Cryptic individual-level variation in propensity for selfing in a hermaphroditic snail

Anja Felmy[1,2,3], Alena Streiff[2,3]& Jukka Jokela[2,3]

[1]University of Zurich, Department of Evolutionary Biology and Environmental Studies, 8057 Zurich, Switzerland.

[2]EAWAG, Swiss Federal Institute of Aquatic Science and Technology, Department of Aquatic Ecology, 8600 Dübendorf, Switzerland.

[3]ETH Zurich, D-USYS, Institute of Integrative Biology, 8092 Zürich, Switzerland.

Corresponding author: Anja Felmy, anja.felmy@uzh.ch

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Abstract

For mating-system evolution, individual-level variation is essential. In self-compatible hermaphrodites, individuals may vary in their lifetime propensity for selfing, which consists of an individual and environmental component. According to the reproductive assurance hypothesis explaining partial selfing, a key environmental factor is mate availability, which fluctuates with population density.

We quantified individual variation in selfing propensity in a hermaphroditic snail by manipulating mate availability, recording mating behaviour, estimating selfing rates from progeny arrays, and measuring female lifetime fitness. We found four classes of individuals: pure outcrossers, pure selfers, and two types of plastic individuals. These classes only became apparent in the laboratory; the field population is outcrossing. All classes were present both under low and increased mate availability. This large variation in selfing propensities rendered effects of differential mate availability on selfing rates non-significant despite large effect sizes and sufficient statistical power.

We suspect that selfing propensities have a genetic component and when selected on cause mean selfing rates to evolve. We propose that heritable variation in selfing propensities offers a reconciliation between the reproductive assurance hypothesis and its weak empirical support: distributions of selfing propensities vary temporally and spatially, thus obscuring the relationship between population density and realised selfing rates.

Background

The mating system is the way a population or species is structured with respect to sexual behaviour. In hermaphroditic species capable of self-fertilisation (hereafter selfing), it primarily describes the relative frequency of selfing *versus* outcrossing. The mating system adopted by hermaphroditic species determines the quantity and genetic quality of offspring (1). Consequently, it has pervasive effects on individual fitness and on the evolutionary and demographic properties of populations and species (2-4).

Variation in selfing rates has often been studied at the species level (5-8), where the distribution of selfing rates has been found to be bimodal, with more outcrossers and selfers than mixed maters ($0.2 <$ selfing rate $s < 0.8$). Recent studies have also documented variability between populations of a single species, particularly in plants (e.g., 9, 10). For example, in 51% of 105 surveyed plant species, populations fell into several mating-system categories (11). Our knowledge on animal populations is sparser, but where selfing rates or the delay in the onset of reproduction when selfing (termed “waiting time”, 12) have been estimated across populations, inter-population variation was the norm (13-20). It thus is important to characterise species by more than one population estimate, lest crucial differences between populations are overlooked.

We postulate that variation in selfing rates among individuals is equally essential. Evolutionary change primarily happens at the population level; among-individual variation constitutes the raw material natural selection acts upon. If individuals within populations of simultaneous hermaphrodites were uniform in their ability to self and had equal opportunities to outcross, the selection gradient for the selfing rate would be zero: selection can only act on traits that vary (21).

Despite their importance, estimates of variation in individual selfing rates are rare. Existing studies have shown that selfing rates varied within populations of trees (22, 23) and snails (15-17, 24-28). However, many of these estimates rely on small numbers of weakly polymorphic loci, which hampers the detection of outcrossing events, and so may be biased (29, 30). Substantial differences among individuals were also found in proxies of selfing rates, such as in animals' waiting time (19, 31, 32) and in the presence of seeds in self-pollinated plants (33, 34).

Individual selfing rates likely depend on genetic factors, environmental conditions, and genotype-by-environment interactions. Genetic self-incompatibility systems are well-known in plants (35, 36) and have recently been discovered in ascidians (37) and oomycetes (38). Differences in waiting times among families, suggestive of genetic variation in selfing rates, have been found in snails (12, 19, 31) and flatworms (32). A key environmental determinant of individual selfing rates is mate availability. Selfing provides reproductive assurance when mates are scarce, but may be unpreferred if outcrossing is possible, due to inbreeding depression and other costs of selfing (39, 40). Yet, to date there are no direct tests of how individual selfing rates in animals respond to differential mate availability; existing studies (41, 42) lack genetically estimated selfing rates. Here we aim to close this knowledge gap.

Individual variation in selfing can be assessed by estimating individuals' lifetime propensity for selfing, here defined as a biological property of an individual (the ability and readiness to self) and its environment (mate availability), loosely following Brandon's propensity interpretation of fitness (43). This definition allows a separate exploration of the individual (supposedly genetic) *versus* ecological determinants of variation in selfing rates, the need for which recent studies have abundantly shown (9, 11, 20, 44).

The freshwater snail *Radix balthica* is an ideal species to study within-population variation in selfing propensity. Laboratory studies have confirmed that self-compatibility is geographically

widespread yet not omnipresent within populations (45, 46). There is also tentative evidence for between- and within-population variation in molecularly estimated selfing rates (26, 27, 47). The population studied here is almost fully outcrossing in the field (30), in accordance with its high density.

We derived individuals' lifetime propensity for selfing for two laboratory environments with differential mate availability. We first ascertained how successfully 274 laboratory-reared, unmated snails selfed in isolation. We then assigned them to one of two mating treatments, simulating (i) a low population density and scarcity of mating opportunities, or (ii) conditions in the ancestral field population, where density was high and multiple paternity within egg clutches common (48). We recorded individual mating behaviour, the lifetime production of eggs in isolation (necessarily selfed) and thereafter (potentially outcrossed), and the proportion of developing eggs. For 57 individuals, we estimated individual selfing rates in eggs produced post-isolation from progeny arrays.

Assuming variation in post-isolation selfing rates, we had two *a priori* hypotheses: Selfing post-isolation is more common under low mate availability (hypothesis 1) and in snails that selfed in isolation (hypothesis 2). Hypothesis 1 posits that selfing has evolved to provide reproductive assurance at low density but is unfavoured when outcrossing opportunities are abundant (39, 40). If supported, it suggests selfing propensities have a strong ecological component. Hypothesis 2 postulates that a period of isolation reveals classes of individuals with different propensity for selfing, which remain largely unaffected by environmental change. It thus assumes selfing propensities to be stable, fundamental properties based in genetic differences. The hypotheses are not mutually exclusive; ecological and genetic factors can jointly shape individual selfing rates. We further explored how variation in selfing propensities is maintained by investigating their association with individuals' sexual drive and female lifetime reproductive success.

Methods

Study system

R. balthica is a simultaneously hermaphroditic snail inhabiting the shallow littoral zone of European lakes (47, 49). In Lake Zurich, Switzerland, it is an annual with non-overlapping generations. Snails hatch from eggs in spring, reach sexual maturity in winter, reproduce from March to May, and then die (48). During the single breeding season, individuals may copulate repeatedly in both sexual roles and lay hundreds of eggs in distinct egg clutches (48). Copulation is unilateral. Lymnaeid snails can store allosperm and should not be allosperm-limited even if they do not mate continuously (50).

Experimental snails

We estimated the selfing rate of 57 first-generation (F1) laboratory-born snails. They belonged to a group of 274 F1 snails subjected to laboratory mating trials, all derived from 38 snails (P0 mothers) collected in Uerikon, Lake Zurich, Switzerland (details in 45). Snails with estimated selfing rates originated from 22 P0 mothers. F1 snails were reared in sibling groups and individually from age 19.4 ± 4.1 weeks onwards (mean ± SD) to preserve their virginity. This start of isolation is very early; paired control snails only began to mate at age 41.3-45.7 weeks.

Mating trials

Mating trials began in early May 2014 when F1 snails were 52.1 ± 1.0 weeks old and 38.7% had selfed in isolation. In the ancestral field population, breeding started in early March (48), and laboratory control snails had started to copulate several weeks earlier (see above). The experiment then continued for seven weeks, until mortality began to increase reflecting the snails' natural, annual life cycle.

Two types of mating trials were conducted: 131 snails had one mating opportunity with one partner (n = 33/57 snails with selfing rate estimates), and 137 had six opportunities during six weeks, each with a different partner (n = 24/57). Each mating opportunity lasted for 10.3 ± 0.8 hours, long enough for copulations in both sexual roles. Mating trials were recorded on time-lapse movies (one frame/30 sec). From these we extracted the number of partners each snail had copulated with as a male and as a female. We also measured females' lifetime number of eggs and how many of these contained developed embryos 17 days after oviposition, both separated into an isolation and post-isolation phase (details in 45).

Genetic analysis

We selected snails (n = 57) for selfing rate estimation that successfully reproduced post-isolation. We genotyped F2 offspring produced post-isolation, but, for logistic reasons, did not genotype any juveniles from eggs laid shortly before the experiment was terminated. Hence, the genotyped offspring of repeatedly paired snails originate from eggs laid before mating opportunity 4 (n = 6 snails), 5 (n = 15 snails), and 6 (n = 3 snails), respectively. Offspring of these snails can thus be sired by three to five non-maternal fathers. F2 offspring were reared in sibling groups until the age of 12.3 ± 0.5 weeks, when some were used in a field experiment and the rest frozen for genetic analysis. By that time, 65.9 ± 16.0% of offspring born post-isolation had died. Because of this pre-sampling mortality, we here estimated "secondary" selfing rates, after the expression of potential in- or outbreeding depression in early development.

We aimed to genotype 15 randomly chosen juveniles per F1 snail, or ≥ 80% of available juveniles when families were smaller, resulting in 13.7 ± 4.3 genotyped juveniles per family. We also genotyped all P0 and F1 snails to estimate population-level inbreeding coefficients using Genetix version 4.05.2 (51) and to ascertain whether F1 snails themselves were selfed

(Table S1). Snails were genotyped for ten highly polymorphic microsatellite loci (GenBank Accession No. KX830983-KX830992) developed specifically for this population by Ecogenics GmbH (Zurich, Switzerland). The genotyping and scoring protocol is described elsewhere (30). One locus showed a non-negligible frequency of null alleles (locus Rb_3, KX830985) and was excluded from all analyses.

Paternity analysis

Paternity analyses were performed using a custom-built R routine. We excluded 44 juveniles (5.6% of 783 juveniles genotyped) from the analysis because they did not meet the inclusion criteria (< 6 loci of both mother and offspring genotyped successfully, OR genotypes lacking a maternal allele at > 2 loci, OR genotypes with 6 successfully genotyped loci but 2 loci without a maternal allele). For the remaining 739 juveniles, we counted the loci consistent with being outcrossed, i.e., those with one non-maternal allele. We then compared each juvenile's genotype to those of all its non-maternal candidate fathers, defined as all the potential mates of a juvenile's mother, irrespective of whether copulations had been observed. A candidate father was considered a perfect match if he possessed a juvenile's paternal allele at every locus successfully genotyped in the juvenile, the mother, and himself. Candidate fathers lacking a juvenile's paternal allele at 1 ≥ loci were considered non-perfect matches and deemed increasingly unlikely fathers the higher the number of father-offspring mismatches. Additionally, we repeated all paternity analyses using COLONY version 2.0.5.9 (52, see Supporting Methods), known for its highly accurate parentage assignments (53).

Using these procedures, paternity was successfully assigned to 731 (98.9%) juveniles with high-quality genotypes, originating from 56 F1 snails (13.1 ± 3.8 assigned offspring per F1 snail). Paternity assignments were inconclusive for the offspring of one F1 snail. For 719 juveniles, the R routine and COLONY produced identical assignments. Of the remaining twelve juveniles, five

were left unassigned by the R routine, four by COLONY, and three were assigned by both but to different fathers; here we gave preference to the R routine (details in Table S2). Allelic diversity was high across loci (Table S1), yielding powerful paternity analyses. The mean probability of being selfed for offspring identified as selfed was 0.80 ± 0.22, while that of offspring identified as outcrossed was 0.00 ± 0.00 (Table S2). Outcrossed offspring were assigned to a father with a probability of 0.92 ± 0.16 (Table S2).

Statistical analysis

We used two-tailed Pearson's Chi-square tests for count data, using either 2x2, 3x2, or 4x2 contingency tables, and generalised linear mixed models (GLMMs) where we could construct a response-predictor structure.

The likelihood of F1 snails producing at least some selfed juveniles post-isolation (no/yes) was analysed using a GLMM with binomial errors. Fixed effects were the experimental treatment (once- vs. repeatedly paired), the number of developed embryos produced in isolation, and the number of male and female partners snails copulated with. P0 mother identity was used as a random intercept.

We used GLMMs to test if snails with different propensities for selfing differed in their total number of eggs (female LRS), total number of developed embryos, or proportion of undeveloped embryos. We estimated these models for F1 snails with successfully genotyped offspring (n = 51; five outcrossers were excluded due to low sample size) and for all female fertile snails (n = 126). We fitted GLMMs using negative binomial errors (nbinom 1 family) for response variables 1 and 2 and Gaussian errors for response variable 3 as it was normally distributed. Adult body size was used as a continuous covariate, the propensity for selfing as a factor (three levels, see results), and P0 mother identity as a random intercept.

Finally, we tested if snails that mated in both sexual roles (no/yes) differed in their propensity for selfing using a GLMM with binomial errors and including snails with successfully genotyped offspring only (n = 51, see above). The propensity for selfing (four levels, see results), adult body size (continuous covariate) and P0 mother identity (random intercept) were used as predictors.

Analyses were performed using R v. 4.0.5 (54). GLMMs were fitted using "glmmTMB" (55). The model fit was assessed using a simulation-based approach implemented in "DHARMa" (56). We tested the significance of the random effect with log-likelihood ratio tests (full model vs. model without random effect). Values are given as mean ± SD. Scatter plots were prepared using "beeswarm" (57) and depict data points superimposed on boxplots. Binomial standard errors were computed according to Zar (58) as $SE = \sqrt{p(1-p))/(n-1)}$, where p was the proportion tested and n the total sample size. The entire code and model output is provided as an R Markdown document (59).

Results

Frequency of selfing and multiple paternity

Most genotyped F2 families (73.2%) contained 20% or fewer selfed offspring (Fig. 1a). At least one selfed offspring was found in 44.6% of families; 26.8% of families consisted of 83% or more selfed offspring (Fig. 1a). Overall, 23.0% of offspring were identified as selfed (168/731). No family showed an intermediate selfing rate ($0.20 < s < 0.80$). There was no evidence of selfing in either the F1 or P0 generation. All 57 F1 snails were outcrossed, i.e., they possessed a non-maternal allele at $1 \geq$ loci (mean: 4.8 ± 1.5 loci; Table S1). P0 mothers were likely outcrossed too: their genotypes did not show reduced heterozygosity (F_{IS} = -0.0009, 95% CI: -0.075, 0.046; Table S1). P0 mother identity did not explain any variance in F1 snails' probability of selfing post-isolation (Table 1).

Multiple paternity was significantly more common among the offspring of repeatedly paired (45.8%) than once-paired snails (15.6%, Pearson's Chi-squared test with Yates' continuity correction: observed counts: 5, 27, 11, 13, expected counts: 9.1, 22.9, 6.9, 17.1, $\chi^2_1 = 4.74$, p = 0.0294; Fig. 1a). On average, the progenies of repeatedly and once-paired snails had 1.7 ± 0.9 (max. = 4) and 1.2 ± 0.4 (max. = 2) fathers, respectively.

Effect of mate availability on selfing

Mean selfing rates did not differ significantly among once- (0.36 ± 0.47) and repeatedly paired snails (0.19 ± 0.36; Wilcoxon rank sum test with continuity correction: W = 1452.5, p = 0.21), nor did their variance (Levene's test for homogeneity of variance: $F_{1,24}$ = 2.48, p = 0.12; Fig. 1b). Repeated mating opportunities did not significantly reduce the likelihood of a snail producing at least some selfed offspring (Table 1), nor the proportion of families with non-zero selfing rates (once-paired: 50.0%, repeatedly paired: 37.5%, Pearson's Chi-squared test: observed counts: 16,

16, 9, 15, expected counts: 14.3, 17.7, 10.7, 13.3, χ_1^2 = 0.87, p = 0.35; Fig. 1c). We thus did not find strong support for hypothesis 1, which predicted that selfing is more common at low mate availability. However, as a group, once-paired snails produced significantly more selfed offspring than repeatedly paired snails: 33.6% vs. 8.9% (Pearson's Chi-squared test: observed counts: 140, 227, 28, 286, expected counts: 90.5, 276.5, 77.5, 236.5, χ_1^2 = 77.80, p < 0.0001; Fig. 1d). Nevertheless, the distribution of selfing rates was similarly bimodal among both treatment groups (Fig. 2a).

Infertility in isolation was linked to outcrossing post-isolation

Predominant selfing after mating trials had started was restricted to snails that had selfed successfully in isolation, while snails without prior selfing exclusively outcrossed post-isolation (Fig. 2b). Yet, 70.6% of snails that selfed in isolation switched to complete or near-complete outcrossing as soon as they could mate (Fig. 2b). Consequently, selfing in isolation did not strongly predict continued selfing; its effect was only statistically significant when an extremely prolific snail was included (Table 1) and non-significant otherwise (b = 0.01, z = 1.93, p = 0.05). We therefore rejected hypothesis 2, which posited that selfing post-isolation is more common in snails with a history of selfing, while noting that the lack thereof reliably predicted outcrossing.

Individual-level variation in propensity for selfing

Snails varied in their propensity for selfing. For snails with successfully genotyped offspring (n = 56), we ascertained the selfing propensity from the presence/absence of selfing in isolation and the post-isolation selfing rate, revealing four groups of snails: 8.9% "outcrossers" that never selfed, 46.4% "plastic switchers" that selfed in isolation but outcrossed as soon as they were paired, 17.9% "plastic mixers" that combined selfing in isolation with predominant yet incomplete outcrossing post-isolation, and 26.8% "selfers" that selfed fully or mostly throughout (Fig. 2c).

The subsample with genotyped offspring underrepresented snails that reproduced only before or only after mating trials started (Fig. S1). To estimate the frequency of selfing propensities, we thus considered all snails (n = 274). We ascertained the apparent selfing propensity of snails without genotyped offspring based on when they laid eggs and how they mated. This revealed three groups: "apparent outcrossers" that only reproduced post-isolation and copulated as a female, "apparent plastic snails" that reproduced both before and after being paired and copulated as a female, and "apparent selfers" that only reproduced in isolation, or in isolation and post-isolation yet never copulated as a female. The selfing propensity of snails without developed embryos (n = 148) is unknown. Among all snails with ascertained selfing propensity (n = 126), outcrossers and apparent outcrossers made up 31.0%, plastic and apparent plastic snails 42.9%, and selfers and apparent selfers 26.2%.

The pairing treatment did not influence the propensity for selfing of snails with genotyped offspring (Pearson's Chi-squared test: observed counts: 11, 5, 15, 1, 4, 5, 11, 4, expected counts: 8.6, 5.7, 14.9, 2.9, 6.4, 4.3, 11.1, 2.1, $\chi^2_3 = 4.63$, $p = 0.20$). However, when using the full dataset, selfing propensities differed among experimental groups (observed counts: 29, 26, 11, 11, 20, 27, expected counts: 21.3, 24.5, 20.2, 18.7, 21.5, 17.8, $\chi^2_2 = 15.17$, $p = 0.0005$). Low mate availability led to more apparent selfers (43.9% vs. 19.0%) and fewer apparent outcrossers (16.7% vs. 46.6%) yet did not affect apparent plastic snails (39.4% vs. 34.5%), lending a small amount of support to hypothesis 1.

Reduced female LRS in selfers

Selfing decreased female LRS (number of developed embryos) significantly, by 46.0% compared to plastic mixers and by 26.8% compared to plastic switchers (Fig. S2a, Table S3). In part, this was because selfers produced significantly fewer eggs – 37.4% and 19.1% fewer than plastic mixers and switchers, respectively (Table S3). Additionally, selfers laid more eggs that

failed to develop (28.3%) than plastic mixers (17.9%); the difference to plastic switchers (21.4%) was non-significant (Fig. S2b, Table S3). Outcrossers were excluded because of low sample size (n = 5).

Analyses of all female fertile snails showed that selfers had reduced female LRS also compared to outcrossers (Fig. 3a, Table 2). Selfers and apparent selfers produced 62.7% fewer developed embryos than plastic and apparent plastic snails, and 53.6% fewer than outcrossers and apparent outcrossers. Again, this was both due to a reduced fecundity (reductions by 56.8% and 46.3%, respectively) and increased proportion of undeveloped embryos in selfers (36.8% vs. 25.5% and 27.8%, respectively; Fig. 3b, Table 2). These results were corrected for effects of body size and P0 mother identity (Table 2).

Mating as a female did not prevent selfing

A strong propensity for selfing was not linked to a lack of sexual drive. Neither the number of male (potential sperm donors) nor female mating partners (potential sperm recipients) was significantly associated with the likelihood of selfing post-isolation (Table 1). Most selfers (53.3%) copulated in the female role and so might have received allosperm. Four out of five selfers (80.8%) mated as a male, potentially siring outcrossed offspring. Of snails that selfed post-isolation, 72.0% mated as a female.

However, selfing propensity and sexual activity were not entirely independent. Significantly fewer selfers (40.0%) than plastic switchers (92.3%) copulated in both sexual roles (b = 2.88, z = 3.18, p = 0.0015; Fig. 4); the difference to plastic mixers was non-significant (70.0%, b = 1.27, z = 1.45, p = 0.15). Outcrossers (80.0%) were excluded from the model due to low sample size (n = 5, Fig. 4). Body size (b = -0.03, z = -0.15, p = 0.88) and P0 mother identity (χ^2_1 = 0.0, p = 1.00) did not affect the likelihood of mating in both sexual roles.

Discussion

We found that propensities for selfing differed substantially within a snail population. Individual-level variation in selfing propensity has not been fully acknowledged in the mating-system literature, which has traditionally been focused on the species (5-8) or population level (10, 11). The variation discovered here was cryptic in two ways. First, it only became apparent in the laboratory, owing to an initial period of isolation followed by controlled pairings. By contrast, both direct and indirect selfing rate estimates of the ancestral field population were near-zero, although obtained across three years and throughout the breeding season (30). Using the field results only, the population would have been deemed outcrossing and its considerable potential for selfing missed.

Second, much variation in individual propensity for selfing would have been overlooked had we only recorded selfing, or lack thereof, during an isolation treatment, without estimating post-isolation selfing rates. This approach, used in pioneering earlier studies (e.g., 60, 61), merely reveals individuals' ability to self. It does not uncover their propensity for selfing, which must be assessed in the face of outcrossing opportunities and using genetic markers. Here we show just how variable the propensity is, comprising two types of plastic individuals, selfers, and outcrossers. Selfing-outcrossing thus is a gradient, not a dichotomy – also, and perhaps especially, at the individual level.

Variation in selfing propensity only partially resulted from variation in mate availability. As expected, selfing was most prevalent when snails were isolated, yet often persisted post-isolation. Moreover, pairing snails repeatedly rather than once had a surprisingly subtle effect, significantly affecting neither the mean selfing rate, the proportion of selfing individuals, nor the likelihood of selfing. This was despite large effect sizes and sufficient statistical power: doubling our sample size would not have rendered any of these effects significant (see R

Markdown document). Instead, treatment effects were non-significant because variation among snails was large even within treatment groups. The only significant consequences of pairing snails repeatedly were a lower overall number of selfed offspring, showing that increased mate availability reduced selfing in high-fitness individuals, and an overabundance of apparent outcrossers and scarcity of apparent selfers when considering the full dataset.

Superficially, the subtlety of these effects contradicts ecological models of selfing, which assume an advantage and thus higher prevalence of selfing at low population density (39, 40). Earlier studies have struggled to find empirical support for the reproductive assurance hypothesis of partial selfing in animals. Selfing rates were not increased in low-density, disturbed, or temporary habitats (13, 16, 18, 25), neither the frequency nor duration of outcrossing depended on the length of a mating opportunity (41), and snails did not adjust their waiting time to the perceived density of conspecifics (42). And yet, it is hard to imagine that the capacity for independent reproduction does not feature among the primary evolutionary drivers of selfing.

Our study offers a potential reconciliation between the reproductive assurance hypothesis and its weak empirical support. First, reproductive assurance matters, but only in extreme situations. Consequently, there is an ascertainment bias: if a population is dense enough to be sampled, it might be too dense for selfing to be common. This is illustrated by the near-complete lack of selfing in our ancestral field population (30) despite its evident self-fertility. At this stage, we do not know why some snails selfed post-isolation: either because they were mate-limited even if paired repeatedly, or because the initial isolation phase primed them for selfing. A study where snails are paired as soon as they reach maturity could disentangle these options.

The second part of the reconciliation is understanding that a population's selfing rate depends on the distribution of individual selfing propensities among its members. As selfing

propensities consist of an individual (e.g., genotype) and environmental component (e.g., population density), the relationship between density and population-level selfing rates is complicated. It depends on the relative frequency of individuals that can respond to density variation with increased or decreased selfing, i.e., on the frequency of plastic individuals and selfers compared to outcrossers. Consequently, a population's selfing rate can be low because it mainly consists of outcrossers that either outcross or fail to reproduce, or because density is high enough for outcrossing to occur even in plastic individuals and selfers. Interpreting high population-level selfing rates is perhaps easier, as they necessitate a sizeable proportion of self-fertile individuals. However, also in highly selfing populations density is difficult to predict, as the threshold density beneath which selfing occurs may be population- or species-specific.

Our results hint at a genetic background to selfing propensities, in line with findings in other hermaphroditic animals (12, 19, 31, 32, 37). As many as 37% of female fertile snails, and 9% of those with genotyped offspring, did not reproduce in isolation. These individuals all had post-isolation selfing rates of zero. An aversion to selfing so strong as to risk reproductive failure when deprived of mates is so clearly maladaptive that a genetic self-incompatibility mechanism appears plausible. Other snails occupied the opposite end of the spectrum, extensively selfing post-isolation and even after mating as a female. Invariably, these snails successfully selfed in isolation. Lastly, we found intermediate, plastic snails that selfed in isolation but not, or only a little, when outcrossing was possible. Our results thus imply that a detailed investigation of the genetic basis of variation in self-compatibility would be interesting in this population.

So, what maintains self-compatibility alleles in our study population, and why have they not gone to fixation? The cost of lacking them is obvious – an occasional crash of population size will quickly wipe out any individuals without self-compatibility alleles. The benefit of lacking them, as demonstrated here, is a higher female LRS, resulting from increased fecundity (likely due to increased clutch size, 45) and decreased embryo mortality (potentially caused by early-

acting inbreeding depression, 4). Male LRS might be higher too, particularly if selfers have sperm discounting (62) or reduced energy allocation to the male function (63). However, the strong sexual drive of selfers documented here (80% mated in the male role) suggests that reductions in siring success when selfing might be more modest. Additionally, selfed progeny may suffer from inbreeding depression. In our population, inbreeding depression has not yet been investigated, but has been found in other populations (64) and in a close relative (60, 61).

Finally, it is worth mentioning the dangers of assuming that pairing individuals results in their offspring being outcrossed, as done in some early work (e.g., 60, 61). In our study, 45% of paired snails selfed at least partially, and 72% of snails that selfed post-isolation copulated as a female. Hence, outcrossing is neither guaranteed by pairing individuals once or repeatedly, nor by observing female copulations; to verify outcrossing events, genetic paternity analysis is unavoidable. Risks of failing to do so include underestimating the frequency and extent of selfing, and underestimating the strength of inbreeding in studies that compare obligately selfed individuals produced by isolated parents with purportedly outcrossed (though potentially selfed) individuals produced by paired parents (e.g., 44, 65, 66, 67). We here add to a growing number of studies that tested the assumption of (near-)exclusive outcrossing after pairing empirically, and found it occasionally confirmed (68), yet more often refuted (28, 50, 69, 70).

Ethics

We performed our study in accordance with national laws.

Data accessibility

Microsatellite markers: GenBank Accession nos. KX830983-KX830992. All data are available from Dryad X.

Authors' contributions

A.F. designed the study, performed experiments, carried out molecular work, and analysed the data. A.F. and A.S. drafted the manuscript, with input from J.J. All authors contributed to manuscript revision and gave final approval for publication.

Competing interests

We declare we have no competing interests.

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References

1. Barrett SCH. Evolution of mating systems: outcrossing versus selfing. In: Losos J, editor. The Princeton Guide to Evolution. Princeton, New Jersey: Princeton University Press; 2014. p. 356-62.
2. Noël E, Jarne P, Glémin S, MacKenzie A, Segard A, Sarda V, et al. Experimental evidence for the negative effects of self-fertilization on the adaptive potential of populations. Current Biology. 2017;27(2):237-42.
3. Baker HG. Self-compability and establishment after "long-distance" dispersal. Evolution. 1955;9(3):347-9.
4. Charlesworth D, Charlesworth B. Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst. 1987;18:237-68.
5. Moeller DA, Briscoe Runquist RD, Moe AM, Geber MA, Goodwillie C, Cheptou P-O, et al. Global biogeography of mating system variation in seed plants. Ecology Letters. 2017;20(3):375-84.
6. Jarne P, Auld JR. Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals. Evolution. 2006;60(9):1816-24.
7. Goodwillie C, Kalisz S, Eckert CG. The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. Annual Review of Ecology Evolution and Systematics. Annual Review of Ecology Evolution and Systematics. 36. Palo Alto: Annual Reviews; 2005. p. 47-79.
8. Vogler DW, Kalisz S. Sex among the flowers: the distribution of plant mating systems. Evolution. 2001;55(1):202-4.
9. Latron M, Arnaud J-F, Ferla H, Godé C, Duputié A. Effects of contemporary shifts of range margins on patterns of genetic structure and mating system in two coastal plant species. Heredity. 2020;124(2):336-50.
10. Christopher DA, Karron JD, Semski WR, Smallwood PA, Trapnell DW, Mitchell RJ. Selfing rates vary with floral display, pollinator visitation and plant density in natural populations of *Mimulus ringens*. Journal of Evolutionary Biology. 2021;34(5):803-15.
11. Whitehead MR, Lanfear R, Mitchell RJ, Karron JD. Plant mating systems often vary widely among populations. Frontiers in Ecology and Evolution. 2018;6(APR):38.
12. Tsitrone A, Jarne P, David P. Delayed selfing and resource reallocations in relation to mate availability in the freshwater snail *Physa acuta*. The American Naturalist. 2003;162(4):474-88.
13. Kupfernagel S, Rusterholz HP, Baur B. Variation in multiple paternity and sperm utilization patterns in natural populations of a simultaneous hermaphrodite land snail. Biological Journal of the Linnean Society. 2010;99(2):350-61.

14. Mackiewicz M, Tatarenkov A, Taylor DS, Turner BJ, Avise JC. Extensive outcrossing and androdioecy in a vertebrate species that otherwise reproduces as a self-fertilizing hermaphrodite. Proceedings of the National Academy of Sciences. 2006;103(26):9924-8.
15. Viard F, Doums C, Jarne P. Selfing, sexual polymorphism and microsatellites in the hermaphroditic freshwater snail *Bulinus truncatus*. Proceedings of the Royal Society B-Biological Sciences. 1997;264(1378):39-44.
16. Trouvé S, Degen L, Renaud F, Goudet J. Evolutionary implications of a high selfing rate in the freshwater snail *Lymnaea truncatula*. Evolution. 2003;57(10):2303-14.
17. Charbonnel N, Rasatavonjizay R, Sellin E, Brémond P, Jarne P. The influence of genetic factors and population dynamics on the mating system of the hermaphroditic freshwater snail *Biomphalaria pfeifferi*. Oikos. 2005;108(2):283-96.
18. Puurtinen M, Knott KE, Suonpaa S, Nissinen K, Kaitala V. Predominance of outcrossing in *Lymnaea stagnalis* despite low apparent fitness costs of self-fertilization. Journal of Evolutionary Biology. 2007;20(3):901-12.
19. Escobar JS, Facon B, Jarne P, Goudet J, David P. Correlated evolution of mating strategy and inbreeding depression within and among populations of the hermaphroditic snail *Physa acuta*. Evolution. 2009;63(11):2790-804.
20. Giannakara A, Ramm SA. Evidence for inter-population variation in waiting times in a self-fertilizing flatworm. Invertebrate Reproduction and Development. 2020;64(2):158-68.
21. Lande R, Arnold SJ. The measurement of selection on correlated characters. Evolution. 1983;37(6):1210-26.
22. Feres JM, Sebbenn AM, Guidugli MC, Mestriner MA, Moraes MLT, Alzate-Marin AL. Mating system parameters at hierarchical levels of fruits, individuals and populations in the Brazilian insect-pollinated tropical tree, *Tabebuia roseo-alba* (Bignoniaceae). Conservation Genetics. 2012;13(2):393-405.
23. Gauzere J, Klein EK, Oddou-Muratorio S. Ecological determinants of mating system within and between three *Fagus sylvatica* populations along an elevational gradient. Molecular Ecology. 2013;22(19):5001-15.
24. Coutellec-Vreto MA, Madec L, Guiller A. Selfing and biparental inbreeding: a mating system analysis in *Lymnaea peregra* (Gastropoda: Lymnaeidae). Heredity. 1997;79:277-85.
25. Henry PY, Bousset L, Sourrouille P, Jarne P. Partial selfing, ecological disturbance and reproductive assurance in an invasive freshwater snail. Heredity. 2005;95(6):428-36.

26. Jokela J, Wiehn J, Kopp K. Among- and within-population variation in outcrossing rate of a mixed-mating freshwater snail. Heredity. 2006;97(4):275-82.
27. Wiehn J, Kopp K, Rezzonico S, Karttunen S, Jokela J. Family-level covariation between parasite resistance and mating system in a hermaphroditic freshwater snail. Evolution. 2002;56(7):1454-61.
28. Meunier C, Hurtrez Boussès S, Jabbour-Zahab R, Durand P, Rondelaud D, Renaud F. Field and experimental evidence of preferential selfing in the freshwater mollusc *Lymnaea truncatula* (Gastropoda, Pulmonata). Heredity. 2004;92(4):316-22.
29. Bernatchez L, Duchesne P. Individual-based genotype analysis in studies of parentage and population assignment: How many loci, how many alleles? Canadian Journal of Fisheries and Aquatic Sciences. 2000;57(1):1-12.
30. Bürkli A, Sieber N, Seppälä K, Jokela J. Comparing direct and indirect selfing rate estimates: when are population-structure estimates reliable? Heredity. 2017;118(6):525-33.
31. Escobar JS, Epinat G, Sarda V, David P. No correlation between inbreeding depression and delayed selfing in the freshwater snail *Physa acuta*. Evolution. 2007;61(11):2655-70.
32. Ramm SA, Vizoso DB, Schärer L. Occurrence, costs and heritability of delayed selfing in a free-living flatworm. Journal of Evolutionary Biology. 2012;25(12):2559-68.
33. Himes SL, Wyatt R. Costs and benefits of self-fertility in *Asclepias exaltata* (Apocynaceae). Journal of the Torrey Botanical Society. 2005;132(1):24-32.
34. Weber JJ, Weller SG, Sakai AK, Nguyen A, Tai ND, Domínguez CA, et al. Purging of inbreeding depression within a population of *Oxalis alpina* (Oxalidaceae). American Journal of Botany. 2012;99(5):923-32.
35. Muñoz-Sanz JV, Zuriaga E, Cruz-García F, McClure B, Romero C. Self-(in)compatibility systems: target traits for crop-production, plant breeding, and biotechnology. Frontiers in Plant Science. 2020;11(195).
36. Fujii S, Kubo K-i, Takayama S. Non-self- and self-recognition models in plant self-incompatibility. Nature Plants. 2016;2(9):16130.
37. Sawada H, Yamamoto K, Yamaguchi A, Yamada L, Higuchi A, Nukaya H, et al. Three multi-allelic gene pairs are responsible for self-sterility in the ascidian *Ciona intestinalis*. Scientific Reports. 2020;10(1):2514.
38. Dussert Y, Legrand L, Mazet ID, Couture C, Piron M-C, Serre R-F, et al. Identification of the first oomycete mating-type locus sequence in the grapevine downy mildew pathogen, *Plasmopara viticola*. Current Biology. 2020;30(20):3897-907.

39. Jarne P, Charlesworth D. The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annu Rev Ecol Syst. 1993;24:441-66.
40. Uyenoyama MK, Holsinger KE, Waller DM. Ecological and genetic factors directing the evolution of self-fertilization. In: Futuyma D, Antonovics J, editors. Oxford surveys in evolutionary biology 9. Oxford: Oxford University Press; 1993. p. 327-81.
41. Vianey-Liaud M, Nassi H, Lancastre F, Dupouy J. Duration of pairing and use of allosperm in *Biomphalaria glabrata* (Gastropoda, Planorbidae). Memorias do Instituto Oswaldo Cruz. 1989;84(1):41-5.
42. Henry P-Y, Vimond L, Lenormand T, Jarne P. Is delayed selfing adjusted to chemical cues of density in the freshwater snail *Physa acuta*? Oikos. 2006;112(2):448-55.
43. Brandon RN. Adaptation and evolutionary theory. Studies in History and Philosophy of Science. 1978;9(3):181-206.
44. Escobar J, Auld J, Correa A, Alonso J, Bony Y, Coutellec M-A, et al. Patterns of mating-system evolution in hermaphroditic animals: Correlations among selfing rate, inbreeding depression, and the timing of reproduction. Evolution. 2011;65(5):1233-53.
45. Felmy A, Weissert N, Travis J, Jokela J. Mate availability determines use of alternative reproductive phenotypes in hermaphrodites. Behavioral Ecology. 2020;31(4):1003-16.
46. Johansson MP, Ermold F, Kristjánsson BK, Laurila A. Divergence of gastropod life history in contrasting thermal environments in a geothermal lake. Journal of Evolutionary Biology. 2016;29(10):2043-53.
47. Pfenninger M, Salinger M, Haun T, Feldmeyer B. Factors and processes shaping the population structure and distribution of genetic variation across the species range of the freshwater snail *Radix balthica* (Pulmonata, Basommatophora). BMC Evolutionary Biology. 2011;11:135.
48. Bürkli A, Jokela J. Increase in multiple paternity across the reproductive lifespan in a sperm-storing, hermaphroditic freshwater snail. Molecular Ecology. 2017;26(19):5264-78.
49. Lawton SP, Lim RM, Dukes JP, Kett SM, Cook RT, Walker AJ, et al. Unravelling the riddle of *Radix*: DNA barcoding for species identification of freshwater snail intermediate hosts of zoonotic digeneans and estimating their inter-population evolutionary relationships. Infection, genetics and evolution. 2015;35:63-74.
50. Nakadera Y, Blom C, Koene JM. Duration of sperm storage in the simultaneous hermaphrodite *Lymnaea stagnalis*. Journal of Molluscan Studies. 2014;80(1):1-7.
51. Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations.

Montpellier, France: Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II; 1996-2004.

52. Jones O, Wang J. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources. 2010;10(3):551-5.

53. Harrison H, Saenz Agudelo P, Planes S, Jones G, Berumen M. Relative accuracy of three common methods of parentage analysis in natural populations. Molecular Ecology. 2013;22(4):1158-70.

54. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/2021.

55. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, et al. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal. 2017;9(2):378-400.

56. Hartig F. DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.1 ed. http://florianhartig.github.io/DHARMa/2021.

57. Eklund A. beeswarm: the bee swarm plot, an alternative to stripchart. R package version 0.2.3 ed. https://CRAN.R-project.org/package=beeswarm2016.

58. Zar JH. Sampling a binomial population. In: Fisher S, Snavely SL, editors. Biostatistical Analysis. 3 ed. New Jersey, Upper Saddle River, NJ, USA: Prentice-Hall, Inc.; 1996. p. 523.

59. Allaire JJ, Xie Y, McPherson J, Luraschi J, Ushey K, Atkins A, et al. rmarkdown: Dynamic Documents for R. R package version 2.7 ed. https://rmarkdown.rstudio.com2021.

60. Jarne P, Delay B. Inbreeding depression and self-fertilization in *Lymnaea peregra* (Gastropoda: Pulmonata). Heredity. 1990;64(2):169-75.

61. Coutellec-Vreto MA, Jarne P, Guiller A, Madec L, Daguzan J. Inbreeding and fitness in the freshwater snail *Lymnaea peregra*: An evaluation over two generations of self-fertilization. Evolution. 1998;52(6):1635-47.

62. Porcher E, Lande R. The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. Journal of Evolutionary Biology. 2005;18(3):497-508.

63. Winkler L, Ramm SA. Experimental evidence for reduced male allocation under selfing in a simultaneously hermaphroditic animal. Biology Letters. 2018;14(10):20180570.

64. Evanno G, Castella E, Goudet J. Evolutionary aspects of population structure for molecular and quantitative traits in the freshwater snail *Radix balthica*. Journal of Evolutionary Biology. 2006;19(4):1071-82.

65. Henry PY, Pradel R, Jarne P. Environment-dependent inbreeding depression in a hermaphroditic freshwater snail. Journal of Evolutionary Biology. 2003;16(6):1211-22.
66. Puurtinen M, Hytönen M, Knott KE, Taskinen J, Nissinen K, Kaitala V. The effects of mating system and genetic variability on susceptibility to trematode parasites in a freshwater snail, *Lymnaea stagnalis*. Evolution. 2004;58(12):2747-53.
67. Weeks SC, Bernhardt RL. Maintenance of androdioecy in the freshwater shrimp, *Eulimnadia texana*: Field estimates of inbreeding depression and relative male survival. Evolutionary Ecology Research. 2004;6(2):227-42.
68. Pélissié B, Jarne P, David P. Sexual selection without sexual dimorphism: Bateman gradients in a simultaneous hermaphrodite. Evolution. 2012;66(1):66-81.
69. Njiokou F, Bellec C, N'Goran EK, Yapi Yapi G, Delay B, Jarne P. Comparative fitness and reproductive isolation between two *Bulinus globosus* (Gastropoda: Planorbidae) populations. Journal of Molluscan Studies. 1992;58(4):367-76.
70. Doums C, Viard F, Pernot AF, Delay B, Jarne P. Inbreeding depression, neutral polymorphism, and copulatory behavior in freshwater snails: A self-fertilization syndrome. Evolution. 1996;50(5):1908-18.

Tables

Table 1

GLMM on the probability of selfing post-isolation. Results are shown for F1 snails with genotyped offspring (n = 56). The response variable was the likelihood of F1 snails producing at least one selfed juvenile after having been paired (no/yes), modelled using binomial errors. Fixed effects were the pairing treatment (once- vs. repeatedly; test of hypothesis 1), the number of developed embryos produced in isolation (test of hypothesis 2), and the number of male (potential sperm donors) and female partners (potential sperm recipients) a snail copulated with. P0 mother identity was included as a random intercept; its effect was non-significant ($\chi^2_1 = 0.0$, $p = 1.0$). Results are provided on a logit scale. β: estimate, S.E.: standard error, z: z-value, p: p-value.

Predictor	β (S.E.)	z	p
Intercept	-0.13 (0.80)	-0.17	0.87
Treatment (once- vs. repeatedly paired)	1.47 (1.73)	0.85	0.40
# dev. embryos produced in isolation	0.01 (0.01)	2.16	0.0310
# male mating partners	-0.77 (0.55)	-1.40	0.16
# female mating partners	-0.16 (0.40)	-0.40	0.69

Table 2

GLMMs on female LRS and its components. Results are shown for three models using all female fertile F1 snails (n = 126). Response variables were the lifetime number of developed embryos (female LRS) and eggs, and the lifetime proportion of undeveloped embryos. For response variables 1 and 2, negative binomial errors were fitted, and results are provided on the log scale. For response variable 3, Gaussian errors were fitted, as it was approximately normally distributed, as judged from QQ-plots and two-sided Kolmogorov-Smirnov tests (D = 0.08, p = 0.36). Fixed effects were propensity for selfing (reference level: apparent selfers) and body size (shell length). P0 mother identity was included as a random intercept; its effect was significant ($\chi^2_1 \geq 5.50$, $p \leq 0.0190$). Results were similar when computed for snails with genotyped offspring only (Table S3). β: estimate, S.E.: standard error, z: z-value, p: p-value, PS.: propensity for selfing.

	Response:								
	# dev. embryos (LRS)			# eggs			% undev. embryos		
	β (S.E.)	z	p	β (S.E.)	z	p	β (S.E.)	z	p
Predictor:									
Intercept	2.55 (0.63)	4.06	<0.0001	2.71 (0.59)	4.60	<0.0001	31.07 (14.39)	2.16	0.0308
PS (plastic)	1.08 (0.18)	6.13	<0.0001	1.01 (0.17)	6.12	<0.0001	-12.67 (3.80)	-3.34	0.0009
PS (outcr.)	0.92 (0.18)	4.96	<0.0001	0.85 (0.17)	4.95	<0.0001	-11.57 (3.90)	-2.97	0.0030
Body size	0.14 (0.04)	3.35	0.0008	0.16 (0.04)	3.90	<0.0001	0.46 (1.03)	0.44	0.66

Figure legends

Figure 1

Experimental variation in mate availability did not affect individual-level selfing rates. Snails had either one mating opportunity with one partner, or three to five, each with a different partner. (a) Paternity distributions among the progeny of 57 F1 snails. Each vertical bar depicts one F2 family, with differently coloured sections corresponding to juveniles sired by different fathers. Father 1 refers to F1 snails' first and father 5 to their last mating partner; identical colours in different F2 families do not indicate identical father identities. Within pairing treatments, families are arranged according to selfing rate and number of genotyped juveniles. (b) Differences in mean selfing rates between once- and repeatedly paired snails were not statistically significant. White triangles on boxplots show group means. (c) The proportion of F2 families with non-zero selfing rates was similar among once- and repeatedly paired snails. (d) However, once-paired snails produced significantly more selfed juveniles overall. Error bars are binomial standard errors (± 1 SE).

Figure 2

Variation in individual propensity for selfing. We ascertained the selfing propensity of snails with genotyped offspring based on the presence/absence of selfing in isolation and the post-isolation selfing rate. (a) The distribution of post-isolation selfing rates was bimodal among both once- and repeatedly paired snails. (b) Successful selfing in isolation did not predict continued selfing: most previously selfing snails switched to near-complete outcrossing once they could mate. (c) There were four classes of snails with differential selfing propensity: "outcrossers" that never reproduced via selfing, "plastic switchers" that selfed in isolation but

outcrossed after being paired, “plastic mixers” that mixed selfing in isolation with a low level of selfing post-isolation, and “selfers” that selfed fully or predominantly throughout their lives.

Figure 3

Reduced female LRS in preferential selfers. Individuals with a strong propensity for selfing had the fewest developed embryos (a) and the highest proportion of embryos that failed to develop (b). Shown are snails with (black circles) and without genotyped offspring (empty circles). We ascertained the apparent propensity for selfing of snails without genotyped offspring based on the timing of egg production and the mating behaviour. White triangles on boxplots show group means.

Figure 4

Poor correspondence between propensity for selfing and sexual drive. Although selfers (n = 15) were less likely to mate in both sexual roles than plastic switchers (n = 26), they showed considerable sexual activity, with 53.3% mating as a female and 80.0% as a male. Error bars are binomial standard errors (± 1 SE).

Figures

Figure 1

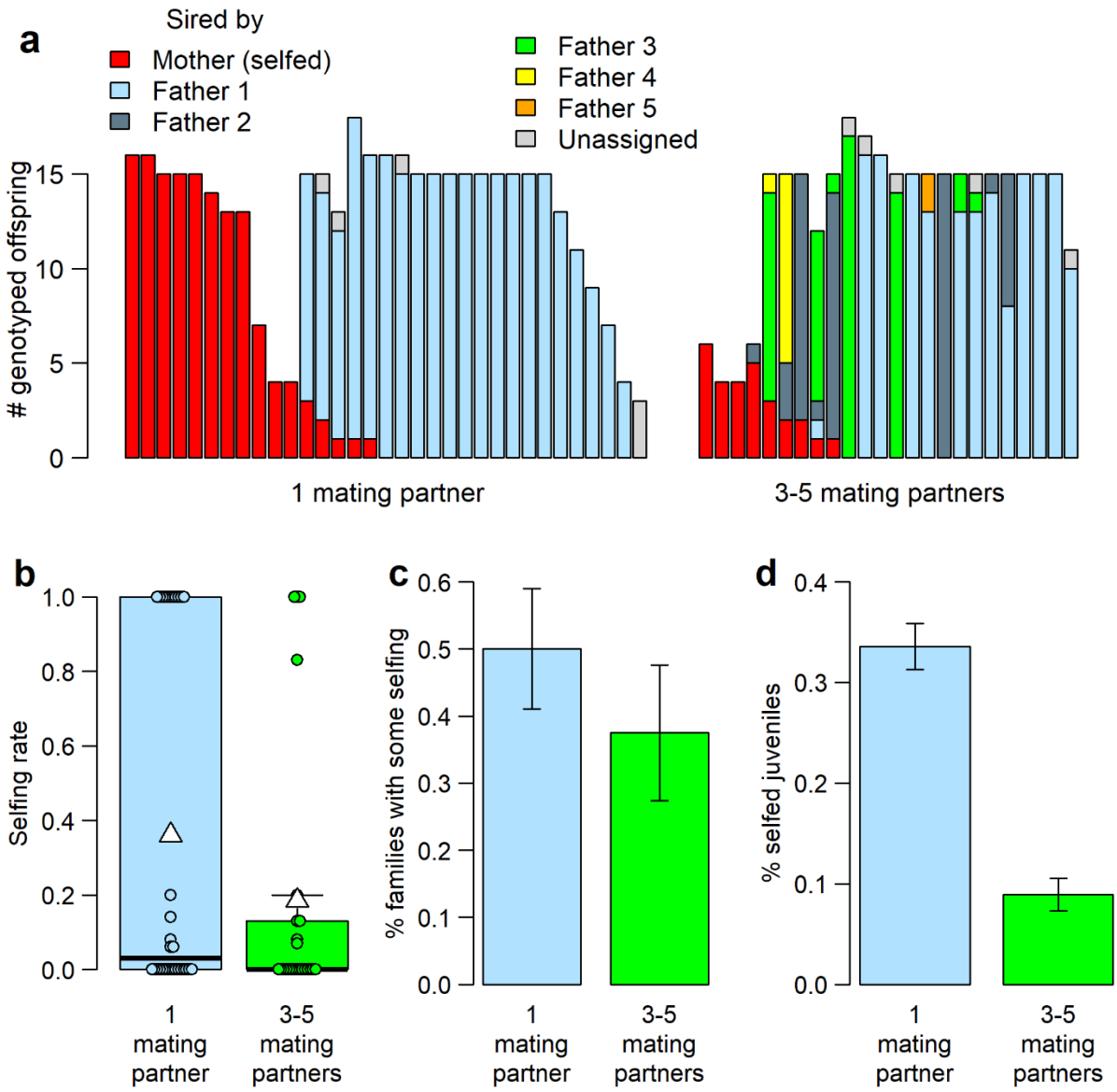


Figure 2

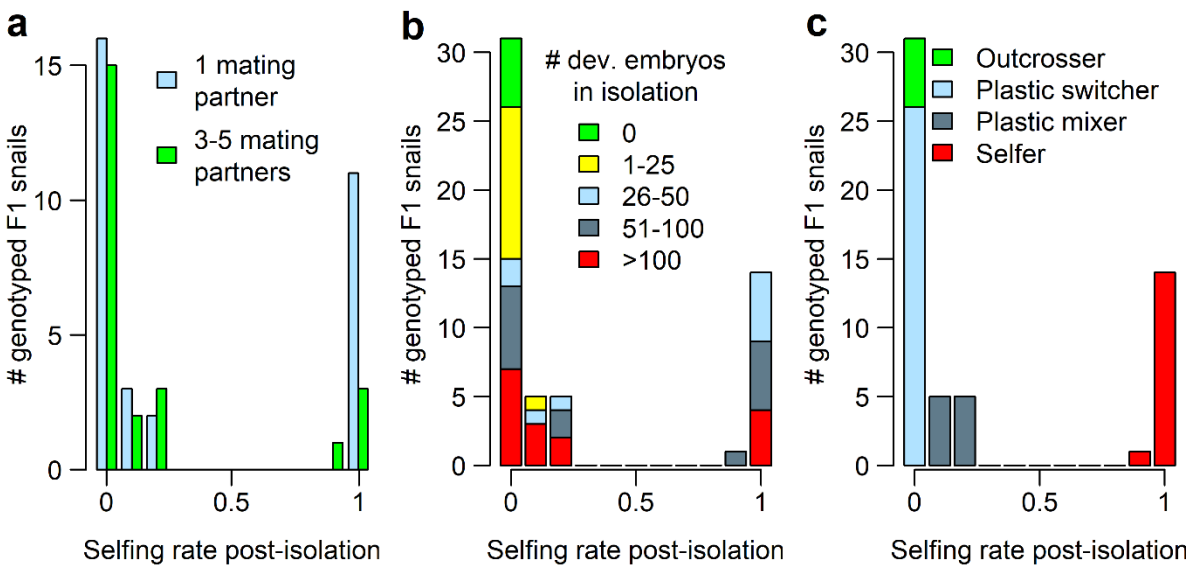

a
b
c
genotyped F1 snails
Selfing rate post-isolation
1 mating partner
3-5 mating partners
dev. embryos in isolation
0
1-25
26-50
51-100
>100
Outcrosser
Plastic switcher
Plastic mixer
Selfer

Figure 3

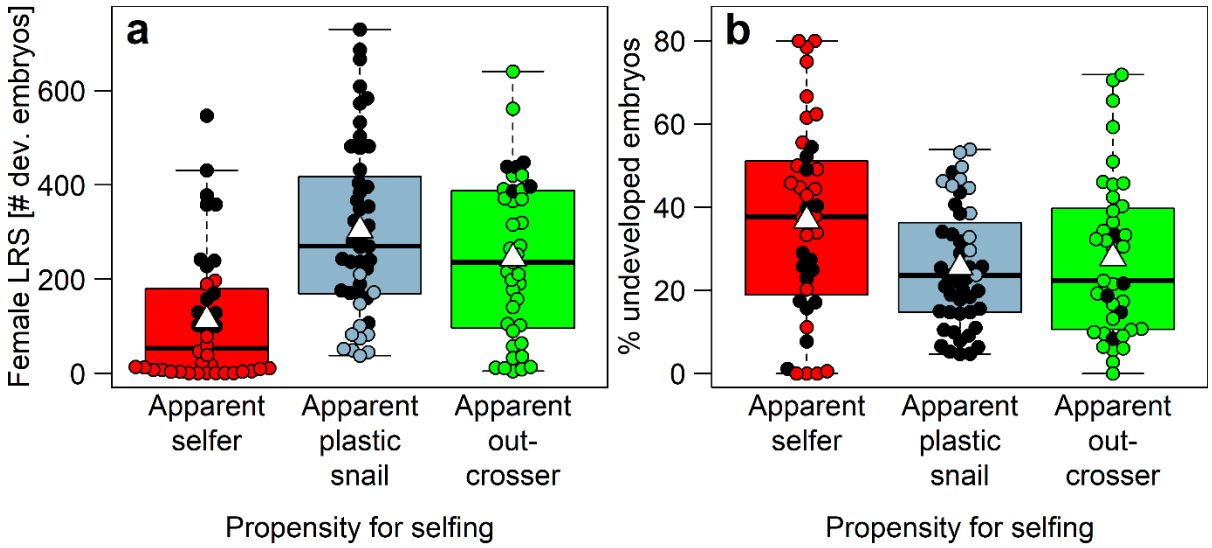
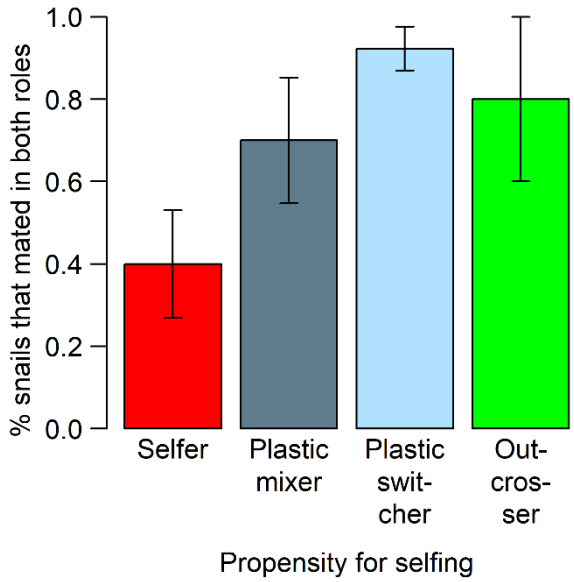

a
Female LRS [# dev. embryos]
0
200
400
600
Apparent selfer
Apparent plastic snail
Apparent out-crosser
Propensity for selfing
b
% undeveloped embryos
0
20
40
60
80
Apparent selfer
Apparent plastic snail
Apparent out-crosser
Propensity for selfing

Figure 4


% snails that mated in both roles
1.0
0.8
0.6
0.4
0.2
0.0
Selfer
Plastic mixer
Plastic swit-cher
Out-cros-ser
Propensity for selfing