

STRONG INBREEDING DEPRESSION IN A *DAPHNIA* METAPOPOPULATION

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Abstract.—The deleterious effects of inbreeding have long been known, and inbreeding can increase the risk of extinction for local populations in metapopulations. However, other consequences of inbreeding in metapopulations are still not well understood. Here we show the presence of strong inbreeding depression in a rockpool metapopulation of the planktonic freshwater crustacean *Daphnia magna*, which reproduces by cyclical parthenogenesis. We conducted three experiments in real and artificial rockpools to quantify components of inbreeding depression in the presence and the absence of competition between clonal lines of selfed and outcrossed genotypes. In replicated asexual populations, we recorded strong selection against clones produced by selfing in competition with clones produced by outcrossing. In contrast, inbreeding depression was much weaker in single-clone populations, that is, in the absence of competition between inbred and outbred clones. The finding of a competitive advantage of the outbred genotypes in this metapopulation suggests that if rockpool populations are inbred, hybrid offspring resulting from crosses between immigrants and local genotypes might have a strong selective advantage. This would increase the effective gene flow in the metapopulation. However, the finding of low inbreeding depression in the monoclonal populations suggests that inbred and outbred genotypes might have about equal chances of establishing new populations.

Key words.—Clonal competition, heterosis, migration, rapid evolution, selfing.

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Inbreeding depression, the reduced fitness of offspring resulting from mating between relatives, has many evolutionary implications. It is thought to be important for the evolution of mating systems (Charlesworth and Charlesworth 1987; Jarne and Charlesworth 1993), as well as for a wide range of traits thought to promote inbreeding avoidance (Forster Blouin and Blouin 1988; Jarne and Charlesworth 1993; Pusey and Wolf 1996). A population's risk of extinction may also be affected by inbreeding depression (Lande 1988, 1994; Hedrick 1994; Frankham 1995; Lynch et al. 1995; Newman and Pilson 1997; Frankham 1998; Saccheri et al. 1998). In metapopulations local populations may be small, and genetic bottlenecks during recolonization events may reduce the effective population size even further. If this is the case, immigration might reduce the extinction risk of local populations (Westemeier et al. 1998; Madsen et al. 1999; Richards 2000) and increase the effective gene flow between populations (Ingvarsson and Whitlock 2000; Ebert et al. 2002). This, in turn, might promote the maintenance of genetic diversity (Charlesworth et al. 1997) and reduce population differentiation (Pamilo et al. 1999; Whitlock et al. 2000).

There is ample evidence that inbreeding depression is a common phenomenon in natural populations (Charlesworth and Charlesworth 1987; Husband and Schemske 1996; Crnokrak and Roff 1999). But its strength depends on the number of deleterious alleles and on the distribution of selection coefficients against them. The latter may be a function of the environment. For example, selection coefficients may be larger under natural conditions than in the laboratory (e.g., Dudash 1990; Chen 1993; Jimenez et al. 1994; but see Armbruster et al. 2000), or could be increased by stressful environmental situations (e.g., Keller et al. 1994; Bijlsma et al. 1999, 2000; Coltman et al. 1999). In particular, it has been

suggested that competition increases the expression of inbreeding depression (Wolfe 1993; López Bueno et al. 1993; Latter and Sved 1994; Carr and Dudash 1995; Cheptou et al. 2000; Meagher et al. 2000). However, the generality of these results is not yet clear. To understand the role inbreeding depression plays in populations with frequent inbreeding, it is therefore important to assess its consequences under natural conditions and in the presence and absence of competition.

The aim of this study was to assess the fitness of outbred clones relative to inbred clones in a *Daphnia* metapopulation with possibly high levels of inbreeding and to assess whether the strength of inbreeding depression under competitive conditions depends on the presence of outbred competitors. To answer these questions, we conducted experiments by manipulating entire populations (natural and seminatural), with the unit of replication being the local population.

The Metapopulation System

We investigated inbreeding depression in a rockpool metapopulation of *Daphnia magna*, a filter-feeding freshwater crustacean (Cladocera) that inhabits small to medium pools. *Daphnia magna* is the largest European species of the genus, reaching an adult size of up to 5 mm. It reproduces by cyclical parthenogenesis, in which phases of asexual reproduction are intermitted by sexual generations. During sexual reproduction, resting stages (ephippia) are produced, and ephippia can only be produced sexually. They usually contain two eggs (but often only one is viable) that are resistant to drought and freezing and act as a dispersal stage, carried by wind, water, and birds (Ranta 1979).

Rockpools are small water-filled depressions in the bare rock and are often found along the Baltic Sea coast. They are a patchily distributed and discrete environment, and mostly freeze solid in winter (Ranta 1979; Pajunen 1986; Bengtsson 1989, 1991; Bengtsson and Ebert 1998). *Daphnia* survive

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the winters in rockpools as ephippia. In spring, females hatch and start to reproduce by parthenogenesis. Because the resting eggs of *D. magna* have been formed by sexual reproduction, each of these ex-ephippial females represents one genetically unique individual and is thus a founder of a unique clone. These clones can quickly form populations of more than 10^5 individuals. There are typically about eight to 12 asexual generations per season, but only one sexual generation. The production of males and sexual females is triggered by the environment (Hobæk and Larsson 1990; Kleiven et al. 1992), and the sexually produced resting eggs hatch only after a resting period. Because males are produced asexually (Hebert and Ward 1972), mating within a clone is genetically equivalent to selfing.

Daphnia rockpool populations form a metapopulation system with discrete habitat patches and with frequent extinctions and colonizations (Ranta 1979, 1982; Hanski and Ranta 1983; Pajunen 1986; Bengtsson 1989, 1991; Bengtsson and Ebert 1998; Ebert et al. 2001, 2002). In any given year, about 20% of all rockpools in our study area contain *D. magna* populations. These populations have a yearly extinction rate of about 20% (Pajunen 1986; unpubl. data covering 19 years). The number of local extinctions is on average balanced by the number of new colonizations, that is, the probability of colonization of an empty pool is about 5% per year (Pajunen 1986). Because *Daphnia* reproduce clonally, colonization may happen only by one or a few individuals, most likely as a result of dispersing ephippia. Thus, new populations might go through severe genetic bottlenecks, sometimes as small as being founded by a single clone or a pair of full-sibling clones from the same ephippium. This observation is supported by electrophoretic data. In 1998, about half of the populations were fixed for one multilocus genotype (five allozyme loci, polymorphic at the level of the metapopulation). Furthermore, 26 of 29 newly founded populations were monomorphic and five of these were fixed for a heterozygous genotype (Ebert et al. 2002).

When a single clone founds a new population, selfing is unavoidable for the production of resting eggs to survive the winter. Even with less severe bottlenecks, inbreeding is expected to occur as a consequence of founder effects.

The finding that inbreeding seems to be common in the rockpool *D. magna* metapopulation raised the question about the consequences of inbreeding in local rockpool populations. In this study we investigated two scenarios. First, we investigated inbreeding depression as measured by the success of inbred clones relative to outbred clones when in competition with each other. Second, we studied inbreeding depression in single-clone populations by comparing the number of ephippia produced by inbred and outbred monoclonal populations. The first scenario could arise after two unrelated clones colonized an empty pool or after the immigration of a clone into an inbred population of *D. magna*. The second scenario arises if a pool is colonized by a single clone, whose success might depend on whether it is inbred or outbred.

MATERIALS AND METHODS

The Study Area

Our study area lies in the archipelago of southern Finland at Tvärminne on the Hanko Peninsula (59°50'N, 23°15'E).

The area is about 30 km² and contains more than 60 islands with rockpools harboring *D. magna* populations. The number of populations per island varies from one to more than 30.

Experiment 1: Competition between Inbred and Outbred Clones in Natural Rockpools

In 1998, samples of *D. magna* were taken from 12 pools on nine islands. Single females were used to create clonal cultures in the laboratory (isofemale lines). Each clone was genetically characterized for five polymorphic allozyme markers using cellulose acetate electrophoresis (Hebert and Beaton 1993). These five loci were aspartate amino transferase (*Aat*, enzyme commission number EC 2.6.1.1), fumarate hydratase (*Fum*, 4.2.1.2), glucose-6-phosphate isomerase (*Gpi*, 5.3.1.9), lactate dehydrogenase (*Ldh*, 1.1.1.27), and phosphoglucomutase (*Pgm*, 5.4.2.2).

Twenty-two natural rockpools on six islands were chosen for the experiment. These rockpools either had no *D. magna* populations or the resident populations were removed. In the latter case, the pools were emptied and the sediment containing the ephippia (resting eggs) of the resident populations was removed. The pools were then left to be refilled by rainwater. In no case was the experimental pool identical to the pools from which we obtained the clones for the experiment.

In August 1998, about 200 individuals from each of two clones from our laboratory cultures were released into each of the 22 experimental pools. In all cases the two clones released together had no common alleles at one or more of the marker loci. They originated from pools on different islands, separated by distances of 1100–7000 m. Each pair of clones was released into two experimental pools. Until the end of the season they grew asexually and underwent sexual reproduction under natural conditions. Sexual matings occurred within and between clones, and thus inbred as well as outbred resting eggs were produced. Because inbred eggs were produced by mating between genetically identical males and females, the inbreeding coefficient, F , of the inbred offspring is 0.5, equivalent to one generation of selfing, or higher, depending on the inbreeding history of the parent clones. In contrast, all outbred offspring have $F = 0$ (see also Fig. 1).

In May 1999, *D. magna* were present in eight of the 22 experimental rockpools. In all populations we found a bimodal body size distribution, probably resulting from the fact that the animals present were either fully grown hatchlings of resting eggs (ex-ephippial females) or their first asexual offspring. Because the two experimental clones (parent clones) that had been released together into a pool did not have common alleles at least at one of the five marker loci (the diagnostic locus), offspring could be identified, because outbred offspring were heterozygous at this locus for alleles from both parents, whereas inbred offspring were homozygous or heterozygous for alleles from only one parent (see also Fig. 1). Even though we had cleaned the pools, we found in two populations formerly resident genotypes and crosses between these and our experimental clones. These genotypes could be distinguished, because, according to previous screening of the formerly resident populations, they did not share marker alleles with the introduced genotypes. These

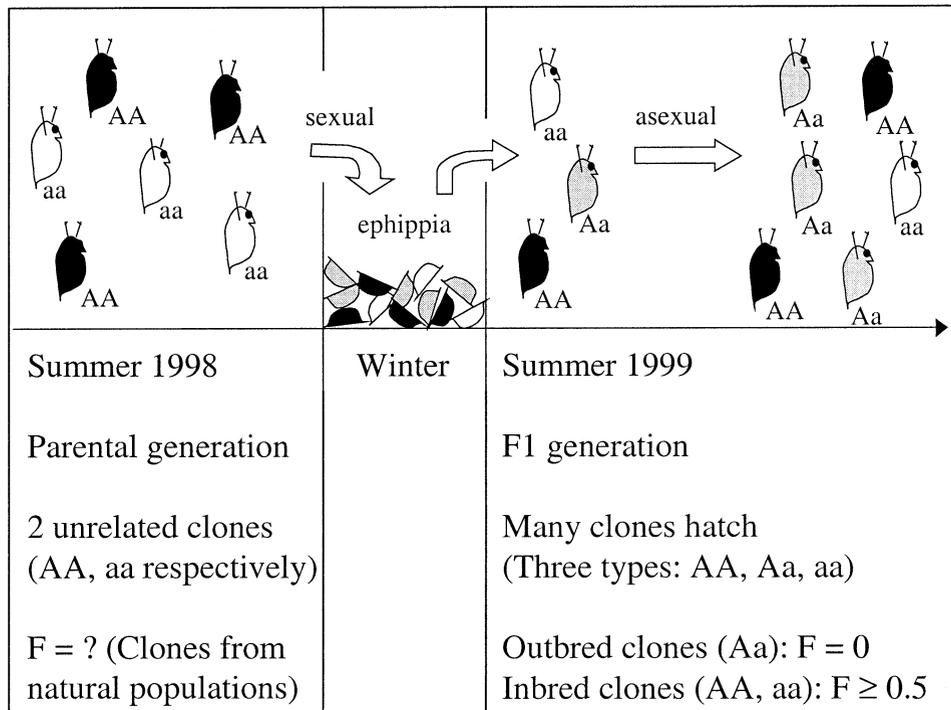


FIG. 1. Design of experiment 1, showing the changes in one of the experimental pools over time. On the right side (year 1999) the black and the white genotypes are inbred, and the gray genotypes are outbred. The genotype at the marker locus is given for every individual. For simplicity, a situation is shown in which both parental clones were homozygous at the marker locus. However, even in cases when the parental clones were heterozygous, the F_1 types could be distinguished, because parental clones never shared alleles (see text).

genotypes were excluded from the analysis. In four other populations, at least one allele at each locus was shared between the formerly resident population and one of the introduced clones, and thus contamination could not be detected. However, because the other introduced clone was in all cases distinct from the formerly resident population, the genotypes classified as outbred were indeed outbred ($F = 0$). Comparing the frequency of the outbred with the frequency of the inbred clones could therefore at most underestimate the effect of inbreeding.

We screened random samples of 65 to 182 ex-ephippial females from all eight populations for the diagnostic loci to establish the frequencies of inbred and outbred hatchlings. From the allele frequencies, we calculated the expected frequencies of the three classes of genotypes (one outbred class and one inbred class of each of the two parent clones) assuming Hardy-Weinberg frequencies. In all pools the number of ex-ephippial females was more than 1000, except for one, in which there were only about 200 ex-ephippial females present. In this case, we took a sample to the laboratory, raised an isofemale line from each female, and carried out electrophoresis on their (genetically identical) offspring. The females, together with most of their offspring were then put back in the field. Note that even though there were only three classes of genotypes present in these pools, each ex-ephippial female is produced by sexual reproduction and is therefore unique.

In mid-July and in the end of August 1999, we again screened random samples from each of the eight populations.

The population sizes at these sampling dates were higher than 10^5 and the sample sizes were 54–154 individuals per population in July and 60–235 in August. The total time span between the first and the last sample was 102 days, which is about two-thirds of the whole season. The inbreeding coefficient of the three classes of genotypes did not change during this period because all individuals present in the population were asexual offspring of the ex-ephippial females (also true for the other two experiments). The set-up of experiment 1 is shown in Figure 1.

During the establishment of inbred and outbred isofemale lines from the May sample (see below), we found that some females produced no or only very few (<5) asexual eggs, whereas most females produced large clutches (>5, but mostly >20 eggs). These low-fecundity females (which were of about the same size as the high-fecundity females) were mostly inbred females. We investigated this unplanned observation further by comparing more females with high and low fecundity from our cloning procedure and by comparing the genotypes of females with and without eggs taken from an additional field sample obtained from one of the populations (ALO-11).

Experiment 2: Competition between Inbred and Outbred Sister-Clones in Artificial Pools

Because of the possibility of confounding or biasing effects of contamination with resident clones and to facilitate comparison with experiment 3, we carried out a competition ex-

periment under seminatural conditions in artificial pools. For this experiment, some of the ex-ephippial females collected in May 1999 were cloned before electrophoresis to obtain inbred and outbred clones from our experimental populations, from which we selected 12 pairs of clones. Each pair consisted of one inbred and one outbred sister-clone. Only seven of these pairs were completely independent, that is, did not share a parent between pairs. Therefore, the analysis was carried out on the complete as well as on a reduced set of data consisting of only these seven pairs.

On 12 July 1999, 200 individuals of each of the clones of a pair were released together into a 10-L plastic bucket, containing 6 L of 20- μ m-filtered rockpool water and placed on one of the islands next to natural rockpools. One week later (19 July) 72 individuals from each bucket were screened electrophoretically. The populations then grew and competed for 2 months (57 days). No food was added, but population size remained over 100, except for one population. On 13 September, another sample of 78–288 individuals per pair was screened. The frequency of the outbred clone in each pair on 13 September was compared with its frequency on 19 July.

Experiment 3: Inbred and Outbred Single Clone Populations in Artificial Pools

To investigate the success of inbred and outbred single-clone populations (i.e., without competition from other clones), we compared their performance by measuring ephippia production in the same type of artificial pools as in experiment 2. The set-up differed from experiment 2 only in a few details. To include possible effects of the initial growth phase, populations were started with only five adult females. The experiment was also run for a longer period, from the end of June until the end of September (86 days), to span most of the period during which ephippia are produced in natural populations. In this experiment it was especially important to use containers placed in the field, because ephippia production requires natural stimuli for the induction of sexuality. At the end of September, the accumulated sediment on the bottom of the buckets was collected and ephippia were counted. To facilitate counting of ephippia, buckets were covered with perforated, light-transparent gauze throughout the experiment, which permitted small organic particles and therefore nutrients to enter, but prevented entry of blown-in sediment. We selected 11 outbred and 14 inbred parasite-free clones for this experiment and used four replicate buckets per clone. Ten pairs of the inbred and outbred clones shared a parent; thus, these data points are not strictly independent.

The effects of the clone and the breeding status (inbred or outbred) were evaluated using a nested ANOVA. Ephippia counts were ln-transformed to meet the assumptions of ANOVA. A Satterthwaite correction was employed because of the unbalanced nature of the data. To further test for an effect of the parent clone (some of the clones shared one parent), we analyzed the correlation in the ephippia counts between the inbred and outbred clones in 10 matched pairs. For this dataset, the effect of inbreeding was analyzed by determining whether the outbred clone of each pair produced more ephippia than the inbred clone, using a paired *t*-test.

Analysis of Inbreeding Depression and Measurement of Fitness

Inbreeding depression was measured in all three experiments as

$$\delta = 1 - \frac{\bar{w}_{in}}{\bar{w}_{out}} \quad (1)$$

(Lande and Schemske 1985), where \bar{w}_{in} and \bar{w}_{out} represent the mean fitness of inbred and outbred clones respectively. In experiments 1 and 2, fitness was measured by a formula linking relative fitness to change in frequency during clonal competition (Hartl and Clark 1997):

$$t \cdot \ln(w) = \ln\left(\frac{out_t}{in_t}\right) - \ln\left(\frac{out_0}{in_0}\right). \quad (2)$$

Here, w is the fitness of the outbred relative to the inbred clone and out_t , out_0 , in_t , and in_0 are the frequencies of the outbred and the inbred clones at times t and 0, respectively. From this formula the relative fitness per unit time can be calculated and the δ -value is easily obtained from the relative fitness ($\delta = 1 - [1/w]$). For experiment 3, the fitness of a clone was measured by the mean number of ephippia produced (Taylor and Gabriel 1993). Means for each clone were calculated using ln-transformed ephippia counts, because of the log-normal distribution of the original data. δ -values were calculated for every population (experiment 1), pair (experiment 2), or matched pair (experiment 3). Standard errors for the mean inbreeding depression were calculated across the populations or pairs.

To compare inbreeding depression among the three experiments, the relative fitness values were scaled to a time interval of one day according to equation (2). However, it was not possible to scale δ -values for those populations in which the inbred clones became extinct (measured δ -value = 1.0). In these cases we used $1/n$ (n , number of *Daphnia* genotyped) as an estimator of the frequency of the inbred clone, which will lower the estimate of inbreeding depression.

Because inbreeding depression increases with increasing inbreeding coefficients (F) of the inbred individuals (Falconer and Mackay 1996), a corrected value of inbreeding depression, $b_{w_{out}}$ (Crnokrak and Roff 1999), was obtained by dividing δ by the inbreeding coefficient, which was always ≥ 0.5 (one generation of selfing) in our experiments. This was done to facilitate comparison with other studies.

The statistical analysis was carried out using the program JMP-IN (SAS Institute 1999). For the ANOVA we used SAS proc GLM and proc VARCOMP (SAS Institute 1990). Frequency data were arcsine-transformed to meet the assumptions of parametric tests.

RESULTS

Experiment 1: Competition between Inbred and Outbred Clones in Natural Rockpools

Genotype frequencies of first generation (ex-ephippial) females from the eight experimental rockpools revealed no consistent deviation from Hardy-Weinberg expectations. In two pools, there was an excess of outbred clones, and in one pool, inbred clones were more frequent. These comparisons re-

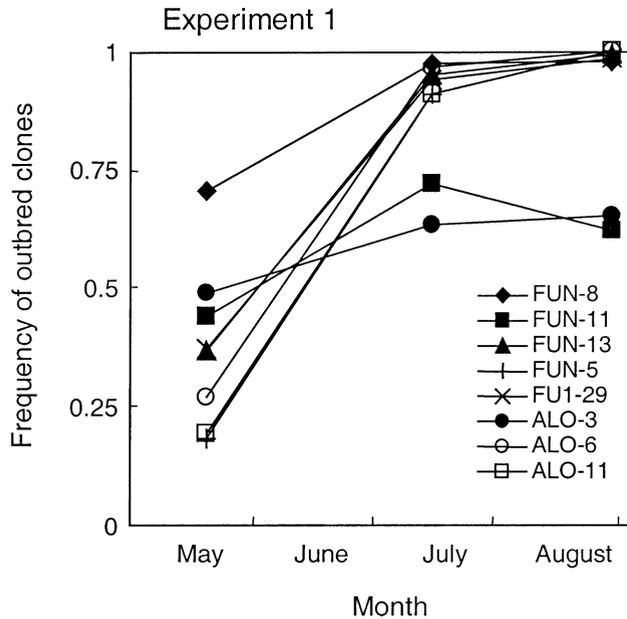


FIG. 2. Changes in frequencies of outbred clones in each population of experiment 1. Sampling dates were 20 May, 16 July, and 30 August 1999.

mained significant after correcting for multiple tests. Averaged over all populations, genotype frequencies did not deviate from Hardy-Weinberg expectations.

During the course of the season, outbred clones increased in frequency in all eight populations and became fixed in three of them (Fig. 2). The mean \pm SE frequency rose from 0.377 ± 0.061 in May to 0.875 ± 0.045 in July to 0.904 ± 0.058 in August. The increase from May to July as well as from July to August was significant (paired t -tests, first interval: $t = 6.18$, $P = 0.0005$, second interval: $t = 2.42$, $P = 0.046$).

The mean \pm SE observed inbreeding depression δ was 0.86 ± 0.08 for the entire period (102 days, Table 1). The per-day fitness of the outbred clones relative to the inbred clones was 1.045. Thus, outbred clones had an average fitness advantage of 4.5% per day (95% confidence interval = 2.3–6.7%).

In the cloning procedure as well as in the additional field sample from population ALO-11, inbred females exhibited reproductive problems more often than outbred females (cloning procedure: 14.4% vs. 3.2%, Fisher's exact test, $P = 0.0199$; ALO-11: 24.4% vs. <4.8%, $P = 0.0074$; Table 2).

TABLE 1. Fitness (w) of outbred relative to inbred individuals, coefficients of inbreeding depression (δ), and standardized coefficient of inbreeding depressions ($b_{w_{out}} = \delta/F$) for the three experiments. The values are given both for the duration of the experiment and per day. The standard errors (in parentheses) and significance are only given for the δ -values. The duration of the three experiments is given. Under natural conditions one asexual generation of *Daphnia magna* lasts about 10–15 days.

	Duration of experiment				Per day		
	w	δ (SE)	$b_{w_{out}}$	days	w	δ (SE)	$b_{w_{out}}$
Experiment 1 mean	27.01	0.861 (0.077)****	1.72	102	1.045	0.043 (0.009)****	0.086
Experiment 2 mean	5.85	0.668 (0.100)****	1.34	56	1.032	0.031 (0.007)****	0.062
Experiment 3 mean	1.611	0.207 (0.159)	0.41	86	1.006	0.006 (0.009)	0.011

**** $P < 0.0001$.

TABLE 2. Numbers of high-fertility and low-fertility females among the inbred and outbred females in the first sample in experiment 1. According to Fisher's exact test, $P = 0.0079$ for population ALO-11, $P = 0.0199$ for the other populations. Females were classified as low fertility if they did not carry eggs in their brood pouch at the time of sampling (ALO-11) or if they produced less than six offspring in the cloning procedure (other populations). Contributions by populations: ALO-3: 10 inbred, six outbred; ALO-6: 12 inbred, three outbred; FU1-29: 20 inbred, four outbred; FUN-5: 15 inbred, two outbred; FUN-8: seven inbred, 23 outbred; FUN-11: 10 inbred, four outbred; FUN-14: 37 inbred, 21 outbred.

	High fertility	Low fertility	Total
Population ALO-11			
Inbred	90	29	119
Outbred	21	0	21
Total	111	29	140
Other populations			
Inbred	95	16	111
Outbred	61	2	63
Total	156	18	174

Experiment 2: Competition between Inbred and Outbred Sister-Clones in Artificial Pools

During the 56 days of this experiment, the frequency of the outbred clone increased in 11 of 12 pairs. Overall, their mean \pm SE frequency was 0.55 ± 0.04 in July and 0.80 ± 0.05 in September (paired t -test, $t = 5.37$, $df = 11$, $P = 0.0002$; Fig. 3). The increase in frequency of the outbred clones in the seven completely independent pairs was still highly significant (paired $t = 4.31$, $df = 6$, $P = 0.005$).

The mean \pm SE inbreeding depression δ was 0.67 ± 0.10 (Table 1), with a 3.2% (95% confidence interval = 1.6–4.8%) mean daily advantage of outbred clones. The estimate of inbreeding depression in this experiment did not differ significantly from the one obtained in experiment 1 ($t = 1.05$, $df = 18$, $P = 0.31$).

Experiment 3: Inbred and Outbred Single Clone Populations in Artificial Pools

Single-clone populations produced between zero and 1310 (median = 94) ephippia (resting stages) during the 86 days of the experiment. The means of the clones differed substantially (ranging from 6.4 to 500.7), and the effect of clone in a nested ANOVA was highly significant ($F_{23,74} = 2.88$, $P = 0.0003$; Fig. 4) and explained 32% of the total variance. However, the means of the inbred and outbred clones did not differ significantly (mean of outbred clones = 96.0, mean of inbred clones = 72.2, $F_{1,23.01} = 0.503$, $P = 0.486$; Fig. 4).

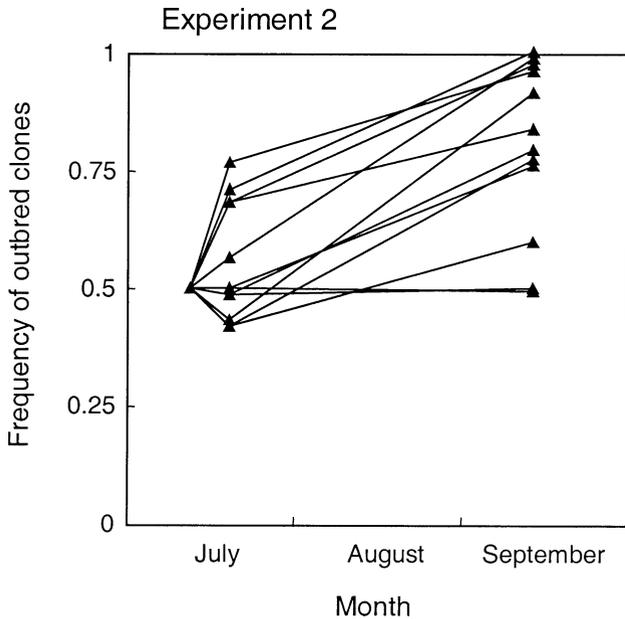


FIG. 3. Changes in frequencies of outbred clones in experiment 2. Sampling dates were 19 July and 13 September 1999. The experiment was started on 12 July 1999.

This difference in means was mainly caused by two (of 14) inbred clones with exceptionally low means and one (of 11) outbred clone with an exceptionally high mean (Fig. 4).

Ephippia counts of inbred and outbred clones within matched pairs correlated positively ($r_s = 0.55$, $n = 10$, $P = 0.10$). Even though the correlation was not significant, it seemed more powerful to calculate the effects of inbreeding based on matched pairs. However, the difference between the inbred and outbred clones of a pair was not significant (paired t -test, $t = 1.86$, $df = 9$, $P = 0.096$).

In this experiment, the mean \pm SE inbreeding depression δ , based on the matched pairs, was 0.21 ± 0.16 (Table 1). The daily advantage of the outbred genotypes was 0.6% (95% confidence interval = -0.01 – 1.2%). Estimates based on inbred and outbred clones treated as independent observations yielded very similar, although slightly lower, values (not shown). The inbreeding depression, δ , in this experiment was significantly lower than in experiments 1 and 2 (t -test with unequal variances, comparison with experiment 1: $t = 3.85$, $df = 16$, $P = 0.0027$; with experiment 2: $t = 3.39$, $df = 20$, $P = 0.0032$).

DISCUSSION

We found strong inbreeding depression in the presence of competition between inbred and outbred genotypes. In experiments 1 and 2, outbred genotypes increased in frequency very rapidly, in many cases eliminating the inbred clone or nearly so during a short period. Inbreeding depression was caused at least partially by the reduced fertility of inbred genotypes. In contrast, inbreeding depression was much weaker in the absence of competition with outbred clones.

Strong Inbreeding Depression in the Presence of Competition with Outbred Clones

When inbred and outbred clones competed (experiments 1 and 2), estimates of inbreeding depression were among the highest observed in natural populations (Crnokrak and Roff 1999). Even after correcting δ for our relatively high inbreeding coefficient of $F = 0.5$, we obtained very high standardized estimates of inbreeding depression (Table 1).

Moderate to high levels of inbreeding depression have also been found in several other cyclical parthenogens, mostly *Daphnia* (Birky 1967; Innes 1989; De Meester 1993; Innes and Dunbrack 1993; Lynch and Deng 1994; Deng 1997; Deng

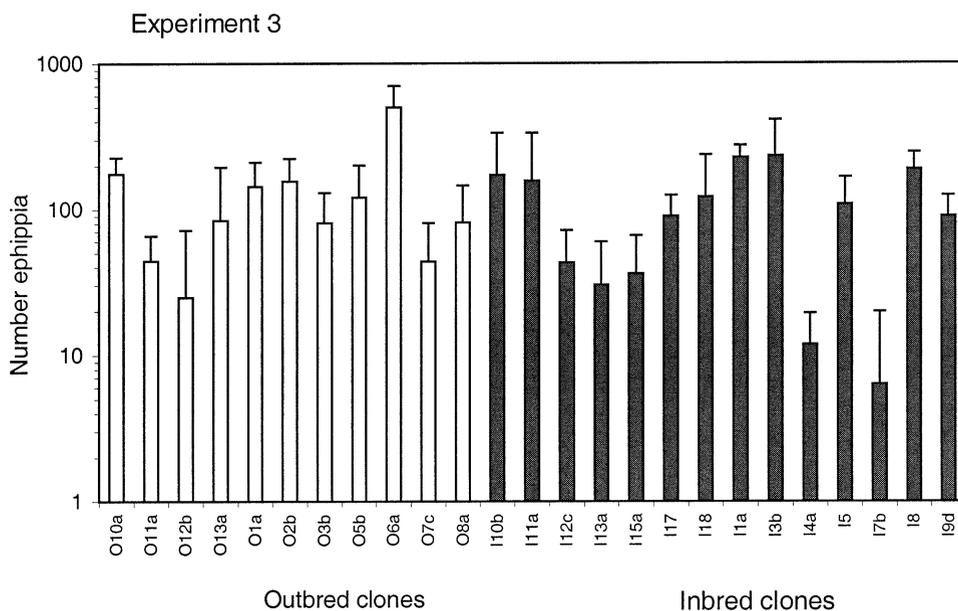


FIG. 4. Mean numbers of ephippia produced by each clone in experiment 3. Error bars are ± 1 SE. Means and standard errors are calculated on log-transformed ephippia counts. The y-axis is presented on a log-scale.

and Lynch 1997). Furthermore, theoretical work by Muirhead and Lande (1997) suggests that inbreeding depression in cyclical parthenogens is expected to be high, mainly due to accumulation of recessive mutations during the asexual generations, followed by only infrequent sexual recombination. It is not known whether inbreeding depression in cyclical parthenogens is indeed higher than in obligate sexuals, but if so, this might be an explanation for the high levels of inbreeding depression found in this study.

In experiment 1, two populations (ALO-3 and FUN-11; see Fig. 2) showed much lower levels of inbreeding depression than the others. We can only speculate about the reasons for this. In the case of ALO-3, there might have been a contamination with formerly resident genotypes, and some of the individuals classified as inbred might actually have been crosses between one of the experimental clones and formerly resident genotypes. FUN-11, on the other hand, was the smallest of all populations and once almost dried out. This might have led to a bottleneck, restricting the number of clones in competition and thus increasing the possibility of chance events.

The outbred individuals in our experiments were produced by crossing clones from different pools. The offspring of such crosses are sometimes referred to as hybrids rather than outbred individuals. Thus, we consider the whole metapopulation as the base population for estimating inbreeding coefficients and inbreeding depression. From an ecological point of view, this is appropriate because we are interested in between-population processes and because inbreeding in our metapopulation probably occurs after a bottleneck and not as a consequence of nonrandom mating within populations.

Reduced Fertility of Inbred Lines

The proportion of high-fertility females in our May sample was reduced by about 20% among the inbred females (Table 2). Similarly, Deng (1997) and Deng and Lynch (1997) reported an inbreeding depression in fertility in three species of *Daphnia* with a δ of around 0.25. The reduction of fertility is probably at least partially responsible for the observed inbreeding depression in our experiments. However, sterile females, who probably contributed to the results of experiment 1, did not contribute to the results of experiments 2 and 3 because the clones used in these experiments were known to reproduce. Thus, inbreeding depression in these experiments is likely to occur because of deleterious alleles with mild or intermediate effects, as inbred lines expressing lethal or strongly deleterious alleles are unlikely to be included in these experiments.

Low or No Inbreeding Depression in the Absence of Competition with Outbred Clones

Inbreeding depression in single-clone populations (experiment 3) was much lower than in experiment 2 (Table 1), which took place under otherwise very similar environmental conditions. Because fitness was measured in a different way in the two experiments (change of clone frequency during competition and production of resting eggs respectively), the estimates of δ may not be directly comparable. However, the differences are so strong that it seems justified to conclude

that inbreeding depression was weaker in the absence of competition with outbred clones. This finding is in agreement with other studies showing that competition between inbred and outbred individuals might enhance the expression of inbreeding depression (Wolfe 1993; Latter and Sved 1994; Meagher et al. 2000) and supports the general idea that the expression of inbreeding depression is environment dependent (e.g., Bijlsma et al. 1999).

The difference between experiments 2 and 3 was not the occurrence of competition itself, but rather the specific type of competition. In experiment 2 inbred and outbred individuals competed with each other (intraclonal and interclonal competition), whereas in experiment 3 all individuals competing with each other were of the same genotype (only intraclonal competition). Thus, it is probable that inbreeding affected competitive ability, and, whereas there was genetic variance in competitive ability under interclonal competition, this variance was absent under intraclonal competition. In summary, our experiments suggest that interclonal competition between inbred and outbred genotypes can have drastic effects on the expression of inbreeding depression, probably because of inbreeding effects on competitive ability.

Expected Effects of Inbreeding at the Metapopulation Level

Our study shows that inbreeding has high fitness costs in this *D. magna* metapopulation. Although further work is needed to assess how often local populations are inbred, data on migration and genetic structure suggest that bottlenecks are common (see introduction). If two unrelated clones simultaneously colonize a pool or if an immigrant clone successfully breeds with an inbred resident population, strong selection is expected to occur in the following season. Assuming masking of recessive alleles in hybrids, Whitlock et al. (2000) showed that an influx of new genetic material into an inbred population will confer a selective advantage to the immigrants, who will initially be rare and thus have mainly hybrid offspring (Whitlock et al. 2000). In the year following the experiments presented here, we could show a strong selective advantage of hybrids between immigrants and residents for the Tvärminne *D. magna* metapopulation (Ebert et al. 2002). In such a scenario, the immigrant genes increase in frequency not because they carry selectively superior genes, but because they carry different alleles than the local residents. The resulting hybrid vigor shown by outbred clones could increase gene flow in the metapopulation (Ingvarsson and Whitlock 2000; Ebert et al. 2002) and might even prevent local extinctions (Richards 2000). It is not clear how frequently secondary colonizations occur. The colonization rate of empty pools is about 5% per year (Pajunen 1986; V. I. Pajunen, unpubl. ms.), and allelic diversity increases with the persistence time of a population (about 10% increase in number of alleles per year; C. Haag, M. Riek, V. I. Pajunen, and D. Ebert, unpubl. ms.), suggesting that immigration does take place. Our results suggest, however, that if a single clone colonizes a rockpool, that is, in absence of competition between inbred and outbred genotypes, inbreeding may have little or no impact on population persistence.

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LITERATURE CITED

- Armbruster, P., R. A. Hutchinson, and T. Linnell. 2000. Equivalent inbreeding depression under laboratory and field conditions in a tree-hole-breeding mosquito. *Proc. R. Soc. Lond. B* 267: 1939-1945.
- Bengtsson, J. 1989. Interspecific competition increases local extinction rate in a metapopulation system. *Nature* 340:713-715.
- . 1991. Interspecific competition in metapopulations. *Biol. J. Linn. Soc.* 42:219-237.
- Bengtsson, J., and D. Ebert. 1998. Distribution and impacts of microparasites on *Daphnia* in a rockpool metapopulation. *Oecologia* 115:213-221.
- Bijlsma, R., J. Bundgaard, and W. F. van Putten. 1999. Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *J. Evol. Biol.* 12:1125-1137.
- Bijlsma, R., J. Bundgaard, and A. C. Boerema. 2000. Does inbreeding affect the extinction risk of small populations? Predictions from *Drosophila*. *J. Evol. Biol.* 13:502-514.
- Birky, C. W., Jr. 1967. Studies on the physiology and genetics of the rotifer *Asplancha*. III. Results of outcrossing, selfing, and selection. *J. Exp. Zool.* 164:105-116.
- Carr, D. E., and M. R. Dudash. 1995. Inbreeding under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity* 75:437-445.
- Charlesworth, B., M. Nordborg, and D. Charlesworth. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genet. Res. Camb.* 70:155-174.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18:237-268.
- Chen, X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic *Arianta arbustorum* (L.) (land snail). *Heredity* 71: 456-461.
- Cheptou, P.-O., E. Imbert, J. Lepart, and J. Escarre. 2000. Effects of competition on lifetime estimates of inbreeding depression in the outcrossing plant *Crepis sancta* (Asteraceae). *J. Evol. Biol.* 13:522-531.
- Coltman, D. W., J. G. Pilkington, J. A. Smith, and J. M. Pemberton. 1999. Parasite-mediated selection against inbred Soay sheep in a free-living, island population. *Evolution* 53:1259-1267.
- Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. *Heredity* 83:260-270.
- De Meester, L. 1993. Inbreeding and outbreeding depression in *Daphnia*. *Oecologia* 96:80-84.
- Deng, H.-W. 1997. Increase in developmental instability upon inbreeding in *Daphnia*. *Heredity* 78:182-189.
- Deng, H.-W., and M. Lynch. 1997. Inbreeding depression and inferred deleterious-mutation parameters in *Daphnia*. *Genetics* 147:147-155.
- Dudash, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution* 44:1129-1139.
- Ebert, D., J. W. Hottinger, and V. I. Pajunen. 2001. Temporal and spatial dynamics of parasites in a *Daphnia* metapopulation: Which factors explain parasite richness? *Ecology* 82:3417-3434.
- Ebert, D., C. Haag, M. Kirkpatrick, M. Riek, J. W. Hottinger, and V. I. Pajunen. 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. *Science* 295:485-488.
- Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow, U.K.
- Forster Blouin, S., and M. Blouin. 1988. Inbreeding avoidance behaviors. *Trends Ecol. Evol.* 3:230-233.
- Frankham, R. 1995. Inbreeding and extinction: a threshold effect. *Conserv. Biol.* 9:792-799.
- . 1998. Inbreeding and extinction: island populations. *Conserv. Biol.* 12:665-675.
- Hanski, I., and E. Ranta. 1983. Coexistence in a patchy environment: three species of *Daphnia* in rock pools. *J. Anim. Ecol.* 52: 263-279.
- Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics. 3rd ed. Sinauer Associates, Sunderland, MA.
- Hebert, P. D. N., and M. J. Beaton. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, TX.
- Hebert, P. D. N., and R. D. Ward. 1972. Inheritance during parthenogenesis in *Daphnia magna*. *Genetics* 71:639-642.
- Hedrick, P. W. 1994. Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* 73:363-372.
- Hobæk, A., and P. Larsson. 1990. Sex determination in *Daphnia magna*. *Ecology* 71:2255-2268.
- Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54-70.
- Ingvarsson, P. K., and M. C. Whitlock. 2000. Heterosis increases the effective migration rate. *Proc. R. Soc. Lond. B* 267: 1321-1326.
- Innes, D. J. 1989. Genetics of *Daphnia obtusa*: genetic load and linkage analysis in a cyclical parthenogen. *J. Hered.* 80:6-10.
- Innes, D. J., and R. L. Dunbrack. 1993. Sex allocation variation in *Daphnia pulex*. *J. Evol. Biol.* 6:559-575.
- Jarne, P., and D. Charlesworth. 1993. The evolution of the selfing rate in functionally hermaphroditic plants and animals. *Annu. Rev. Ecol. Syst.* 24:441-466.
- Jimenez, J. A., K. A. Hughes, G. Alaks, L. Graham, and R. C. Lacy. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* 266:271-273.
- Keller, L. F., P. Arcese, J. N. M. Smith, W. M. Hochachka, and S. C. Stearns. 1994. Selection against inbred song sparrows during a natural population bottleneck. *Nature* 372:356-357.
- Kleiven, O. T., P. Larsson, and A. Hobæk. 1992. Sexual reproduction in *Daphnia magna* requires three stimuli. *Oikos* 65:197-206.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* 241:1455-1460.
- . 1994. Risk of population extinction from the fixation of new deleterious mutations. *Evolution* 48:1460-1469.
- Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24-40.
- Latter, B. D. H., and J. A. Sved. 1994. A reevaluation of data from competitive tests show high levels of heterosis in *Drosophila melanogaster*. *Genetics* 137:509-511.
- López Bueno, J. A., A. Moya, and F. González-Candelas. 1993. The effect of periodic bottlenecks on the competitive ability of *Drosophila pseudoobscura* lines. *Heredity* 70:60-66.
- Lynch, M., and H.-W. Deng. 1994. Genetic slippage in response to sex. *Am. Nat.* 144:242-261.
- Lynch, M., J. Conery, and R. Burger. 1995. Mutation accumulation and the extinction of small populations. *Am. Nat.* 146:489-518.
- Madsen, T., R. Shine, M. Olsson, and H. Wittzell. 1999. Restoration of an inbred adder population. *Nature* 402:34-35.
- Meagher, S., D. J. Penn, and W. K. Potts. 2000. Male-male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci. USA* 97:3324-3329.
- Muirhead, C. A., and R. Lande. 1997. Inbreeding depression under

- joint selfing, outcrossing, and asexuality. *Evolution* 51:1409–1415.
- Newman, D., and D. Pilson. 1997. Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. *Evolution* 51:354–362.
- Pajunen, V. I. 1986. Distributional dynamics of *Daphnia* species in a rock-pool environment. *Ann. Zool. Fenn.* 23:131–140.
- Pamilo, P., S. Pálsson, and O. Savolainen. 1999. Deleterious mutations can reduce differentiation in small, subdivided populations. *Hereditas* 130:257–264.
- Pusey, A., and M. Wolf. 1996. Inbreeding avoidance in animals. *Trends Ecol. Evol.* 11:201–206.
- Ranta, E. 1979. Niche of *Daphnia* species in rock pools. *Arch. Hydrobiol.* 87:205–223.
- . 1982. Animal communities in rock pools. *Ann. Zool. Fenn.* 19:337–347.
- Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. *Am. Nat.* 155:383–394.
- Saccheri, I. J., M. Kuusari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly metapopulation. *Nature* 392:491–494.
- SAS Institute. 1990. SAS/STAT. Ver. 6.06. SAS Institute, Cary, NC.
- . 1999. JMP-IN. Ver. 3.2.6. SAS Institute, Cary, NC.
- Taylor, B. E., and W. Gabriel. 1993. Optimal growth of *Daphnia* in a seasonal environment. *Funct. Ecol.* 7:513–521.
- Westemeier, R. L., J. D. Brawn, S. A. Simpson, T. L. Esker, R. W. Jansen, J. W. Walk, E. L. Kershner, J. L. Bouzat, and K. N. Paige. 1998. Tracking the long-term decline and recovery of an isolated population. *Science* 282:1695–1698.
- Whitlock, M. C., P. K. Ingvarsson, and T. Hatfield. 2000. Local drift load and the heterosis of interconnected populations. *Hereditas* 84:452–457.
- Wolfe, L. M. 1993. Inbreeding depression in *Hydrophyllum appendiculatum*: role of maternal effects, crowding, and parental mating history. *Evolution* 47:374–386.

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