Size-dependent discrimination of mating partners in the simultaneous hermaphroditic cestode *Schistocephalus solidus*

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The cestode *Schistocephalus solidus* is a simultaneous hermaphrodite that reproduces in the gut of birds, or for this study in an *in vitro* system that simulates the gut of the bird. Like many other helminth parasites, *S. solidus* can reproduce by self- and cross-fertilization. Hermaphrodites are expected to mate not primarily to get their own eggs fertilized, but rather to get the opportunity to fertilize a partner's eggs. Because *S. solidus* has a size-dependent sex allocation (i.e., larger worms are more biased toward female allocation and produce more egg mass), we expect larger individuals to be attractive mating partners for smaller ones. However, this may be a one-directional preference, as smaller individuals may not be attractive to larger ones. We tested this experimentally by studying the reaction of focal worms of different sizes to a compartment containing a potential mating partner that was either smaller or larger than the focal worm. The focal worms were, on average, closer to the compartment containing the stimulus than to an empty control compartment. Moreover, they indeed showed a preference for larger stimulus worms than for smaller ones. They even tended to avoid being close to stimulus worms of very small size compared to themselves. This may reveal a general preference for cross-fertilization over selfing, but it also indicates that all the genetic benefits from outcrossing do not necessarily outweigh the costs of mating with a relatively small individual and that the worms may take this into account in their reproductive decisions. *Key words:* cross-fertilization, hermaphrodite's dilemma, inbreeding avoidance, *Schistocephalus solidus,* self-fertilization, simultaneous hermaphroditism, size-dependent preference, two-player games. [*Behav Ecol* 13:254–259 (2002)]

I n hermaphrodites as well as in species with separate sexes, the fecundity of the female function is normally limited by the amount of energy available for egg production, and the fecundity of the male function is normally limited by the number of eggs available (Bateman, 1948; Charnov, 1979). Therefore, simultaneous hermaphrodites are expected to mate not primarily to get their own eggs fertilized, but rather to get the opportunity to fertilize a partner's eggs. If two hermaphrodites meet for reproduction, they are normally expected to be in a conflict over who is allowed to give how much sperm (e.g., Dugatkin and Reeve, 1998; Fischer, 1980; Leonard, 1990; Michiels, 1998). Because of this kind of social dilemma, simultaneous hermaphrodites are expected to be choosy about who they mate with (Michiels, 1998), especially when individuals vary in quality (e.g., fecundity), copulations are costly, and mate choice is possible due to high density (Ridley, 1983).

For a number of cestode species, multiple infection in the final host results in multiple mating between the individual parasites (Nollen, 1983). Moreover, many parasite helminths are not completely outbreeding when given the opportunity. Often, some of the sperm they collect in their receptaculum seminis are their own, indicating some selfing even in the presence of a potential mate (Nollen, 1983, 1997; Trouvé et

al., 1996). Apart from this, however, not much is known about the potential of cestodes for mate-choice decisions. This is surprising because hermaphroditic cestodes may face social dilemma situations (Leonard, 1990) that are probably easier to specify than in other systems (Dugatkin and Reeve, 1998). Their mate-choice decisions and any phenotypic plasticity in egg production may reveal evolved solutions to two-player or multiplayer nonzero-sum games (Colman, 1995; Leonard, 1990). Moreover, the techniques to study cestodes in *in vitro* systems that replace their final hosts are often well developed and tested.

The tapeworm *Schistocephalus solidus* is a simultaneous hermaphrodite that is able to reproduce by selfing and cross-fertilization (Clarke, 1954). Its entire reproduction (i.e., sperm and egg production, mating, and egg release) lasts only few days and takes place in the gut of birds shortly after infection (Clarke, 1954; Schärer and Wedekind, 1999). Birds get infected by eating infected sticklebacks (*Gasterosteus aculeatus*), which get the parasite by eating infected copepods (a three-host cycle that includes two intermediate hosts). Reported percentages of infected sticklebacks are often high, sometimes up to 100% (Abildgaard, 1790; Dick, 1816; Hickey and Harris, 1948; Hopkins and Smyth, 1951; Jääskeläinen, 1921; Pallas, 1761; Smyth, 1947; Vik, 1954). Infected sticklebacks often contain more than one plerocercoid (cestode larvae), and the total parasite weight can even exceed the fish net weight (Smyth, 1994; Vik, 1954; Wedekind, unpublished data). Worm growth is positively correlated with fish size at infection (Schärer et al., 2001); in other words, worm size is largely conditionally determined. Adult worm weight ranges from around 50 mg (Tierney and Crompton, 1992) up to at least 1029 mg (Wedekind, personal observation). In birds, up

to 300 adult worms per individual host have been observed (Nybelin, 1919; Pallas, 1761; Vik, 1954).

The *in vitro* technique that replaces the final host has been established by Smyth (1954) and was modified by Wedekind (1997)and Schärer and Wedekind (1999). *S. solidus* is therefore a potential model for the study of evolved solutions to social dilemmas that can be experimentally specified (Wedekind et al., 1998).

Female fecundity of *S. solidus* is positively correlated with body size (Schärer and Wedekind, 1999; Wedekind et al., 1998), as it is in many invertebrates (Coadwell and Ward, 1982; Greenspan, 1980). Moreover, sex allocation in *S. solidus* is size dependent: larger worms are more biased toward female allocation (Schärer et al., 2001). Therefore, larger worms are expected to be very attractive mating partners to smaller ones (i.e., sperm donors are expected to prefer matings with high fecundity sperm acceptors).

In mate choice experiments, two stimulus animals who differ in a trait the experimenter is interested in are normally offered to a focal animal who is then allowed to choose. This method has a general problem that is often neglected: it cannot distinguish between a potential attraction of the focal animal toward one stimulus individual and a potential avoidance of the other one. To disentangle attraction and avoidance, we presented only one stimulus animal to each focal individual. This allowed us to test (1) whether *S. solidus* is able to locate and actively move toward a potential mating partner, and (2) the prediction that, given the choice between no partner and a potential mating partner, relatively larger stimulus worms are more attractive than smaller ones.

There are a number of potential benefits for outbreeding as compared to selfing in *S. solidus*. Selfing is an extreme form of inbreeding that normally causes a fitness reduction through inbreeding depression and a general reduction of heterozygosity and of genetic diversity in the offspring (Charlesworth and Charlesworth, 1987). Genetic diversity among the offspring can be very advantageous in parasite–host interactions (Baer and Schmid Hempel, 1999). Wedekind and Rüetschi (2000) have used *S. solidus* and its first intermediate host as a model to experimentally test the effect of genetic diversity among the parasites in a multiple exposure. They found that copepods are more susceptible to infection when exposed to a genetically more heterogeneous set of parasites than to a more homogeneous set (i.e., increased genetic diversity among the offspring offers an advantage against the defense of the first intermediate host). We therefore expect cross-fertilization to be generally preferred over self-fertilization in *S. solidus*. However, when the potential mating partner is smaller than the focal worm, the focal worm is expected to weigh the benefits of outcrossing with the disadvantage of mating with a smaller mate. We studied the reaction of focal worms of different sizes to a compartment containing a potential mating partner that is either smaller or larger than the focal worm.

METHODS

The plerocercoids came from sticklebacks that had been infected naturally, but under controlled conditions (methods described in Wedekind and Milinski, 1996), and had been kept in the laboratory for about 8–12 months. To obtain the worms, the fish were killed by a cerebrospinal cut and the plerocercoids were removed aseptically from the stickleback. Each fish contained one to four plerocercoids. The worms were weighed on a balance to the nearest milligram and kept in cell culture medium at room temperature for a maximum of 4 h. This culture medium consisted of sterilized minimum essential medium with Earle's salts, L-glutamine, 25 mM HE-
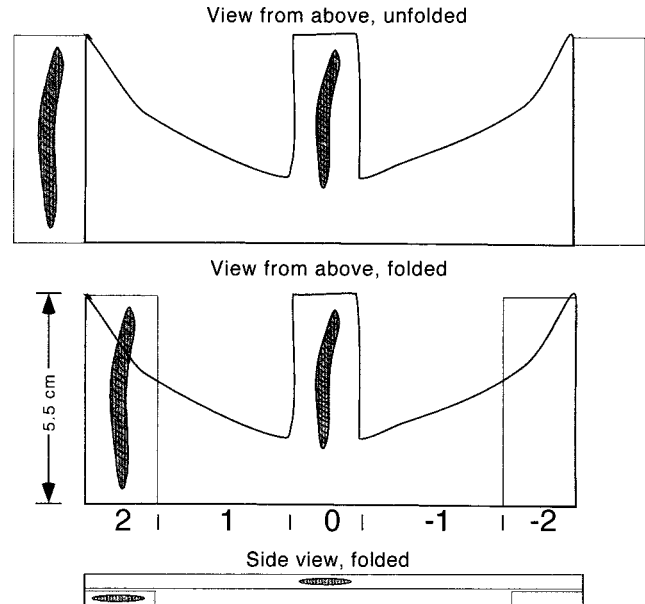

Figure 1
Schematic view from above (unfolded and folded) and from the side of the fork-shaped nylon bag. The numbers are the a priori classification and scoring of the sections the focal worms were assigned to during the course of the experiment. The focal worm was placed in the middle prong and the stimulus worm could be placed in either one of the two side prongs. The focal worm could position itself anywhere between positions 2 and −2 (positive values indicate a preference for the side with the stimulus worm), while the stimulus worm could not leave its compartment. As the decisive criteria, the position of the head of the focal worm was assigned to one of the five sections at each time lap. Anterior and posterior end of an individual could be distinguished by the direction of the individual's movements.

PES buffer (Sigma) and additives (per liter of medium: 1 ml penicillin/streptomycin, 6.5 g D-glucose), and had been titrated with NaOH to a pH of 7.5.

We used fork-shaped, nylon mesh bags (200 μm mesh size) that consisted of three compartments for the preference tests. The three compartments were separated by seams and were folded in a way that allowed the focal worm in the middle compartment to position itself right above the stimulus worm, without the two worms touching each other (Figure 1). The mesh bags had been watered for at least 4 days before sterilization and use.

Pairs of focal and stimulus worms came from different donor fish. Moreover, because we had been keeping different family lines of *S. solidus* over several generations in the laboratory, the pairs of focal and stimulus worms were chosen from different family lines [i.e., they belonged to the same original population (a pond near Bochum, Germany) but had different parents and different grandparents].

In 7 of the 45 original experimental pairs, the pH dropped below the tolerance level (as indicated by the color of the pH indicator), and the worms died before the end of the experiment. In two other cases, the focal worm entangled itself in the seam of the nylon net and was therefore not able to change position from this moment on. Neither the mean weight nor the weight ratio of these nine pairs were significantly different from the remaining sample (weight: $t = -0.41$, $p = .69$; weight ratio: $t = -1.17$, $p = 0.25$). These nine pairs were excluded from all further analyses.

In 20 of the 36 experimental pairs, we chose a focal worm that was heavier than the stimulus worm. In the remaining
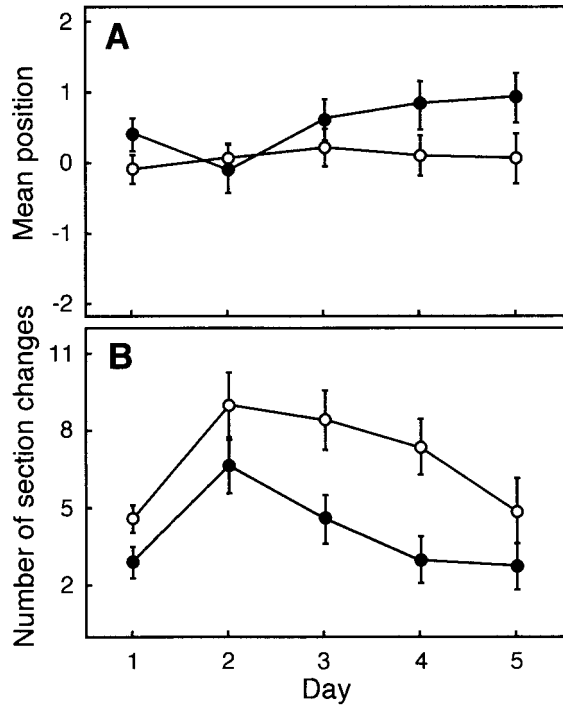
Figure 2
Behavior of focal worms in the fork-shaped nylon bag. Filled circles = the stimulus worm is larger than focal worm ($n = 16$); open circles = the stimulus worm is smaller than focal worm ($n = 20$). (A) Mean daily positions ($\pm$SE) of focal worms during the 5 days of observation. Positive values indicate a preference for the side with the stimulus worm. (B) Mean number of section changes ($\pm$SE) of focal worms during the 5 days of observation.

Figure 3
Mean position of focal worms (means of 120 hourly measurements during the 5 days of observation) plotted against (A) focal worm weight and (B) weight ratio (i.e., focal worm weight/stimulus worm weight). Total number of section changes (during 5 days) of the focal worms plotted against (C) their weight, and (D) the weight ratio. Filled circles = the stimulus worm is larger than focal worm; open circles = the stimulus worm is smaller than focal worm. The figures give the regression lines, the Pearson correlation coefficients (r) and the p values (p_2 = two-tailed, p_{dir} = directed, see Rice and Gaines, 1994).

pairs the focal worms was lighter. We randomly assigned the worms to these two experimental groups. The average weight and the average weight difference among the two groups was similar (focus worm heavier than stimulus worm: mean weight $\pm$ SE = 411.40 $\pm$ 27.5 mg, mean weight difference = 123.10 $\pm$ 17.66 mg; focus worm lighter than stimulus: mean weight = 448.60 $\pm$ 37.42 mg, mean weight difference = 132.25 $\pm$ 19.35 mg; t tests: $t < 0.82$, $p > .4$). Overall, focal worm weights ($\mu = 432.75 \pm 23.77$ mg) did not differ significantly from stimulus worm weights ($\mu = 423.14 \pm 27.52$ mg; $t = 0.264$, $p = .79$).

We placed the focal worm in the middle prong of the nylon mesh bag, and the stimulus worm was placed at random in one of the two side prongs. All three openings of the bag were then closed by melting the ends of the nylon layers with a lighter.

One bag with a stimulus and a focal worm each was placed in a translucent glass container, which was then filled with 400 ml culture medium. After covering the containers with a lid, they were placed into a water bath at 40°C. We then continuously recorded the focal worm's behavior with a video camera for 5 days. Previous studies have shown that within 5 days of incubation at 40°C, worms have produced 95% of their total egg mass (Schärer and Wedekind, 1999).

The culture medium in the glass containers was exchanged every 2 days. This was done with prewarmed medium without taking the containers out of the water (i.e., the worms remained in a constant temperature environment during the whole experiment).

For data analysis, five sections in the fork-shaped nylon bag were defined a priori and arbitrary scores were given (Figure 1). We determined the position of the head of the focal worm
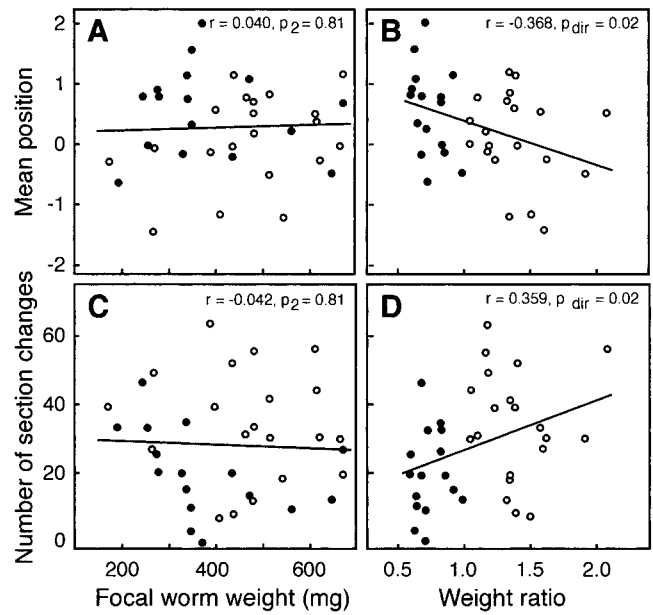
after every 1-h sequence of film. From these scores, the mean position and a measure of daily activity could be determined. We defined daily activity as total number of changes of sections within the 24 observations per day. The weight ratio of each worm pair was computed as focal worm weight divided by stimulus worm weight. This weight ratio ranged from 0.52 to 2.08.

The data were analyzed with the JMP IN 3.2.1. statistics package (Sall and Lehman, 1996). We used parametric statistics after data plots indicated that the assumptions of such statistics were not violated. Directed statistics (Rice and Gaines, 1994) were performed when clear a priori predictions about the direction of the effects existed.

RESULTS

Overall, the focal worms showed on average a preference for the side that contained the stimulus worm ($t = 2.16$, $n = 36$, $p = .02$, directed, Figure 2a). Focal worms that were smaller than the stimulus worm showed a stronger preference for the side of the stimulus worm than relatively larger focal worms (one-way ANOVA with repeated measurement, using Huynh-Feldt correction: effect of treatment group: $F = 3.45$, df = 1, $p < .05$, directed). The mean positions did not differ significantly between the days of observation (effect of day: $F = 1.31$, df = 4, $p = .19$), nor was the interaction between treatment group and day significant ($F = 1.16$, df = 4, $p = .33$; Figure 2a).

The weight of the focal worms did not significantly influence their behavior in the test apparatus (Figure 3a,c). However, the effect of our experimental treatment was confirmed by the finding that the actual weight ratio (focal worm weight divided by stimulus worm weight) influenced the preference

of the focal worms in the experimental setup (Figure 3b). This effect could not be explained by mere differences in the absolute weight of pairs (partial correlation coefficient, controlling for absolute weight of pairs, $r_{xy.z} = .347$, $p = .03$, directed).

To test whether the threshold for focal worms to either approach or withdraw from a stimulus worm is different from equal-weight level, the origin in Figure 3b was set to the mean position 0 and the weight ratio 1. The intercept of the regression line was then positive (0.357) and significantly different from this new origin ($t = 2.821$, $p = .008$). This suggests that the focal worms have a preference for outcrossing even if the offered mating partner was a bit smaller than the focal worm itself. The regression line drops below the mean position of 0 at a weight ratio of about 1.5.

Focal worms that were smaller than their respective stimulus worm were less active in the experimental setup (repeated-measures ANOVA using Huynh-Feldt correction; effect of treatment group: $F = 8.70$, df $= 1$, $p = .006$, two-tailed; Figure 2b). Again, the more detailed analysis using the actual weight ratios confirmed this result (Figure 3d). The absolute weight of worm pairs would not explain this result (partial correlation coefficient, controlling for weight of pairs, $r_{xy.z} = .334$, $p = .05$, two-tailed). The mean number of section changes of the two groups of focal worms was significantly different between days (repeated-measures ANOVA; effect of day: $F = 9.03$, df $= 4$, $p < 0.0001$, two-tailed; Figure 2b). The focal worms appeared to be increasing their activity from day 1 to day 2 and decreasing it thereafter. However, there did not seem to be a final resting point during the 5 days of observation. The two experimental groups did not differ significantly with respect to time (interaction treatment group $\times$ day: $F = 0.99$, df $= 4$, $p = .42$, two-tailed).

DISCUSSION

Darwin (1871) assumed that intersexual selection would not occur in hermaphroditic species because the two sexes are combined in one individual. This assumption has been disproved by Bateman (1948), Charnov (1979), Morgan (1994), and Michiels (1998). Sexual selection can be present in hermaphrodites, although it is generally agreed to be weaker than in species with separate sexes (Greeff and Michiels, 1999a). There may be several reasons for this. The most important one is that if two sexes are combined in one individual, selection on one sex function cannot be independent of the other one (Charnov, 1979; Greeff and Michiels, 1999a; Morgan, 1994). Another reason is the often limited access to mating partners in hermaphrodites due to sessile life forms or low population densities (Charnov, 1979; Morgan, 1994), or severe sperm competition and sperm digestion (Greeff and Michiels, 1999b). These confounding effects could be the reason that several empirical studies in the past have failed to detect mate choice in simultaneous hermaphrodite species (Baur et al., 1998; Peters and Michiels, 1996; Trouvé and Coustau, 1999). One exception was the study by Vreys and Michiels (1997), which demonstrated mate choice in the hermaphroditic flatworm *Dugesia gonocephala*. This flatworm cannot reproduce by selfing, in contrast to *S. solidus*. Greeff and Michiels (1999a) have shown that sexual selection is expected to be even weaker in hermaphrodites that do not necessarily need a partner to reproduce. Nevertheless, we found that *S. solidus* responds differently to potential mating partners and that this response depends on the size differences between the two worms.

A sexual conflict arises when the interests of the mating partners do not coincide. Hermaphroditic individuals are normally assumed to have a preferred sexual role. Therefore, the mating of two hermaphrodites can often be seen as a conflict that may lead to a dilemma situation. Leonard (1990) called it "the hermaphrodite's dilemma," which summarizes several nonzero-sum games that are each defined by the payoff matrices of the two actors. The evolutionarily stable strategy to such games may sometimes be a mating system based on reciprocity with occasional attempts of cheating in the preferred role (Axelrod and Hamilton, 1981; Leonard, 1990, 1999). Reciprocity can be achieved through either egg or sperm trading depending on the preferred sexual role and has been found for several taxa (egg trading: Fischer, 1984; Petersen, 1995; Sella et al., 1997; Sella and Lorenzi, 2000; sperm trading: Leonard and Lukowiak, 1984; Michiels and Bakovski, 2000; Vreys and Michiels, 1998). *S. solidus* does not have any structures for sperm digestion. The female reproductive success is, in contrast to the male function in this species, constrained by size (Schärer et al., 2001; Schärer and Wedekind, 1999; Wedekind et al., 1998), and the reproductive output of the male function seems restricted only by the number of available eggs (Schärer and Wedekind, unpublished results). This suggests that the male role is, in general, the preferred sexual role in *S. solidus*. Mate avoidance is therefore likely to be explained by a strategic decision related to the female function.

A large worm has much egg mass to offer but will get only little in return if it mates with a small worm. There are good reasons that *S. solidus* is expected to weigh outbreeding against the number of offspring and the degree of relationship to these offspring. On one hand, Wedekind et al. (1998) observed a better hatching rate in eggs of paired worms than in selfed eggs, and Wedekind and Rüetschi (2000) found that genetical heterogeneous progeny (that would result from outcrossing) can infect a broader range of hosts and have, overall, higher fitness. On the other hand, selfing reduces the costs of mating (Schärer and Wedekind, 1999) and increases the degree of relatedness between parent and offspring. Moreover, inbreeding depression is expected to be less severe in species with frequent selfing than in obligate outbreeders (due to purging; see Charlesworth and Charlesworth, 1987).

We found evidence for the kind of one-directional preference that could be predicted from an asymmetry of payoffs: Focal worms preferred to be near large stimulus worms rather than small ones. Furthermore, worms that were offered a relatively small stimulus worm spent more time moving around than those that were offered a large worm.

Our findings also demonstrate that communication takes place between individual *S. solidus* and that it is based on tactile and/or chemical cues. Pheromonal mate attraction has been suggested for other helminth parasites (e.g., Fried et al. 1980; Imperia et al., 1980; Miller and Dunagan, 1985). This leads to an alternative potential explanation of our results that needs to be considered. Greeff and Michiels (1999a) showed that hermaphrodites should not invest too much in mate finding, but rather exploit mate finding of other conspecifics, thus saving their own resources. In our study species, large individuals probably produce more metabolic signals than small ones, and therefore large worms could play a sit-and-wait-to-be-found strategy. However, Greeff and Michiels' model is based on substantial costs of mate finding. Mate finding is probably not very costly in *S. solidus* because adults of this parasite must constantly counteract the movement of the gut, as this species has no adhesive structure (hocks or suckers) to attach to the gut tissue. Moreover, *S. solidus* has been found in high numbers compacted in the lower intestine of bird hosts (Vik, 1954). Moreover, Greeff and Michiels' (1999a) model predicts that worms that produce more pheromones play a more passive role in mate finding than others. We cannot find any evidence for this prediction in our study species,

as the number of section changes of focal worms did not correlate with the worms' weight.

The worms who seemed to avoid a potential mate either waited for a better opportunity or started to reproduce by selfing. Schärer and Wedekind (1999) tested whether *S. solidus* waits for a partner if isolated. They found no difference in the start of egg release between paired and isolated worms. After the start of egg release, isolated worms shed eggs at an even faster rate than paired worms (Schärer and Wedekind, 1999). For *S. solidus,* waiting in the final host is assumed to be costly because constantly counteracting the movements of the gut is probably quite energetically demanding. Therefore, while waiting, individuals probably use energy they could otherwise allocate to reproduction. This may be the main reason that waiting does not seem to be an option that adult *S. solidus* play. The behavior we observed in the present study indicates that *S. solidus* even prefers selfing over cross-fertilization if their potential mating partner is much smaller than they are.

There are at least two approaches that can be used to investigate the advantages of outbreeding. First, one can measure the fitness of different types of progeny, as done by Wedekind and Rüetschi (2000) for the present system. Second, one can measure the advantages of outbreeding by studying the decisions that parents make about their reproduction, assuming that these decisions are evolved under natural selection. The size-related behavior of focal worms, as we observed it here, indicates that outbreeding is not always the preferred way of reproduction. It could reflect the intention of *S. solidus* to outcross depending on how much the enclosed partner can offer in terms of egg mass, with the preference to outcross becoming stronger the more foreign eggs could potentially be fertilized relative to own egg mass. We observed a kind of switching point (i.e., a switch from a general tendency of preferring to be near the stimulus worm toward a general tendency of avoiding the stimulus worm) when the focal worm was about 1.5 times heavier than the stimulus worm. However, this first quantitative result has to be interpreted with care because it is possible that our experimental setup and the *in vitro* system may not adequately reflect the natural situation in all respects (e.g., movement, volume, and composition of the medium). The *in vitro* system may yield different values for an optimal inbreeding/outbreeding ratio (i.e., for a cutpoint where selfing wins over outcrossing) than an *in vivo* system. Moreover, mean positions of focal worms are only a first approximation of mate preferences.

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