

Mate availability determines use of alternative reproductive phenotypes in hermaphrodites

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Abstract

In many species individuals can employ alternative reproductive phenotypes, with profound consequences for individual fitness and population dynamics. This is particularly relevant for self-compatible hermaphrodites, which have exceptionally many reproductive options. Here we investigated the occurrence of reproductive phenotypes in the simultaneously hermaphroditic freshwater snail *Radix balthica* under experimentally simulated conditions of low vs. moderate population density. We captured all mating behavior on camera and measured individual female lifetime reproductive success. We found every possible reproductive phenotype: (1) both male and female (i.e. truly hermaphroditic) reproduction, (2) purely female and (3) purely male reproduction, (4) male reproduction combined with self-fertilization and (5) female mating activity, (6) pure self-fertilization without mating and (7-8) two types of reproductive failure. Variation in alternative reproductive phenotypes was explained by mate availability (10.8%) and individual condition, approximated by a snail's mean daily growth rate (17.5%). Increased mate availability resulted in a lower diversity of reproductive phenotypes, in particular increasing the frequency of true hermaphrodites. However, it lowered phenotype-specific fecundities and hence reduced the population growth rate. Snails in better condition were more likely to reproduce as true hermaphrodites or pure females, while low-condition snails tended to suffer reproductive failure. Overall, we show substantial variation in alternative reproductive phenotypes in a hermaphrodite, which is possibly in part maintained by fluctuations in population density and thus mate availability, and by variation in individual

37 condition. We also provide evidence of an almost two-fold increase in clutch size that can
38 be ascribed specifically to mating as a female.

39 **Key words**

40 growth rate, mate availability, mating-system evolution, *Radix*, selfing, simultaneous
41 hermaphroditism

Introduction

Mating systems evolve in response to the lifetime costs and benefits of alternative reproductive tactics (Gross, 1996; Barrett, 1998; Shuster and Wade, 2003; Taborsky and Brockmann, 2010). When different reproductive tactics confer different fitnesses on the individuals that employ them, the type and number of reproductive tactics in a population can affect the numerical dynamics of a population (Smallegange et al., 2018; Croll et al., 2019). Those dynamics can, in turn, feed back onto the selective pressures, favouring some tactics over others, which could create an ongoing eco-evolutionary dynamic (Smallegange and Coulson, 2013).

The reciprocal feedback between numerical dynamics and the distribution of reproductive tactics places a premium on understanding which factors affect the expression of alternative reproductive tactics and what are the consequences of different combinations of tactics for the distribution of fitness and the dynamics of populations. Specifically, which lifetime reproductive tactics from among those available do individuals actually use? Which factors determine the frequencies of tactics and are those factors based on characteristics of individuals or of the population itself? How does the use of a tactic affect individual fitness and how does the distribution of tactics affect the mean fitness of a population?

Some of these questions have been asked in the large number of species that display alternative reproductive tactics (e.g. insects: Brockmann, 2008; reptiles: Calsbeek and Sinervo, 2008; birds: Krüger, 2008; fish: Taborsky, 2008; amphibians: Zamudio and Chan, 2008). Typically, these studies report the coexistence of two or three reproductive tactics

63 within a population. Hermaphrodites, which represent 5-6% of all metazoans (about 33%
64 when arthropods are excluded; Jarne and Auld, 2006), offer a more difficult challenge for
65 understanding the evolutionary origin and ecological consequences of alternative
66 reproductive tactics because they have, in principle, considerably more reproductive
67 options (Nakadera and Koene, 2013; Ramm, 2017; Schärer, 2017). Hermaphrodites can
68 function in both sexual roles, either sequentially or simultaneously. They are expected to
69 adjust their sex allocation dynamically, reflecting the currently anticipated fitness returns
70 for different points along the gradient of pure maleness over equal investments into both
71 sexual functions to pure femaleness (Charnov et al., 1976).

72 Self-compatibility increases the number of possible reproductive tactics even further. In
73 theory, a self-compatible individual can combine any amount of self-fertilization (hereafter
74 “selfing”) with any amount of male, female or hermaphroditic mating behavior. In reality,
75 not all combinations may be equally likely. For example, limited energy budgets may cause
76 a trade-off between male and female reproduction (Charnov, 1979), a constraint found in
77 some species (De Visser et al., 1994; Yund et al., 1997; Schärer et al., 2005; data in De
78 Visser et al. 1994 reanalyzed by Koene, 2006), but not in others (Locher and Baur, 2000;
79 Baeza, 2007; Hoch and Levinton, 2012). Similarly, any reproductive fitness gained through
80 selfing will reduce the amount of sperm that can be transferred to a prospective mating
81 partner if there is sperm discounting (Porcher and Lande, 2005; Busch and Delph, 2012;
82 Barrett, 2014), and the number of eggs that can be outcrossed if female gametes are
83 limiting. Individuals may also be constrained in their reproductive tactic by their innate
84 propensity for certain types of behavior (e.g. Ramm et al., 2012) or by their overall

reproductive capability, often approximated by variation in body size (e.g. Nakadera et al., 2015) or fertilization status (i.e. whether individuals are outcrossed or selfed and hence inbred, e.g. Janicke et al., 2014).

In many cases of alternative reproductive tactics, the availability of mates and the condition of individuals can affect which tactics an individual expresses (Gross, 1996; Oliveira et al., 2008; Taborsky and Brockmann, 2010). While these same factors are known to affect reproductive performance in hermaphrodites (mate availability: e.g. Jarne and Delay, 1990; Doums et al., 1994; Coutellec-Vreto et al., 1998; Koene et al., 2006; Auld and Relyea, 2010) (condition: e.g. Koene et al., 2007; Dillen et al., 2010; Yu and Wang, 2013), their associations with alternative reproductive tactics in hermaphrodites have rarely been studied.

The simultaneously hermaphroditic freshwater snail *Radix balthica* has an exceptionally broad spectrum of reproductive options. This species copulates unilaterally, so an individual's male and female mating activity can vary almost independently, although time spent in one role may affect the odds of playing the other role. Multiple mating is widespread in the field, with ample variation in the degree of polyandry and polygyny both between individuals and across the reproductive season (Bürkli and Jokela, 2017). Additionally, *R. balthica* is self-compatible (Pfenninger et al., 2011; Haun et al., 2012), although under natural conditions selfing is exceedingly rare in the population studied here (selfing rate s 0.000-0.095 depending on the sample and estimation method, with only 1/16 estimates significantly different from zero; Bürkli et al., 2017). To self-fertilise their eggs, snails do not physically copulate with themselves, but instead transfer autosperm to their

eggs internally. Finally, snails can most likely store obtained sperm and use it for fertilization months after receiving it, or alternatively digest it (shown in related species: Cain, 1956; Madsen et al., 1983; Vianey-Liaud et al., 1989; Koene et al., 2009b; Nakadera et al., 2014).

In *R. balthica*, alternative reproductive phenotypes – here defined as discontinuous variation in an individual’s lifetime mating and egg-laying behavior – can thus arise from (at least) three binary options (see fig. 1). First, snails can mate with another individual as a male, or not; second, regardless of what they do as a male, they can mate with another individual as a female, or not; and third, regardless of how they have mated, they can lay eggs, or not. Assuming that these options are independent of each other, eight potential alternative reproductive phenotypes exist (i.e. 2^3). We deliberately refer to them as alternative reproductive phenotypes, rather than alternative reproductive strategies, as the term “strategies” implies a certain degree of active choice on the part of snails, and may give a flavor of optimality. However, alternative reproductive phenotypes may result from (a series of) chance events, influenced by both external (e.g. the number of available mating partners) and internal factors (e.g. individual condition). Alternative reproductive phenotypes are thus “optimal” at best in the relative sense of “making the best of a bad job”.

Six alternative reproductive phenotypes in *R. balthica* can result in the production of offspring, while two represent instances of reproductive failure. Each reproductive phenotype entails a unique combination of selfed eggs, outcrossed eggs, and/or sired offspring. We also consider reproductive failure an integral part of reproductive diversity.

Non-reproductive individuals exist in many populations and species (see e.g. table S1 for an overview of the frequency of female infertility in pulmonate gastropods), and their presence may impact both the population genetic and evolutionary dynamics in a population, e.g. by reducing the effective population size (Crow and Kimura, 1970; Hartl and Clark, 2007). We therefore retain all alternative reproductive phenotypes in our analyses, including those leading to reproductive failure. We distinguish between two types of reproductive failure: no egg production despite (exclusively) female mating activity (type 1), and neither egg production nor mating activity of any kind (type 2). While sterile individuals of type 1 act as a dead end for received sperm, thereby potentially decreasing their mating partners' siring success, sterile individuals of type 2 only interfere with other individuals' reproductive efforts by reducing the availability of mating partners.

Here, we used mating trials in the laboratory to empirically test five hypotheses about the use of alternative reproductive phenotypes and their connection to individual and population mean fitness:

H1: Snails will not employ every possible alternative reproductive phenotype. Specifically, phenotypes with potentially low (pure selfer) or zero fitness (reproductive failure type 1 and 2), as well as such with potentially non-adaptive components (female-mating males, which gain no fitness as a female despite female matings) should be absent.

H2: The availability of mates will affect the distribution of alternative reproductive phenotypes. Specifically, we expect to see more true hermaphrodites and female-mating

149 males at high mate availability (as these phenotypes are characterised by maximum mating
150 activity).

151 H3: The condition of an individual will affect which particular reproductive phenotype it
152 employs. Specifically, we predict that true hermaphrodites and pure females are in better
153 average condition than other individuals (as these are the only phenotypes capable of
154 producing outcrossed eggs).

155 H4: The population growth rate (i.e. mean female lifetime reproductive success) increases
156 with increasing mate availability (as reproductive success typically correlates positively
157 with mating success).

158 H5: The increase in population growth rate, i.e. mean female lifetime reproductive success,
159 will be caused by two factors: an increase in the mean fecundity of particular reproductive
160 phenotypes and a shift toward increased frequencies of those reproductive phenotypes that
161 have higher levels of fecundity.

162 The relationship between mating success and reproductive output captures sexual selection
163 (Bateman, 1948). We found substantial differences among individuals in their propensity
164 for promiscuity in the ancestral field population of the snails studied here, along with a
165 frequent skew of paternity shares in field-collected egg clutches (Bürkli and Jokela, 2017),
166 both of which suggest that sexual selection may be an important evolutionary force in this
167 population. Hence we also tested whether female lifetime reproductive success was
168 associated with a snail's overall mating activity or sex bias when mating. Finally, we
169 analyzed variation in the size of individual egg clutches, to gain a more direct

understanding of how individual mating behavior prior to oviposition affected female reproductive output.

Materials and Methods

Study system

Radix balthica is a simultaneously hermaphroditic freshwater snail of the shallow littoral of lakes throughout Europe (Cordellier and Pfenninger, 2009; Pfenninger et al., 2011; Lawton et al., 2015). In Lake Zurich, Switzerland, eggs hatch in spring and snails reach sexual maturity at the end of the year. Individuals reproduce in spring and then die, resulting in non-overlapping generations and a generation time of one year (Bürkli and Jokela, 2017). During the egg-laying period (March to May) snails may copulate repeatedly in both sexual roles and lay hundreds of eggs in distinct egg clutches (Bürkli and Jokela, 2017). Copulation is unilateral. This species is self-compatible (Pfenninger et al., 2011; Haun et al., 2012), but selfing was found to be extremely rare in our population (Bürkli et al., 2017).

Experimental snails

We collected 86 adult snails (hereafter “P0 snails”) at peak breeding season (24.04.2013) in Uerikon, Lake Zurich, using snorkelling equipment. In the laboratory at Eawag-Duebendorf, Switzerland, they were kept individually in 200 ml plastic cups filled with aged tap water (room temperature 18°C). Snails were fed *ad libitum* with organic lettuce. Water was changed once a week. When P0 snails died of natural causes in late spring 2013, their heads were preserved at -80°C. As *R. balthica* is phenotypically variable (Pfenninger

et al., 2006; Brönmark et al., 2011; Rundle et al., 2011; Schniebs et al., 2011; Ahlgren et al., 2013), we confirmed the taxonomic identity of all P0 snails by sequencing the mitochondrial cytochrome oxidase subunit I (COI) gene (for details see Bürkli et al., 2017). The one individual that was not *R. balthica* and all its offspring were excluded from further analyses.

Each clutch laid by isolated P0 snails was placed in a separate water-filled 40 ml plastic cup. After 17 to 21 days hatching started and clutches were transferred to larger 200 ml cups. After about 19 weeks (11.6-27.3, mean 19.4) juveniles (hereafter “F1 snails”) were placed in individual 200 ml cups to maintain their virginity. This is a very early start of isolation, as in our laboratory paired snails started to copulate at age 41.3-45.7 weeks. Throughout isolation, F1 snails were fed a mixture of finely ground chalk, flakes of fish food and *Spirulina* powder, from age 37.9-42.3 weeks onwards increasingly supplemented and eventually replaced by organic lettuce. Before, between and after mating trials F1 snails were kept in individual cups from which new clutches were removed at least once a week. When hatching was imminent, we counted all eggs in each clutch and ascertained how many eggs contained developed embryos using a dissection microscope. We measured the adult shell length of F1 snails in week two of the mating trials (on 14.05.2014), when 75% of snails had been paired once. At this point, 25% of snails had been paired for the second time, just one and five days before being measured, respectively. The shortness of this period makes an effect of the number of mating opportunities on shell length very unlikely. Shell lengths were measured to the nearest 0.1 mm using digital callipers. Mean individual daily growth rates (our measure of individual condition) were then calculated by

dividing adult shell length by the snails' age at that time, measured from the day when the egg clutch was laid from which the snail hatched.

All F1 snails used in this study were demonstrably outcrossed, as all of them contained an allele not present in their P0 mothers at least at 1/9 highly polymorphic microsatellite loci (see Bürkli et al., 2017 for details of markers and genotyping routines).

Design of mating trials

Mating trials were started on 02.05.2014 with 274 F1 snails from 38 P0 mothers. By then, F1 snails were 52.1 ± 1.0 weeks old (mean \pm SD, range 48.3-53.7) and had a shell length of 12.8 ± 2.2 mm (range 7.0-18.2). We increased the likelihood that most snails had reached sexual maturity when first meeting a mating partner by starting the mating trials several weeks after laboratory-reared control snails had begun to mate (first mating of control snails: age 41.3-45.7 weeks), and long after copulating pairs were observed in the ancestral field population (Bürkli and Jokela, 2017; Bürkli et al., 2017). At that time, >30% of experimental snails had reproduced through selfing. On the day before the start of mating trials, we marked snails individually by writing a number on a spot of their shell previously grounded with white correction fluid. Marking snails in this way affects neither survival nor shell growth (see Supporting Results 1 and fig. S1).

Mating trials were conducted in two ways. F1 snails either had six subsequent mating opportunities over the course of six weeks, each with a different partner, or one mating opportunity with one partner. A choice of six mating partners may come close to what happens in the ancestral field population, where egg clutches collected throughout the

reproductive season had 2.1 fathers on average, with a range of one to nine (Bürkli and Jokela, 2017). The average number of potential mating partners a snail encounters in its life is probably a multiple of 2.1. For instance, a snail may not copulate with all its potential partners or may mate exclusively as a female with some of them, sperm transfer may be unsuccessful, or siring success may be hampered by sperm competition, cryptic female choice, or genetic incompatibility between sperm and egg. We thus chose to use six potential mating partners in our experimental treatment of moderate mate availability, in the knowledge that this may not reflect conditions in the field fully accurately. By contrast, the second treatment simulates conditions at low population density, such as in newly colonized or ecologically disturbed habitats.

For practical reasons, mating trials were conducted in four temporal blocks, always during daylight hours. Dates and sample sizes are provided in table S2. A mating opportunity lasted for 10.3 ± 0.8 hours (mean \pm SD, range 9.0-12.4), which is long enough for copulations in both sexual roles. During a mating opportunity, pairs of snails were placed in small, water-filled plastic containers (58 x 38 x 23 mm) covered by a screen of acrylic glass to prevent snails from escaping. We terminated the experiment one week after the sixth mating opportunity, when the mortality rate began to increase in accordance with these snails' annual life cycle.

The first mating partner of each snail was size-matched to ensure that copulation was not constrained by anatomical or developmental differences between mating partners (mean absolute size difference between mating partners \pm SD: 1.2 ± 0.8 mm; difference between hypothetical random pairs: 2.3 ± 1.7 mm). Snails were paired with partners from the same

block. When the number of snails in a block was uneven, one snail was paired with a snail from a temporally parallel block (blocks 1 and 4) or with one of two additional snails not part of blocks 1-4. However, except in 5/465 pairings (1.1%), paired snails had had equal numbers of previous mating opportunities, ensuring that they showed the same eagerness to mate. Mating pairs were set up randomly with respect to whether they were siblings. As a consequence, in 22/465 pairings (4.7%) the mating partners had the same P0 mother (i.e. full- or half-siblings). Hence, 40 F1 snails had one and two snails had two potential mating partners from the same mother. Of these 42 snails, 37 had repeated mating opportunities and thus were also paired with unrelated snails, diminishing potential effects of mating partner relatedness. The five snails solely paired with a sibling were excluded from all analyses. Another once-paired snail was excluded due to missing data on female reproductive output, reducing the sample size to 268 snails. During mating opportunities, snails were not fed.

All behavior during mating trials was recorded on time-lapse movies using a *Nikon COOLPIX P330* camera mounted 30-40 cm above groups of plastic containers (resolution of 12 Mpx in 24 bit RGB-color (red-green-blue) space, macro mode). The camera took a picture automatically every 30 seconds, which is sufficiently short to capture all mating behavior. The resulting time-lapse movies told us whether each snail had copulated with its partner and in which sexual role. We then assigned each snail to one of four lifetime mating types: (1) did not mate, (2) only mated as a male, (3) only mated as a female, and (4) mated in both roles. Copulations were categorized as “verified” or “potential”, depending on whether penis insertion was clearly visible on time-lapse movies or not (bearing in mind

that a visible penis insertion is no guarantee for the successful transfer of sperm). Accordingly, the lifetime mating type of each snail was inferred twice, once including and once excluding “potential” copulations. We only used snails with consistent classification in analyses that involved mating information, reducing the sample size in most analyses to 215 snails. In analyses looking exclusively at female reproductive output, the sample size is 268 snails.

For each snail we also extracted its male mating success, measured as the number of verified female mating partners (i.e. snails possibly inseminated by the focal snail) and its female mating success, measured as the number of verified male mating partners (i.e. snails that possibly inseminated the focal snail). Using values of male and female mating success, we then performed a principal component analysis (PCA) to obtain uncorrelated measures of the overall mating activity and the sex bias when mating. Specifically, we calculated non-standardized principal components using equations A2a and A2b in Appendix A of Anthes et al. (2010). We used a non-standardized PCA because the variance in male and female mating success was similar ($\chi^2 = 0.02$, $df = 1$, $p = 0.90$, assessed using a nonparametric Fligner-Killeen median test (Conover et al., 1981), which is robust against departures from normality). Principal component 1 (PC₁) represents overall mating activity positively, while PC₂ represents a female bias in mating activity. The variance explained by PC₁ vs. PC₂ was computed by dividing the variance in PC₁ or PC₂, respectively, by the sum of the variance in PC₁ plus the variance in PC₂.

The movies also enabled us to assign egg clutches that were laid during mating opportunities to their mothers (45/2118 clutches, i.e. 2.1%). To further improve

consistency, all movies were analyzed by N. Weissert, and all detected copulations in the 50% of movies analyzed first were reconfirmed after completion of analyses to prevent potential detection bias.

Statistical analysis

We tested for an association between the observed frequencies of alternative reproductive phenotypes and the experimental treatment (a factor with two levels: 1 vs. 6 mating opportunities; test of H2), mean individual daily growth rates (a covariate; test of H3), and temporal blocks (a factor with four levels) using a multinomial log-linear regression and function “multinom” in R-package “nnet” (Venables and Ripley, 2002). The response had seven rather than eight levels because we excluded pure selfing due to low sample size ($n = 1$). As reference level for the response we chose true hermaphroditism, because it represents the highest possible level of reproductive activity. This means that, for each predictor (e.g. 1 vs. 6 mating opportunities), the log odds of exhibiting a given alternative reproductive phenotype will always be compared to this reference level. *P*-values were computed using two-tailed *z* tests. The proportion of variance in reproductive phenotypes explained by each predictor was calculated by dividing the residual deviance of a reduced model without the predictor in question by the residual deviance of the full model.

We also used a multinomial log-linear regression to test for an effect of experimental treatment on the mating activity snails showed at mating opportunity 1. Here the response had four levels (mated in both roles, only as a female, only as a male, and non-mated), with

mating activity in both roles as reference level. No other predictors were included in this model. *P*-values were computed as in the previous model.

To account for the large number of snails with zero eggs, female lifetime reproductive success 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). We also ran, and ruled out due to higher AIC values or convergence problems, a negative binomial model using the NB2 parameterization, a Poisson model, and these three models with zero-inflation. As fixed effects we included the experimental treatment (test of H4), the mean individual daily growth rate, the temporal block, and two principal components which represent uncorrelated measures of an individual’s total mating activity and the sex bias when mating, respectively (details in “Design of mating trials”). Of principal components we also included the squared terms, as plots of these predictors against the response showed curvilinear relationships. Note that temporal block is included as a fixed effect because it is a nuisance factor, rather than a “block” in the technical sense. As random effects (i.e. random intercepts) we included P0 mother, to correct for potential effects of relatedness, and the “pair identity” on mating opportunity 1. The latter accounts for the non-independence of sexual functions within pairs of once-paired snails (e.g. it was impossible for a once-paired snail to remain unmated while its sole assigned mating partner mated, or for two snails solely paired with each other to both mate as a male, but not as a female). *P*-values for random effects were obtained by means of log-likelihood ratio tests comparing the full model to one without the random effect in question. We also fitted an identical model after excluding the 22 snails that did

not mate at all, and hence had values of zero for both principal components 1 and 2. The output of the full and reduced models differed only minimally, and so we retained non-mating snails.

To test hypothesis H5, that is to assess the relative contributions of changes in mean fecundities of alternative reproductive phenotypes and changes in the frequencies of phenotypes, we compared the observed population growth rates under both treatments to those estimated under two hypothetical scenarios (table 1). Observed population growth rates for treatments 1 (paired once) and 2 (paired six times) were computed as $M_1 = \sum_{i=1}^8 \frac{n_{i1} \times x_{i1}}{s_1}$ and $M_2 = \sum_{i=1}^8 \frac{n_{i2} \times x_{i2}}{s_2}$, with n_{i1} the observed number of individuals of phenotype i under treatment 1, x_{i1} the observed mean lifetime number of eggs produced by an individual of phenotype i under treatment 1, s_1 the total number of individuals in treatment 1, and n_{i2} , x_{i2} and s_2 the analogous quantities for treatment 2. In scenario 1, we kept the frequencies of reproductive phenotypes constant across treatments, with $\bar{f}_i = \frac{\frac{n_{i1}}{s_1} + \frac{n_{i2}}{s_2}}{2}$ the average frequency of reproductive phenotype i across both treatments. Mean population growth rates under scenario 1 were then obtained from $M_{1,sc1} = \sum_{i=1}^8 \bar{f}_i \times x_{i1}$ and $M_{2,sc1} = \sum_{i=1}^8 \bar{f}_i \times x_{i2}$. By contrast, in hypothetical scenario 2, we kept the phenotype-specific fecundities constant across treatments, with average fecundities calculated as $\bar{x}_i = \frac{x_{i1} + x_{i2}}{2}$ for each reproductive phenotype i , and mean population growth rates $M_{1,sc2} = \sum_{i=1}^8 \frac{n_{i1} \times \bar{x}_i}{s_1}$ and $M_{2,sc2} = \sum_{i=1}^8 \frac{n_{i2} \times \bar{x}_i}{s_2}$. As there was a single pure selfer under treatment 1 but not 2, all mean observed and hypothetical population growth rates were computed twice, once including and once excluding pure selfers.

The distribution of clutch size was zero-inflated. We used generalized linear mixed models with negative binomial errors (NB1 parameterization) and a log link function, using function “glmmTMB” in R-package “glmmTMB” (Brooks et al., 2017). As in our other analyses, models with negative binomial (NB2 parameterization) and Poisson errors, and the same three models with zero-inflation, either had higher AIC values or did not converge. We fitted two models to investigate variation in clutch size. In model 1, we tested whether clutches laid in isolation differed from those laid by once-paired snails after mating trials began, and by repeatedly paired snails after mating trials began (fixed effect with three levels). In model 2, we tested whether clutch size varied in relation to the snails’ actual mating activity (fixed effect with four levels: laid by so-far unmated mothers, or laid by mothers that so far had only mated as a male, as a female, or in both roles).

Both models additionally included the mean individual daily growth rate and the temporal block as fixed effects. Random effects (i.e. random intercepts) were P0 mother, snail identity, which accounts for repeated measurements (i.e. multiple clutches laid by the same snail), and the date when a clutch was collected, to correct for temporal changes in mean clutch size. Dates of collection are at most seven days later than dates of clutch-laying. We did not add the pair identity on mating opportunity 1 as a random effect because doing so resulted in convergence problems. *P*-values for random effects were computed as in the previous model. Adjusted *p*-values for pairwise contrasts between levels of categorical predictors were obtained from function “lsmeans” in R-package “lsmeans” using the Tukey method (Lenth, 2016).

We compared frequencies of alternative reproductive phenotypes and frequencies of snails with different mating or egg-laying behavior using two-tailed chi-square tests. For 2 x 2 contingency tables, Yates' continuity correction was used, as some cell frequencies were below five. Analyses testing for effects of size-matching first mating partners are detailed in the Supporting Methods 1.

The 37 repeatedly paired snails with one or two mating partners from the same P0 mother were not more or less likely to produce eggs ($\chi^2_1 = 1.2, p = 0.27$) or developed embryos ($\chi^2_1 = 3.3, p = 0.07$), or to mate as a male ($\chi^2_1 = 0.0, p = 1.0$) or as a female ($\chi^2_1 = 2.3, p = 0.13$), than snails whose mating partners were all from different mothers. We therefore did not include mating partner relatedness in statistical analyses.

For simplicity, we only present results based on the number of eggs, but we also counted the number of developed embryos for each snail. Both measures of total female reproductive output were strongly and positively correlated, both when including snails that did not lay eggs (Pearson's product-moment correlation: $r = 0.98, t_{266} = 76.4, p < 0.0001$) and when excluding them ($r = 0.97, t_{136} = 43.6, p < 0.0001$, see figs. S2, S4, S6 and S7).

Statistical analyses were performed using R v. 3.4.0 (R Core Team, 2017). Values are given as mean \pm SD. Scatter plots were prepared using R-package "beeswarm" (Eklund, 2016).

Results

Distribution of alternative reproductive phenotypes

We found that every single alternative reproductive phenotype was present among snails, including a pure selfer, female-mating males and both types of reproductive failure, thus leading us to reject H1 (fig. 2, see legend for numbers of snails per reproductive phenotype). The distribution of phenotypes varied significantly between treatments. When mate availability was limited, the distribution of snails amongst alternative reproductive phenotypes was more even (Pielou's evenness, 0.88 vs. 0.70), and fewer phenotypes were rare (i.e. used by <5% of snails, 3 vs. 5). Consequently, the frequencies of repeatedly-paired snails significantly deviated from a 50:50 ratio at several bifurcations of the flowchart (fig. 1), while the mating and egg-laying behaviour of once-paired snails was mostly consistent with random binary processes (Supporting Results 2).

The experimental treatment (i.e. one vs. six mating opportunities) significantly affected the frequency of alternative reproductive phenotypes, accounting for 10.8% of the observed variation (fig. 2A, full model results in table S3). Most notably, as predicted by H2, repeated mating opportunities more than doubled the number of truly hermaphroditic snails, and more than quintupled the number of female-mating male snails. Consequently, the log odds of being a female-mating male vs. a true hermaphrodite (the latter being the response's reference level in the statistical model), were not significantly different between both experimental treatments ($p = 0.37$). True hermaphroditism and female-mating male reproduction are the only two alternative reproductive phenotypes that entail copulations in

both sexual roles, showing that repeated mating opportunities clearly increased the probability of mating both as a male and female. By contrast, four reproductive phenotypes were more common among once-mated snails: The log odds of male reproduction while selfing, pure female reproduction, and both types of reproductive failure, when compared to true hermaphroditic reproduction, respectively, were significantly lower when snails were paired six times rather than once (all $p \leq 0.0142$). The single purely selfing snail was excluded from the model due to low sample size.

Also individual condition, here approximated by a snail's mean daily growth rate, was significantly associated with alternative reproductive phenotypes, explaining 17.5% of the observed variation (fig. 2B, table S3). This association must be considered correlative, as we did not manipulate growth rates. As predicted by H3, we found that truly hermaphroditic snails – i.e. snails characterized by maximum reproductive activity – had significantly faster growth rates than most other snails. An increase in shell growth was associated with a decrease in the log odds of male reproduction while mating as a female, male reproduction while selfing, pure male reproduction, and both types of reproductive failure, when compared to true hermaphroditism (all $p \leq 0.0031$). The exceptions were pure females, which grew as fast as true hermaphrodites ($p = 0.65$). This result is also consistent with H3.

Finally, 4.3% of the variance in alternative reproductive phenotypes could be attributed to the four temporal blocks in which mating trials were conducted (table S3).

Mating activity

As expected, there was no difference in mating activity between once- and repeatedly paired snails on mating opportunity 1: the log odds of pure female, pure male and no mating activity vs. mating activity in both roles (the response's reference level) were not significantly different between experimental treatments (all $p \geq 0.37$, full model results in table S4, fig. 3A). However, in repeatedly paired snails mating activity decreased over time, from 75.0% of snails that mated on mating opportunity 1 to 37.0% on opportunity 6. The decline in overall mating activity was accompanied by a decrease in reciprocal copulations, from 26.8% to 0.0%. Most repeatedly paired snails mated early on, with very few that mated for the first time on mating opportunities 3 or later (fig. 3B), suggesting that most snails were sexually mature at the beginning of the mating trial.

A snail's mating activity was also associated with its partner's shell length (Supporting Results 3). In brief, pairs of snails were unlikely to mate when they were very dissimilar in size, or when both snails were small. When a mating occurred, however, sexual roles were not predicted by shell length. A potential bias introduced by providing once-paired snails exclusively with a size-matched partner is therefore small at most.

Female lifetime reproductive success

Experimentally manipulated mate availability (1 vs. 6 mating opportunities) had a significant effect on female lifetime reproductive success ($b = -1.76$, $p < 0.0001$). Contrary to H4, which predicted a higher population growth rate under increased mate availability, we found that repeatedly paired snails laid, on average, 20.6% fewer eggs than snails paired

only once (table 1, fig. 4A, full model results in table S5). The reduced population growth rate under increased mate availability is attributable to a reduction in the average fecundities of alternative reproductive phenotypes when snails were paired six times, thus leading to a rejection of the first half of H5. Most importantly, true hermaphrodites laid 34.3% fewer eggs when paired repeatedly rather than once (259.3 ± 211.2 vs. 394.6 ± 199.2 eggs).

If the distribution of alternative reproductive phenotypes had been equal among experimental treatment groups, the contrast in population growth rates would have been even larger, with 46.2% fewer eggs per repeatedly paired vs. once-paired snail (table 1). However, as shown earlier (Fig. 2A), reproductive phenotypes were not equally common under both treatments. In particular, increased mate availability more than doubled the frequency of true hermaphrodites (53.6% vs. 24.3%). As true hermaphrodites had the highest mean fecundity under both treatments (apart from the true selfer), increased mate availability thus resulted in a higher frequency of high-fecundity phenotypes, in agreement with the second part of H5. The population growth rate would actually have been higher among repeatedly paired than once-paired snails, had the fecundities of reproductive phenotypes been equal among both treatment groups (table 1).

In summary, these results show that pairing snails repeatedly affected population growth rates via two effects that act in opposition: a reduction in reproductive phenotype-specific fecundities, but an increase in the frequency of high-fecundity phenotypes.

In addition, there was a strong association between female lifetime reproductive success and a snail's mean daily growth rate ($b = 139.63$, $p < 0.0001$, fig. 4B, tables S5): snails that grew very slowly tended to lay no eggs, while the highest number of eggs was laid by fast-growing snails. A positive correlation between body size and female reproductive success has been found in other species of freshwater snails (e.g. Pélissié et al., 2012). Female reproductive output was also significantly associated with both a snail's overall mating activity (PC₁, capturing 67.7% of the total variance in the number of mating partners, $b = 1.78$, $p < 0.0001$, quadratic term: $b = -0.20$, $p < 0.0001$, fig. 4C) and their sex bias when mating (PC₂, capturing 32.3% of the variance, $b = -0.12$, $p = 0.41$, quadratic term: $b = -0.12$, $p = 0.0262$, fig. 4D). In both cases, the relationship was curvilinear, with snails that showed an intermediate level of mating activity and mated similarly often in both sexual roles laying the most eggs. Consequently, female reproductive output also showed hump-shaped relationships with a snail's number of male mating partners (i.e. its degree of polyandry, Fig. 4E) and female mating partners (i.e. its degree of polygyny, Fig. 4F, note that these two variables are shown for illustrative purposes only).

Temporal blocks did not significantly affect egg production (all $p \geq 0.09$). Among the random effects, P0 mother identity was significant ($\chi^2 = 10.50$, $p = 0.0012$), while the pair identity on mating opportunity 1 was not ($\chi^2 = 2.07$, $p = 0.15$).

Female infertility and self-fertility

Almost half of the snails (48.5%) did not lay eggs (female-mating males, pure males, and snails suffering from reproductive failure type 1 and 2, see Supporting Results 4 and figs. S5 and S6). Female infertility was equally common among snails paired once or six times

(46.6% vs. 50.4%, $\chi^2_1 = 0.2$, $p = 0.62$). As two thirds of female infertile snails mated as a female (66.3%, female-mating males and snails of reproductive failure type 1), female infertility did not result from female virginity, although we do not know how often copulations resulted in the successful transfer of sperm. While 38.2% of female infertile snails mated as a male and hence may have gained fitness through their male function (female-mating and pure males), the majority of female infertile snails did not mate as a male (reproductive failure type 1 and 2). Accordingly, 25.6% of experimental snails had zero lifetime reproductive success.

Self-fertility was widespread. Overall, 38.8% of snails laid eggs in isolation before entering the mating trials, and of female fertile snails even 75.4%. Snails that selfed in isolation were equally likely to mate as a female as snails that only laid eggs after the mating trials began (78.1% vs. 71.4% female-mating snails, $\chi^2_1 = 0.9$, $p = 0.34$).

Clutch size

The number of eggs per clutch almost doubled after snails entered the mating trials, from 10.9 ± 12.0 eggs per clutch laid in isolation to 20.1 ± 19.3 eggs laid by once-paired snails, and 20.7 ± 18.0 eggs per clutch laid by repeatedly paired snails (fig. 5A, full model results in table S6). This increase was highly significant for both treatment groups (paired once: $b = 0.54$, $p < 0.0001$; paired repeatedly: $b = 0.50$, $p < 0.0001$). Post-isolation clutches laid by once- vs. repeatedly paired snails did not differ in size (pairwise contrast: $b = 0.04$, $p = 0.83$). Clutch size was strongly associated with a snail's mean daily growth rate ($b = 64.76$, $p < 0.0001$, fig 5B), similar to findings in other Basommatophoran freshwater snails (e.g. Koene et al., 2007; Yu and Wang, 2013). It also slightly differed between temporal blocks

526 1 and 2 ($b = 0.33$, $p = 0.0479$), as well as between P0 mothers ($\chi^2 = 6.16$, $p = 0.0131$),
527 individual snails ($\chi^2 = 442.05$, $p < 0.0001$) and clutch laying dates ($\chi^2 = 111.08$, $p <$
528 0.0001 , fig. 5C).

529 We next analyzed the association between clutch size and maternal mating activity before
530 laying a clutch (fig. 5D, full model results in table S7). We found that clutches laid by
531 unmated (i.e. obligately selfing) mothers were significantly smaller than clutches laid by
532 mothers that had mated as a female ($b = 0.70$, $p < 0.0001$) and in both roles ($b = 0.67$, $p <$
533 0.0001), but not smaller than clutches laid by mothers that had only mated as a male and
534 hence must also be selfed ($b = 0.14$, $p = 0.25$). Looking at pairwise contrasts revealed that
535 both types of selfed clutches were significantly smaller than both types of (supposedly)
536 outcrossed clutches (all $p \leq 0.0001$), with no differences within groups (both $p \geq 0.66$).
537 Overall, clutches laid after mating as a female (22.1 ± 18.5 eggs) were nearly twice the size
538 of clutches laid without prior female mating (11.4 ± 13.4 eggs). Also in this model, clutch
539 size was associated with the mean daily growth rate ($b = 55.39$, $p < 0.0001$), and differed
540 between temporal blocks 1 vs. 2 ($b = 0.36$, $p = 0.0252$), P0 mothers ($\chi^2 = 7.67$, $p =$
541 0.0056), individual snails ($\chi^2 = 357.48$, $p < 0.0001$) and clutch laying dates ($\chi^2 = 91.96$, p
542 < 0.0001).

Discussion

Distribution of alternative reproductive phenotypes

We found that among individuals of a single population of the simultaneously hermaphroditic freshwater snail *R. balthica* all biologically possible alternative reproductive phenotypes were present, eight in total, including phenotypes with potentially low (pure selfing) or zero fitness (two types of reproductive failure), and phenotypes with potentially non-adaptive components (male reproduction while also mating as a female). Variation in reproductive phenotypes depended, to some extent (10.8%), on the degree of mate availability. When snails were given moderate access to mating partners, potentially reflecting what happens in the ancestral field population (Bürkli and Jokela, 2017), some alternative reproductive phenotypes were rare (e.g. purely male and purely female reproduction) or absent (pure selfing), while others were very common (e.g. truly hermaphroditic reproduction). As expected, repeated mating opportunities increased the likelihood of mating in both sexual roles. By contrast, when snails had a single opportunity to mate, simulating conditions at low population density, snails expressed alternative reproductive phenotypes more evenly. As a result, all phenotypes were present, but none was overwhelmingly common.

A second factor that explained variation in alternative reproductive phenotypes (17.5%) was individual condition, measured here as the mean individual daily growth rate. As we knew the size of snails at maturity (mating trials) and their precise age, we were able to calculate exact growth rates for the full pre-reproductive lifespan, and for a good part of the

reproductive lifespan. We found that, as hypothesized, truly hermaphroditic snails and pure females grew fastest, while snails that neither laid eggs nor copulated grew slowest. This suggests that the full hermaphroditic potential (i.e. fitness gains through both sexual functions) can only be tapped by individuals in good condition, while the increased reliance on male reproduction appears to be a valid alternative for snails of suboptimal condition. Complete reproductive inactivity, however, seems to be restricted to snails in poor condition.

Our experiment suggests that a wide spectrum of reproductive options may be maintained in *R. balthica* when population density varies between generations. Fluctuations of population size are common in many species of freshwater snails, including *R. balthica* (Henry et al., 2005; Trouvé et al., 2005; Evanno et al., 2009). Demographic stochasticity may affect the stability of alternative reproductive phenotypes in numerous biological systems, yet is often ignored in studies of the maintenance of polymorphism (Moulherat et al., 2017). In addition, we found that reproductive phenotypes associated with high fitness (e.g. truly hermaphroditic reproduction) require a high investment. Hence variation in individual condition may cause low-condition individuals to exhibit reproductive phenotypes that are suboptimal, but the best these individuals can achieve.

Female lifetime reproductive success

Contrary to our expectations, female lifetime reproductive success was lower, rather than higher, under increased mate availability. This was the result of two factors acting in opposition. Although elevated mate availability increased the frequency of alternative reproductive phenotypes endowed with above-average female fecundity, most importantly

increasing the frequency of true hermaphroditism, it carried a cost in the form of a reduction of phenotype-specific rates of egg production. This cost proved to outweigh the benefit of a larger proportion of high-fitness phenotypes in the population. The coupled effect of population density on the population growth rate and the diversity of reproductive phenotypes may result in a feedback loop between population dynamics and the expression of alternative reproductive phenotypes. As population density is high, fewer reproductive phenotypes will be employed, phenotypes will have lower average reproductive output, and population density will go down. The resultant scarcity of mating partners will, in turn, increase both the diversity and mean fecundity of alternative reproductive phenotypes in the population, which will increase population density again. Over time, this eco-evolutionary feedback loop could maintain variation in reproductive phenotypes even in the absence of extrinsic (i.e. stochastic) causes of fluctuations in population density. Eco-evolutionary feedback effects were found to contribute to the maintenance of two alternative reproductive tactics in male mites (Smallegange et al., 2018; Croll et al., 2019).

The fecundity cost of being paired repeatedly may be linked to the observed hump-shaped relationship between total egg number and a snail's overall mating activity. Snails with more than two male mating partners, or more than four female mating partners, tended to have reduced fecundity. At this stage, we cannot say whether the reduction is due to physical damage, physiological harm, decreased foraging time, increased energy expenditure or some other cost of promiscuity. A female fitness optimum for an intermediate number of mating opportunities was found in a hermaphroditic sea slug with traumatic mating (Lange et al., 2012), while studies in other snails reported no decrease of

either male or female reproductive fitness at high mating rates (Anthes et al., 2010; Minoretti et al., 2011; Péliissié et al., 2012).

Conversely, the hump-shaped relationship between total female reproductive success and mating activity meant that also unmated snails laid fewer eggs. A reduced fecundity was associated with a lack of both female and male copulations. The direction of causality remains unclear for copulations in the male role (i.e. did snails lay fewer eggs because they did not mate as a male, or did snails with low fecundity fail at mating in the male role?), but our analysis of clutch size sheds some light on the link between fecundity and mating in the female role. We found that (supposedly outcrossed) egg clutches laid after a female mating were nearly twice the size of (necessarily selfed) clutches laid before. Female reproductive success and/or clutch size is often reported to be higher in paired than in isolated or rarely paired snails of self-compatible species (e.g. Jarne and Delay, 1990; Jarne et al., 1991; Wethington and Dillon, 1997; Coutellec-Vreto et al., 1998; Koene et al., 2006; Dillen et al., 2009; Lamy et al., 2012), although also the opposite effect has been found (e.g. Doums et al., 1994; Gutiérrez et al., 2001; Auld and Relyea, 2010). While the effects on isolated snails may be related to what we saw, it is important to bear in mind that an isolation treatment, or a severe limitation of mate availability, can affect snails in several ways. First, it obviously prevents snails from mating as a female. It also prevents them from mating as a male, and largely deprives them of the social, physical and physiological stimuli of intraspecific contacts. In contrast, our study shows an increase in clutch size following female mating activity in snails that had access to mating partners, and so, to the

best of our knowledge for the first time, we can ascribe the increase specifically to copulating as a female.

This is interesting because egg production should not require a female mating in a population where 75.4% of egg-laying snails are evidently self-fertile. The frequency of self-fertility may be higher still, considering that many species only self-fertilize after a prolonged time of isolation (termed "waiting time"; Tsitrone et al., 2003; Escobar et al., 2011), which in our study may not have been long enough for all self-fertile snails to be detected. If selfing is a viable option, then why does it reduce female fecundity? There are at least five possible, mutually non-exclusive explanations for this observation. In the following, we will discuss the fit to our data for each of them.

First, the reduction in female fecundity in selfing individuals could result from the very early action of inbreeding depression (Charlesworth and Charlesworth, 1987). If lethal recessive alleles segregate in the population, zygotes homozygous for such an allele may be aborted soon after fertilization, before they ever become part of an egg clutch. Second, snails may actively reduce the amount of energy invested in selfing in order to re-allocate it to any outcrossed offspring they may produce in future. In that case, the reduced fecundity when selfing and especially the smaller size of selfed clutches may represent another form of resistance to selfing, similar to the "waiting time". A third potential explanation is sperm limitation when selfing. In most species, reproduction is assumed to be limited by female gametes as a consequence of their large size in relation to male gametes (Bateman, 1948). However, simultaneous hermaphrodites are predicted to invest most of their resources in eggs because of a saturating male fitness gain curve (Charnov, 1982), and so they may, on

an individual level, experience (auto)sperm limitation. In our study, this appears implausible, as pure females did not lay more eggs than snails that mated both as males and females – even though the latter presumably gave away some of their autosperm. Fourth, egg production when selfing could be reduced because snails that lack female copulations also do not receive seminal fluid proteins, which were shown to increase egg production in *Drosophila melanogaster* (Ram and Wolfner, 2007). However, based on findings in a relative of *R. balthica*, where snails injected intravaginally with seminal fluid were less likely to lay eggs (Koene et al., 2009a) or laid fewer eggs (Koene et al., 2010), this explanation also seems unlikely. Finally, a fifth potential explanation is the lack of possible benefits gained from the digestion of received sperm, which has been suggested to represent a source of nutrients for egg production (Michiels, 1998; Greeff and Michiels, 1999; Yamaguchi et al., 2012). Although sperm digestion has been found in related species of snails (Cain, 1956; Madsen et al., 1983; Vianey-Liaud et al., 1989; Koene et al., 2009b; Nakadera et al., 2014), it has not yet been studied in *R. balthica*. Moreover, a recent study in two snail species suggests that investment into ejaculates is negligible when compared to eggs (Lodi et al., 2017), casting doubt on this hypothesis as well.

To conclude, we consider the early action of inbreeding depression and an attempt to save resources for potential future outcrossing the most likely reasons for the reduced female fecundity of selfing snails in our study, but clearly more research is needed on this subject.

Female infertility

Finally, we found that almost half of the snails in our experiment were female infertile. Based on theoretical models that postulate a fixed energy budget allotted to reproduction

(Charnov, 1979), one might expect that the absence of egg production goes along with an increased investment in the male function. However, only 38.2% of female infertile snails mated as a male (the female-mating males and the pure males). The remaining 61.8% of female infertile snails did not mate as a male, and so relinquished any chance of compensating for a lack of eggs by siring more offspring (reproductive failure type 1 and 2). Hence, to put it bluntly, the majority of female infertile snails were not preferential males that forgot to take care of their female function, but rather genuinely non-reproductive individuals.

Limitations

In our study, we accounted for numerous potential confounding factors that could have affected mating behavior and female reproduction. We used the first-generation lab-reared offspring of field-collected snails from a single population, and so we knew that all experimental snails were outcrossed, fed *ad libitum* and uninfected by parasitic trematode worms that often castrate these snails in natural populations (Wulfschleger and Jokela, 1999; Wiehn et al., 2002). The fact that mating activity was highest during the first mating opportunity and declined subsequently also suggests that most snails were sexually mature when mating trials started. We presented each snail with at least one size-matched mating partner of equal age and mating experience, and not closely related to the focal snail, to ensure that snails were constrained as little as possible in their reproductive behavior by the identity of their mating partner(s). We monitored the reproductive lifespan to the largest part, from the very beginning of egg-laying until snails were more than a year old and, in agreement with their annual life cycle, began to show an increased mortality rate. For these

reasons, we are confident that our estimates of mating activity and of female lifetime reproductive success are accurate for the study conditions – acknowledging that a laboratory environment will never perfectly simulate natural conditions.

However, two limitations need to be addressed explicitly. First, this study is in large part oblivious to the individual variation in fitness gains via a snail's male function. While we did record male mating behavior, and therefore know the total reproductive output of all non-mating and purely female-mating snails (pure females, pure selfers, and snails suffering from reproductive failure type 1 and 2; 33.5% of snails), male-mating snails may have an unknown number of sired offspring. Second, eggs laid by female-mating snails may not be fully outcrossed. If some eggs were selfed, they would carry twice as many copies of the maternal genome as outcrossed eggs (Fisher, 1941), thereby doubling a mother's contribution to the next generation. On the downside, selfed eggs may suffer from inbreeding depression (Charlesworth and Charlesworth, 1987) or other fitness disadvantages (Higgins and Lynch, 2001; Morran et al., 2011). In our study population, inbreeding depression has not yet been investigated, but evidence from other populations (Evanno et al., 2006) and a closely related species (Jarne and Delay, 1990; Coutellec-Vreto et al., 1998) suggests that selfed individuals of *R. balthica* may face at least some costs. A thorough quantification of the mean reproductive fitness associated with alternative reproductive phenotypes will thus require genetic paternity analyses of all eggs laid by female-mating snails, but also knowledge of the relative fitness of selfed vs. outcrossed offspring. This will be the subject of future research. Pioneering studies of both mating and reproductive success in both sexual roles, albeit only for a part of the reproductive lifespan,

717 have been conducted in three primarily outcrossing snail species (Anthes et al., 2010;
718 Minoretti et al., 2011; Péliissié et al., 2012).

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724 **Data Accessibility**

725 Analyses reported in this article can be reproduced using the data provided by Felmy et al.
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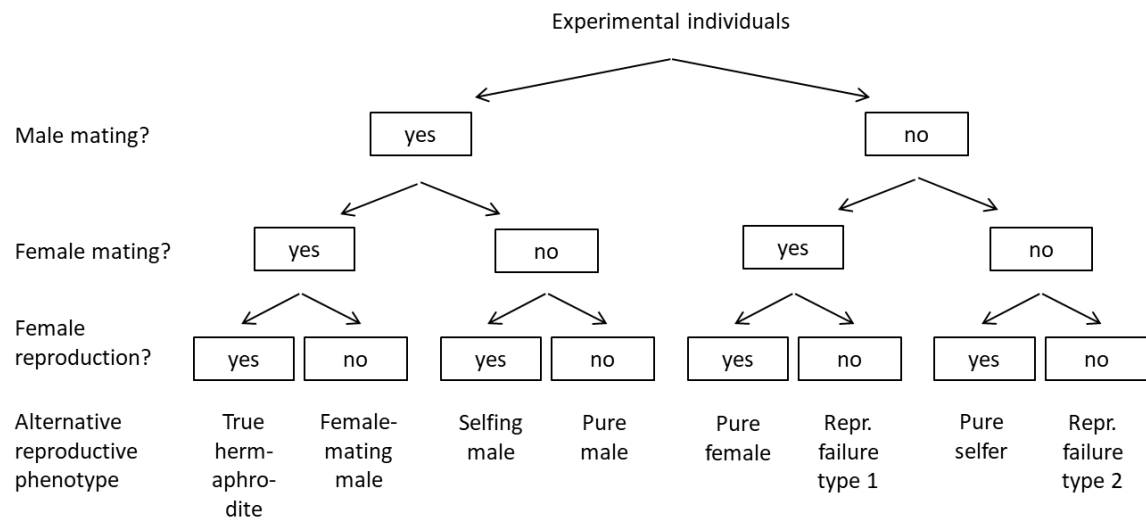
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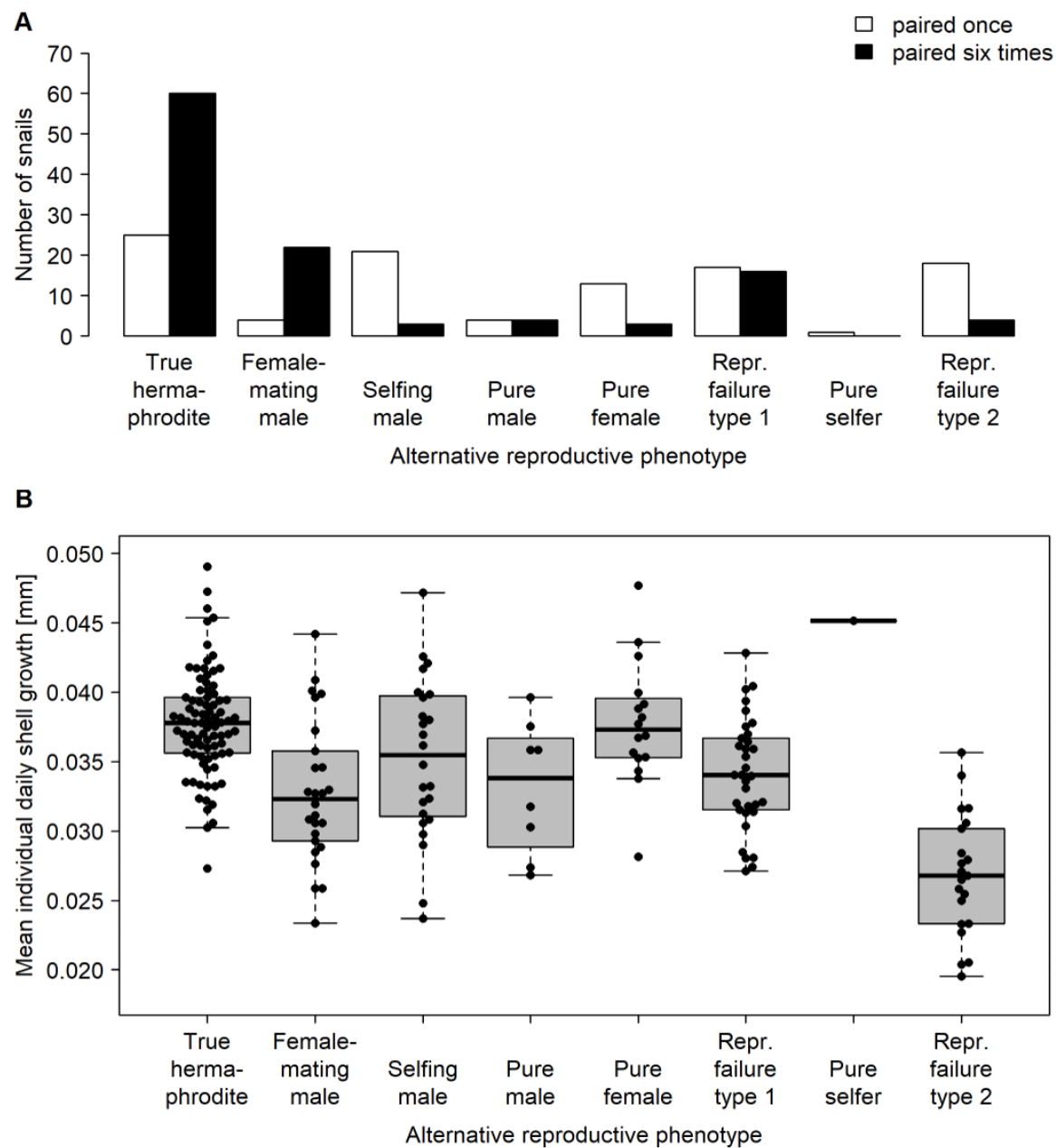
977 **Figures**

978 **Figure 1**



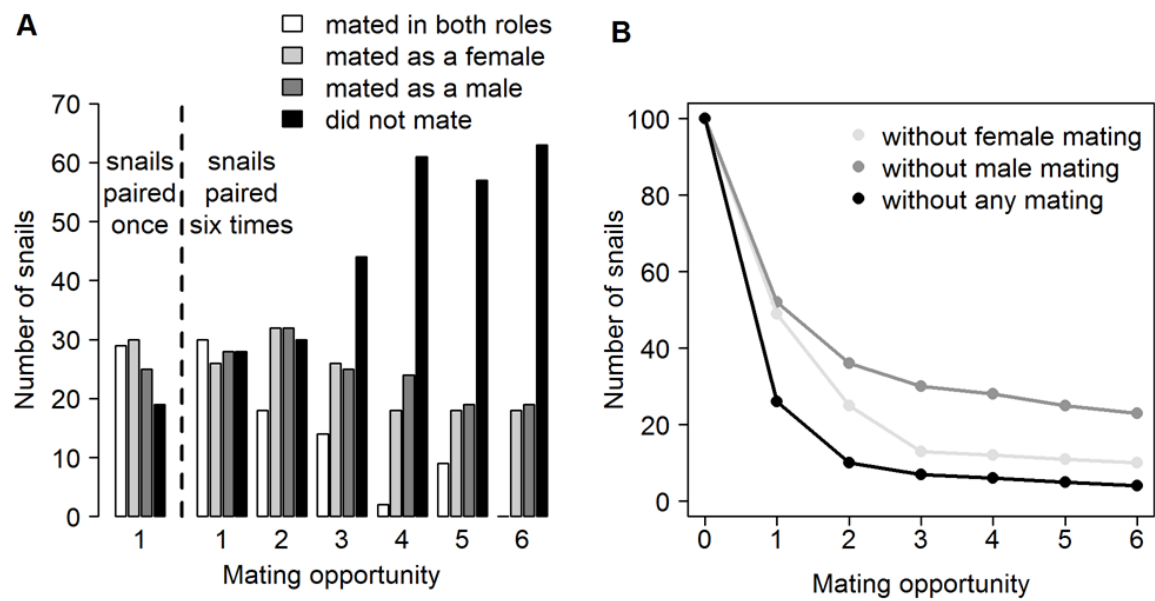
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980 **Figure 2**

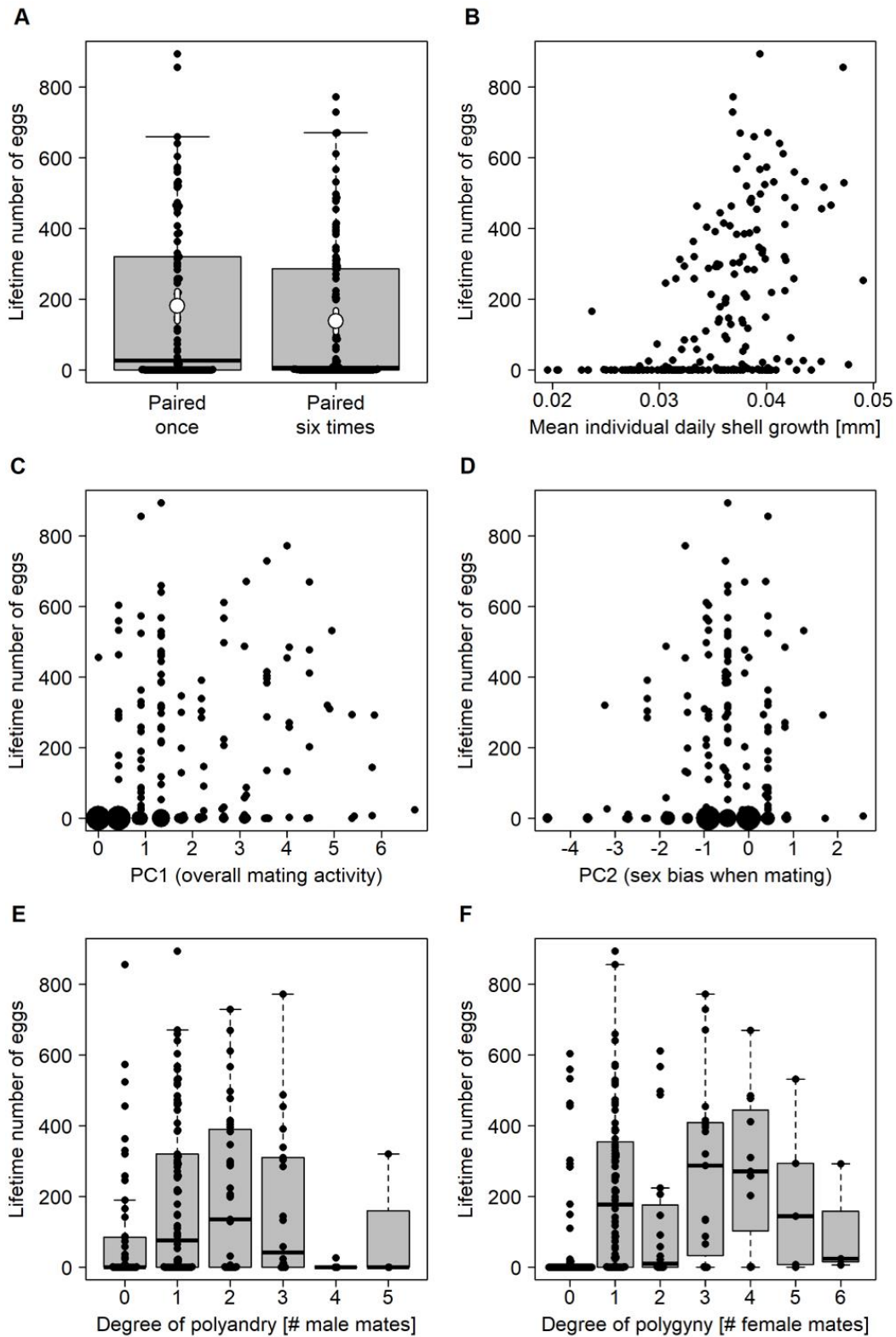


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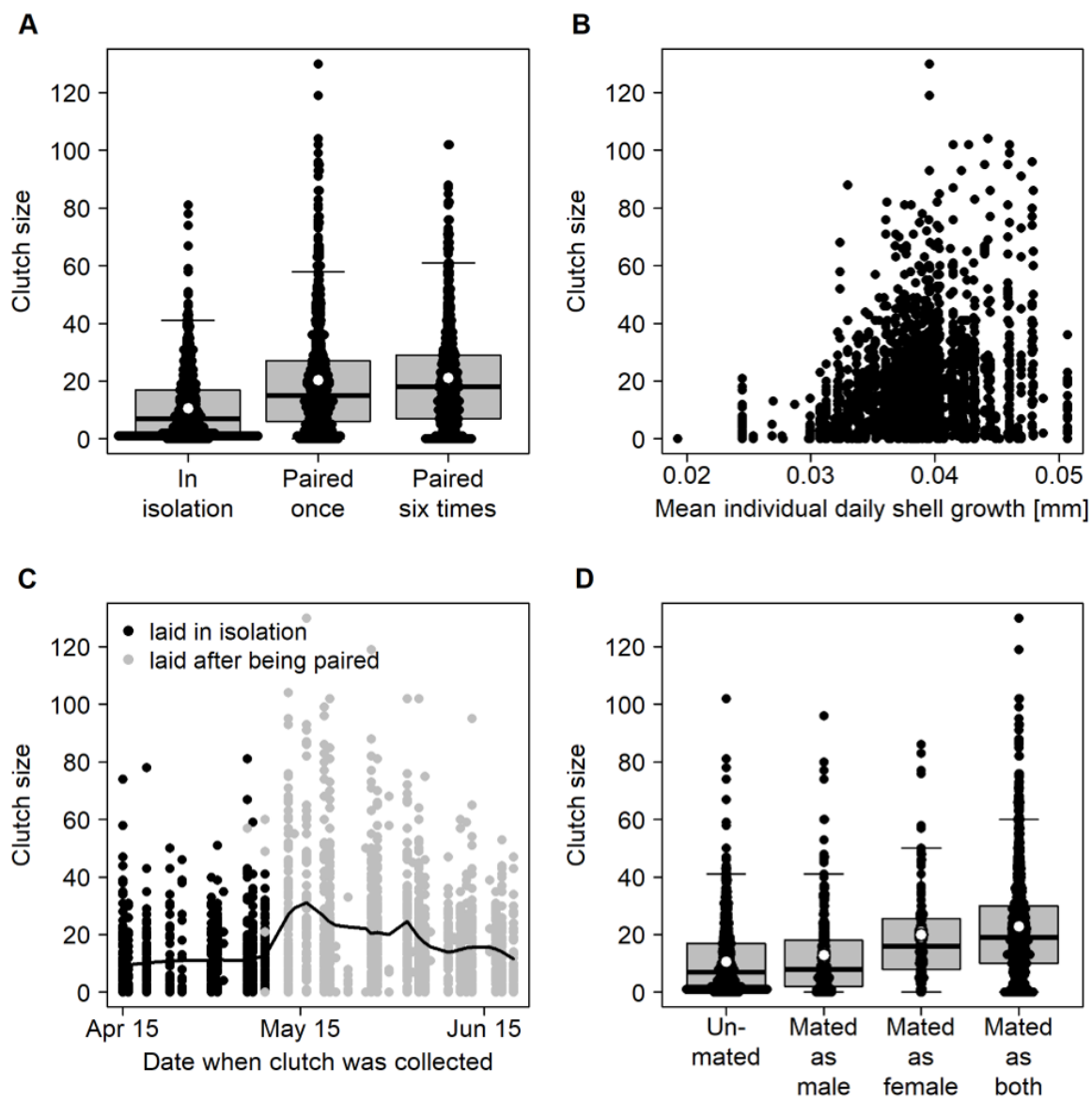
982 **Figure 3**



983



985 **Figure 5**



986

Figure legends

Figure 1

Reproductive options in a self-compatible, simultaneous hermaphrodite, giving rise to eight alternative reproductive phenotypes. Male reproduction was not included as a separate option because the fate of transferred sperm depends largely on the sperm recipient, e.g. in the case of cryptic female choice or when the sperm recipient is unable to produce eggs.

Figure 2

Causal link between observed alternative reproductive phenotypes and experimentally manipulated mate availability (A), and correlative association with mean individual daily growth rate (B). The numbers of snails per reproductive phenotype are as follows (from left to right): 60, 22, 3, 4, 3, 16, 0 and 4 for snails paired six times, and 25, 4, 21, 4, 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. See also fig. S2 and table S3.

Figure 3

Mating activity during six consecutive mating opportunities (A), and cumulative number of unmated snails after zero to six mating opportunities (B). Snails were paired once or six times, each time with a different mating partner. Shown are verified copulations only (i.e. copulations with visible penis insertion). Snails are included only if their mating behavior could be assessed with very high accuracy (80.2% of snails). Consequently, in (A)

the numbers of solely male- and solely female-mating snails are not necessarily identical on a given mating opportunity. Figure (B) is restricted to repeatedly paired snails that survived through all six mating opportunities ($n = 100$). See also fig. S3 and table S4.

Figure 4

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).

Shown are raw data (black), and box plots based on raw data (gray). In (A) we added predicted means with 95% confidence intervals extracted from the negative binomial model (white). 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. See also fig. S4 and table S5.

Figure 5

Causal link between clutch size 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 (A, D) we added predicted means with 95% confidence intervals extracted from negative binomial models (white). Note that 95% CIs are too small to be seen. (C) Clutches were collected from snails at least once a week, and so 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). Figure (D) only includes clutches laid by snails whose mating behavior could be assessed with very high accuracy (80.2% of snails). See also fig. S7 and tables S6 and S7.

1039 **Tables and table legends**

1040 **Table 1**

1041 **Average female lifetime reproductive output per experimental treatment group.**

	Paired once	Paired six times
Observed	176.4 ± 227.2	140.0 ± 200.8
Without the pure selfer	173.7 ± 226.6	-
Hypothetical (1), assuming equal frequencies of alternative reproductive phenotypes in both treatments	200.3	107.7
Without pure selfers	198.1	105.4
Hypothetical (2), assuming equal average fecundities of alternative reproductive phenotypes in both treatments	125.8	182.1
Without the pure selfer	121.4	-

1042 Provided is the mean lifetime number of eggs laid per snail (\pm SD where applicable), for
 1043 snails paired once or six times. In addition to observed values, we computed hypothetical
 1044 values by keeping either the frequencies of alternative reproductive phenotypes (scenario
 1045 1), or the average fecundities of each reproductive phenotype (scenario 2), constant across
 1046 treatments. For details, see Methods.