

Supporting Information for:

Mate availability determines use of alternative reproductive phenotypes in hermaphrodites

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Supporting Methods

Supporting Methods 1: Statistical analyses testing for effects of size-matching first mating partners

We used a simple linear model to test whether the absolute size difference between repeatedly paired mating partners on mating opportunities 2-6 (when snails were not size-matched) was associated with a pair's mating activity (a predictor with three levels: non-mating, mating in one direction, mating in both directions).

We then tested whether pairs with different mating activity had different average adult shell lengths, now including size-matched pairs. The sole predictor in this model was again a pair's mating activity, but now the response was the mean adult shell length of both mating partners. In both analyses, adjusted p -values for pairwise contrasts were computed using function "glht" with the "single-step" method in R-package "multcomp" (Hothorn et al. 2008).

The size differences between the male- and the female-acting snail in pairs that mated in one direction only were normally distributed, both for size-matched snails ($W = 1.0$, $p = 0.08$) and for snails paired at random ($W = 1.0$, $p = 0.67$). We thus compared the male-female size differences to zero using one-sample t -tests.

Supporting Results

Supporting Results 1: Effects of marking on survival and growth

To test whether our method of marking snails affected their ability to survive and grow, we conducted an experiment on juvenile F1 snails not included in any other analysis. We took experimental snails out of the water, dried their shells using paper towels, applied white correction fluid to a part of their shell, and put snails foot-down on a piece of wet paper towel for five minutes until the correction fluid had dried. Then we wrote a number on the grounded spot using a waterproof felt-tipped pen, left the snails to dry for another five minutes like before, and returned snails to their plastic cups ($n = 30$ snails). Control snails were removed from the water, dried like marked snails, left on wet paper towels for ten minutes, and returned to their plastic cups ($n = 20$ snails). Shell length was measured to the nearest 0.1 mm using a digital calliper. At the beginning of the experiment, snails were 27.0 ± 1.3 weeks old (mean \pm SD, time since egg clutches were laid) and 6.5 ± 1.7 mm long, with no difference between marked and control snails (age: $W = 232.5$, $p = 0.18$, shell length: $W = 287$, $p = 0.80$, Wilcoxon rank sum tests). Growth and survival were monitored for two weeks. After this time, similar numbers of marked ($2/30$, i.e. 6.7%) and control snails had died ($1/20$, i.e. 5.0%, two-tailed chi-squared test with Yates' continuity correction: $\chi^2_1 = 0.0$, $p = 1.00$). Also shell growth did not differ between marked and control snails ($W = 352.5$, $p = 0.06$, Wilcoxon rank sum test, see fig. S1).

Supporting Results 2: Randomness of reproductive patterns

We tested whether observed frequencies of snails at each bifurcation of the flowchart shown in fig. 1 (main manuscript) deviated from a random 50:50 ratio using two-tailed chi-square tests with Yates' continuity correction. The analysis was done separately for once- and repeatedly paired snails. For example, we tested whether the observed numbers of once/paired snails that did vs. did not mate as a male (54 vs. 49) were significantly different from an even distribution (51.5 vs. 51.5), which they were not ($\chi^2_1 = 0.0$, $p = 0.83$). We found that several frequencies deviated significantly from a random 50:50 ratio. Specifically, snails paired six times were much more likely to mate as a male than expected by chance ($\chi^2_1 = 20.0$, $p < 0.0001$). They were also significantly more likely to mate as a female, both when ($\chi^2_1 = 36.4$, $p < 0.0001$) and when not mating as a male ($\chi^2_1 = 4.1$, $p = 0.0426$). Moreover, if mating in both sexual roles, repeatedly paired snails were disproportionately likely to lay eggs ($\chi^2_1 = 8.4$, $p = 0.0039$). In contrast, the mating behavior shown by once-paired snails was consistent

with random binary processes. The only frequencies that deviated from 50:50 ratios pertained to the production of eggs. Egg-laying was much more likely if once-paired snails mated as a male, irrespective of female matings (female mating yes: $\chi^2_1 = 7.2$, $p = 0.0074$; female mating no: $\chi^2_1 = 5.1$, $p = 0.0241$). In addition, once-paired snails that did not mate in any role were exceedingly likely to forgo egg production ($\chi^2_1 = 7.4$, $p = 0.0065$).

Supporting Results 3: Effect of size-matching first mating partners

Each snail's first mating partner was size-matched, while mating partners 2-6 were chosen at random with respect to shell length. Snails with a single mating opportunity thus could only mate with a partner of similar size. We therefore here explore potential effects of matching snails by size on mating behavior.

We first looked at the absolute size difference between mating partners on mating opportunities 2-6, because these pairs were not size-matched. We found significant variation in size difference between pairs with differing mating activity ($F_{2, 305} = 5.5$, $p = 0.0044$). Specifically, non-mating pairs had larger size differences than both pairs that mated in one direction ($b = -0.6$, $t = -3.0$, $p = 0.0075$) and pairs that mated in both directions ($b = -0.7$, $t = -2.4$, $p = 0.0428$). Pairs that mated in one vs. both directions had similar size differences ($b = -0.1$, $t = -0.5$, $p = 0.88$). This shows that reducing size differences between mating partners increased mating probability.

Next we tested for an effect of the mean size of the two snails in a pair on the pair's mating activity, including all (also size-matched) pairs of snails. We found significant differences in average adult shell length between non-mating, unidirectionally mating and (sequentially) reciprocally mating pairs ($F_{2, 435} = 38.1$, $p < 0.0001$). Non-mating pairs were smallest, differing significantly from both pairs mating in one direction ($b = 1.1$, $t = 6.9$, $p < 0.0001$) and pairs mating in both directions ($b = 1.7$, $t = 8.0$, $p < 0.0001$). Pairs that mated in one direction were intermediate in size, and pairs that mated in both directions largest ($b = 0.6$, $t = 3.1$, $p = 0.0061$). Hence, mating was relatively unlikely when mating partners differed substantially in size, but also when both mating partners were small. Further support for this conclusion comes from the eleven repeatedly paired snails that did not mate on mating opportunity 1, but mated on a later occasion. Four of them had very large (5.1-6.7 mm) size differences to their first mating partners (with which no mating occurred), while the partners they eventually mated with were closer to their own size (size differences: 0.3-5.0 mm). The size differences between the other seven snails and their non-copulating first partners were

more moderate (0.0-2.8 mm), but in all except one case here the eventual mating partner was larger than the first one.

Finally, we tested whether adult shell length predicted sexual roles. To do that, we only considered pairs that mated in one direction and asked whether the size difference between the male- and female-acting partners was on average significantly different from zero. It was not, neither during first mating opportunities where partners were size-matched (mean difference \pm SD: 0.1 ± 1.4 mm, one-sample t test: $t_{72} = 0.6$, $p = 0.52$), nor during later mating opportunities (0.3 ± 2.6 mm, $t_{153} = 1.4$, $p = 0.16$), showing that the snail playing the male role was sometimes larger and sometimes smaller than its mating partner, without a strict rule.

To sum up, snails tended to remain unmated when they were small or when their partner was very dissimilar in size, but when a mating occurred sexual roles were taken randomly with respect to shell length. Hence, we consider the bias introduced by size-matching first mating partners negligible.

Supporting Results 4: Relationship between female infertility and mating activity

Many snails did not produce any eggs throughout their lives (48.5%). This is not an artifact of snails dying prematurely. In fact, fewer snails without eggs than snails with eggs died before the end of the experiment (5.4% vs. 18.8%, $\chi^2_1 = 10.0$, $p = 0.0016$). The frequency of female infertility was equal among snails paired once or six times (fig. S5A). This is surprising, considering that once-paired snails remained without a female mating significantly more often than repeatedly paired snails (42.7% vs. 9.8%, $\chi^2_1 = 28.8$, $p < 0.0001$), and were thus more often forced to rely on self-fertilization. Within treatment groups, female infertility was also not significantly associated with female virginity (fig. S5B). Only when pooling all snails female infertility was significantly more common among snails that did not mate as a female (54.5%) than among female-mating snails (36.9%, $\chi^2_1 = 4.6$, $p = 0.0326$). Altogether, two thirds (66.3%) of female infertile snails mated as a female.

Somewhat counterintuitively, *male* mating activity was positively associated with female fertility. In fact, mating activity in the male role predicted female fertility much better than female mating activity did. Overall, only 23.8% of snails that mated as a male remained without eggs, compared to 76.4% of snails that did not mate as a male ($\chi^2_1 = 52.5$, $p < 0.0001$). This pattern was repeated within treatment groups (fig. S5C). Although we did not

measure male reproductive output, we know that 61.8% of female infertile snails did not mate as a male, and so truly have a reproductive fitness of zero (reproductive failure type 1 and 2).

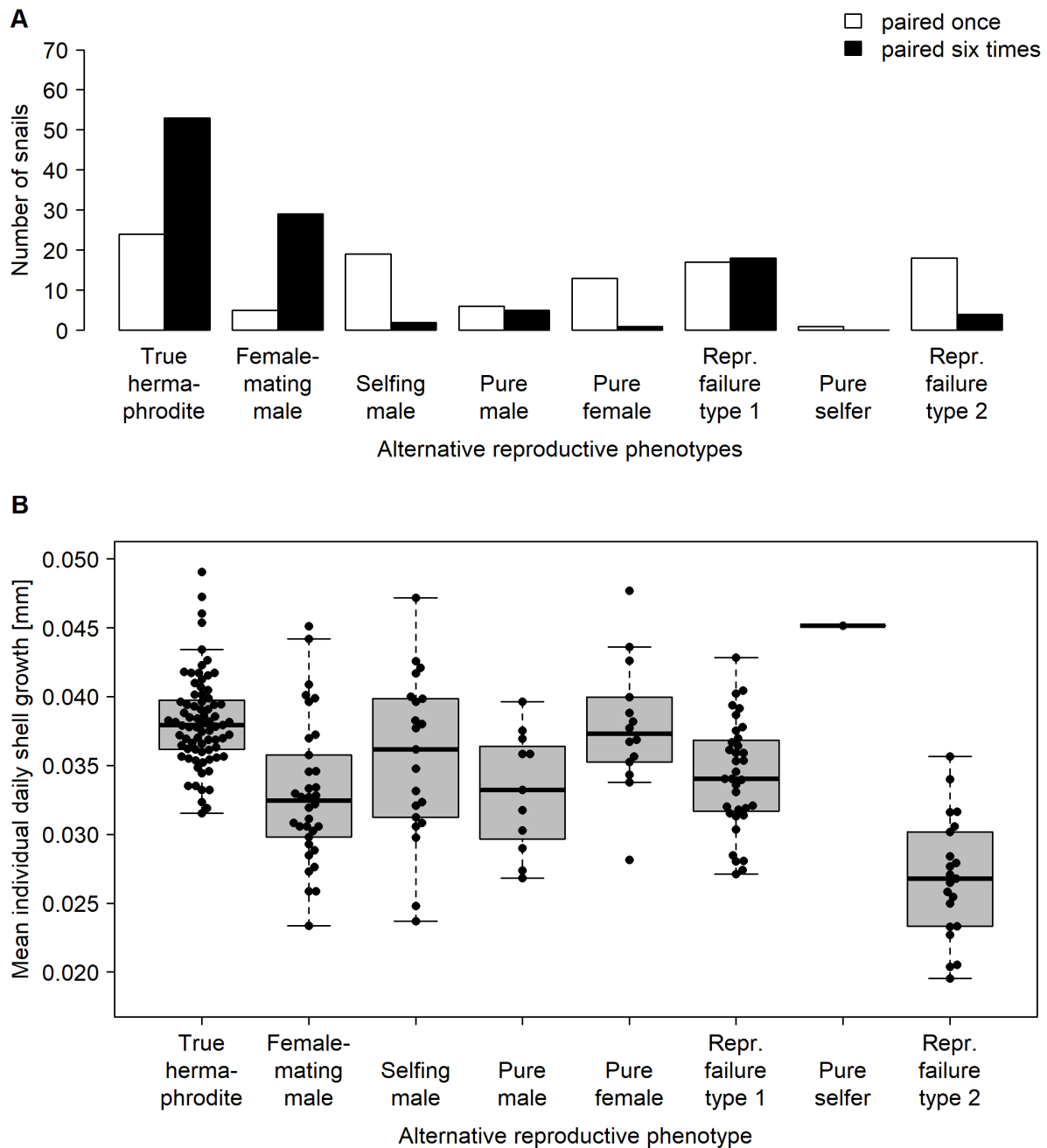


Fig. S2. Dependence of observed alternative reproductive phenotypes on experimentally manipulated mate availability (A), and correlative association with mean individual daily growth rate (B) – when measuring female reproductive success as the number of developed embryos

The numbers of snails per reproductive phenotype are as follows (from left to right): 53, 29, 2, 5, 1, 18, 0 and 4 for snails paired six times, and 24, 5, 19, 6, 13, 17, 1 and 18 for snails paired once. Note that of two snails included in (B) the growth rate could not be measured, reducing the sample size here to 213 snails.

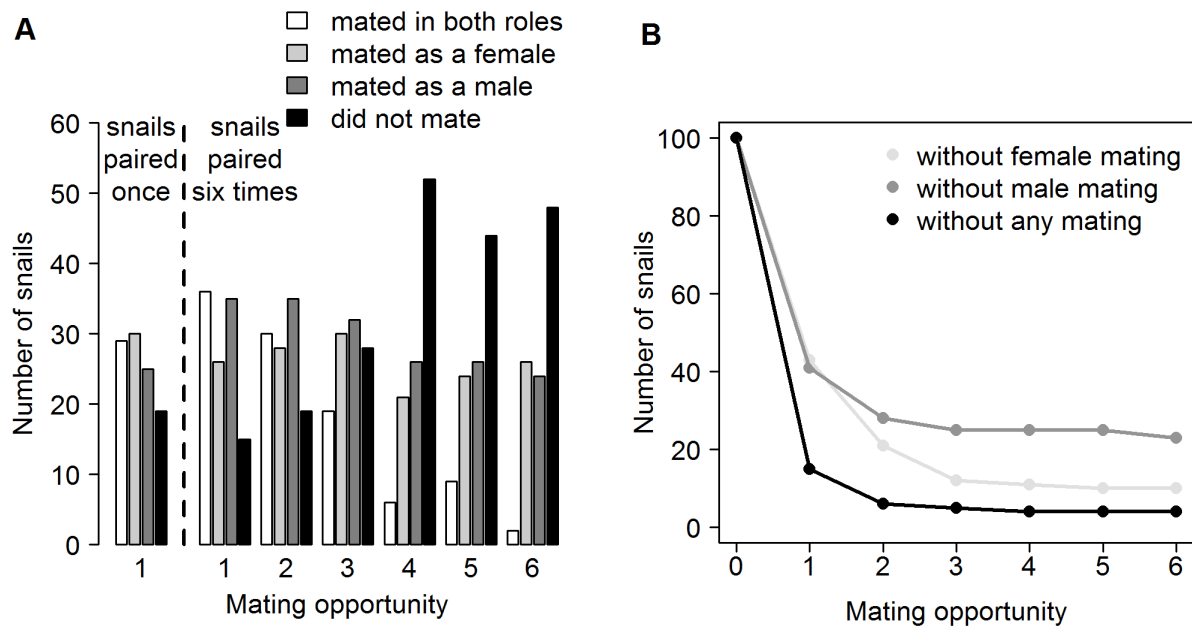


Fig. S3. Mating activity during six consecutive mating opportunities (A), and cumulative number of unmated snails after zero to six mating opportunities (B) – when considering both verified and unverified matings

Snails were paired once or six times, each time with a different mating partner. Snails are included only if their mating behavior could be assessed with very high accuracy (80.2% of snails). Consequently, for a given mating opportunity, in (A) the numbers of solely male- and solely female-mating snails are not necessarily identical. Figure (B) is restricted to repeatedly paired snails that survived through all six mating opportunities ($n = 100$).

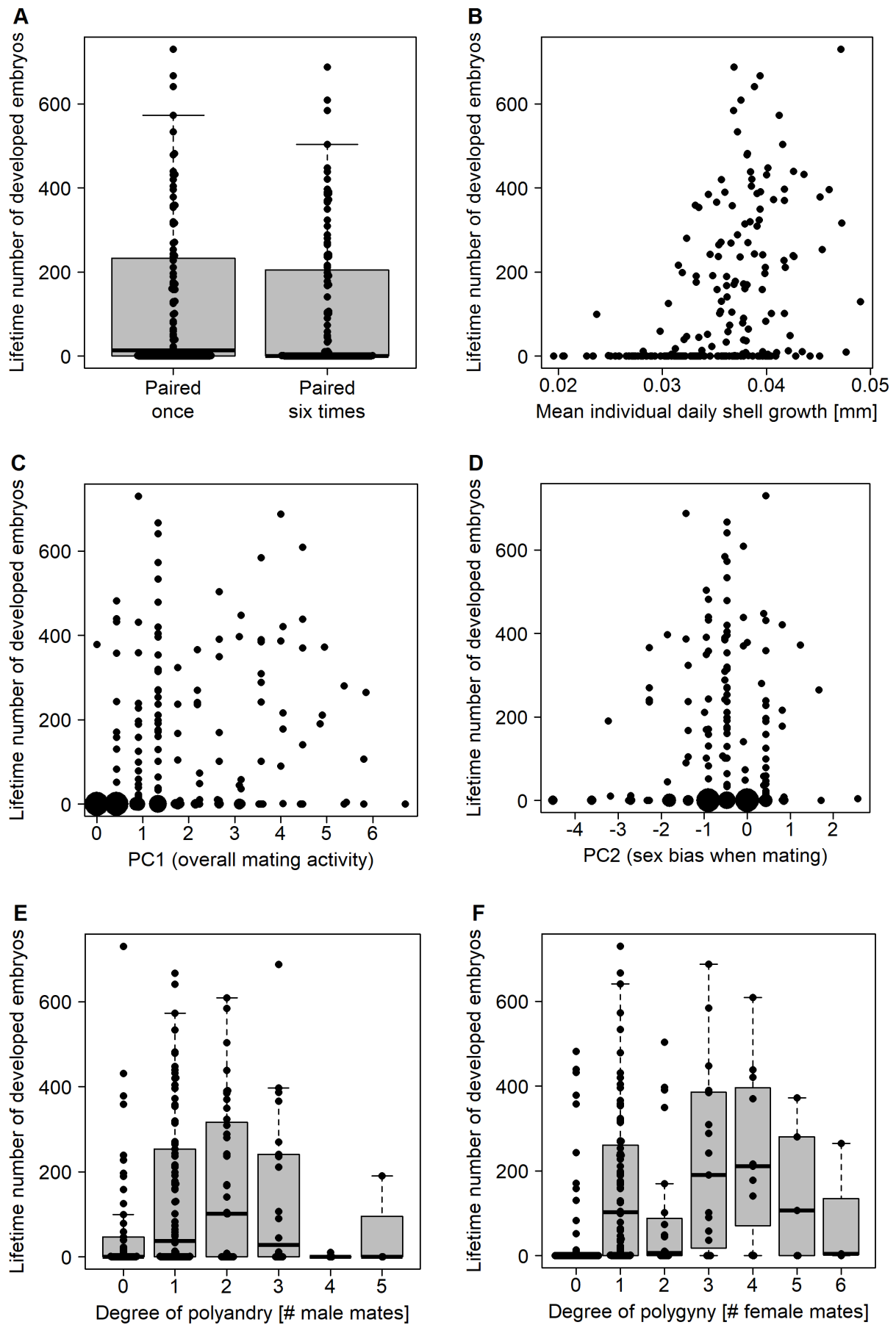


Fig. S4. Causal link between female lifetime reproductive success and experimentally manipulated mate availability (A), and correlative association with mean individual daily growth rate (B), overall mating activity (C), and the sex bias when mating (D) – when measuring female reproductive success as the number of developed embryos

Shown are raw data (black), and box plots based on raw data (gray). In (C-F), we only show mating partners with which verified copulations occurred (i.e. copulations with visible penis insertion). In (C-D) the size of data points is proportional to their frequency. Principal component 1 (PC_1) represents overall mating activity positively, while PC_2 represents a female bias in mating activity. Principal components were computed following an approach outlined in Anthes et al. (2010). A snail's degree of polyandry (E) and polygyny (F) are shown for illustrative purposes only and were not included as predictors in the statistical model. Eggs produced by the single non-copulating snail that reproduced as a female were selfed, as verified by microsatellite genotyping.

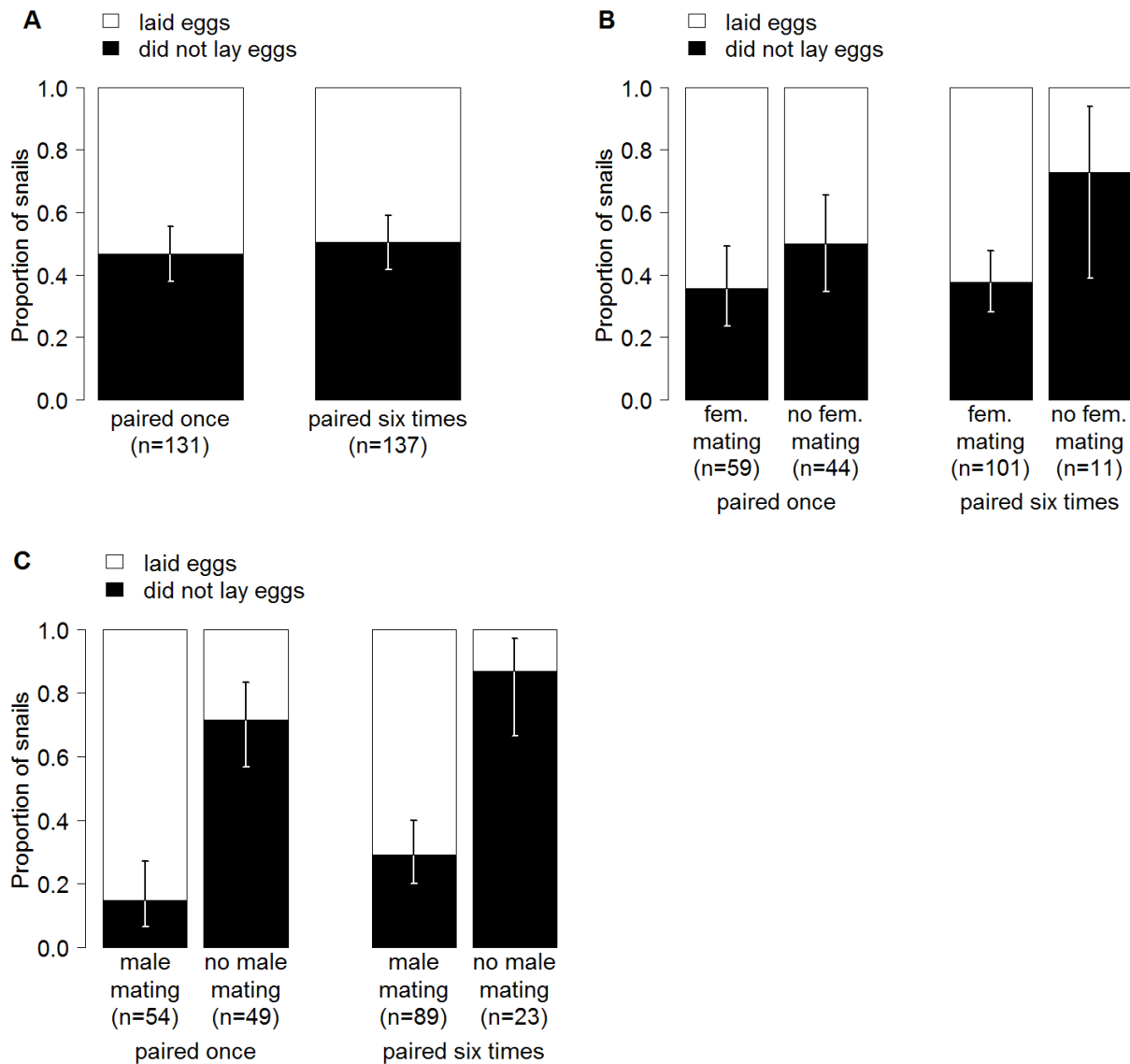


Fig. S5. Association between female infertility and mating activity

In A-C, results are shown separately for snails paired once vs. six times. Sample sizes are provided in parentheses. Error bars show 95% confidence intervals computed according to Zar (1996).

(A) No difference in the frequency of snails that did not lay eggs between snails paired once or six times (46.6% vs. 50.4%, $\chi^2_1 = 0.2$, $p = 0.62$).

(B) No difference in the frequency of snails that did not lay eggs between snails that did or did not mate as a female (snails paired once: 35.6% vs. 50.0%, $\chi^2_1 = 1.6$, $p = 0.21$; snails paired six times: 37.6% vs. 72.7%, $\chi^2_1 = 3.7$, $p = 0.05$). Note that statistical power is low in snails paired six times, as only eleven snails remained without a female mating.

(C) Significantly lower frequency of snails that did not lay eggs among snails that mated as a male (snails paired once: 14.8% vs. 71.4% female infertile snails, $\chi^2_1 = 31.6$, $p < 0.0001$; snails paired six times: 29.2% vs. 87.0% female infertile snails, $\chi^2_1 = 22.8$, $p < 0.0001$).

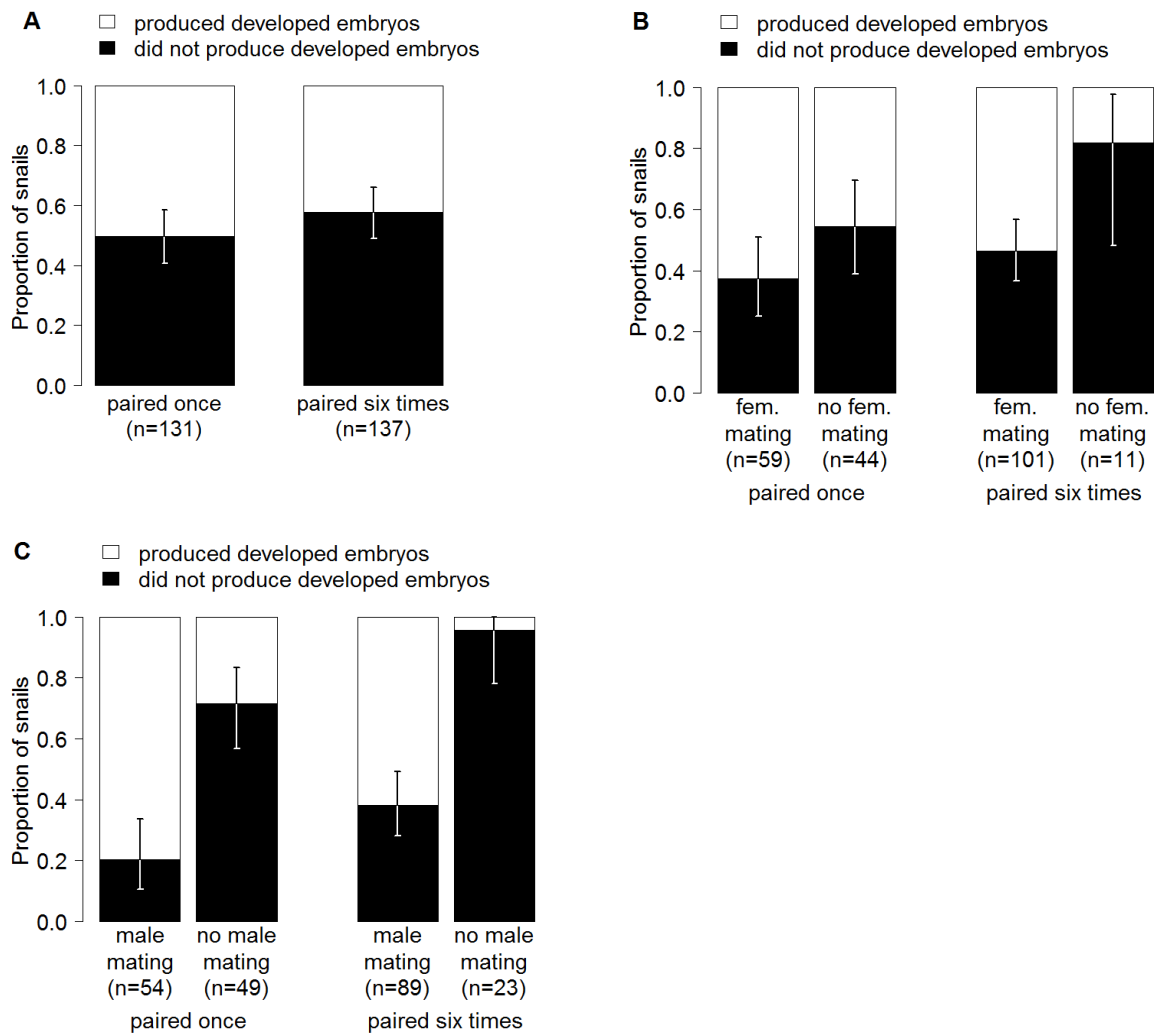


Fig. S6. Association between female infertility and mating activity – when measuring female reproductive success as the number of developed embryos

Results are shown separately for snails paired once vs. six times. Sample sizes are provided in parentheses. Error bars show 95% confidence intervals computed according to Zar (1996).

(A) No difference in the frequency of snails that produce developed embryos between snails paired once or six times (49.6% vs. 57.7%, $\chi^2_1 = 1.4$, $p = 0.23$).

(B) No difference in the frequency of snails that did not produce developed embryos between snails that did or did not mate as a female (snails paired once: 37.3% vs. 54.5%, $\chi^2_1 = 2.4$, $p = 0.12$; snails paired six times: 46.5% vs. 81.8%, $\chi^2_1 = 3.6$, $p = 0.06$). Note that statistical power is low in snails paired six times, as only eleven snails remained without a female mating.

(C) Significantly lower frequency of snails that did not produce developed embryos among snails that mated as a male (snails paired once: 20.4% vs. 71.4% female infertile snails, $\chi^2_1 =$

25.1, $p < 0.0001$; snails paired six times: 38.2% vs. 95.7% female infertile snails, $\chi^2_1 = 21.9$, $p < 0.0001$).

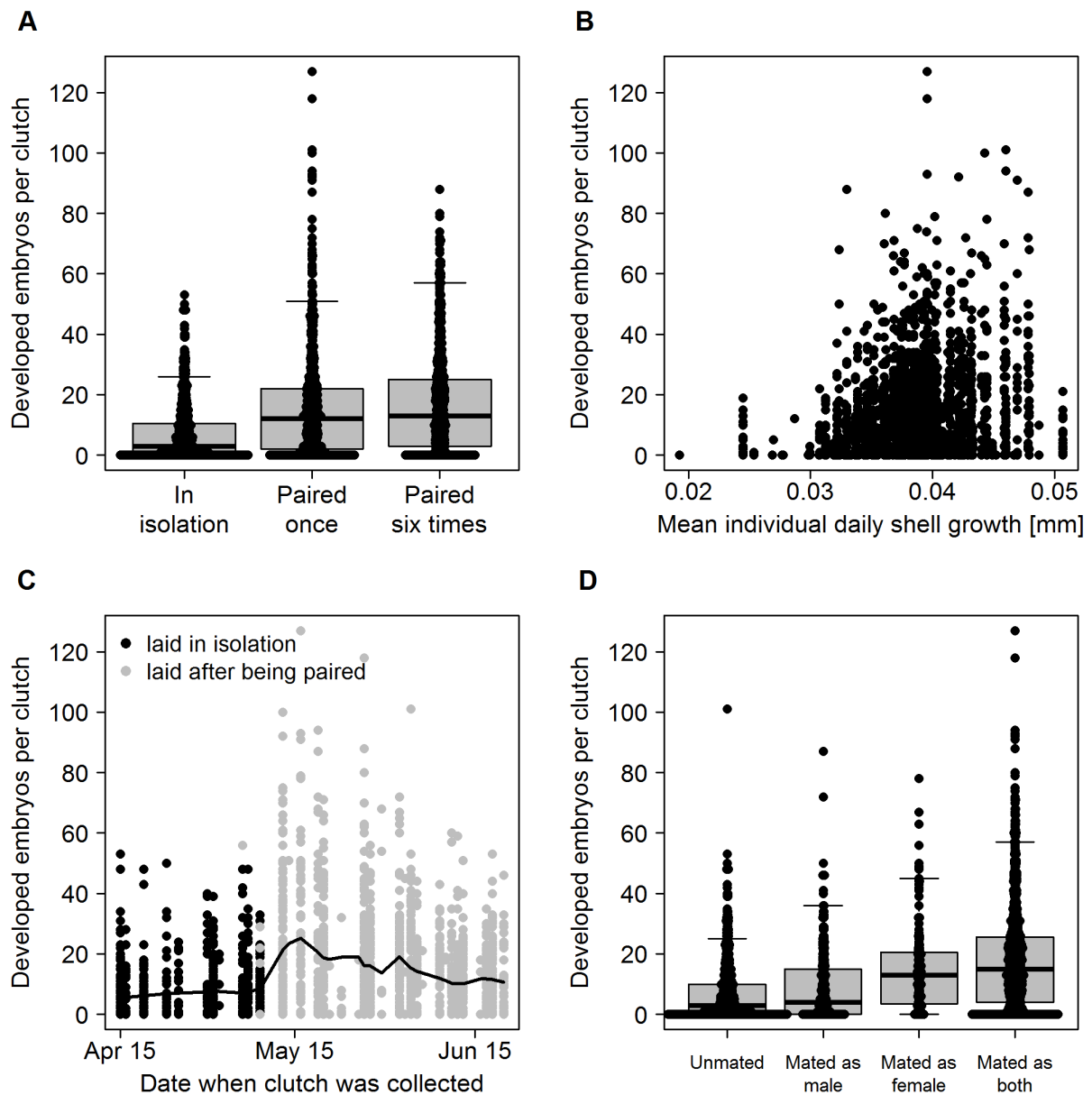


Fig. S7. Causal link between the number of developed embryos per clutch and experimentally manipulated mate availability (A), and correlative association with mean individual daily growth rate (B), time (C), and maternal mating activity before laying a clutch (D)

Shown are raw data (black), and box plots based on raw data (gray). Multiple data points may stem from the same snail, and are therefore not independent. Clutches laid without prior female mating must be selfed. In (C) dates of collection are at most seven days later than dates of clutch-laying. A loess line is superimposed on the data points (smoothing parameter α set to 0.25).

1 **Supporting Tables**2 **Table S1: Frequency of female infertility in pulmonate gastropods**

| Species | Opportunity to mate? | Born and reared in the laboratory? | Reproduction monitored until/for | Number of snails | Proportion female infertile (%) | Reference |
|------------------------------|----------------------|------------------------------------|----------------------------------|------------------|---------------------------------|-----------------------------|
| <i>Ancylus fluviatilis</i> | no | no | death | 18 | 16.7 | (Städler et al. 1993) |
| | yes | no | death | 72 | 51.4 | |
| | yes | no | 21 days | 33 | 0.0 | (Städler et al. 1995) |
| <i>Arianta arbustorum</i> | yes | yes | 4 months | 86 | 64.0 | (Baur and Baur 1997) |
| | yes | yes | 4 months | 78 | 0.0 | (Chen 1993) |
| | yes | yes | 4 months | 78 | 59.0 | |
| | yes | no | 30 days | 16 | 6.3 | (Janssen and Baur 2015) |
| | yes | no | 30 days | 16 | 0.0 | |
| | yes | no | 45 days | 13 | 69.2 | |
| | yes | no | 45 days | 15 | 73.3 | |
| | yes | no | 60 days | 21 | 0.0 | (Kupfernagel and Baur 2011) |
| | yes | no | 60 days | 40 | 10.0 | (Locher and Baur 2000) |
| | yes | no | 56 days | 24 | 62.5 | (Minoretti et al. 2011) |
| <i>Biomphalaria glabrata</i> | yes | yes | exhaustion of | 370 | 7.8 | (Vianey-Liaud et al. |

| Species | Opportunity to mate? | Born and reared in the laboratory? | Reproduction monitored until/for | Number of snails | Proportion female infertile (%) | Reference |
|---|----------------------|------------------------------------|----------------------------------|------------------|---------------------------------|-------------------------------|
| | | | allosperm | | | 1987) |
| <i>Cornu aspersum</i> | yes | no | 3.5 months | 78 | 53.8 | (Evanno et al. 2005) |
| | yes | no | about 2 months | 44 | 15.9 | (Garefalaki et al. 2010) |
| | yes | no | unknown | 64 | 40.6 | (Rogers and Chase 2002) |
| <i>Drepanotrema depressissimum</i> | no | yes | death | 83 | 28.9 | (Lamy et al. 2012) |
| | yes | yes | production of >10 eggs | 100 | 11.0 | |
| <i>Galba truncatula</i> | no | yes | 3 months | 13 | 15.4 | (Meunier et al. 2004) |
| | no | yes | 3 months | 10 | 70.0 | |
| <i>Helisoma duryi</i> | no | yes | 9 weeks | 10 | 100.0 | (Madsen et al. 1983) |
| | yes | yes | 5 months | 15 | 0.0 | |
| <i>Lymnaea ovata</i> (now probably <i>Radix balthica</i>) | yes | yes | 27 weeks | 20† | 85.0 | (Wulschleger and Jokela 2002) |
| <i>Lymnaea peregra</i> (now probably <i>Radix lagotis</i>) | yes | yes | 27 weeks | 20† | 10.0 | (Wulschleger and Jokela 2002) |
| <i>Lymnaea stagnalis</i> | no | yes | 25 days | 22 | 90.9 | (Koene and Ter Maat 2004) |
| | yes | yes | 25 days | 23 | 30.4 | |
| <i>Physa acuta</i> | yes | no | 21 days | 20 | 10.0 | (Henry et al. 2005) |
| | yes | no | 21 days | 20 | 21.1 | |
| | yes | no | 21 days | 20 | 5.9 | |
| | yes | no | 21 days | 20 | 15.0 | |

| Species | Opportunity to mate? | Born and reared in the laboratory? | Reproduction monitored until/for | Number of snails | Proportion female infertile (%) | Reference |
|---------------------------|----------------------|------------------------------------|----------------------------------|------------------|---------------------------------|------------------------------|
| <i>Physa heterostroph</i> | yes | no | 21 days | 20 | 16.7 | |
| | yes | no | 21 days | 20 | 47.4 | |
| | yes | no | 21 days | 20 | 15.8 | |
| | yes | no | 21 days | 20 | 50.0 | |
| | yes | no | 21 days | 20 | 16.7 | |
| | yes | no | 21 days | 20 | 12.5 | |
| | yes | no | 21 days | 20 | 10.5 | |
| | no | yes | 3 months | 131 | 44.3 | (Jarne et al. 2000) |
| | no | yes | 3 months | 132 | 24.2 | |
| | yes | yes | 10 days | 120 | 8.3 | (Pélissié et al. 2014) |
| | yes | yes | 3 days or more | 55 | 20.0 | (Noël et al. 2016) |
| | yes | yes | 3 days or more | 50 | 14.0 | |
| | yes | yes | 3 days or more | 52 | 19.0 | |
| | yes | yes | 3 days or more | 50 | 16.0 | |
| | yes | no | 60 days | 35 | 17.1 | (Wethington and Dillon 1991) |
| | yes | yes | death | 26 | 0.0 | (Wethington and Dillon 1997) |

3

4 Empirical studies that estimated the frequency of female infertility in pulmonate gastropods. Estimates were only included if they were
5 obtained using sexually mature individuals. This list is not intended to be exhaustive. † Number of snail pairs.

Table S2. Experimental design and number of snails

| | Date (number of snails) | | | |
|--------------------------|-------------------------|-----------------|-----------------|-----------------|
| | Block 1 | Block 2 | Block 3 | Block 4 |
| Marked individually | 01.05.2014 (72) | 05.05.2014 (71) | 06.05.2014 (67) | 08.05.2014 (64) |
| Mating opportunity 1 - S | 02.05.2014 (36) | 06.05.2014 (36) | 07.05.2014 (33) | 09.05.2014 (32) |
| Mating opportunity 1 - R | 02.05.2014 (36) | 06.05.2014 (35) | 07.05.2014 (34) | 09.05.2014 (32) |
| Still alive - S | 09.05.2014 (35) | 13.05.2014 (36) | 14.05.2014 (33) | 16.05.2014 (31) |
| Mating opportunity 2 - R | 09.05.2014 (36) | 13.05.2014 (35) | 14.05.2014 (34) | 16.05.2014 (32) |
| Still alive - S | 16.05.2014 (35) | 20.05.2014 (36) | 21.05.2014 (33) | 23.05.2014 (30) |
| Mating opportunity 3 - R | 16.05.2014 (34) | 20.05.2014 (34) | 21.05.2014 (34) | 23.05.2014 (31) |
| Still alive - S | 23.05.2014 (34) | 27.05.2014 (36) | 28.05.2014 (33) | 30.05.2014 (30) |
| Mating opportunity 4 - R | 23.05.2014 (33) | 27.05.2014 (32) | 28.05.2014 (34) | 30.05.2014 (30) |
| Still alive - S | 30.05.2014 (32) | 03.06.2014 (36) | 04.06.2014 (33) | 06.06.2014 (29) |
| Mating opportunity 5 - R | 30.05.2014 (32) | 03.06.2014 (32) | 04.06.2014 (33) | 06.06.2014 (30) |
| Still alive - S | 06.06.2014 (32) | 10.06.2014 (35) | 11.06.2014 (32) | 13.06.2014 (25) |
| Mating opportunity 6 - R | 06.06.2014 (31) | 10.06.2014 (32) | 11.06.2014 (31) | 13.06.2014 (30) |
| Killed - S | 13.06.2014 (30) | 17.06.2014 (35) | 18.06.2014 (30) | 20.06.2014 (22) |
| Killed - R | 13.06.2014 (23) | 17.06.2014 (30) | 18.06.2014 (29) | 20.06.2014 (29) |

Snails were either given a single mating opportunity with a single mating partner (rows marked with an S), or six sequential mating opportunities, each one with a different mating partner (rows marked with an R). One once-paired snail from block 1 with missing reproductive data, and five once-paired snails whose sole mating partners were from the same mother, were excluded from all further analyses, reducing the total sample size to 268 snails.

Table S3. Multinomial log-linear regression on observed frequencies of alternative reproductive phenotypes

| | Intercept | Treatment (level: paired repeatedly) | Growth rate | Block (level: 2) | Block (level: 3) | Block (level: 4) |
|----------------------|-----------|--|----------------|---------------------|---------------------|---------------------|
| Coefficients: | | | | | | |
| Female-mating male | 8.01 | 0.58 | -278.72 | 0.52 | -0.07 | 0.07 |
| Selfing male | 5.75 | -3.27 | -177.25 | 1.36 | 0.06 | 1.53 |
| Pure male | 7.50 | -1.33 | -266.86 | -0.05 | 0.28 | 1.30 |
| Pure female | -1.14 | -2.64 | -31.96 | 2.83 | 1.94 | 1.44 |
| Repr. failure type 1 | 7.59 | -1.15 | -212.82 | 0.04 | -0.80 | -0.04 |
| Repr. failure type 2 | 17.23 | -2.57 | -567.25 | 1.74 | 1.19 | 1.01 |
| Standard errors: | | | | | | |
| Female-mating male | 2.18 | 0.64 | 60.38 | 0.65 | 0.68 | 0.73 |
| Selfing male | 2.22 | 0.70 | 59.86 | 0.74 | 0.79 | 0.76 |
| Pure male | 3.06 | 0.81 | 86.60 | 1.30 | 1.08 | 1.04 |
| Pure female | 2.85 | 0.73 | 70.32 | 1.16 | 1.15 | 1.31 |
| Repr. failure type 1 | 1.97 | 0.47 | 53.24 | 0.59 | 0.63 | 0.63 |
| Repr. failure type 2 | 2.87 | 0.81 | 89.79 | 0.95 | 1.02 | 1.19 |
| z-values: | | | | | | |
| Female-mating male | 3.71 | 0.90 | -4.62 | 0.79 | -0.10 | 0.09 |
| Selfing male | 2.59 | -4.66 | -2.96 | 1.83 | 0.08 | 2.01 |
| Pure male | 2.45 | -1.64 | -3.08 | -0.04 | 0.26 | 1.26 |
| Pure female | -0.40 | -3.63 | -0.45 | 2.45 | 1.69 | 1.10 |
| Repr. failure type 1 | 3.86 | -2.45 | -3.40 | 0.07 | -1.27 | -0.07 |
| Repr. failure type 2 | 6.01 | -3.19 | -6.32 | 1.83 | 1.18 | 0.85 |
| p-values: | | | | | | |
| Female-mating male | 0.0002 | 0.37 | <0.0001 | 0.43 | 0.92 | 0.93 |
| Selfing male | 0.0096 | <0.0001 | 0.0031 | 0.07 | 0.94 | 0.0446 |
| Pure male | 0.0142 | 0.10 | 0.0021 | 0.97 | 0.79 | 0.21 |
| Pure female | 0.69 | 0.0003 | 0.65 | 0.0142 | 0.09 | 0.27 |

| | | | | | | |
|----------------------|-------------------|---------------|-------------------|------|------|------|
| Repr. failure type 1 | <i>0.0001</i> | <i>0.0142</i> | <i><0.0001</i> | 0.94 | 0.20 | 0.95 |
| Repr. failure type 2 | <i><0.0001</i> | <i>0.0014</i> | <i><0.0001</i> | 0.07 | 0.24 | 0.39 |

Observed frequencies of alternative reproductive phenotypes were analyzed using a multinomial log-linear regression and the function “multinom” in R-package “nnet” (Venables and Ripley 2002). The response had seven rather than eight levels, as we excluded pure selfing due to low sample size ($n = 1$). The reference level for the response are the true hermaphrodites. *P*-values in multinomial models were computed using two-tailed z tests. Italics: $p < 0.05$.

Table S4. Multinomial log-linear regression on mating activity on mating opportunity 1

| | Intercept | Treatment (level: paired repeatedly) |
|------------------------|---------------|--------------------------------------|
| Coefficients (s. e.): | | |
| Only mated as a female | 0.03 (0.26) | -0.18 (0.37) |
| Only mated as a male | -0.15 (-0.54) | 0.08 (0.38) |
| Did not mate | -0.42 (-1.43) | 0.35 (0.40) |
| <i>z</i> -values: | | |
| Only mated as a female | 0.13 | -0.47 |
| Only mated as a male | -0.54 | 0.21 |
| Did not mate | -1.43 | 0.90 |
| <i>p</i> -values: | | |
| Only mated as a female | 0.90 | 0.64 |
| Only mated as a male | 0.59 | 0.83 |
| Did not mate | 0.15 | 0.37 |

The mating activity on mating opportunity 1 was analyzed using a multinomial log-linear regression and the function “multinom” in R-package “nnet” (Venables and Ripley 2002). The response had three levels, with “mated in both roles” as the reference level. *P*-values in multinomial models were computed using two-tailed *z* tests.

Table S5. Generalized linear mixed model on female lifetime reproductive output

| Predictor | | | |
|---------------------------------------|------------------|------------|----------|
| Fixed effect: | Estimate (s. e.) | z-value | p-value |
| Intercept | -1.84 (0.80) | -2.29 | 0.0222 |
| Treatment (level: paired repeatedly) | -1.76 (0.36) | -4.89 | < 0.0001 |
| Growth rate | 139.63 (19.36) | 7.21 | < 0.0001 |
| Block (level: 2) | 0.38 (0.26) | 1.51 | 0.13 |
| Block (level: 3) | 0.45 (0.26) | 1.71 | 0.09 |
| Block (level: 4) | 0.01 (0.29) | 0.02 | 0.98 |
| PC ₁ | 1.78 (0.29) | 6.05 | < 0.0001 |
| PC ₁ squared | -0.20 (0.04) | -4.75 | < 0.0001 |
| PC ₂ | -0.12 (0.14) | -0.83 | 0.41 |
| PC ₂ squared | -0.12 (0.06) | -2.22 | 0.0262 |
| Random effects: | | χ^2_1 | p-value |
| P0 mother | | 10.50 | 0.0012 |
| Pair identity on mating opportunity 1 | | 2.07 | 0.15 |

Female lifetime reproductive output was analyzed using a generalized linear mixed model with negative binomial errors (NB1 parameterization) and a log link function, using function “glmmTMB” in R-package “glmmTMB” (Brooks et al. 2017). The total number of snails included in the model is 213, the number of P0 mothers is 34, and the number of pair identities on mating opportunity 1 is 121. The overdispersion parameter for the nbinom1 family is 349. The reference level of fixed effect “treatment” is paired just once. The reference level of fixed effect “block” is block 1. PC₁ (principal component 1) represents overall mating activity, with higher

values corresponding to increased mating activity. PC₂ represents a sex bias in mating activity, with higher values corresponding to a female bias when mating. Italics: $p < 0.05$.

Table S6. Generalized linear mixed model on clutch size – model 1

| Predictor | | | |
|---|------------------|------------|----------|
| Fixed effect: | Estimate (s. e.) | z-value | p-value |
| Intercept | -0.51 (0.54) | -0.95 | 0.34 |
| Type (level: post-isolation, paired once) | 0.54 (0.10) | 5.37 | < 0.0001 |
| Type (level: post-isolation, paired repeatedly) | 0.50 (0.11) | 4.65 | < 0.0001 |
| Growth rate | 64.76 (13.40) | 4.83 | < 0.0001 |
| Block (level: 2) | 0.33 (0.17) | 1.98 | 0.0479 |
| Block (level: 3) | 0.03 (0.17) | 0.19 | 0.85 |
| Block (level: 4) | 0.19 (0.18) | 1.02 | 0.31 |
| Random effects: | | χ^2_1 | p-value |
| P0 mother | | 6.16 | 0.0131 |
| Snail identity | | 442.05 | < 0.0001 |
| Collection date | | 111.08 | < 0.0001 |

Clutch size was analyzed using a generalized linear mixed model with negative binomial errors (NB1 parameterization) and a log link function, using function “glmmTMB” in R-package “glmmTMB” (Brooks et al. 2017). The total number of clutches included in the model is 2117, the number of P0 mothers is 34, the number of individual snails is 147, and the number of collection dates is 36. The overdispersion parameter for the nbinom1 family is 8.96. The reference level of fixed effect “type” is laid in isolation. The reference level of fixed effect “block” is block 1. The pairwise contrast between levels “post-isolation, paired once” and “post-isolation, paired repeatedly” was computed from function “lsmeans” in R-package “lsmeans” using the Tukey method (Lenth 2016) and was not significant (estimate (s. e.) = 0.04 (0.08), t -ratio = 0.56, p = 0.83). Dates of collection of egg clutches are at most seven days later than dates of clutch-laying. Italics: p < 0.05.

Table S7. Generalized linear mixed model on clutch size – model 2

| Predictor | | | |
|--|------------------|------------|----------|
| Fixed effect: | Estimate (s. e.) | z-value | p-value |
| Intercept | -0.18 (0.54) | -0.33 | 0.74 |
| Type (level: after mating as a male) | 0.14 (0.18) | 1.15 | 0.25 |
| Type (level: after mating as a female) | 0.70 (0.13) | 5.31 | < 0.0001 |
| Type (level: after mating in both roles) | 0.67 (0.10) | 6.93 | < 0.0001 |
| Growth rate | 55.39 (13.64) | 4.06 | < 0.0001 |
| Block (level: 2) | 0.36 (0.16) | 2.24 | 0.0252 |
| Block (level: 3) | 0.08 (0.17) | 0.46 | 0.65 |
| Block (level: 4) | 0.20 (0.18) | 1.08 | 0.28 |
| Random effects: | | χ^2_1 | p-value |
| P0 mother | | 7.67 | 0.0056 |
| Snail identity | | 357.48 | < 0.0001 |
| Collection date | | 91.96 | < 0.0001 |

Clutch size was analyzed using a generalized linear mixed model with negative binomial errors (NB1 parameterization) and a log link function, using function “glmmTMB” in R-package “glmmTMB” (Brooks et al. 2017). The total number of clutches included in the model is 1956, the number of P0 mothers is 32, the number of individual snails is 133, and the number of collection dates is 36. These numbers are slightly lower than in the previous model because only clutches laid by snails whose mating behavior could be assessed with very high accuracy were included. The overdispersion parameter for the nbinom1 family is 8.93. The reference level of fixed effect “type” is laid without prior mating. The reference level of fixed effect “block” is block 1. Pairwise contrast between levels of fixed effect “type” were computed from function “lsmeans” in R-package “lsmeans” using the Tukey method (Lenth 2016). Pairwise contrasts were significant between levels “after mating as a male” and “after mating as a female” (estimate (s. e.) = -0.57 (0.13), t -ratio = -4.27, p = 0.0001) and between levels “after mating as a male” and

“after mating in both roles” (estimate (s. e.) = -0.53 (0.09), t -ratio = -5.70, $p < 0.0001$), but not between levels “after mating as a female” and “after mating in both roles” (estimate (s. e.) = 0.04 (0.11), t -ratio = 0.33, $p = 0.99$). Dates of collection of egg clutches are at most seven days later than dates of clutch-laying. Italics: $p < 0.05$.

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