Inbreeding and Potential for Evolutionary Rescue after Environmental Change

Honors Thesis
Presented to the College of Agriculture and Life Sciences
Cornell University
in Partial Fulfillment of the Requirements for the
Biology Honors Program

by
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May 2012

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Abstract:

Evolutionary rescue occurs when populations that experience a severe decline in numbers following environmental change adapt evolutionarily and so avoid extinction. However, low population size, even without extinction, can have detrimental effects such as inbreeding depression that may influence the likelihood of evolutionary rescue. The aim of this study was to determine the effect of inbreeding on the propensity for evolutionary rescue. The monogonont rotifer *Brachionus plicatilis* was used as the study species and environmental change was created by increasing the salinity of its medium. All rotifers used in my study were originally derived from a laboratory stock culture that had been previously forced to a population genetic bottleneck with some lineages then maintained clonally while others were induced to sexual reproduction which led to inbreeding. Populations were later subjected to the environmental change and their resulting mortality and growth during recovery were observed. Inbreeding was found to significantly decrease the ability of the populations to recover from the environmental stress.

Introduction

As anthropogenic effects on ecosystems increase due to local stresses, as well as global climate change, it is important that the factors influencing the persistence of populations in altered environments are understood (Bell and Collins 2008). The current extinction rate is estimated to be higher than that of any of the five mass extinctions in the fossil record, a result attributable primarily to the synergistic action of various anthropogenic effects, including altered climate dynamics, increased habitat fragmentation, and overexploitation of natural resources (Barnosky et al. 2011). At the same time, there are numerous examples of contemporary evolution in nature, where populations experience measurable genetic changes on the scale of years and decades (Hendry and Kinnison 1999; Hairston et al. 2005; Fussmann et al. 2007), often in response to anthropogenic environmental change (Hendry et al 2007; Palumbi 2001).

Evolutionary rescue is a process whereby populations avoid extinction by evolving after a detrimental change in their environment that causes an initial population decline. If the population had not evolved, it would have gone extinct. All current work on evolutionary rescue references the theoretical foundation presented by Gomulkiewicz and Holt (1995). Researchers have examined the implications of evolutionary rescue on conservation (Kinnison and Hairston 2007; Bell and Collins 2008), and have confirmed aspects of the theory through experiments (Bell and Gonzalez 2009; Collins and de Meaux 2009; Moser 2010; Bell and Gonzalez 2011).

Historically the possibility of rapid evolutionary processes such as evolutionary rescue received little attention because biologists assumed that the timescale of evolution would not allow for adequately swift adaptation (Fisher 1930; Slobodkin 1961; Hoffmann and Blows 1993). Populations may instead persist through various strategies of evasion, which includes alteration of behavior, migration, range expansion, or changes in resource use (Lynch and Lande 1993). Experimental approaches to understanding the role of evolution are especially important because the only record of organisms that survived environmental change is found in the fossil record, which typically cannot reveal adaptation of the physiological and life history traits that are often essential in evolutionary rescue (Travis and Futuyma 1993).

The initial genetic variation present in a population before an environmental change is critical to how a population responds (Bell and Collins 2008). Some amount of genetic variation is necessary to allow a population to evolve at all (Lynch and Lande 1993), and a population bottleneck can decrease genetic variation to such an extent that the population is less able to

evolve to a change in environment (Frankham et al. 1999). Population bottlenecks occur when the effective population size decreases to a dramatically reduced size for one or more generations.

One effect of a population bottleneck in a sexually reproducing population is an increase in the amount of inbreeding (Frankham et al. 1999). Elevated homozygosity due to inbreeding means that diploid organisms have more genes with alleles that have identity by descent than would be expected under Hardy-Weinberg equilibrium. A decrease in fitness may result, called inbreeding depression (Wright 1977). This effect primarily occurs because zygotes are more likely to be homozygous for deleterious recessive alleles (Charlesworth and Charlesworth 1999). The affected genes can influence any aspect of the organism's life cycle, but the overall result of decreased fitness is necessary for an effect of inbreeding to be considered inbreeding depression (Armbruster and Reed 2005). In addition, gene expression levels may be different after an environmental change, so even previously neutral loci may experience positive or negative selection after environmental change (Hoffmann and Blows 1993).

While the effects of inbreeding depression might be expected to be particularly pronounced in stressful environments, such as those where evolutionary rescue might occur, this relationship is far from well-established. Armbruster and Reed (2005) surveyed the literature describing experiments designed to measure inbreeding depression in either stressful or benign environments, and found that only 48% of experiments show a significant increase in inbreeding depression in a stressful environment relative to benign environment. A separate meta-analysis by Fox and Reed (2011) found that more inbreeding depression is found in environments that are more stressful. They posit the binary categorization of environments as either benign or stressful as the main shortcoming of Armbruster and Reed (2005). In environments as extreme as those giving rise to evolutionary rescue, however, it is possible that the existence of the rare individuals that are able to survive the change will be more important than population's mean fitness.

My experiments used the planktonic monogonont rotifer *Brachionus plicatilis* to compare the propensity for evolutionary rescue in response to severe osmotic stress in non-inbred and inbred lineages. All lineages began from a single individual in the laboratory, which created an intentional bottleneck effect. The population was not initially inbred, however, because *Brachionus* reproduce parthenogenetically in most conditions. Such populations can be inbred

though sexual reproduction, which would lead to the increase in homozygosity that generally occurs in inbreeding, and subsequent exposure of deleterious alleles.

The life cycle of monogonont rotifers facilities the straightforward induction of sexual reproduction in the laboratory (Figure 1). When there is a high density of conspecifics, asexually reproducing (amictic) females are induced to produce sexually reproducing (mictic) females (Snell et al. 2006). Mictic females produce haploid eggs which can have one of two fates. Unfertilized mictic eggs develop into haploid males. Mictic eggs that are fertilized by a male become diploid diapausing eggs that can remain for years in the sediments and provide a bank of genetic variation when they hatch at the beginning of subsequent growing seasons (Hairston 1996; Ellner et al. 1999, Gomez and Carvalho 2001). When a diapausing egg hatches, it yields a diploid amictic female that is a different genotype from either of the rotifer lineages that contributed to it. It might be expected that haploid male stage would serve to purge the effects of inbreeding, because it would expose deleterious recessive genes. However, *B. plicatilis* has been shown to experience inbreeding depression in experimental conditions, likely because males' shorter life spans and inability to reproduce asexually mean they experience very few of the selection pressures faced by females, and also express different genes (Tortejada et al. 2009).
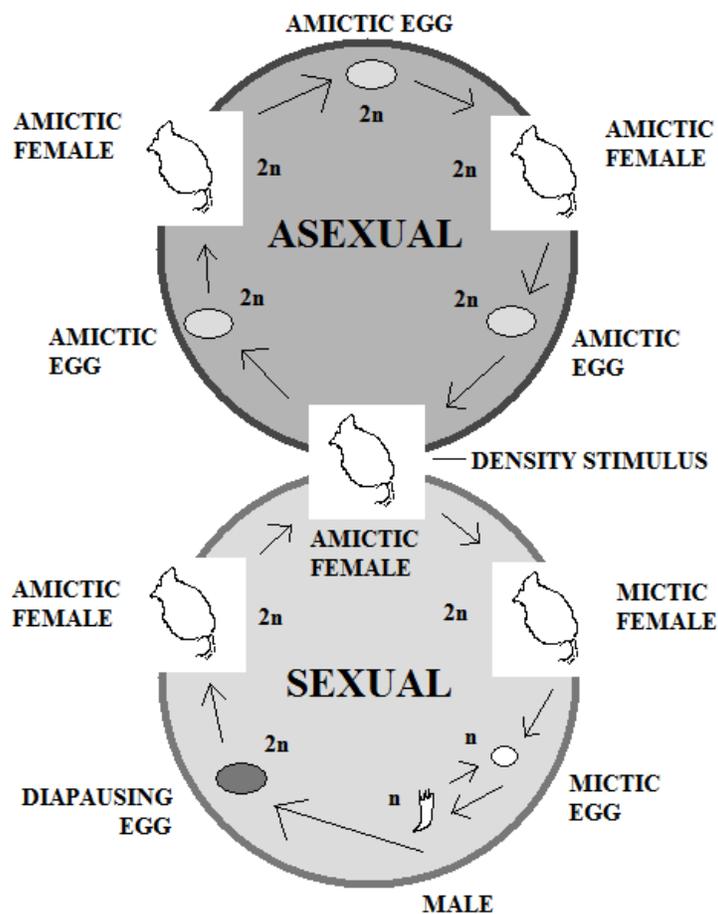
Figure 1: *Brachionus plicatilis* life cycle. (Modified from Becks and Agrawal 2010)

I chose elevated salinity as the means of creating an environmental change that would stress the rotifers. In nature, *B. plicatilis* is found in brackish water, with full strength sea water (35 g L^{-1}) the highest possible salinity to which it would normally be exposed. *B. plicatilis* is an osmoregulator (Lowe et al. 2005), so it is able to deal with a wide range of salinities. Previous experiments with rotifers originating from the same stock in our lab showed that growth rate was significantly lower in 65 g L^{-1} than in 45 g L^{-1} or below, even though the rotifers had been incrementally eased into those salinities (K. Blackley unpublished data). This trend is confirmed by other salinity tolerance studies of *B. plicatilis* (Lowe et al. 2005, Yin and Zhao 2008). These same studies found that rotifers grew well at 16 g L^{-1}, which is why I chose that salinity as the benign environment in this study.

Previous experiments attempting to demonstrate the role of initial genotypes in evolutionary rescue have used the alga *Chlamydomonas reinhardtii,* at a much larger population size than was the case for the rotifers in my study, so that novel mutations after the environmental stress is applied may have overwhelmed any effect of standing genetic variation (Moser 2010). In many natural populations that are the target of conservation efforts, population sizes are small and reproduction occurs less frequently relative to the rate of the environmental change (Bell and Collins 2008). Rotifers are therefore a model organism better suited for the exploration of evolutionary rescue as it relates to standing variation, especially in the context of conservation and the effect of inbreeding depression.

Methods

Environmental change experiment

I maintained rotifers in two separate conditions before testing them in the same environmental change experiment. In one condition, the single rotifer clone was induced to sexual reproduction, which led to inbreeding in those populations. In the other condition, the rotifers only reproduced asexually, so they did not become inbred although they had originated generations in the past from a single clone. After rotifers had lived for approximately 50 generations in these conditions, I increased suddenly the salinity of their medium and observed their response.

To establish my initial populations, I sampled from a continuous chemostat culture of *B. plicatilis* descended from a single individual chosen randomly from a lab stock that was originally generated from diapausing eggs obtained from Florida Aqua Farms (FL, USA). I subsequently maintained rotifers in semi-continuous batch culture for three months in 16 g L^{-1} medium without phosphorus and fed *Chlorella autotrophica* grown in a chemostat maintained at 35 g L^{-1} medium. The culture media were prepared using Tropic Marin ® (Dr. Biener GmbH, Wartenberg, Germany) supplemented with 882 µM nitrate, 36.2 µM phosphate, and vitamins and trace metals from f/2 medium (Guillard and Ryther 1962, Guillard 1975), except where the omission of phosphorus is specifically stated. Phosphorus is omitted from the rotifer medium to prevent the growth of the algae once it was added as food.

I determined the threshold for male production by placing 90 individual amictic females from the bottlenecked population into 3 mL of 16 g L^{-1} medium with high N and P and excess

food in 5 mL wells of 12-well tissue culture plates, and then noting the population density when the first male appeared. I selected ten wells in which males appeared at a relatively low population density to begin the experimental clones, and their thresholds for male production were used to determine the appropriate feeding schedule for the inbreeding populations (Figure 2). The appearance of males in all experimental clones also demonstrated that there were no obligate parthenogens among the experimental clones. Because the transition from cyclical to obligate parthenogenesis is controlled by homozygosity at a single locus in a related species, *Brachionus calyciflorus* (Stelzer et al. 2010), it was essential to confirm that all clones could be induced to mixis.

I combined 100-300 rotifers from each of the ten experimental lineages into three separate 2 L flasks containing 1L of 16 g L^{-1} medium without phosphorus (Figure 2) and fed them weekly with 1×10^7 cells mL^{-1} of *C. autotrophica,* or 2.81×10^4 mg C L^{-1} (*C. autotrophica* carbon content is 5.5×10^{-6} μg $cell^{-1}$). The high density of food allowed the rotifer population to grow to densities of approximately 100 female rotifers mL^{-1}, which was a sufficiently high density once a week that male production was induced, and male concentration would peak at more than three males mL^{-1}. About one quarter of *B. plicatilis* diapausing eggs hatch within one month in constant light conditions (Hagiwara and Hino 1989), so cultures were maintained in 24-hour light and partially renewed once a month. To partially renew a given population, I thoroughly mixed the flask, then poured off about ⅔ to ¾ of the medium along with any rotifers, algae, and diapausing eggs suspended in it. I then filled the flask with fresh medium so that it returned to the original volume. Many rotifers were also observed carrying amictic eggs during this period, which indicates that asexual reproduction was also occurring, but these populations were still becoming more inbred than asexually reproducing lineages.

Each of the clones that comprised the inbreeding populations was also maintained in isolation in 50 mL centrifuge tubes (Figure 2). Once a week, I renewed these cultures by transferring 10-20 rotifers from each clone into a new tube filled with fresh medium and 5×10^5 cells/mL *C. autotrophica*. Frequent transfer ensured that mixis would not be induced in the non-inbreeding lineages. Because the clones were all reproducing asexually, the small number of individuals transferred each week did not affect the level of inbreeding in the population.
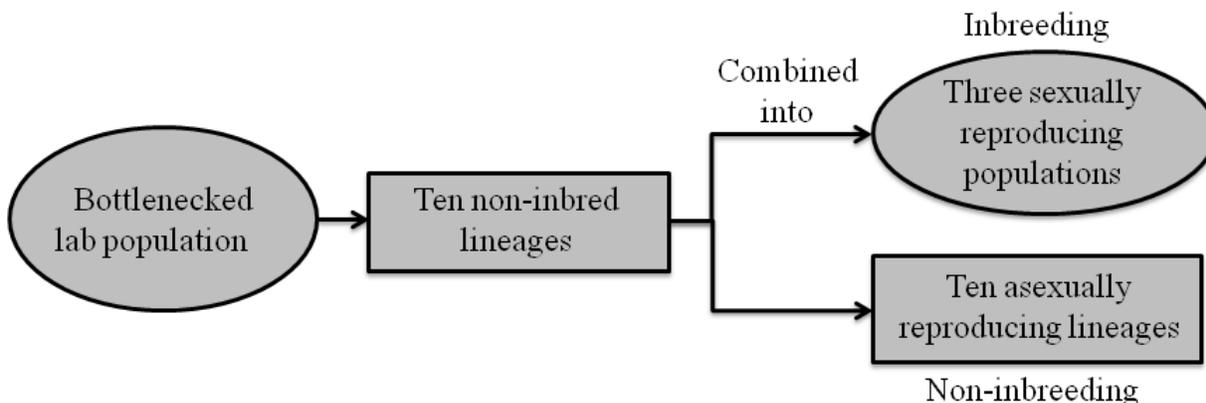
Figure 2: Flowchart showing the origins of the experimental populations and lineages

To prepare for the salinity elevation experiment, I placed 50-100 rotifers from each non-inbred lineage and each inbred population into individual 125 mL flasks containing 100 mL of 16 g L^{-1} medium without phosphorus and about 2×10^6 cells mL^{-1}. After five days of growth, I diluted each culture to reach the same population density of approximately 70 rotifers mL^{-1}. I then added rotifers from each culture to 17 mL wells in 6-well tissue culture plates, and counted females, their eggs, and males. I added different salinities of media without phosphorus calibrated so that wells reached final salinities of 16, 42, 46, 50, 54, and 58 g L^{-1}, and all wells had a total volume of 9 mL. The range of elevated salinities was chosen based preliminary tests, which showed that these salinities caused high mortality. Each well also contained 3×10^6 g L^{-1} of *C. autotrophica*. I counted females, their eggs, and males once a day for the following nine days using a dissecting scope. Males were observed in many of the wells that reached high population densities, but because there was not sufficient time to allow diapausing eggs to hatch, all rotifers were reproducing asexually throughout the experiment. Each treatment was replicated three times for each lineage or population.

Growth rate experiment in benign environment

The inbred populations and non-inbred lineages were further compared to quantify intrinsic growth rates in the benign environment of 16 g L^{-1} medium without phosphorus. Based on previous results, I considered two non-inbred lineages and one inbred population as representative of their respective treatments for this experiment. I placed ten rotifers from each group into wells filled with 16 g L^{-1} medium without phosphorus and 3×10^6 cells mL^{-1} of *C.*

autotrophica. After approximately 24 h, I counted female rotifers and eggs, then randomly selected ten individuals from each well to begin the experiment anew in a well containing fresh medium and food. This was continued in all wells for four consecutive days.

Follow-up environmental change experiment

Because the results of the initial experiment did not reveal any difference in salinity tolerance among the non-inbred lineages, I carried out a second experiment at a narrow range of high salinities to provide a more definitive test. Six non-inbred lineages from the previous experiment were chosen for an additional evolutionary rescue experiment in which the salinities were 47, 48, and 49 g L^{-1}.The earlier environmental change experiment showed that these salinities should be around the threshold above which the lineages would not be able to recover. Methods were the same as those described above.

Analysis

Analyses of variance (ANOVA) were used to compare differences in mortality and growth rates among the rotifers in both 42 g L^{-1} and 46 g L^{-1}. To examine mortality, ANOVAs were used with inbreeding or not inbreeding as the predictor and mortality as the response. To examine in the effects on daily population growth rates of inbreeding in 42 g L^{-1}, two linear mixed effects models were compared using a likelihood ratio test (LRT). To compare average daily population growth rates of non-inbred lineages, ANOVAs were used with non-inbred lineage as the predictor and average daily population growth rate as the response. To examine differences in daily population growth rates due to inbreeding in the 16 g L^{-1}growth rate experiment, two linear mixed effects models were compared using an LRT. Analyses were carried out using R version 2.12.2 (R Development Core Team).

Results

In the benign environment at 16 g L^{-1}, as measured using the growth rate experiment, there was no significant effect of inbreeding on growth rate (LRT against null: $p > 0.1$) (Figure 3).
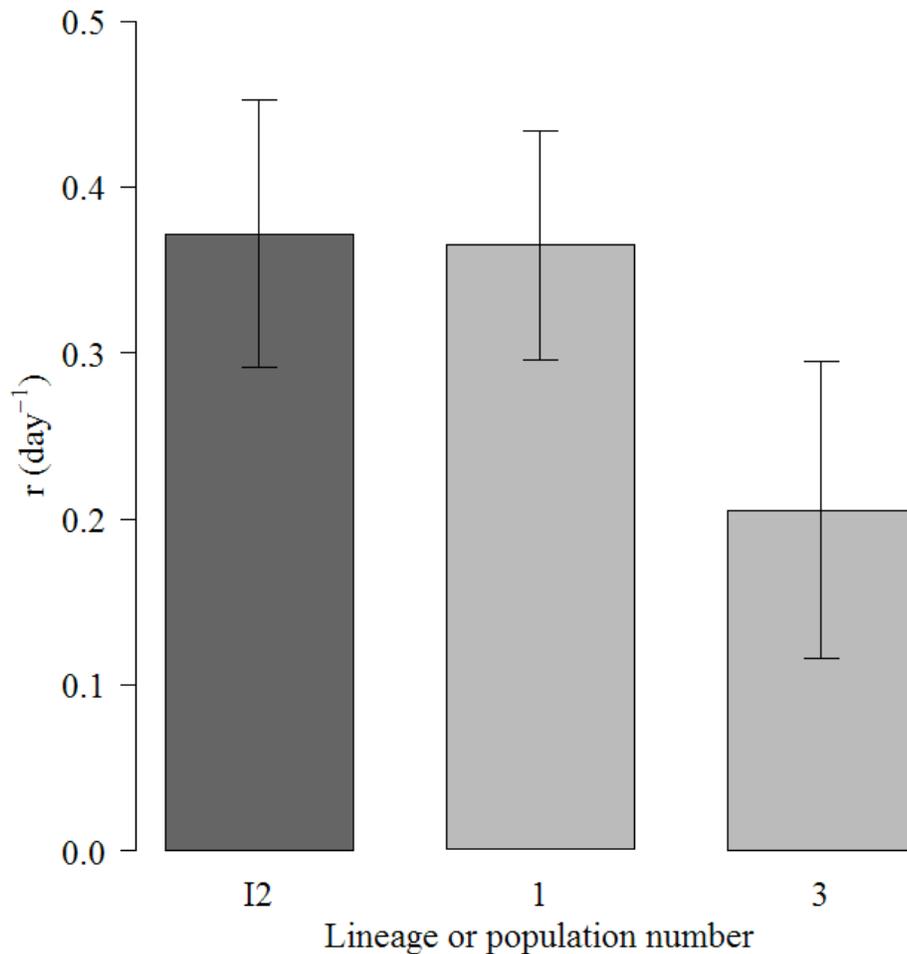
Figure 3: Mean daily population growth rates in 16 g L^{-1} medium ($\pm$ 1 s.e.) The dark bar is inbred population #2. The light bars are non-inbred lineages 1 and 3. Error bars show standard error.

In the environmental change experiment, rotifer population sizes initially decreased in 16 g L^{-1} presumably due to a combination of handling stress and age structure (Figure 4). The rotifers that were added to the experiment had grazed down their food and were therefore carrying very few eggs. Five days after the initial salt addition, however, the rotifer populations in 16 g L^{-1} medium had approximately doubled (Figure 4). Because these rotifer populations never reached a very low population, they were limited by food density through most of the experiment, and so there are no statistical analyses of their growth rates.

At 42 g L^{-1}, most of the rotifers in each replicate died, but in only one replicate was extinction observed. Many of the replicates returned to their initial density about 10 days after the salinity increase. In 46 g L^{-1}, most of the rotifers in each replicate died, and some replicates

became extinct. By the end of the experiment, some of the persisting replicates had begun to grow again, while others maintained a small population that was not growing. Within two days all the populations at 54 and 58 g L^{-1} were extinct. Because 16 g L^{-1} is the reference and 54 and 58 g L^{-1} ultimately held no rotifers, statistical analyses of the environmental change experiment were performed on data from 42 g L^{-1} and 46 g L^{-1}.

In both salinities, there was a significant effect of inbreeding on mortality. Within 24 hours of the salt addition mortality was significantly higher in the rotifers from inbred populations than in those from the non-inbred lineages in both 42 g L^{-1} ($F_{1,11} = 33.422$, $p < 0.001$) and 46 g L^{-1} ($F_{1,11} = 11.430$, $p < 0.01$) (Figure 3). The non-inbred lineages were compared using lineage as the factor and mortality as the independent variable. There was not a significant effect of lineage in either 42 g L^{-1} ($F_{1,8} = 0.6156$, $p > 0.4$) or 46 g L^{-1} ($F_{1,8} = 0.0743$, $p > 0.7$).
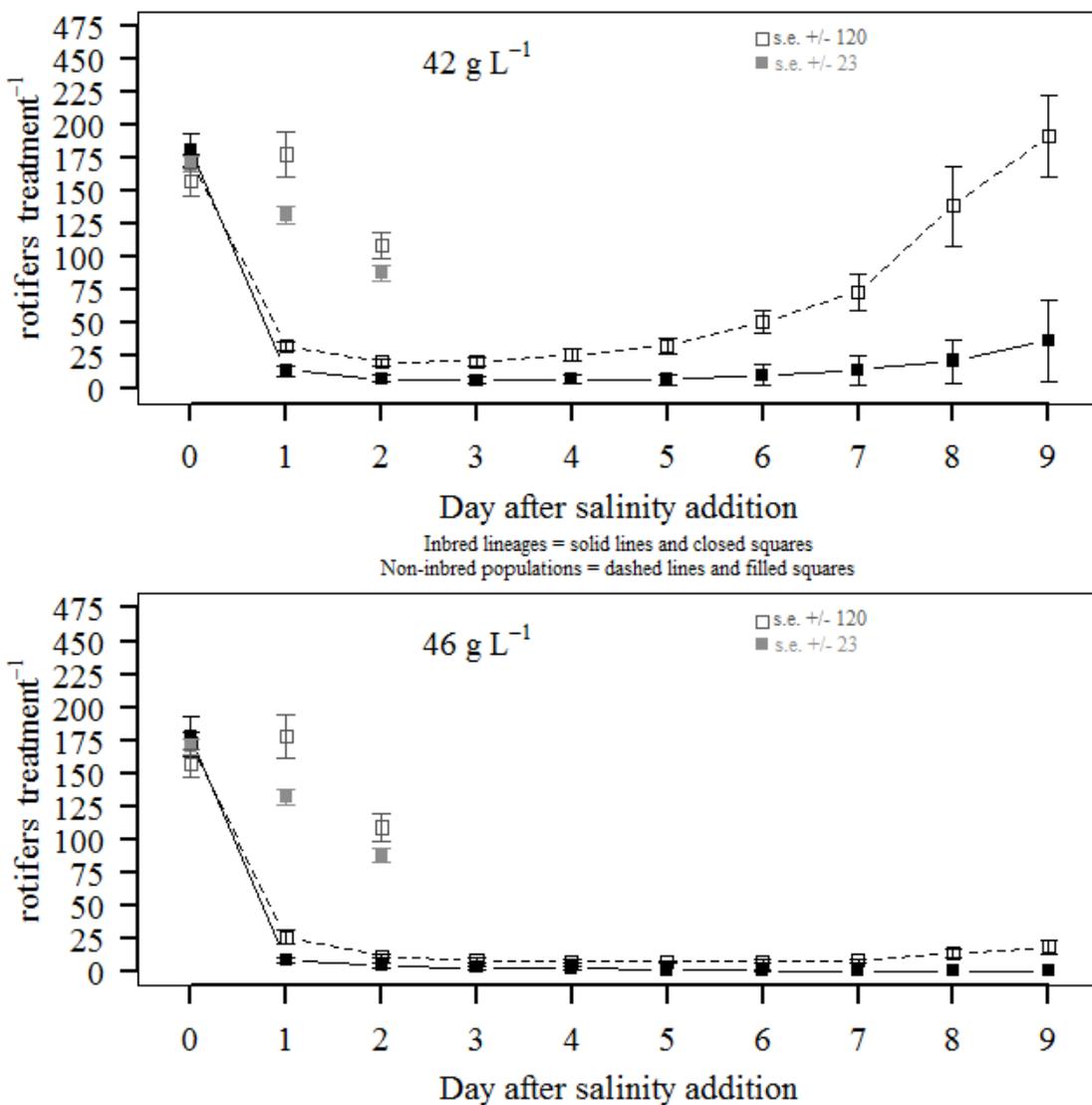
Figure 4. Female rotifers per well (± 1 s.e.) after sudden increases of salinity to (a) 42 g L^{-1} and (b) 46 g L^{-1} medium. Dashed lines and open squares= Non-inbred lineages. Solid lines and filled squares= Inbred populations. Light gray open squares: non-inbred lineages in16 g L^{-1}. Dark gray open squares: inbred populations in 16 g L^{-1}.

The average daily population growth from days four to nine was significantly higher for the rotifers from the non-inbred lineages than for the rotifers from the inbred populations in 42 g L^{-1} (LRT against null: $p < 0.01$) (Figure 5). Inbreeding could not be used as a factor in 46 g L^{-1} (Figure 3b) because most of the inbred populations were extinct before day nine. The non-inbred lineages were compared using lineage as a factor and average daily population growth rate as the

response, but no significant effect of lineage was detected in either 42 g L^{-1} (F$_{9, 40}$ = 0.3237, $p >$ 0.9) or in 46 g L^{-1} (F$_{9, 40}$ = 0.9632, $p >$ 0.4).
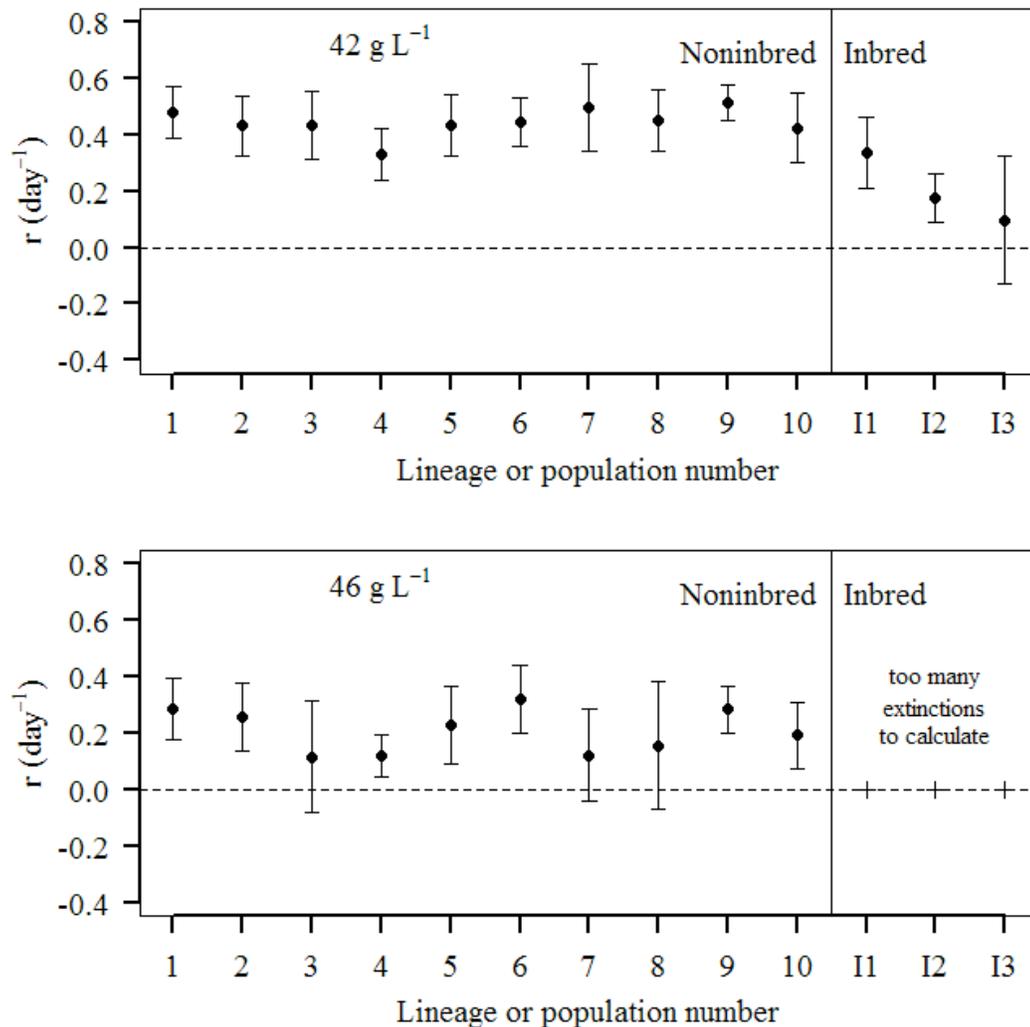

Figure 5. Daily average population growth rates (± 1 s.e.) in the non-inbred lineages and the inbred populations from day 4 to day 9 at (a) 42 g L^{-1} and (b) 46 g L^{-1}. The inbred populations (I1-I3) do not appear in panel b because they were extinct after day 6.

In the follow-up experiment using six non-inbred lineages tested at 47, 48, and 49 g L^{-1}, populations went extinct in all wells at 49 g L^{-1}. A single amictic female survived and reproduced in one well at 48 g L^{-1}. In 47 g L^{-1}, three of the six non-inbred lineage replicates became extinct, while three survived.

Discussion:

Inbred populations, the result of sexual reproduction in a population driven to extremely low numbers, were found to have a severely reduced ability to recover from environmental stress. The inbred populations of rotifers experienced higher mortality in 42 g L^{-1} and 46 g L^{-1} than the non-inbred lineages. The surviving rotifers from the inbred populations also grew at a lower growth rate than the non-inbred lineages. Both of these parameters affect a species' likelihood of extinction (Gomulkiewicz and Holt 1995), and it is clear that in my study inbreeding influenced the number of rotifers that survive in a treatment on a given day.

There was a negative effect of inbreeding, when compared with the non-inbred lineages, both of which had undergone the same population bottleneck (reduction to a single clonal lineage) in the past. While at 16 g L^{-1}, a salinity at which the rotifers performed best, there was no inbreeding effect on growth rate, there was a marked effect at the more stressful 42 g L^{-1}.

In every comparison, including growth rates in 16, 42, and 46 g L^{-1}, and mortalities in 42 and 46 g L^{-1}, there were no differences among the ten non-inbred lineages I tested. The non-inbred lineages were maintained in containers separate from each other for four months before the experiment began, enough time for approximately 50 generations of asexual reproduction. At 46 g L^{-1}, all non-inbred lineages survived, while 49 g L^{-1} was so osmotically stressful that none of the six tested lineages survived. One explanation for the lineages' similarity is that mutations that would be beneficial after the environmental change will likely be quickly lost in the benign environment, especially since mortality was imposed through weekly partial renewal of cultures to limit population density.

There was variation within each non-inbred lineage in the response to the environmental change, since most but not all of the individuals died in the first day after salinity was increased. This could be because the non-inbred lineages may have some genetic diversity due to mutations, although it is not likely that mutations would have led to ten lineages which are so phenotypically similar. Differences in survival within non-inbred lineages are more readily explained by factors besides genetics, such as age. As the mortality and the recovery processes occurred within lineages that only had a single genotype, their recovery should not be interpreted as instances of evolutionary rescue, because evolution requires a change in genotype frequencies.

Nevertheless, this experiment measured survival and growth after a sudden and stressful environmental change, which are important parameters for predicting whether evolutionary rescue will occur (Gomulkiewicz and Holt 1995).

It is not possible to quantify the precise amount of inbreeding represented by the inbred lines in this experiment because too much remains unknown about the original population from which the non-inbred lineages were selected, as well as the amount of sexual reproduction that actually occurred in the inbreeding populations. Male production and diapausing eggs were observed, but the diapausing eggs were always found inside the lorica of dead rotifers and did not hatch when placed alone in wells for observation. An earlier study of inbreeding in *B. plicatilis* (Tortejada et al. 2009) showed that the proportion of diapausing eggs that hatched was significantly less in an inbred than in non-inbred lineages, so it is possible that the level of inbreeding in the population stabilized over the course of the experiment, although this was not measured.

One cost of the sexual reproduction, by which inbreeding occurs, is that it may break up beneficial gene complexes through outbreeding depression, which is the disassociation of allele combinations that previously conferred high fitness (Templeton et al. 2005). Because of the earlier bottleneck in the rotifer population used, it is likely that the rotifers in my experiment were too similar genetically for any substantial outbreeding effects on fitness. In addition, it is not likely that the rotifers used in this experiment would have beneficial gene complexes that would be affected by sexual reproduction. For three or more years before the experiment, the rotifer populations lived in media ranging from 3 g L^{-1} to 35 g L^{-1}, which is consistent with the range of environments they would have experienced in nature.

I have documented a clear effect of inbreeding depression on rotifer performance following a stressful environmental change. When predicting a natural population's chance of surviving due to evolutionary rescue, it is important to consider whether the population may be inbred, perhaps due to a low effective population size or local isolation. Unfortunately, this describes many species which are of particular interest in conservation biology. It may be that species for which the potential for evolutionary rescue would be most valuable as a means of persistence will also have population genetics that make it less likely to occur.

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