSV. After use in a mating experiment, reproductive maturity in putative NPSV snails was verified by the production of viable pigmented young and, when the partner was also a virgin, the induction of pigmented offspring from the partner.

Such NPSV snails, producing pigmented young after pairing with a complementary or pigmented partner, were redesignated not previously selfing mated (NPSM) snails. Previously selfing mated (PSM) snails were PSV individuals subsequently paired with a complementary or pigmented partner and currently producing pigmented offspring. Both PSM and NPSM snails were held with a mature partner, complementary or pigmented, to ensure that allosperm stores remained undepleted. It is more difficult to monitor stores of autosperm. We usually had direct observations of our PSM and NPSM snails copulating as males, a behaviour pattern that seems to occur regularly given a partner of suitable age and size. We therefore assumed that both classes of previously mated snails had mated recently in both capacities, and were carrying somewhat depleted autosperm stores.

Procedures

We paired snails between all four categories of reproductive history to 10 pairings per comparison, 100 pairings overall (Wethington 1992). Each comparison involved 10 different pairs of line 7 and line 29 snails, split evenly. For example, the 10 tests of PSM × NPSV included five in which line 7 served as PSM and five in which line 29 served as PSM. We measured snails with an ocular micrometer and matched each with a partner of similar shell size as closely as possible. The shell of each individual in line 7 was marked with white correction fluid for identification.

We placed pairs of snails in a clean petri dish filled with 65 ml of aerated, filtered pond water, facing each other at a distance of 7 cm. By constant observation, we clocked time until encounter, duration of encounter and duration of mating (if applicable). We defined mating in this situation as the complete insertion of the penis into the mantle cavity of a partner in the vicinity of the gonopore. We recorded the snail acting as male, whether the pair exchanged roles during mating, and whether either snail displayed any evasive behaviour. We paid particular attention if the bottom individual (mounted as female) displayed any of the evasive behaviours previously described in *Physa* by DeWitt (1991): shell swinging, shell jerking, increasing distance to gonopore and head/preputium contact. If involving a virgin in the female role, we verified insemination by the production of pigmented young by the putative female upon re-isolation several days later.

Most encounters did not culminate in mating. If the pair disengaged without mating, we removed them to a clean petri dish of water facing 7 cm apart and re-started the clock. Any pair not mating in 30 encounters was judged incompatible. We have described substantial levels of functional sterility (both self and out-cross) in these laboratory lines and evidence suggests that such sterility may be associated with a lowered likelihood of copulation. We therefore felt it necessary to eliminate any pairs of snails for which no mating was observed after 30 encounters from calculations of mating probability. We summed the results of all unilateral matings across all reproductive categories and used chi-squared goodness-of-fit tests to determine whether individual size or line affected gender choice.

Analysis of Male Function

The two hypotheses of male function differ in their predictions regarding the outcome of PSV × NPSV pairings. Under the autosperm build-up hypothesis, we expected PSV snails to be more likely to mate as males than NPSV snails. If the onset of self-fertilization brings a reduction in the autosperm available (the autosperm re-allocation hypothesis), however, PSV snails might be expected to show less tendency to mate as male than NPSV snails. They might even show a lower probability of initiating copulation as males than PSM or NPSM. By examining the polarity of unilateral mating results, especially the PSV × NPSV pairs, we determined whether the autosperm build-up or the autosperm re-allocation hypothesis might pertain.

Because results ultimately matched the autosperm build-up hypothesis and proved strikingly counter to prediction from re-allocation, we judged that the likelihood of mating as male, estimated from either NPSV or PSV, should always be greater than or equal to that likelihood displayed by NPSM or PSM. We therefore pooled the two virgin categories, and the two mated categories, to distinguish between the four hypotheses of female behaviour. We re-designated the experiments PSV × PSV, PSV × NPSV, and NPSV × NPSV as V × V, the experiments PSV × PSM, PSV × NPSM, NPSV × PSM, and NPSV × NPSM as M × V, and the experiments PSM × PSM, PSM × NPSM, and NPSM × NPSM as M × M.

Analysis of Female Function

Our first test of female behaviour was for complete receptivity. Under this hypothesis, the probability that either a virgin or a previously mated snail mates as a female is simply the probability that its partner donates sperm. All other hypotheses predict that previously mated snails will be less likely to receive sperm than virgin snails. We therefore estimated the probability that virgin males inseminated previously mated females by counting all occasions where a mated partner served as female to a virgin male, either unilaterally or during the course of a swap, in all M × V pairs ultimately proving compatible. Because each encounter affords two opportunities to mate (in the event of swapping), the denominator of this probability was twice the total encounters. Under the completely receptive female hypothesis, this probability should be equivalent to the likelihood that a virgin male may inseminate a virgin female, which we established from the frequency that virgin partners served as females to virgin males, either unilaterally or as during the course of a swap, in compatible V × V pairings. Under the other three hypotheses of female behaviour, however, the likelihood that a virgin male inseminates a previously mated female should be less than that calculated for a virgin meeting a virgin. We performed a one-tailed Fisher's exact test on this prediction.

We next examined the data on female behaviour for evidence that females may increase their likelihood of insemination as required by the inductive and inductive/rejective hypotheses. The most immediately testable prediction of these hypotheses is that virgin snails should (by some unspecified mechanism) increase their likelihood of receiving sperm above the probability of donating sperm that a second snail brings to an encounter. Under the autosperm build-up hypothesis of male behaviour, the probability that a previously mated snail mates at any encounter is expected to be low, and thus a snail encountering a PSM or NPSM partner is presented with a low likelihood of receiving sperm. We estimated the expected frequency at which previously mated snails serve as male as the fraction of encounters between compatible M × M snails in which previously mated snails served as males, either unilaterally or during the course of a swap. We compared this frequency to the observed frequency at which previously-mated snails were induced to mate as males by virgin partners in compatible M × V pairings, either unilaterally or during the course of a swap. We used Fisher's exact method to test the one-tailed hypothesis that previously-mated snails mated as males more often with virgin partners

		Snail 2			
		High autosperm High allosperm	**High autosperm Low allosperm**	**Low autosperm High allosperm**	Low autosperm Low allosperm
Snail 1	High autosperm High allosperm	G = common S = common			
	High autosperm Low allosperm	G = common S = rare	**V × V** **G = common** **S = none**		
	Low autosperm High allosperm	G = none S = common	**M × V** **G = none** **S = common**	**M × M** **G = none** **S = rare**	
	Low autosperm Low allosperm	G = none S = rare	G = none S = none	G = none S = rare	G = none S = none

Figure 2. The model of Fig. 1 applied to the particular case of basommatophoran pulmonates with varying reproductive histories. G indicates gender conflict, S indicates sexual conflict, and the modifiers common, rare and none refer to the likelihood of a mating in which evasive behaviours due to these conflicts are predicted to be displayed. The experimental design included only those comparisons highlighted in bold.

than with other previously mated partners. Rejection of the null hypothesis in this direction would constitute evidence that virgin snails may indeed use some mechanism to increase their likelihood of being inseminated, as required by the inductive and inductive/rejective hypotheses.

Analysis of Conflict

Our observations were consistent only with the autosperm build-up hypothesis of male behaviour and the rejective hypothesis of female behaviour. Female–female gender conflict is not expected under these circumstances, because *Physa* seem to have no mechanism to increase their likelihood of insemination. Male–male gender conflict may be expected, however, when two snails with large autosperm stores encounter one another, and male–female sexual conflict may be expected when at least one partner has a large store of allosperm, and therefore tends to reject suitors (Fig. 2).

The experimental design did not provide for the production of snails with high or low reservoirs of both autosperm and allosperm. (Such individuals could be obtained by artificial disruption of mating.) Male–male gender conflict is expected to be most intense when a pair of virgin snails encounter one another, and sexual conflict should be negligible because virgin snails are expected to have little tendency to reject suitors (Fig. 2). Sexual conflict is expected in our M × V encounters, because a virgin snail 2 will seek to assume the male role, but mated snail 1 will tend to reject further insemination. Gender conflict should be negligible in M × V encounters, because a previously mated snail 1, stored with a ready partner, will have recently mated and thus have low autosperm reserves. We used two different sets of observational data from comparisons of these two categories to estimate the relative strengths of male–male gender conflict and sexual conflict.

First we hypothesized that conflict (of either origin) might be observable in elongated periods of grappling or positioning between compatible snails (those that ultimately mated) not resulting in mating on a particular occasion. We thus defined non-mating contact time as that period after a pair of compatible snails encountered each other but before they disengaged without mating. To estimate the relative strengths of gender and sexual conflicts, we used a *t*-test (Welch's approximation, assuming unequal variances) to compare the mean non-mating contact time over all encounters between compatible V × V pairs to the mean from all encounters between M × V pairs. Pairs mating upon first encounter were necessarily excluded. Significantly greater non-mating contact time between V × V pairs implies that gender conflict is the stronger force, and the opposite result favours sexual conflict.

Similarly, we hypothesized that such evasive behaviours as shell shaking might occur with

Table I. The numerators give the total matings observed in 10 pairs of *Physa* representing four different categories of reproductive history, and the denominators show the encounters observed between pairs of snails ultimately mating

	NPSM	NPSV	PSM	PSV
NPSM	4/83			
NPSV	10/67	10/17		
PSM	2/24	9/50	4/64	
PSV	2/27	10/41	5/40	9/38
Frequency	0.090	0.223	0.112	0.178

NPSM: not previously selfing mated, NPSV: not previously selfing virgin; PSM: previously selfing mated, PSV: previously selfing virgin.
Where $N<10$ matings were observed, this implies that $10-N$ pairs were incompatible, i.e. did not mate in 30 encounters. Mating frequencies are calculated table-wide, for each of the four classes.

Table II. That subset of the matings shown in Table I that involved role-swapping

	NPSM	NPSV	PSM	PSV
NPSM	1			
NPSV	1	5		
PSM	1	0	0	
PSV	1	4	2	6
Frequency	0.22	0.26	0.15	0.50

NPSV, NPSM, PSV, and PSM as in Table I.
Frequencies are calculated table-wide for each class, as proportions of observed matings.

Table III. That subset of the matings reported in Table I that were unilateral

Unilaterally mating as female	Unilaterally mating as male			
	NPSM	NPSV	PSM	PSV
NPSM	3	7	1	1
NPSV	2	5	2	5
PSM	0	7	4	1
PSV	0	1	2	3
Frequency	0.28	0.51	0.45	0.38

NPSM, NPSV, PSM, and PSV as in Table I.
For on-diagonal data (involving snails matched in reproductive history), we counted matings where participants did not swap gender roles. Off-diagonal, the gender assumed by snails of differing reproductive history can be determined by reference to the margins. The frequencies at which each class unilaterally mated as male are calculated as a proportion of total observed matings.

greater frequency in V × V than in M × V pairings if gender conflict were more severe, but that the reverse should be true if sexual conflict is the stronger. Thus, as a second comparison of the two sources of conflict, over each pair of V × V and M × V snails (including incompatibles as well as compatibles) we tallied the total encounters during which an evasive behaviour was noted, regardless of whether that particular encounter resulted in a mating. (We often observed mating in spite of evasive behaviours.) We categorized a pair as evasive if either snail displayed any of the four behaviour patterns described by DeWitt (1991) in 50% or more of their encounters. We calculated Fisher's exact probability to compare the proportion of evasive pairs, over 30 V × V and 40 M × V observations.

RESULTS

The first tests were for potentially confounding effects due to line and size. We observed 65 matings between snails over all 100 pairings (Table I), and the partners swapped roles without disengaging on 21 of these occasions (Table II). In 19 of the 44 non-swapping cases, the line 29 snail assumed the male role, and in 25 cases the line 7 snail served as male, yielding a ratio not significantly different from 1:1 ($\chi^2=0.82$). In 24 of these 44 cases the larger individual served in the male role, and in 19 it served in the female role; one pair of snails was indistinguishable in size. This result is again not significantly different from 1:1 ($\chi^2=0.58$). Thus we can discern no line or size effects in these data.

Male Function

All 10 PSV × NPSV pairings ultimately resulted in a mating, but four of these were swaps (Tables I, II). In five of the unilateral matings remaining, PSV served as male, and on only one occasion did PSV unilaterally serve as female (Table III). This observation is counter to expectation from the autosperm re-allocation hypothesis of male mating probability, and consistent with the autosperm build-up hypothesis.

We found two other trends with regard to gender choice (Table III). In NPSV × PSM

Table IV. Mean ± SD duration (min) of various stages in encounters between compatible virgin (V) and previously mated (M) snails

	V × V	V × M	M × M
Time to encounter	7.84 ± 7.54	8.61 ± 8.13	7.93 ± 11.95
(*N*)	(95)	(184)	(171)
Mating time	13.81 ± 9.97	14.74 ± 11.88	13.49 ± 13.21
(*N*)	(28)	(26)	(10)
Non-mating contact time	2.08 ± 4.09	1.80 ± 4.46	1.45 ± 2.71
(*N*)	(67)	(158)	(161)
Total observation time	22.82 ± 26.64	16.32 ± 21.04	11.41 ± 18.70
(*N*)	(95)	(184)	(171)

pairings, the NPSV took the male role seven times and the female role only two times, with one incompatible pair observed. In NPSV × NPSM pairings, the NPSV again adopted a unilateral male role seven times and again a female role only twice (one swap). Then ranking reproductive categories from left to right in order of the likelihood they mate as male:

$$PSV > NPSV > NPSM \text{ and } PSM$$

This hierarchy agrees with expectation if male mating probability is a function of autosperm stores building up asymptotically with time, with no re-absorption or re-allocation of sperm.

Because there was no evidence of sperm re-allocation, we combined the previously selfing and not previously selfing categories. Snails (of any reproductive history) required an average of about 7–8 minutes before encountering one another in our petri dishes (Table IV). They then either disengaged after 1–2 minutes or mated for 13–14 minutes. Note that the standard deviations of these times were very large, approaching or exceeding the means in most cases.

Female Function

Our first test of female reproductive behaviour was a comparison of the predictions of the completely receptive hypothesis to those of the other three hypotheses. We observed 184 encounters between compatible virgin and previously mated snails, resulting in 26 matings (Table I). In 16 unilateral matings, the previously mated snail served as female, and in 6 matings, the previously mated individual served unilaterally as a male, leaving 4 swaps in the remainder (Table II, III). The observed frequency at which previously mated snails mate as females with a virgin suitor is thus $(16+4)/2(184)=0.054$. By comparison, across the three V × V experiments, 29 matings were observed in 96 encounters, including 15 swaps (Table I, II). Then the observed frequency at which virgin snails mate as females when encountering other virgin snails would be $(29+15)/2(96)=0.229$. This mating frequency is significantly greater than that observed for previously mated females mating with virgin males ($P<0.0001$, Fisher's exact method). Because virgin males appear to be significantly less likely to mate successfully with previously mated females than with virgin females, the complete receptivity hypothesis of female reproductive behaviour cannot be supported.

We next examined the data for evidence that snails can be inductive in the female role. As noted above, 6 of the 184 encounters between compatible virgin and previously mated snails resulted in the virgin mating unilaterally as a female. When combined with the four swaps, the observed frequency at which virgin snails would seem to have induced mating in the female capacity was $(6+4)/2(184)=0.027$. By comparison, we observed 10 matings between compatible pairs of previously mated snails in 171 encounters, including two swaps (Tables I, II). Then the background frequency at which previously mated snails mate as male is $(10+2)/2(171)=0.035$. The 2.7% observed incidence of successful induction by virgin snails is in fact lower than the 3.5% background rate at which previously mated snails mate between each other, although not significantly so ($P=0.67$, Fisher's exact method). Thus neither the inductive nor the inductive/rejective hypotheses or female reproductive behaviour can be supported. A hypothesis of female reproductive behaviour

Table V. The occurrence of identifiable evasive behaviours in 50% or more of encounters between pairs of virgin snails (V × V) and between pairs of virgin and previously mated snails (M × V)

	Evasive	Not evasive	Total
V × V	24	6	30
M × V	9	31	40
Total	33	37	70

Fisher's exact probability; $P<0.0001$, two-tailed.

that is rejective (only) alone seems to fit our observations.

Conflict

We observed all evasive behaviours described by DeWitt (1991) for *P. gyrina* during the course of these *P. heterostropha* mating experiments. Evasion was noted in 50% or more of the encounters between 24 (of 30) pairs of virgin snails observed (Table V). We suggest that such behaviour must be due to male–male gender conflict, because sexual conflict is not predicted between virgin snails by the rejective hypothesis of female reproductive behaviour. We noted a frequency of evasive behaviour greater than or equal to 50% in only nine of 40 comparisons between virgin and previously mated snails. Our hypotheses attribute evasion in this situation to sexual conflict between one snail serving as female and another serving as male. Because the frequency of evasion is significantly greater in V × V than in M × V comparisons (Fishers's exact $P<0.0001$, two-tailed), we conclude that male–male gender conflict may be a substantial force in some circumstances.

Eleven of the 29 compatible V × V pairs mated upon first encounter, but only 4 of the 26 M × V compatible pairs did so. The mean non-mating contact time of 2.08 minutes observed for the remaining 18 V × V compatible pairs was not significantly different from the 1.80 minutes observed for the 22 M × V pairs, over 67 and 158 encounters, respectively (Welch's approximate $t=0.457$, $df=134$; Table IV). Judging by time spent positioning or grappling in unsuccessful mating attempts, sexual conflict and male–male gender conflict seem to be of equal consequence.

DISCUSSION

We detected no potentially confounding effects of relative size on the gender selected by our experimental animals. Neither have such effects been observed in *Navanax* or terrestrial pulmonates (Leonard & Lukowiak 1985; Baur 1992). Although the phenomenon has been reported in *P. gyrina* (DeWitt 1991), it may have been minimized here by our deliberate selection of pairs approximately matched in size. A line effect was also not detectable in our experiments, although Rollinson et al. (1989) reported that individuals from an isofemale line of the freshwater pulmonate *Bulinus* (homozygous for a particular allozyme allele) were more likely to serve in the female role than other individuals.

Our results were consistent with the autosperm build-up hypothesis of male reproductive behaviour in *Physa*. In encounters between PSV and NPSV snails, the PSV individuals (isolated for the greatest length of time) were more likely to assume the male role unilaterally. NPSV snails were more likely to mate as males in encounters with snails whose sperm stores were depleted by previous mating. The likelihood of mating as a male apparently increases in *Physa* up to 20–30 weeks of isolation in culture, probably much greater than typical life span in the wild. If there is any re-allocation of energetic resource from sperm to egg production at onset of self-fertilization, we can see little behavioural evidence of it. Our observation that PSV snails were less successful in mating with previously mated snails than were the younger NPSV individuals suggests that the overall likelihood of mating may lessen with age, however.

Our results were consistent with the rejective hypothesis of female behaviour. The observed probability that a virgin snail receives sperm from a virgin partner was 22.9%, very significantly greater than the 5.4% observed for previously mated snails encountering virgin partners. Snails, especially previously-mated ones, can apparently reject partners, perhaps exercising some choice regarding mate quality (DeWitt 1991). We could see no evidence that snails have mechanisms to induce insemination from otherwise unresponsive partners. The observed 2.7% probability that a virgin snail receives sperm from a previously-mated partner shows no improvement over the 3.5% background probability at which previously-mated snails receive sperm from others previously mated.

Leonard (1990, 1991) and Leonard & Lukowiak (1985) predicted that most encounters among simultaneous hermaphrodites should involve swapping, regardless of reproductive history. Sella (1990) also predicted an exchange of mating roles in situations where self-fertilization is deleterious or precluded. We observed swapping in about half of the matings between pairs of *Physa* from most reproductive categories. The phenomenon occurred at much lower frequencies in NPSV × NPSM pairings (only one of 10 matings) and in NPSV × PSM pairings (none of nine matings). In both cases, the virgin snail usually mated as male unilaterally with the previously mated snail. The rarity of swapping in encounters between virgin and previously mated snails is further evidence against induction in the female role. Evidently, even over many minutes of physical contact with a previously mated partner, virgin snails themselves are only inseminated at background frequencies.

We have separately analysed our data on time-to-encounter, the period elapsed between the start of each trial and the first contact between pairs of snails (R. T. Dillon, E. R. Eastman & A. R. Wethington, unpublished data). These times seem to be a simple function of the sizes (and hence speeds) of the snails involved, with no effect directly attributable to trail-following or other environmental cue. These observations again suggest that the snails are not inductive.

Thus snails of no reproductive history seem to seek the female role in our experimental conditions. Two virgin snails, upon first meeting, are both, however, each expected to seek the male role. Our observation of evasive behaviours in 50% or more of the encounters between 24 of 30 virgin pairs does not seem attributable to male–female sexual conflict, because both virgin snails would benefit from insemination. Rather, this result constitutes, to our knowledge, the first recognition of male–male gender conflict in simultaneous hermaphrodites. The demonstration that evasive behaviours are significantly more common in V × V pairs than in M × V pairs, where they are attributable to sexual conflict, implies that male–male gender conflict may be strong in some circumstances.

Rudolph & Bailey (1985) mated 16 pairs of the basommatophoran pulmonate *Bulinus* daily for 14 consecutive days, allowing only one partner to serve as male. Attempts by the designated female to copulate as male were artificially disrupted, rendering her the high autosperm/high allosperm type shown in Figure 2. Non-receptive female behaviour, as previously described by Rudolph (1979b), was often observed as high/high individuals evaded (probably) high/low partners. At very low frequency (six occasions of 224 encounters) the high/high designated female successfully frustrated copulation. Although Rudolph and Bailey designed their experiment with other questions in mind, we interpret their results as evidence that, given a large sample size, male–male gender conflict (rather than non-receptive female behaviour per se) might have a detectable effect on mating probability.

At our sample sizes we could find, however, no evidence that male–male gender conflict affected the probability of successful mating in *Physa*. Encounters between pairs of virgin snails were more likely to culminate in successful mating than encounters between snails of any other reproductive history. Gender conflict does not seem to prolong non-mating contact times (the periods of apparent grappling between compatible snails not on that occasion culminating in copulation) beyond those normally observed when a virgin snail attempts to mate as male with a previously-inseminated (and hence rejective) partner. Thus gender conflict in these snails seems to be of minor evolutionary consequence. Because the opportunity to swap roles is ordinarily available, the loser in a male–male gender conflict will generally also gain copulation as a male, when the winner's autosperm stores, and hence its drive to mate as male, are lowered.

Gamete trading has been proposed as a method of resolving gender conflict between hermaphrodites (Leonard 1984, 1990). Egg trading is expected in animals with external fertilization (sea basses, polychaetes) and sperm trading in animals with internal fertilization, such as the marine slug *Navanax*. We saw no evidence of the alternation of sexual roles that has been interpreted as sperm trading in *Navanax*, however (Leonard & Lukowiak 1985). The 29 V × V matings that we observed included 14 that were unilateral and 15 that involved single reversals. A single one (of the six) swaps we observed between M × V or M × M snails involved two reversals (a PSV mated as male–female–male with a PSM). This constitutes no evidence of sperm trading.

Perhaps it is to mitigate gender conflict that induction in the female role has not evolved. Although a virgin snail may incur some cost in departing from a mating uninseminated, the evolution of a more active role for the female would promote female–female gender conflict as well as male–male. Ultimately, mating may be more likely in a population of snails under the autosperm build-up/female rejective model than under any other.

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