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Sex in crowded places: population density regulates reproductive strategy

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Keywords: *Daphnia galeata*, population density, reproductive strategy, population growth,
ephippia, reliable environmental cue

Abstract

Changing reproductive strategy from investment in current (asexual eggs) to future (sexual ephippia) reproduction depending on environmental cues is an important fitness trait in zooplankton species like water fleas, *Daphnia*. Different cues are reported for ephippia production, for example food limitation, changes in photoperiod and temperature, the presence of predators, nutrient depletion or signals of crowding. However, the reliability of these cues strongly depends on the local habitat. In *Daphnia galeata*, ephippia production might not be subject to any of the environmental cues outlined. Here, we directly test whether changes in crowding alone provides a reliable cue for *D. galeata* to switch reproductive mode. We conducted life history assays using 10 genotypes of *D. galeata* from a Swiss Lake to investigate density dependent reproduction and production of ephippia. We started populations at different densities and eliminated competition for food by systematically increasing food availability with population size. Our results suggest that population density depresses population growth rate via a change in energy allocation from current into future reproduction in *D. galeata*. Thus, signals of crowding produced at high population density constitute a reliable and sufficient cue for *D. galeata* to switch from asexual to sexual reproduction.

Introduction

Daphnia (planktonic crustacean water fleas) play a key role in aquatic ecosystems, they feed on the primary producers (algae) and are themselves food for invertebrate predators and fish (Lampert, 2011). In that way they can potentially have a large impact on aquatic ecosystems. In response to environmental cues, for example those of predators, *Daphnia* can alter their behaviour, produce defended phenotypes and change their life history (Lass & Spaak, 2003). Typically, changes in life history affect immediate population growth as the summation of individual fecundities in a given population, or the prospect of survival beyond proximate unfavourable conditions (Lynch, 1984; Spitze, 1991; Miner et al., 2012).

Daphnia usually reproduce parthenogenetically, but are able to switch to sexual reproduction in unfavourable environments. The resting eggs produced during sexual reproduction can withstand harsh environments like droughts (Zaffagnini, 1987; Hobaek & Larsson, 1990; Jarnagin et al., 2000), but incur a considerable cost both physiologically (Lynch, 1983) and in terms of missed reproductive opportunity as only two resting eggs are produced, interrupting the potentially fast population growth that occurs during asexual reproduction. The production of resting eggs therefore constitutes a trade-off between current and future reproduction.

How important sexual reproduction is for population survival differs strongly among *Daphnia* species. For species (e.g. *Daphnia magna*) living in ephemeral ponds such as rock pools, that dry up completely, the production of resting eggs is essential to survive drought, while *Daphnia* species of large lakes that do not freeze can potentially survive winter as parthenogenetic individuals. Furthermore, habitats differ in several other aspects like food availability, temperature fluctuation and predators. Therefore, it can be expected that also

the cues that induce sexual reproduction differ among species adapted to temporal and permanent habitats.

The timing of the switch to (sexual) future reproduction has long been of interest to researchers since the work of Woltereck (1911). Previous work showed that in natural populations switching is governed by an ensemble of cues such as changes in photoperiod or temperature (*Daphnia pulex*, Stross & Hill, 1965), decreasing food availability (*D. magna*, Kleiven et al., 1992), nutrient depletion (*D. magna*, Kleiven et al., 1992), predators (*D. magna*, Pijanowska & Stolpe, 1996) and signals of crowding (i.e. population density, Stross & Hill, 1965; Kleiven et al., 1992). In laboratory experiments with *D. magna*, the shift to sexual reproduction was shown to be the result of three environmental cues: high population density, decreasing day length and food limitation (Kleiven et al., 1992).

Recent work in natural populations of *D. magna* suggests that the frequency of switching to sexual reproduction increases as the demographic cost decrease; when the population is nearing carrying capacity (Gerber et al., 2018). Laboratory based studies support that increasing or decreasing photoperiod is a reliable cue in *D. magna*, but only in locally adapted populations with strong seasonal events of summer drought or winter freezing. In permanent and randomly ephemeral ponds, photoperiod is not a reliable signal for sexual reproduction (Roulin et al., 2013, 2016). Clearly, within *Daphnia* there are several cues that induce sexual reproduction and the reliability of these cues vary between species, geographic localities and habitat.

As mentioned above, *D. magna* is a large *Daphnia* species that lives in ponds that often dry out and therefore usually do not contain fish. Lakes are normally inhabited by smaller *Daphnia* species that have adapted their life history strategies to cope with fish predation (Benzie, 2005). In an experimental study on species in the *Daphnia longispina*

complex (which live in lakes and include *Daphnia galeata*), using the same stimuli as used by Kleiven et al (1992), Spaak (1995) found strong differences in the response of different *Daphnia* taxa to cues that induce sexual egg production.

D. galeata invaded eutrophic lakes around the world in the past half century and is now widespread in a variety of seasonal and aseasonal environments and latitudes (Taylor & Hebert, 1993; Jankowski & Straile, 2003; Rellstab et al., 2011; Karabanov et al., 2018). Invasive genotypes of *D. galeata* not only displace resident *D. longispina* but also hybridize with this resident species (Brede et al., 2009). While displacement of resident species can occur through simple competitive exclusion, hybridization is only possible through sexual reproduction, so seasonal cues associated with switching to sexual reproduction might be unreliable in populations of *D. galeata* and other members of the *D. longispina* complex than for more distantly related *Daphnia* species, especially in newly invaded environments. For example, *D. magna* and *D. pulex* are often found in shallow water bodies (i.e. ponds) where changes in photoperiod might be reliable predictors of unfavourable abiotic conditions approaching (pond drying in long summer days, or freezing during dark winters). In deep lakes where *D. galeata* is common, photoperiod might be less relevant as changes in day length are not necessarily predictors of cues like food stress or predation. Therefore, we hypothesize that in such environments crowding is a better indicator of population collapse. However, in natural populations food stress and crowding co-occur and cannot be disentangled, but experimentally this is possible.

D. galeata produces ephippia in spring when photoperiod is increasing and food is limiting because of the mid-summer decline clear water phase when population density is high (Sommer et al., 1986, 2012), and in autumn when food is not really limiting but days are getting shorter (Jankowski & Straile, 2003; Keller et al., 2007; Möst et al., 2015). This

suggests that neither food abundance nor photoperiod changes are reliable cues for *D. galeata* to produce ephippia, and that crowding might be the most reliable signal for *D. galeata* to switch to sexual reproduction.

Here, we directly test whether changes in crowding alone provides a reliable cue for *D. galeata* to switch reproductive mode. We conducted life history assays using ten genotypes of *D. galeata* resurrected from sediment cores from Lake Greifensee, Switzerland, to investigate density dependent reproduction and production of ephippia. For each of ten genotypes, we seeded populations at five different rates (1,2,3,4 or 5 neonates) to generate a range of population densities throughout the experiment and, to eliminate competition for food, we fed each population an algal ration proportional to population size. We hypothesize that *D. galeata* will switch from asexual reproduction to ephippia production as crowding increases, even in the absence of other switching cues that have been reported for other *Daphnia* species.

Materials and Methods

We conducted life history assays using ten *D. galeata* clones hatched from resting eggs which were extracted from dated sediments from Lake Greifensee, Switzerland (N 47°21', E 8°41') using a resurrection ecology approach (Kerfoot et al., 1999). We selected clones spanning 54 years (1958 – 2012) to obtain a representative mix of genotypes across a period during which the lake underwent strong eutrophication and recovery, to ensure our results remain relevant in the face of potential adaptation to different environments. To exclude abiotic cues, experimental cultures were maintained at constant photoperiod (light/dark cycle of 12/12 h) and temperature (18°C). To eliminate potential biotic cues from decreasing food per capita, populations were fed an algal ration proportional to their size

(Supplementary Figure S1). We monitored population size, maturation rates, and reproductive output (egg and ephippia production) over 21 days.

General culturing conditions

Routine culturing and experiments were performed using filtered surface water from Lake Greifensee. To standardize experimental conditions, experimental water was collected at the same time (i.e. a single batch) and stored at 12°C for 10 days to allow degradation of any biological signals (e.g. predator kairomones). Prior to use, aged water was filtered through a 0.45 µm filter and allowed to equilibrate with laboratory temperature.

All *Daphnia* were routinely fed with the green algae *Acutodesmus obliquus* (formerly *Scenedesmus obliquus*) grown in a chemostat and cultured in WC medium (Guillard, 1975). Stock populations received ~1 mg C L⁻¹ day⁻¹.

Establishment of experimental populations

The experimental clones were maintained in our “library” at 12°C and a light/dark cycle of 12/12 h. Prior to the experiment, all genotypes were raised for at least four generations in the laboratory under standardized conditions to minimize maternal and environmental (e.g. crowding) effects. For each genotype, a single individual was raised at a constant temperature of 18-20°C and a light/dark cycle of 12/12 h. To further reduce maternal effects, we discarded the first offspring and established experimental populations with a neonate from the second clutch.

Experimental design and sampling

Experimental populations were seeded with 1,2,3,4, or 5 neonates (< 24 h old) from each

genotype. Each experimental population was contained in a 100mL microcosm (jar). Each genotype and seeding combination was replicated three times. For logistical reasons each replicate was set up and analysed on consecutive days (blocks). This resulted in total 150 microcosms, with 50 microcosms censused on a specific day. Microcosms with males or deaths in the first four days were eliminated. Populations were fed every day with an algal ration proportional to the starting population size and increasing throughout the experiment (Fig. S1).

Each block was censused every four days, i.e. each population was counted and media was exchanged. During counting each individual was assigned to a demographic group (babies, juveniles, asexually reproducing females, non-reproducing females, sexually reproducing females, males). We also recorded asexual clutch sizes and the number of released ephippia.

While populations were small (until about day 13), individuals from each population were transferred individually with a glass pipette into a new microcosm with fresh media. As populations grew this became unfeasible so from day 17 populations were gently filtered (30 μ m mesh size). Individuals were assessed on the filter before being returned to a new microcosm with fresh media. Egg counting was conducted using a microscope (Leica MZ 9.5). At day 21, the experiment was concluded by preserving all populations in 70% ethanol. Prior to placing populations in ethanol each population was narcotized using ice-cold carbonated water. This greatly reduces eggs loss due to carapace deformation (Burns, 1969; Black & Dodson, 2003). Populations were counted and stored in ethanol (70%) at room temperature.

Statistical analyses

Population growth rate (r) was calculated as the intrinsic rate of increase in population size (N) between day 1 and 21 as $r = (\ln(N_{t+1}) - \ln(N_t)) / \Delta t$ (Begon et al., 1986). Growth rate was analysed using linear regression to test whether population growth rates differ among genotypes, blocks, or as a function of our initial seeding rate (model 1: population growth rate $r \sim \text{block} + \text{genotype} + \text{seeding rate}$, see Table 1). Note that because population growth rate is calculated directly from population size, the two measures are not mathematically independent and we do not include population size in our assessment of changes on population growth rate. We ranked population growth rate of genotypes for each seeding treatment for nine of the ten clones (Clone A-1958 was excluded because values for some treatments were missing due to early deaths and males in the starting populations and the test applied requires complete balanced data). A rank of nine indicated the fastest population growth and rank one represented the slowest growth rate. Rank order was analysed with a non-parametric Friedman rank sum test (Friedman, 1937).

To test the main hypothesis whether asexual or sexual reproductive effort of the population differs with population size, the relationship between egg production and population size was analysed using a generalized linear mixed model (GLMM) with a quasi-poisson distribution.

Food availability and population size were included as a fixed-effect and to account for repeated measures, jar, day and genotype were included as random factors (model 2, for model structure see Table 2). We included the food amount (mg carbon) per individual as a fixed covariate to disentangle whether egg or ephippia production was influenced by carbon limitation. The food amount per individual was calculated as the total amount of carbon

supplied to the culture during the four days between media exchanges expressed as its concentration per *Daphnia*.

We quantified ephippia production between sampling events. We modelled the relationship between the number of ephippia produced and population size using a GLMM with a poisson distribution with the same model structure as used in model 2 (model 3, Table 3).

To investigate asexual reproductive output we used a simplification of the Edmondson egg ratio (EER). Edmondson originally defined his egg-ratio as eggs produced per female per day with subsequent calculation of instantaneous birth rates based on temperature dependent development rate of eggs (Edmondson, 1960). As temperature was maintained constant throughout the experiment the temperature dependence was omitted. EER was calculated as $EER = No_{total\ eggs} / No_{total\ adult\ females}$. The change in EER over time was analysed using polynomial regression with population size and genotype as predictors and day as a quadratic predictor (model 4, Table 4).

Modelling strategy

Models were fit as saturated models with interactions, and reduced so that only significant terms were retained. Where higher order interactions were retained, component terms were also retained irrespective of their significance. Seeding rate, genotype, jar and day were treated as factors, whereas population size and carbon per individual were used as continuous variable. Linear models were checked for violations of assumptions of normality using a Shapiro-Wilk test (Shapiro & Wilk, 1965) and of homogeneity of variance using a Levene Test (Levene, 1960). All data manipulation, calculations and statistical analyses were performed in R version 3.4.2 (R Core Team, 2017). For GLMMs, we used the package

glmmTMB (Brooks et al., 2017). The best models predicting egg or ephippia production were selected based on model AIC scores.

Results

Population size and growth rate

Populations initiated with more individuals grew slower than those that started with fewer individuals (Fig. 1A). Population growth rate decreased as the number of founders (i.e. seeding rate) increased (model 1: $F(4, 98) = 15.03$, $p < 0.0001$) and differed between genotypes (model 1: $F(9, 98) = 10.94$, $p < 0.0001$). However, all populations grew exponentially up to densities of 50 – 200 individuals per jar (100 ml) (Fig. 1B).

The rank order of population growth rate between genotypes varied strongly between seeding rates (Fig. S2) and differed significantly between genotypes, (Friedman rank sum test; $\chi^2(8) = 33.87$, $p < 0.0001$), but there was no pattern supporting genotype age affecting growth rate. Because these results were transparent in Figure S2, we did not perform any post hoc tests. Genotype I-2009 had the highest growth rate, followed by H-2000 and in contrast, D-1986 grew the slowest. Unexpectedly, experimental block had an effect on population growth rate (model 1: $F(2, 98) = 10.29$, $p < 0.0001$), namely block 3 had lower growth rates than the other blocks (for growth rate model estimates see Table 1).

Egg and Ephippia production

Both egg and ephippia production increased with increasing population size (GLMM model estimates for model 2 in Table 2, $Z = 7.919$, $p < 0.0001$; and model 3 in Table 3, $Z = 4.946$, $p < 0.0001$). Egg production initially increased with population size but levelled off midway through the experiment (Fig. 2). In contrast, ephippia production was absent in populations

with fewer than 50 individuals, and increased linearly as population size increased in larger populations (Fig. 2).

We calculated food rations based on seeding rate and increased those rations throughout the experiment. At each media exchange we observed surplus algae in every jar, and when food availability was back-calculated per individual it was clear that daphnids were unlikely to experience prolonged conditions where food would be limiting during the experiment ($< 0.25 \text{ mg C L}^{-1} \text{ Daphnia}^{-1}$ Lampert, 1987; and Fig. 3), irrespective of which seeding rate was used to initiate populations (model 2 in table 2, $Z = -3.82$, $p < 0.0001$; model 3 in table 3, $Z = -0.92$, $p = 0.3558$).

Edmondson egg ratio

Over the duration of the experiment EER decreased when population size increased (model 2: $F(1, 661) = 10.69$, $p = 0.001$), and there was a significant effect of day (model 2: day: $F(1, 611) = 905.20$, $p < 0.0001$), poly (day^2): $F(1, 611) = 0.15$, $p = 0.70$); Fig.4 showing EER over the course of the experiment). Initially, EER increased to a maximum around day 9 or 13 before declining, in line with our overall observations of egg production (see Fig. 2). EER differed strongly between genotypes (model 2: $F(9, 611) = 5.92$, $p < 0.0001$; parameter estimates in Table 1), genotype A-1958 had the highest EER when populations were seeded with few (1-3) individuals, but unfortunately populations of this clone were lost at the two highest densities.

Discussion

For *Daphnia* species and populations many different environmental cues are reported to trigger a switch between asexual and sexual reproduction. Here, we show that clones of *D.*

galeata from a deep lake require only signals of crowding to induce a shift in reproductive strategy. Previous studies done with *D. magna* and *D. pulex* suggested that several cues were simultaneously required (Stross & Hill, 1965; Kleiven et al., 1992). More recently, Roulin and colleagues presented strong evidence that a change in photoperiod (day length) alone was sufficient to induce a selective shift in *D. magna* (Roulin et al., 2013). However, even in that study it was clear that selection had been effective only in shallow ponds where photoperiod was a very reliable indicator of impending population collapse (i.e. freezing or drying). In deep lakes that do not freeze or dry, changes in day length are largely decoupled from adverse environmental conditions.

The switch to sexual reproduction in *D. galeata* might not only be associated with avoidance of adverse environmental conditions as it is in other *Daphnia* species. For species such as *D. magna* and *D. pulex*, the principal reason for switching to sexual reproduction is likely to be the production of resting eggs that can endure a period of unfavourable conditions (e.g. pond freezing or drying), and a fortunate by-product is that those resting eggs are usually recombinant, new genotypes. Diapausing eggs prevent local extinction during unfavourable environmental conditions (e.g. Deng, 1996; Cáceres, 1998; Walsh, 2013). In most *Daphnia* species diapausing eggs are tightly linked with sexual reproduction, but species in the *D. pulex* group can also produce resting eggs in the absence of sex, i.e. without recombination (Schurko et al., 2009) which suggests that survival, via diapausing eggs, is the principal objective of ephippia production in these species. In contrast, deep-lake species such as *D. galeata*, where overwintering as adults is possible and drying events uncommon, always require sex to initiate the production of diapausing eggs so the main benefit from switching to sexual reproduction might be recombination, and durable resting eggs the by-product.

Sexual reproduction is theoretically maintained in populations by benefits of recombination; for example in response to antagonistic coevolution between organisms and their pathogens and parasites (Lively & Howard, 1994), but is also costly due to several factors (reviewed by Lehtonen et al., 2012) Because horizontal transmission of parasites is density-dependant in *D. magna* (Ebert, 1995) and in *D. galeata* (Bittner et al., 2002), signals of crowding might be a reliable cue of impending infection, and switching to sexual reproduction a means of escape from parasites (Soper et al., 2014; Auld et al., 2016). Moreover, because in *D. galeata* sex also facilitates hybridization with other species in the *D. longispina* complex, the adaptive benefit of sex would be increased with the likely acquisition of beneficial resistance alleles. As mentioned in the introduction, in natural populations food stress and crowding often co-occur, so producing resting eggs in response to crowding cues is also an effective means of surviving food shortages. Irrespective of the driver of the switch to sexual reproduction, surviving inhospitable environments, declining resources or escaping parasites, it is clear that the energetic and demographic costs require precise timing to maximize benefits, so the reliability and simplicity of the cue is crucial.

We observed an increase in ephippia production as population size increased (Fig 2), and this increase was associated with a decrease in population growth rate. Ephippia production began at a density of 50 individuals, which is over 300 individuals per litre. In Greifensee *Daphnia* densities during ephippia production of 50 per litre have been reported (Keller et al., 2002). However these are densities calculated over the whole water column of 34 meters, where the *Daphnia* in summer are restricted to the upper 5 oxygenated meters of the lake (AWEL Kanton Zürich Switzerland, 2019), meaning that the densities we had in our experiment represent densities that occur in the lake. Recent theoretical work suggests that *Daphnia* should switch reproductive strategies as the cost of that switch decreases

(Bookmythe et al., 2018). In our study we aimed to decouple population density (crowding) from food limitation by adding proportionally more food to the populations that started with higher number of daphnids. Our analysis shows that food availability (Fig. 3) had no influence on ehippia production as it did for parthenogenetic eggs. In earlier studies authors achieved this decoupling by growing *Daphnia* in “crowded water”, in that case only the chemical crowding signal was available in the population (Spaak, 1995; Boersma et al., 1998). The approach in our study gives the daphnids not only the chemical crowding signal but also the mechanical one. Maybe for this reason we could show so clearly that crowding induces ehippia production in lake *D. galeata*.

The observed decrease in population growth rate (i.e. current reproduction), especially at higher starting densities, might be explained through the approximation towards carrying capacity, as r and EER start to decrease close to the end of the experiment. Other studies have shown that stress from environmental factors can change carrying capacity (Sibly et al., 2000). Reaching carrying capacity might trigger the decision to change life history strategy. Three competing life history trade-offs are possible: a trade-off between current reproduction and survival, a trade-off between many small and few large offspring, and finally a trade-off between current and future reproduction. In *Daphnia* the trade-off between current reproduction and survival is often invoked (Stearns, 1989), as *Daphnia* may be able to wait out unfavourable environmental conditions by holding energy in reserve (Cleavers et al., 1997). A trade-off between number and size of offspring (Cleavers et al., 1997) may be occurring, but egg size does not vary with clutch size in these genotypes group (unpublished data). Even though both the above trade-offs are plausible, we argue that in our experiment a trade-off between current vs future reproduction is better explained by conserving energy for future reproduction.

For delayed reproduction not only sexual females but also males need to be produced to fertilize the diapausing eggs within the ephippium. Previous studies found that the production of males was stimulated by crowding (Kleiven et al., 1992; Spaak, 1995; Boersma et al., 1998). Although we observed more males in populations with higher density, our dataset is too small to analyse this aspect in detail.

Our experimental setup limited us to an experimental duration of 3 weeks. The limited duration of the experiment did not allow to study mortality rates which means that the traditional use of Edmondson's Egg Ratio method is limited. We also observed an unexpectedly strong effect of block in the experiment. We can only speculate why this could be. These block effects limit the statistical power of our results, however our main findings are robust and not affected by these block effects.

Conclusion

In this research, we have shown that increasing population density induces ephippial production in *D. galeata* in the absence of increased competition for diminishing resources (i.e. per capita food limitation). We could show this for a large number of clones resurrected from a period of 50 years, which shows that the pattern we observe is a general one and not specific for a certain *Daphnia* clone.

Acknowledgements

We thank Christine Dambone and Esther Keller for support in *Daphnia* and algae culturing, and Nelson Stevens and Robert Dünner for statistical help. This manuscript was improved by the valuable input of two anonymous reviewers. This work was supported in part by the grant "SeeWandel: Life in Lake Constance - the past, present and future" within the

framework of the Interreg V programme “Alpenrhein-Bodensee-Hochrhein
(Germany/Austria/Switzerland/Liechtenstein)” which funds are provided by the European
Regional Development Fund as well as the Swiss Confederation and cantons.

Data availability

All data and processing scripts used in this study will be available on github/lhaltiner.

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Figure legends

Fig. 1 A Population growth rate (mean $\pm$ se) for the different seeding rates. Colours indicate different genotypes. **B** Population size (individuals per 100 ml jar) throughout the experiment (mean $\pm$ se).

Fig. 2 Reproductive output of *Daphnia* populations as a function of population size. Eggs (circles, blue line) and ephippia (triangles, red line). The lines indicate loess functions fitted to the data.

Fig. 3 Per capita food availability for individual *Daphnia* during the experiment. The data are compacted at day five as individuals had not yet begun to reproduce. Data are offset on the x axis for clarity.

Fig. 4 Changes in Edmondson egg ratio (mean of three replicates) for each sampling event. Panels represent initial seeding rates. Colours indicate different genotypes, error bars not shown for clarity.

Table 1 Parameter estimates for model 1: population growth rate. Linear model parameter estimates ($\pm$ std error) are indicated. See main text for details of model fitting.

Model 1: population growth rate $r \sim$ block + seeding rate + genotype				
	F	P	R²	
	11.94	< 0.0001	0.59	
	Estimate	Std. error	t value	P value
Intercept	0.204	0.006	36.742	< 0.0001
Block B2	-0.002	0.003	-0.664	0.5083
Block B3	-0.012	0.003	-3.980	0.0001
Seeding rate 2	-0.014	0.004	-3.613	0.0005
Seeding rate 3	-0.020	0.004	-5.267	< 0.0001
Seeding rate 4	-0.024	0.004	-5.999	< 0.0001
Seeding rate 5	-0.023	0.004	-5.908	< 0.0001
B-1964	-0.004	0.006	-0.548	0.5851
C-1969	0.000	0.006	0.040	0.9679
D-1986	-0.035	0.007	-4.908	< 0.0001
E-1988	-0.024	0.007	-3.596	0.0005
F-1991	-0.015	0.006	-2.361	0.0202
G-1998	-0.003	0.006	-0.480	0.6320
H-2000	0.003	0.007	0.472	0.6381
I-2009	0.006	0.006	0.970	0.3345
J-2012	-0.023	0.006	-3.683	0.0004

Table 2 GLMM model parameter estimates of model 2: asexual reproduction. Egg production estimates ($\pm$ std error) are indicated. See main text for details of model fitting.

Model 2: eggs ~ carbon/individual + population size + (1 jar)+(1 day)+(1 genotype)					
Fixed-effects		Beta	Std. error	Z value	P value
	Intercept	2.143	0.448	4.783	< 0.0001
	Carbon per individual	-0.292	0.076	-3.826	< 0.0001
	Population size	0.009	0.001	7.919	< 0.0001
Random effects		Variance	Std. deviation		
	Jar	0.009	0.094		
	Day	0.949	0.974		
	Genotype	0.032	0.180		
AIC: 3705.7					

Table 3 GLMM parameter estimates of model 3: sexual reproduction. Ehippia production estimates ($\pm$ std error) are indicated. See main text for details of model fitting.

Model 3: ehippia ~ carbon/individual + population size + (1 jar)+(1 day)+(1 genotype)					
Fixed-effects		Beta	Std. error	Z value	P value
	Intercept	-7.068	3.840	-1.841	0.0657
	Carbon per individual	-1.783	1.931	-0.923	0.3558
	Population size	0.017	0.003	4.946	< 0.0001
Random effects		Variance	Std. deviation		
	Jar	0.797	0.893		
	Day	5.021	2.241		
	Genotype	15.036	3.878		
Zero-inflated model		Estimate	Std. error	Z value	P value
		-19.13	5542.6	-0.003	0.9970
AIC: 469.4					

Table 4 Parameter estimates of model 4: Edmondson egg ratio. EER estimates ($\pm$ std error) are indicated. See main text for details of model fitting and calculation of EER.

Model 4: Edmondson egg ratio ~ population size+ genotype + I(day^2) + day				
		F	P	R²
		80.78	<0.0001	0.59
	Estimate	Std. error	t value	P value
Intercept	-1.295	0.546	-2.372	0.0180
Population size	-0.008	0.005	-1.688	0.0919
B-1964	-1.306	0.598	-2.184	0.0293
C-1969	-1.568	0.569	-2.757	0.0060
D-1986	-3.009	0.663	-4.541	<0.0001
E-1988	-2.601	0.607	-4.284	0.0001
F-1991	-2.427	0.571	-4.250	<0.0001
G-1998	-2.616	0.566	-4.621	<0.0001
H-2000	-2.048	0.685	-2.991	0.0029
I-2009	-0.831	0.577	-1.439	0.1505
J-2012	-2.846	0.571	-4.986	<0.0001
I(day^2)	-0.084	0.003	-27.166	<0.0001
Day	1.970	0.065	30.087	<0.0001