

INBREEDING DEPRESSION VARIES WITH INVESTMENT IN SEX IN A FACULTATIVE PARTHENOGEN

Carla E. Cáceres,^{1,2} Cynthia Hartway,^{3,4} and Kimberly A. Paczolt^{5,6}

¹*School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801*

²*E-mail: caceres@life.uiuc.edu*

³*Wildlife Biology Program, University of Montana, Missoula, Montana 59812*

⁴*E-mail: cynthia.hartway@mso.umt.edu*

⁵*Department of Biology, Texas A&M University, College Station, Texas 77843*

⁶*E-mail: kpaczolt@mail.bio.tamu.edu*

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The reproductive mode of facultative parthenogens allows recessive mutations that accumulate during the asexual phase to be unmasked following sexual reproduction. Longer periods of asexual reproduction should increase the accumulation of deleterious mutations within individuals, reduce population-level genetic diversity via competition and increase the probability of mating among close relatives. Having documented that the investment in sexual reproduction differs among populations and clones of *Daphnia pulicaria*, we ask if this variation is predictive of the level of inbreeding depression across populations. In four lake populations that vary in sex investment, we raised multiple families (mother, field-produced daughter, laboratory-produced daughter) on high food and estimated the fitness reduction in both sexually produced offspring relative to the maternal genotype. Inbred individuals had lower fitness than their field-produced siblings. The magnitude of fitness reduction in inbred offspring increased as population-level investment in sex decreased. However, there was less of a fitness reduction following sex in the field-produced daughters, suggesting that many field-collected mothers were involved in outcross mating.

KEY WORDS: Cyclical parthenogenesis, *Daphnia*, ephippia, inbreeding, resting eggs.

The diversity of breeding systems found in nature has long intrigued ecologists and evolutionary biologists (Barrett 2002; Charlesworth 2006; Jarne and Auld 2006). Fundamental to this interest is why sexual reproduction is so common when it has so many apparent costs. All else being equal, an asexual female can produce twice as many daughters as her sexual counterpart, each of which carries her entire genome (Williams 1975; Maynard Smith 1978). Recombination is also costly in the potential break up of coadapted gene complexes, the time and energy required to find a mate, and the risks associated with engaging in sex (e.g., disease transmission) (Bell 1982; Lewis 1987). Proposed benefits of sexual reproduction include creation of varia-

tion, DNA repair, avoidance of Muller's ratchet, and resistance to parasites (both ecological and genetic) (Maynard Smith 1978; Butlin 2002; Schön and Martens 2003). Despite a general consensus that the benefits of sex must outweigh the costs, there is still little agreement as to why sexual reproduction prevails (Kondrashov 1993; Barton and Charlesworth 1998; Otto and Lenormand 2002).

One aspect of breeding system evolution that has received considerable attention is the nearly universal decline in fitness following mating with a close relative (inbreeding depression) (Charlesworth and Charlesworth 1987; Lynch and Walsh 1998). The role of inbreeding depression in mating system evolution

has been particularly well studied in plants (e.g., Husband and Schemske 1996; Kelly 2005). This vast literature suggests that even though inbreeding depression is common, its magnitude varies enormously among populations and species for reasons such as population size, history of inbreeding, and the interaction of genetic effects with the environment (Byers and Waller 1999; Hedrick and Kalinowski 2000; Michaels et al. 2008). This variability complicates predictions of how severe inbreeding depression will be in a particular population.

As a further complication, many organisms only occasionally engage in sex (Hadany and Otto 2007). Here, the potential costs of inbreeding are only realized during times of sexual reproduction. How should the frequency of sexual generations influence the magnitude of inbreeding depression? The answer to this question depends on the interplay of a number of factors. First, mutations accumulate during asexual reproduction resulting in a positive relationship between the genetic load carried by an individual and the age of the clone (Innes 1989; Lynch et al. 1998; Zeyl et al. 2001). The rate at which deleterious mutations are eliminated should depend on the frequency of sex for at least two reasons: frequency of sex determines the number of asexual generations during which selection via clonal competition can act and frequency of sex also determines the number of reproductive cycles allocated to sexual reproduction. Sexual generations can allow recessive mutations to be unmasked, resulting in potentially lower levels of fitness among sexually produced offspring (Carr et al. 1997; Charlesworth and Charlesworth 1999). However, the probability that the switch to sexual reproduction will translate into fitness declines due to inbreeding, depends in part on the probability of mating with a close relative. Longer periods of asexual reproduction (as the result of reduced investment in sex) reduce population-level genetic diversity via clonal competition (Hebert 1974; Vanoverbeke et al. 2007). With fewer genotypes in the population, the probability of encountering a mate that is a close relative should increase.

Facultative parthenogenetic populations of the freshwater crustacean *Daphnia* offer an excellent opportunity to test the prediction that variation among populations in the allocation to sexual reproduction will influence the magnitude of inbreeding depression. Inbreeding depression in *Daphnia* has been documented in a number of populations and species (e.g., Innes 1989; De Meester 1993; Deng and Lynch 1997). The duration of the clonal phase varies as does the relative investment in sex among populations (Innes and Dunbrack 1993; Tessier and Cáceres 2004; De Meester et al. 2006). Clonal selection has been documented repeatedly in natural populations (Hebert 1974; Vanoverbeke et al. 2007). Finally, lower effective population sizes have been found in populations with reduced investment in sex (Allen 2006). These observations support the hypothesis that inbreeding depression should vary among populations that differ in their fre-

quency of sex. These predictions have yet to be tested for natural populations.

We ask how inbreeding influences fitness in populations of *Daphnia pulex* that differ in their frequency of sex. Specifically, we ask (1) among populations, does the average magnitude of inbreeding depression increase as the population-level average allocation to sexual reproduction decreases? and (2) do individuals in each population experience inbreeding depression when reproducing sexually in the field? We addressed these questions by comparing the fitness change following sex in both laboratory-created inbred lines (mothers mated to genetically-identical sons) and in field-produced daughters. Although the sire of the field-produced daughters is unknown, if they are also inbred, then there should be a similar reduction in fitness in the inbred and field-collected animals.

Methods

We investigated the fitness change following sexual reproduction in populations of *D. pulex*. Details of how we quantified variation in sex investment are given in Cáceres and Tessier (2004). Here, we used four natural lake populations for which prior research documented genetic differences in mean levels of sex, and substantial genetic variation in sex investment among clones within each population (Tessier and Cáceres 2004). Baker Lake had the lowest investment in sex, followed by Warner Lake, Three Lakes Two (3L2), and Little Long Lake.

In May 2004, we collected 50–100 females carrying ephippia (the case containing the fertilized eggs) from the four populations. In the laboratory, females released their ephippium and returned to parthenogenetic reproduction (maternal genotype for each family, see Fig. 1). We tracked which ephippium belonged to each mother and females that hatched from these dormant sexual eggs were established into clonal culture (“field” genotype for each family). Because these ephippia were produced in the field, the sire is unknown, but should be representative of the baseline level of fitness change due to sexual reproduction occurring in each population. For each maternal genotype, we created a second sexually produced offspring in the laboratory by mating mothers to their genetically identical sons. We again retained a record of which ephippia containing inbred eggs belonged to which mother and inbred females that hatched were established into clonal culture (“inbred” genotype for each family). Once we isolated all three members (maternal, field-produced, inbred) of a particular family, each genotype was split into two clonal sublines, and each subline was raised individually under high food (4×10^4 cell/mL *Ankistrodesmus falcatus*) at 20°C.

In August 2005, we assayed the fitness of all clonal lines for those families for which we successfully raised the maternal, field-produced, and inbred genotypes. We used a life-table protocol

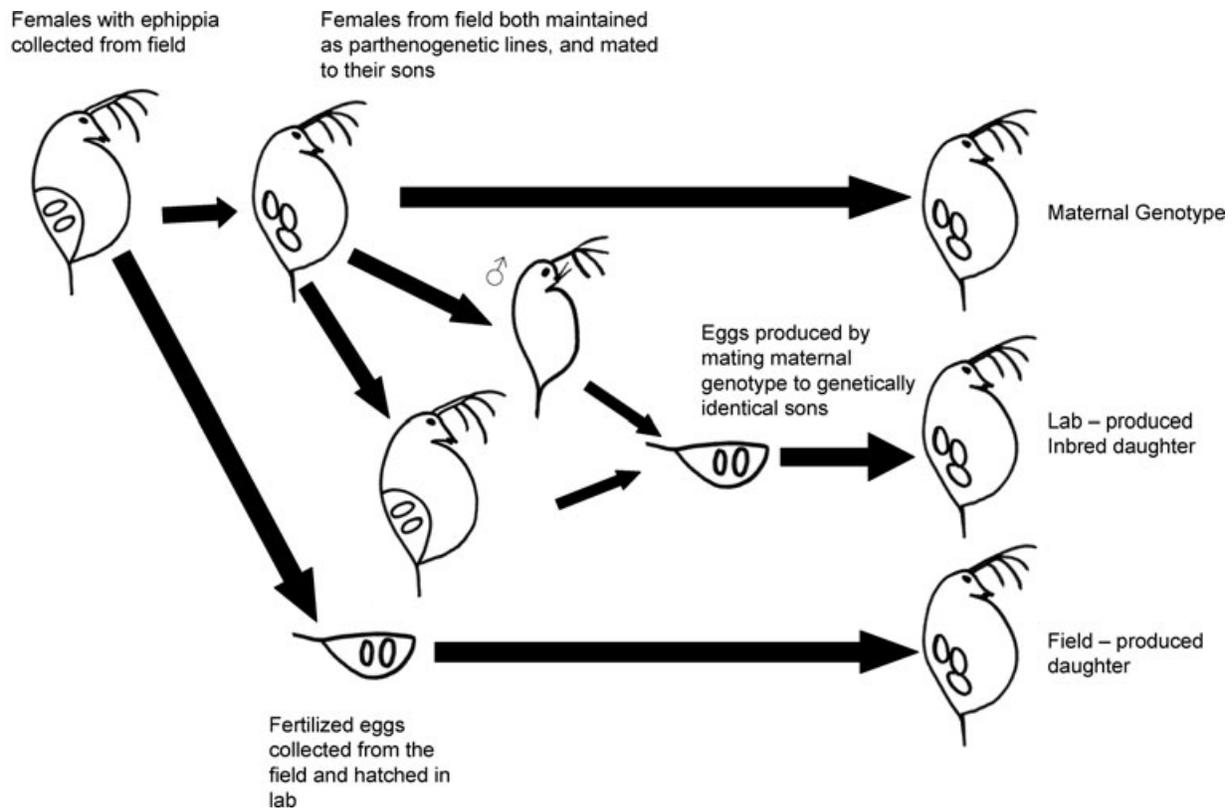


Figure 1. Our three breeding treatments (maternal, inbred, and field-produced genotypes) were established by field collections and laboratory crosses. Individuals used in the life table to estimate growth rates of the maternal genotype and field-produced daughter were descendants of animals collected from the field. Inbred offspring were created by mating descendants of the maternal genotype collected from the field to laboratory-produced males of the same genotype.

modified from Lynch et al. (1989). Briefly, we raised each sub-line under high-food conditions for three asexual generations. Neonates born from the third clutch or later of third generation females were raised individually in 110 mL of filtered lake water and fed 4×10^4 cell/mL *A. falcatus* every other day. We recorded time to maturity and timing and size of the first four clutches. For each genotype, our fitness metric was the average (across replicate sublimes) instantaneous rate of increase (r), which we calculated as an iterative solution to Euler's equation (Desmarais and Tessier 1999). We used two-way analysis of variance ANOVA (Systat 10, 2000) with lake and daughter type (field produced or inbred) to test the hypothesis that inbred genotypes would have lower growth rates than sexually produced offspring collected from the field.

We next asked the degree to which fitness changed following sexual reproduction. Fitness change following sex was calculated as $1 - (\text{offspring}/\text{maternal})$, which is analogous to the standard measurement of $1 - (\text{inbred}/\text{outbred})$ often used as a metric of inbreeding depression, (Charlesworth and Charlesworth 1987). With our design, however, we are only sure of the parentage of the inbred offspring we created in the laboratory (Fig. 1). The field-produced offspring may or may not be inbred, and the maternal genotype may or may not be inbred. We compared this

metric of fitness reduction among lakes and among breeding treatments (field produced vs. inbred) with ANOVA (Systat 10, 2000). Breeding treatment and lake were treated as fixed effects and family (lake) was included as a random term. Of the 56 families available when we started the life table, only 39 families provided data for all three genotypes. As a result, families that are missing values for one of the two treatments were excluded from this analysis. We used a paired t -test to compare the percent of families from each of four lake populations showing fitness reductions following sexual reproduction in either field-produced offspring or inbred offspring. Finally, regression (Systat 10, 2002) was used to test the hypothesis that inbreeding depression is more severe in populations with historically lower investments in sex. Average field estimate of sex were taken from Cáceres and Tessier (2004).

Results

On average, laboratory-produced inbred offspring had lower fitness (as measured by r) than did sexually produced offspring collected from the field ($F_{1,88} = 6.63$, $P = 0.012$, Table 1). Average clonal growth rates of the daughters did not differ significantly

Table 1. Average fitness (as measured by r) (\pm SE) was lower for inbred offspring than field-produced offspring in four populations of *D. pulicaria*. Lakes are arranged from lowest (Baker) to highest (Little Long) frequency of sexual reproduction. Within each lake, clonal lines served as replicates.

Lake	Field-produced daughter	Inbred daughter
Baker	0.234 (0.009)	0.195 (0.012)
Warner	0.206 (0.008)	0.181 (0.009)
3L2	0.211 (0.009)	0.206 (0.010)
Little Long	0.224 (0.014)	0.216 (0.011)
Average \pm 1(SE)	0.219 (0.005)	0.199 (0.006)

among lakes ($F_{3,88} = 2.40$, $P = 0.074$), nor was there a significant lake \times treatment interaction ($F_{3,88} = 1.11$, $P = 0.35$, Table 1).

Scaling the growth rates of these sexually produced offspring relative to the maternal genotype confirmed that the direction and magnitude of fitness change following sex depended on breeding treatment (Fig. 2; Treatment: $F_{1,35} = 4.46$, $P = 0.042$). In addition, fitness reduction following sex differed among lakes (Fig. 2; Lake- $F_{3,35} = 2.94$, $P = 0.047$). Using lakes as replicates, we found that a greater percentage of inbred offspring showed fitness reductions following sexual reproduction than did their field-produced siblings (Fig. 2, paired t -test, $t_3 = -4.18$, $P = 0.025$). Moreover, all inbred lines of the lowest-sex lake (Baker)

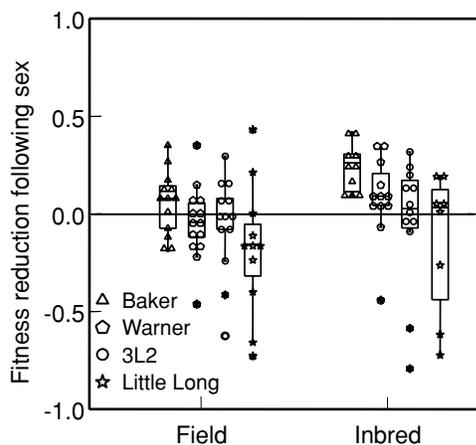


Figure 2. Estimates of the relative reduction in the instantaneous rate of increase (r) of sexually produced daughters (relative to the maternal genotype). Positive values indicate a lower r value for the daughter, negative values indicate that the daughter outperformed the mother. Field-produced offspring were collected from the field and therefore the sire is unknown. Inbred offspring were created in the laboratory by mating mothers to their genetically identical sons. Each point represents one mother-offspring comparison. Boxes are standard box-and-whisker plots with the central line indicating the median and the whiskers encompassing those points that fall within 1.5 interquartile ranges of the box edges. Outliers are indicated by circles and asterisks.

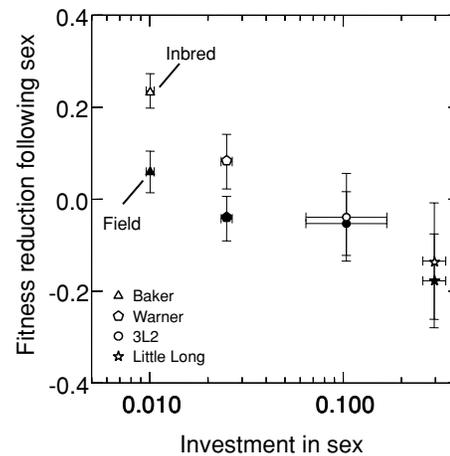


Figure 3. Relationship between the average population-level investment in sex in 1999–2001 (data from Cáceres and Tessier 2004) and the average fitness reduction following sex (inbred lines relative to the maternal genotype open symbols, field collected lines relative to the maternal genotype closed symbols) for genotypes collected from the same lakes in 2004. Error bars are 1 SE. Investment in sex was estimated by calculating the area under the plot of the fraction of clutches that were sexual on each sampling date and adding that to the similarly calculated number for male production. Numbers are scaled to a per-day basis (see Cáceres and Tessier 2004 for further details).

had positive values of inbreeding depression whereas only 45% of families from the highest-sex lake (Little Long) showed fitness reductions following selfing (Fig. 2).

Although our ANOVA did not detect a significant treatment \times lake interaction ($F_{3,35} = 0.98$, $P = 0.41$), Figure 3 hints at possible differences in the effect of sexual reproduction in general, and inbreeding in particular, among the four lakes. In the lowest-sex lake (Baker), both inbred and field-collected offspring show fitness reductions following sex, with the average decline in inbred offspring being almost four times that of the average decline seen in field-produced offspring. In lakes with increased investment in sexual reproduction, not only is there a lower magnitude of inbreeding depression (open symbols) but there is less of a difference between inbred and field collected offspring. In fact, in all but Baker Lake, many of the sexually produced daughters had higher growth rates than their mothers (results in a negative value of fitness reduction). To test the hypothesis that inbreeding depression is in fact more severe in lower-sex lakes, we calculated an effect value for each family by subtracting the estimate of fitness reduction in the field-produced daughter from that of the inbred-daughter. If inbreeding is more severe, on average, in populations with a lower investment in sex, then we would predict a significant negative relationship between our average calculated effect value and sex investment (from low to high). This is, in fact, the relationship we found ($r^2 = 0.995$, $n = 4$, $P = 0.005$).

Discussion

Our results demonstrate that inbreeding depression in these facultatively parthenogenetic populations varies along a gradient of investment in sexual reproduction. Inbred genotypes from Baker Lake (low sex) had the highest average inbreeding depression, with all 10 families experiencing fitness reductions. In contrast, individuals from populations with increasing investments in sex showed reduced inbreeding depression. Moreover, the simultaneous estimates of fitness obtained for field-produced offspring suggest that this pattern is not simply a result of sexual reproduction. Within each lake, more families showed fitness reductions following inbreeding than did the field-produced offspring, suggesting that females are often involved in outcross mating.

We recognize that our estimates of inbreeding include biases of selective sampling; only females engaging in sexual reproduction in the field were included, both the inbred and field eggs had to hatch in the laboratory and all three genotypes had to survive in culture before they could be assayed. As a result of this bias, our estimate can be interpreted as a lower bound of the effect of inbreeding depression in these populations. Inbred eggs often have reduced viability (Innes 1989; De Meester 1993) suggesting that if anything, inbreeding effects are even more severe, because effects that occur at the early stage are often more difficult to assess than at later stages (Keller and Waller 2002). Moreover, the sampling bias was likely most extreme in the lower sex lakes as females that were engaging in sex were rare, and therefore less representative of the majority of the population that continued parthenogenetic reproduction.

Although inbreeding depression and clonal variation in investment in sex and gender allocation are well known for *Daphnia* (e.g., De Meester 1993; Deng and Lynch 1997; Innes and Singleton 2000), the link between inbreeding depression and mating system variation in facultative parthenogens has not always been explicit. Do the potential costs of inbreeding influence the mating system variation seen in *Daphnia*? Our results suggest that they might. Variation among *Daphnia* genotypes in allocation to sex translates into among-clone variation in the number of asexual generations that the maternal genotype collected from the field has experienced. Innes (1989) suggested that the variable load that he found among inbred lines of *D. obtusa* was due to variation in the age of the maternal genotype. However, it has been argued that inbreeding in *Daphnia* in nature is quite rare (e.g., De Meester 1993; Deng and Lynch 1997). Although this may be the case in some populations, it is not necessarily the general rule (e.g., Haag et al. 2005). Moreover, because little is known about mating behavior in *Daphnia* (but see Winsor and Innes 2002), and populations clearly differ in population size and spatial and temporal aggregation or separation of clones, it is difficult to predict a priori the expected level of inbreeding in a given population.

Further evidence for the role of inbreeding depression on mating system evolution in *Daphnia* comes from Innes and Dunbrack (1993). They documented the existence of nonmale-producing genotypes in *D. pulex* and suggested that many populations may be the animal equivalent of gynodioecious plants. Their model suggested that the nonmale-producing trait could be maintained if there was a negative effect of inbreeding on male-producing genotypes. We have conducted two separate assays of gender allocation in these populations, both of which suggest the existence of nonmale-producing genotypes. Tessier and Cáceres (2004) exposed multiple genotypes to conditions such as crowding and temperature-photoperiod conditions known to induce sexual reproduction. In that assay, the frequency of clones that did not make males under the experimental conditions ranged from a low of 6% in the population from Little Long Lake to a high of 56% in Warner Lake. Using a recently developed assay for male-function (Olmstead and Leblanc 2002), we confirmed that some genotypes from these populations have indeed lost the ability to produce males (up to 24% of clones sampled from some of these populations are nonmale producing, C. E. Cáceres, unpubl. data). Because our results suggest that inbreeding depression is higher in populations with a lower investment in sexual reproduction, it seems plausible that a correlation exists between gender specialization and inbreeding risk, such that the frequency of gender specialization among clones will increase in systems with an increased risk of inbreeding.

This study was motivated in part by the striking gradient in allocation to sexual reproduction across 12 populations of *D. pulicaria* (Cáceres and Tessier 2004). As with the cold-tolerant sexually produced eggs of aphids (Simon et al. 2002), part of that variation is ecological; risks in the water column vary across habitats and sex is the only way to produce a form resistant to the adverse environmental conditions. But the production of the dormant stage is not a complete story, hence additional hypotheses for this variation in allocation to sexual reproduction need to be explored. Although asexual reproduction may allow a genotype to dominate in the short term, our documented variation in inbreeding depression also indicates a cost to engaging in long-term asexual reproduction; deleterious mutations are accumulating during the parthenogenetic phase (Lynch et al. 1998; Zeyl et al. 2001). Therefore, going too long without sex may at some point reduce the advantage of asexuality gained via clonal competition. For populations or clones in which sexual reproduction is infrequent, the cost of inbreeding depression becomes a stronger selective force. Therefore, among-lake differences in ecological interactions likely promote clonal variation in gender allocation to reduce inbreeding when sex does occur (De Meester and Vanoverbeke 1999; Innes and Singleton 2000; Tessier and Cáceres 2004). Additionally, Allen and Lynch (2008) used four *Daphnia* populations that vary in sex (three were included in

both studies) and also found that costs associated with sexual reproduction increased with decreased allocation to sexual reproduction. Similar to our results, they found that sex was costly in lakes such as Baker because field-produced offspring showed severe reductions in fitness-related traits relative to their mother. They also measured changes in genetic variance following sex. Here, they found that the benefits of sex, in terms of increased genetic variation, were also higher in low sex lakes. In short, the results from both of these studies illustrate the complex relationship between fitness and allocation to sex in these populations of cyclical parthenogens. Understanding the balance between these various short- and long-term aspects of sexual reproduction in cyclical parthenogens is the focus of ongoing research regarding implications for mating-system evolution.

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