ABSTRACT

Title of Document: POST-BOTTLENECK INBREEDING ACCUMULATION REDUCES FITNESS IN LABORATORY POPULATIONS OF *TRIBOLIUM CASTANEUM* UNDER ENVIRONMENTAL STRESS

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Populations are often driven to extinction due to low genetic diversity. One major cause for loss of genetic diversity in a population is a demographic bottleneck. A demographic bottleneck was imposed on twenty-one populations of *Tribolium castaneum* using multiple strategies. After recovering to original census numbers, the populations were subjected to stressful environments, and fitness was quantified. There was a significant decrease in additive genetic variance in all populations as a result of the bottleneck event ($P<0.05$). As estimated inbreeding accumulation increased, there was a decrease in the mean of fitness related traits, such as adult weight, total progeny, fecundity and survivorship. This relationship was best explained using quadratic models and became even more significant when the populations were under stress. This suggests that both dominance and epistatic gene effects are playing a role in phenotypic expression of traits and that expression may be flexible, supporting survival and fitness.

POST-BOTTLENECK INBREEDING ACCUMULATION REDUCES FITNESS IN LABORATORY POPULATIONS OF *TRIBOLIUM CASTANEUM* UNDER ENVIRONMENTAL STRESS

By

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Dedication

I would like to dedicate this work to my family, and more specifically, my parents. I know that they have had to make many sacrifices over the years in order for me to get to where I am today. The last few years have been particularly difficult with my father's diagnosis of rectal cancer, but through all the chemotherapy, radiation, operations and the pain, he has always supported me, even though I was regrettably not able to be home with him more often. I've completed this work in the hopes of some day being able to give back to them for everything they have done…

…For you, Mom and Dad. I love you!

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Chapter 1: Introduction

Overview

Populations are often unnecessarily driven to extinction due to a lack of genetic variation and reduced ability to adapt to environmental changes. Adaptation occurs through the joint forces of mutation and selection and reflects the result evolution. Evolution can be simply defined as changes in the frequencies of alleles in a population over time. Therefore, in order for evolution to occur, it is critical that genetic variation already exists. Genetic variation at the level of the population includes not only the alleles present, but also the frequency of these alleles across all members of the population. It is these alleles that have an influence on the phenotypes, which allow for diversity within traits. However, gene frequencies within a population are dynamic and change due to differential survival and reproduction through natural selection (Darwin 1859), immigration and emigration, as well as by chance (genetic drift). Populations with low genetic diversity have fewer alleles upon which natural selection can act and thus reduce their chance for persistence through selection for advantageous phenotypes in a changing environment (Lande and Shannon 1996).

It is also thought that heterozygosity, high genetic variation in an individual or population at a single gene locus or multiple gene loci, confers fitness advantages to the individual(s). Many studies have shown heterozygosity to be favorably correlated with fitness (Gaffney et al. 1990, Hoffman et al. 2004, Koehn et al. 1988, Mitton and Grant 1984, Tiira et al. 2006). These advantages are derived from a superior phenotype in the heterozygote (overdominance) as well as masking a lethal or deleterious recessive allele with another allele (complete dominance).

Inbreeding is defined as the mating of individuals who are related by a common ancestor. The probability for inbreeding to occur increases as population sizes become smaller. Inbreeding causes an increase in homozygosity as a result of alleles being identical by decent, and may lead to inbreeding depression: the loss of fitness (Charlesworth and Charlesworth 1999). This may be unavoidable in small populations because the number of potential mates for an individual is limited. Thus, the preservation and adequate management of genetic diversity is a critical component of conservation efforts.

There are two main factors that can cause a loss in genetic variation that are related to the size of the population: founder effects and demographic bottlenecks which result in genetic drift and inbreeding. A demographic bottleneck, which is the focus of this study, is a severe, temporary reduction in the number of breeding individuals in a population. For example, a population of sea birds on a coastal island has 500 individuals, but when a hurricane tears across the island, the population may suffer high mortality until only 50 birds remain. The genetic constitution of this population then depends on these few individuals who survived and will reproduce. If the genes carried by these 50 individuals are not representative of all the genes carried in the original population, the result is a gene pool that has less genetic variance than the original. The degree of genetic variability that is lost will depend on the severity of the bottleneck, its duration and the growth rate of the population after the bottleneck (Nei et al. 1975).

Some studies have been completed to test many of the theories regarding loss of genetic variability in populations post-bottleneck through computer simulations (Fernandez et al. 2004, Luikart et al. 1999), but few empirical studies have been done using data from real populations. Using real populations is a difficult task because collecting genotypic data from all its members

is not always feasible due to collection methods, availability of samples, as well as legal and ethical barriers; therefore, a lot of work is done using small, but presumably representative samples to make inferences about populations of interest. Another challenge has been gathering information for several generations when the generation interval of a species is long. There have been a few exceptions, including Le Page et al. (2000) who showed significantly reduced levels of allelic diversity and heterozygosity in wild populations of Bennett's wallabies in New Zealand in comparison to the source population and also Dunn and Byers (2008) who evaluated survival and fecundity in the National Bison Range pronghorn populations after a drought resulted in a population bottleneck.

The present study used a model organism, *Tribolium castaneum*, to evaluate effects of inbreeding accumulation on additive genetic variance and fitness related traits, owing to population bottleneck events, under laboratory conditions and environmental stressors.

Background Information

Genetic Diversity

Genetic diversity in a population is generally created by mutation. New alleles can be created through point mutations in which a mis-match nucleotide substitution changes the DNA sequence at a single locus or through unequal crossing over resulting in alleles that differ in length. These types of mutation may cause a change in the amino acid sequence of the protein it codes for. A slight change in amino acid sequence can have large consequences on the biochemical properties and function of a protein and this can result in different phenotypes among individuals. Insertions and deletions of tandem repeats or transposable elements can also result in mutation and the creation of new alleles. Likewise, the insertion or deletion of a single nucleotide may cause a mutation; however, this is less common due to the likelihood of this type

of mutation to cause a frame shift in the DNA sequence, often resulting in a non-functional gene or protein which may be lethal to the organism.

There are two main theories in regards to how genetic diversity is maintained within and among populations of a species. The Selectionist Theory asserts that genetic diversity is maintained through natural selection (Freeman and Herron 2001). If natural selection favors a new, rare allele, it will become more represented in the population. If there is either a heterozygote advantage (overdominance) or an effect of masking deleterious alleles by other alleles (complete dominance), then heterozygous individuals may be favored by natural selection, and thus genetic diversity is preserved. It is also thought that different alleles will be favored in different locations and at different times, so natural selection would select for different phenotypes depending on time and space, resulting in diversity over individuals and populations (Freeman and Herron 2001). The Neutral Theory is based on the premise that the alleles at most loci are functionally and selectively equivalent, and therefore genetic diversity is not eliminated through natural selection (Freeman and Herron 2001).

Adequate genetic diversity in a population is important because it is directly proportional to the potential for evolutionary change to occur in a population in response to a changing environment (Fisher 1958). In this study, changes in additive genetic variance due to a demographic bottleneck will be quantified in populations and then the populations' response to stressful environmental conditions will be observed.

Adaptation, Evolution and Fitness

Evolution is a dynamic mechanism through which populations can become better adapted to environments that are constantly changing. The process of adaptive evolution is reliant upon

heritable variation in traits. The more genetic variation that exists in the population, the more likely it is that there will also be variation in the expression of traits in that population. Natural selection, a concept first introduced by Charles Darwin (1859), is a process in which favorable phenotypes are "selected for" in a population. This selection occurs through differential survival and reproduction of individuals in response to a changing environment. Therefore, individuals who exhibit phenotypes that are more adapted to the current environmental conditions perform better and produce more offspring who will reach reproductive age. Those adapted individuals will become more genetically represented in subsequent generations compared to others who will leave fewer progeny. It is said that those individuals have a higher individual fitness. It is through this process that evolution occurs, resulting in a population with a higher mean fitness (Wright 1932, Wright 1988). Alternatively, evolution can also reflect genetic drift in populations which may evolve to have lower fitness over time. Natural selection acts upon phenotypes, but adaptive evolution requires a genotypic basis for the phenotypic variation in traits.

Effective Population Size

Matings cannot always be tracked in experimental populations. One solution is to use the effective population size (N_e) to estimate levels of inbreeding. The effective population size is the size of an ideal, or model population that would have the same loss in genetic diversity due to genetic drift and inbreeding as the population of interest. The effective population size is almost always smaller than the census population size (N_c). Some reasons for this include: individuals dying before they are able to reproduce, sexual selection, unequal sex ratio, family size (unequal genetic contribution of individuals to the next generation) and fluctuating population size. Some of these relationships have been described mathematically, by the following expressions:

$$N_e = \frac{(4N_m \cdot N_f)}{(N_m + N_f)} \qquad [1.1]$$

*adjustment for sex ratio (N_m = number of males, N_f = number of females)

$$N_e = \frac{4N_c}{(\sigma^2 + 2)} \qquad [1.2]$$

*adjustment for family size (σ^2 = variance in family size among females)

$$\frac{1}{N_e} = \frac{1}{t} \cdot \left(\frac{1}{N_1} + \frac{1}{N_2} + \ldots + \frac{1}{N_t} \right) \qquad [1.3]$$

*adjustment for fluctuating population size
(t = time in generations) (Falconer and Mackay 1996, p.67-68)

Newman and Pilson (1997) demonstrated that genetic effective population size can strongly affect the probability of a population's survival and persistence. Conducting their experiment in a natural habitat, they used populations of evening primrose, *Clarkia pulchella*, that were founded by the same number of individuals, but which varied in the relatedness of those individuals (aka. genetic effective population size). Mean population fitness and population growth rate were found to be lower in the low N_e populations and these populations also had an increased probability of extinction than the high N_e populations.

Inbreeding and Inbreeding Depression

Inbreeding is the mating between two individuals with a common ancestor; therefore, it results in an increase in the number of homozygotes in the population (Charlesworth and Charlesworth 1999). The rate at which this happens depends on the average relationship among individuals in a population. The coefficient of inbreeding, *F*, is defined as the probability that

two alleles at a locus are identical by decent, i.e., are direct copies of the same allele in a common ancestor. In a closed population, heterozygosity is proportional to 1 - *F* giving the relationship: $H_F = H_0 (1 - F)$ for which H_F is the heterozygosity of an inbred population and H_0 is the heterozygosity of a non-inbred, random mating population of the same size (Freeman and Herron 2001, p.185).

Inbreeding depression is defined as a reduction in the fitness of individuals or populations as a result of inbreeding. The genetic basis for this reduction in fitness is often due to the increase in homozygosity as a result of alleles being identical by decent because either heterozygotes are superior (overdominance) or because homozygotes for deleterious alleles become more common.

Inbreeding may become unavoidable in small populations, such as those which undergo a demographic bottleneck, because the number of potential mates for an individual is limited. If the population remains small for several generations, then eventually all of the individuals in the population will be related to each other. Therefore, small populations can suffer from inbreeding depression which is a major concern for their conservation, particularly since there is evidence that populations may be more affected by inbreeding depression when in natural environments as opposed to captive ones (Jimenez et al. 1994) making it a large obstacle for reintroduction programs.

Pray and Goodnight (1995) demonstrated that the effect of inbreeding depression can be variable among different genetic lines of a population and also among families of the same line. They also suggested that there is too much variation to predict the level of inbreeding depression in a population based on the level of heterozygosity or the level of inbreeding. However, Pray (1997) showed a trend in *T. castaneum*, in which juvenile mortality was a reasonable measure of

inbreeding depression. A significant correlation between relative fitness and weight in *T. castaneum* was also found, suggesting that weight can be used to evaluate inbreeding depression in populations of this species (Pray 1997).

It is also possible that phenotypic variation could increase after inbreeding due to an increase in a population's sensitivity to the environment (Falconer and Mackay 1996). Pray and Goodnight (1997) showed that phenotypic variance increased as a result of inbreeding in *T. castaneum* for some traits, but not all and in fact decreased in some lines, therefore there is also variation in the response to inbreeding within a single trait.

Theodorou and Couvet (2006) demonstrated that for the same level of inbreeding, larger populations show higher fitness and also, that populations that rebound after a demographic bottleneck have greater fitness compared to populations that did not go through a bottleneck, even as the inbreeding coefficient increases. The level and cost (inbreeding depression) of inbreeding is highly dependent upon the demographic parameters (size, growth rate, and population history e.g. bottlenecks) of the particular population of study.

Heritability

Traits that show continuous variation of phenotypic values in the individuals of a population are considered quantitative traits, such as body weight. The fraction of this variation within the population that can be explained by heritable variation in genes is the heritability, h^2, of a trait. Heritability is always a number between zero and one. A heritability closer to zero indicates variation within the population that is due to variation in the response of individuals to the environment and a heritability closer to one indicates variation within the population that is due to variation in the their genes, with limited environmental influence. Values of heritability

between zero and one indicate variation in the population that is due to both variation in environments and variation in genes of individuals.

Genetic variation in a single locus can be partitioned into additive and dominance components. Additive genetic variance (σ_A^2) is due to the additive effects of genes and dominance genetic variance (σ_D^2) is due to allelic interactions, such as overdominance. The total genetic variance (σ_G^2) is a sum of the additive genetic variance and the dominance genetic variance. Therefore: $\sigma_G^2 = \sigma_A^2 + \sigma_D^2$.

Epistatic variance (σ_I^2) can play a large role in the phenotypic variation within a population. Epistasis occurs when the action of one gene is modified by one or more genes at different loci, which generally leads to variation in gene expression and individual phenotypes.

The total phenotypic variance (σ_P^2) in a population is the sum of all variance components, including additive genetic variance, dominance genetic variance, epistatic variance and environmental variance (σ_E^2) such that: $\sigma_P^2 = \sigma_A^2 + \sigma_D^2 + \sigma_I^2 + \sigma_E^2$.

Most commonly, biologists use what is defined as the narrow-sense heritability. This is the proportion of variation in a trait that is due to the segregation of alleles with additive genetic effects and it is used because it represents the proportion of the total phenotypic variation that is actually transmittable to progeny; dominance and epistatic effects rely on combinations of alleles or genes, which cannot be directly transmitted. It is defined as:

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2} = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_D^2 + \sigma_I^2 + \sigma_E^2} \qquad [1.4]$$

Heritability can also be calculated as the regression of phenotypic values on breeding values in a population. The estimation of the heritability of a trait assumes there is no correlation between genotypes and environments.

Reproductive traits and traits related to fitness generally have low heritabilities, while morphological traits tend to be more highly heritable. However, the heritability of a trait is affected by any changes in genetic or environmental variation. In general, in small populations that are maintained for long enough periods of time, some alleles at certain loci become fixed; it is expected that the heritability will decrease due to a decrease in additive genetic variance (Falconer and Mackay 1996). This would include populations that experience a demographic bottleneck event, where the process of allele fixation is likely accelerated. Also, heritability is expected to decrease with an increase in inbreeding (Falconer and Mackay 1996, Wade et al. 1996). However, these trends will only be seen for traits whose phenotypic values are based on additive genetic variances only (no dominance or epistasis) and when the population is not under selection (Van Buskirk and Willi 2003).

It has been shown that the heritability of a trait may actually increase as a result of a population bottleneck, even though there is an increase in inbreeding (Wade et al. 1996, Bryant et al. 1986, Kaufman et al. 1977). As rare alleles become more represented in a population due to a bottleneck, the non-additivity of allele effects within loci and allele effects among loci can result in an increase in the overall additive genetic variance (Bryant et al. 1986). In this case, if the phenotypic variance remains unchanged, an increase in the heritability of the trait would result. This effect can be very significant, even 50% greater at $F=0.4$ when compared to outbred populations (Van Buskirk and Willi 2003). Bryant et al. (1986) demonstrated in the housefly that heritability and additive genetic variance for morphometric traits increased as a result of a

population bottleneck, although the patterns of increase among bottleneck size varied between traits examined. Despite the increases observed in additive genetic variance following a bottleneck, van Heerwaarden et al. (2008) demonstrated that this does not increase the adaptive potential of populations. This is likely due to the adaptive nature of the originally existing epistatic relationships among genes (Van Buskirk and Willi 2003).

If phenotypic variation decreases due to a demographic bottleneck, but additive genetic variation does not, due to a heterozygote advantage in fitness (as demonstrated by Kaufman et al. 1977), the post-bottleneck heritability will increase as well.

Use of a Model Organism: *Tribolium castaneum*

The red flour beetle, *Tribolium castaneum* (Herbst), is being used for this study because the individuals are small, easily handled and inexpensive to maintain. These beetles are holometabolous insects, undergoing a complete metamorphosis in as few as twenty days after hatch. Determining which beetles are male and female is very easily done at the pupae stage and makes this an ideal species for establishing populations with an equal sex ratio. Also, adults can be removed from the populations when the progeny reaches the pupa stage to ensure that there are no overlapping generations. Laboratory populations kept in an incubator at approximately 33°C and 70% humidity will have a generation interval of approximately 25 days.

Tribolium are polygamous (Pai and Yan 2002a, 2002b), therefore it is likely that a female will lay eggs fertilized by multiple males, and also the males may fertilize eggs in multiple females. This may artificially increase the effective population size, but it is expected that it will be proportional to the number of individuals present and that it will not affect comparisons between populations.

Cannibalism is also a concern when working with these beetles. There is a high rate of cannibalism of adult beetles on pupae and larvae on eggs (Wade 1976) which could affect the number of progeny observed in the populations, particularly since progeny were only counted at the end of every 25 day generation period. However, cannibalism is also an important life history character in *Tribolium* and may be necessary for growth and development (Mertz and Robertson 1970).

Environmental Stressors

It can be hypothesized that if a correlation between heterozygosity and fitness was found in the data, that it would likely be enhanced under environmental stress (Audo and Diehl 1995). Even populations that would otherwise be stable can be greatly affected and even eliminated due to stochasticity in the environment (Dunn and Byers 2008).

One method of introducing a stressor into the environment of the *T. castaneum* populations is addition of an insecticide, such as malathion, to the medium on which the beetles live and feed. Malathion is an organophosphate that inhibits acetylcholine esterase and has been previously shown to reduce *Tribolium* population growth (Sokoloff 1974, Dunbrack et al. 1995).

Another effective method to submit the beetles to an environmental stress is to restrict the atmospheric oxygen concentration (which is required for respiration). Rust et al. (1993) showed that all life stages of *Tribolium confusum* were killed within 96 hours of exposure to a low oxygen atmosphere. In this experiment, beetle populations were placed in chambers which were then flooded with pure nitrogen gas and equipped with Z-1000 Ageless™, an oxygen scavenger, to reduce the oxygen concentration to less than 0.1%. The cause of mortality is suspected to be water loss (Rust et al. 1993).

The Experiment

For the present study, twenty-one populations were established using a laboratory strain of the red flour beetle, *Tribolium castaneum*. The goals of this study were to investigate the consequences of demographic bottleneck events varying in severity, duration and population growth rate (post-bottleneck) on the genetic variation of populations, population fitness, and to measure the ability of those populations to persist under environmental stress. The use of varying strategies for the bottleneck events is based on work by Nei et al. (1975) who showed that the reduction of heterozygosity in a population is not only dependent on the severity of the bottleneck event, but also the rate of population growth after the bottleneck and Day et al. (2003) who showed the intensity and rate of inbreeding to most significantly affect fitness, completely independent of the final level of inbreeding; The loss of alleles in the population, in contrast, appears to be heavily affected by bottleneck severity alone (Nei et al. 1975). By using *T. castaneum* as a model species, the varying bottleneck strategies used in this study could be replicated in a manner such that every individual could be sampled and measured over many generations.

The inbreeding accumulation in the populations was estimated and later correlated to changes in the mean value of fitness related traits, such as pupa weight and progeny numbers. Also, the heritability for pupa weight was estimated in generation two and generation seven. Based on work by Wade (1996), as the level of inbreeding in the populations increases, it is predicted that we will see a decrease in the observed pupae weight heritability, an increase in the variance of fitness related traits, and a decrease in the average number of progeny per female.

According to Falconer and Mackay (1996, p.266) it is possible to predict the decrease in heritability after inbreeding by:

$$h_t^2 = \frac{h_o^2 (1 - F_t)}{1 - (h_o^2 \cdot F_t)} \qquad [1.5]$$

where: h_t^2 is the estimate of heritability of the trait at time t, h_o^2 is the original heritability of the trait and F_t is the inbreeding coefficient in the population at time t. Our results will be compared to estimates we calculate using this equation.

After the populations recover in size following the demographic bottleneck event, they will be subjected to two different environmental stressors: malathion insecticide and restricted atmospheric oxygen concentration. Populations will be challenged in this way in an attempt to magnify any effects of inbreeding accumulation on the fitness of individuals and the populations. The premise behind these experiments was to expose these populations of beetles to severe adverse conditions for which you would only expect individuals carrying rare alleles to be able to withstand, thus only populations with individuals carrying such rare mutations would persist over time. The hypothesis was that populations with greater inbreeding accumulation, due to the difference in bottleneck severity and population size manipulation, would be less likely to have retained rare alleles such as these, and would have higher levels of mortality and poorer reproductive success when exposed to the adverse environmental conditions.

To address the long term effect of a demographic bottleneck on individual fitness, fecundity and fertility will be quantified in generation 19, twelve generations after population size recovery.

Research Questions

In this study, the following questions will be investigated:

a) What is the effect of inbreeding on fitness-related traits in *T. castaneum*?

b) Is there a detectable loss in phenotypic variance or additive genetic variance of traits post-bottleneck and are they dependent on the severity or duration of the bottleneck event or on the growth rate of the population afterwards?

c) After populations recover in size following a demographic bottleneck event, how will stressors introduced into the environment affect survival and reproduction?

d) What are the long-term effects of a historical bottleneck on individual fitness (fertility and fecundity)?

e) Is there a correlation between heterozygosity (measured by estimated inbreeding accumulation) and fitness and if so, what effect does environmental stress have on this correlation?

Chapter 2: Materials and Methods

Structure of the Populations

Six hundred and seventy-two pupa from a laboratory strain of the red flour beetle, *Tribolium castaneum*, obtained from Bloomington, Indiana[1], were randomly assigned to seven different demographic bottleneck strategies (with three replications), each becoming a separate population (labeled A through G) such that each population had sixteen male and sixteen female pupae (Figure 2a). After 25 days, each population was sorted using a mesh to sift out the flour medium and a metal spatula to separate the adults and progeny. Pupae were sexed under a BAYTRONIX® ST60 Series Stereo Microscope. After the pupae were sexed, male and female pupae were randomly selected to maintain the populations. These pupae were placed in new mason jars with fresh medium composed of 95% enriched white flour and 5% brewers yeast (by weight) and covered with appropriately labeled Whatman filter paper. Due to random assignment, the initial variation among populations should be minimal. Populations were maintained separately for 19 generations in a walk-in incubator with a temperature of 33ºC ± 1ºC and 70% ± 2% average humidity.

Table 2.1 illustrates the changes that were imposed on population size over the course of nineteen generations. The seven populations, A through G, represent the different bottleneck strategies for this experiment. The numbers represent the census population size. A 1:1 ratio of

[1] Courtesy of Dr. M. Wade, Department of Biology, Indiana University.

Figure 2a. Completely Randomized Design: Structure of the Populations

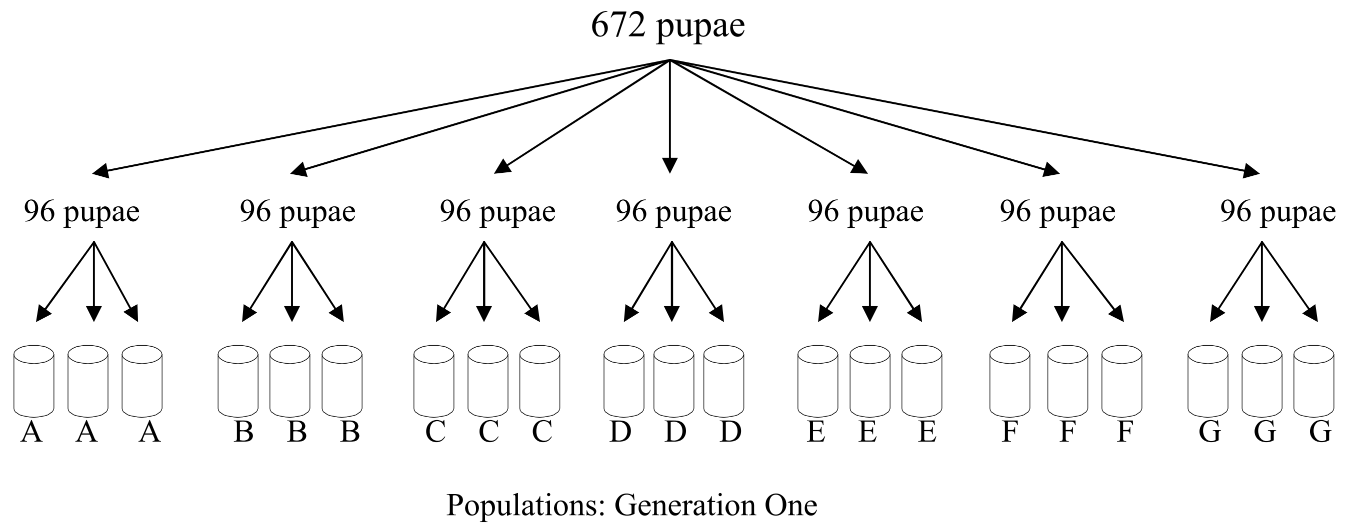


Table 2.1 Experimental Design for Population Size Manipulation

Census Population Size (N)

Malathion Stress

Oxygen Restriction Stress

Fertility and Fecundity Assays

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Population A	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Population B	32	10	10	10	10	10	32	32	32	32	32	32	32	32	32	32	32	32	32
Population C	32	4	4	4	4	4	32	32	32	32	32	32	32	32	32	32	32	32	32
Population D	32	10	12	14	18	24	32	32	32	32	32	32	32	32	32	32	32	32	32
Population E	32	4	10	16	22	28	32	32	32	32	32	32	32	32	32	32	32	32	32
Population F	32	10	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
Population G	32	4	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32

males to females was used in each generation, because an unequal sex ratio changes the effective population size, the effect of which is not being studied in this experiment.

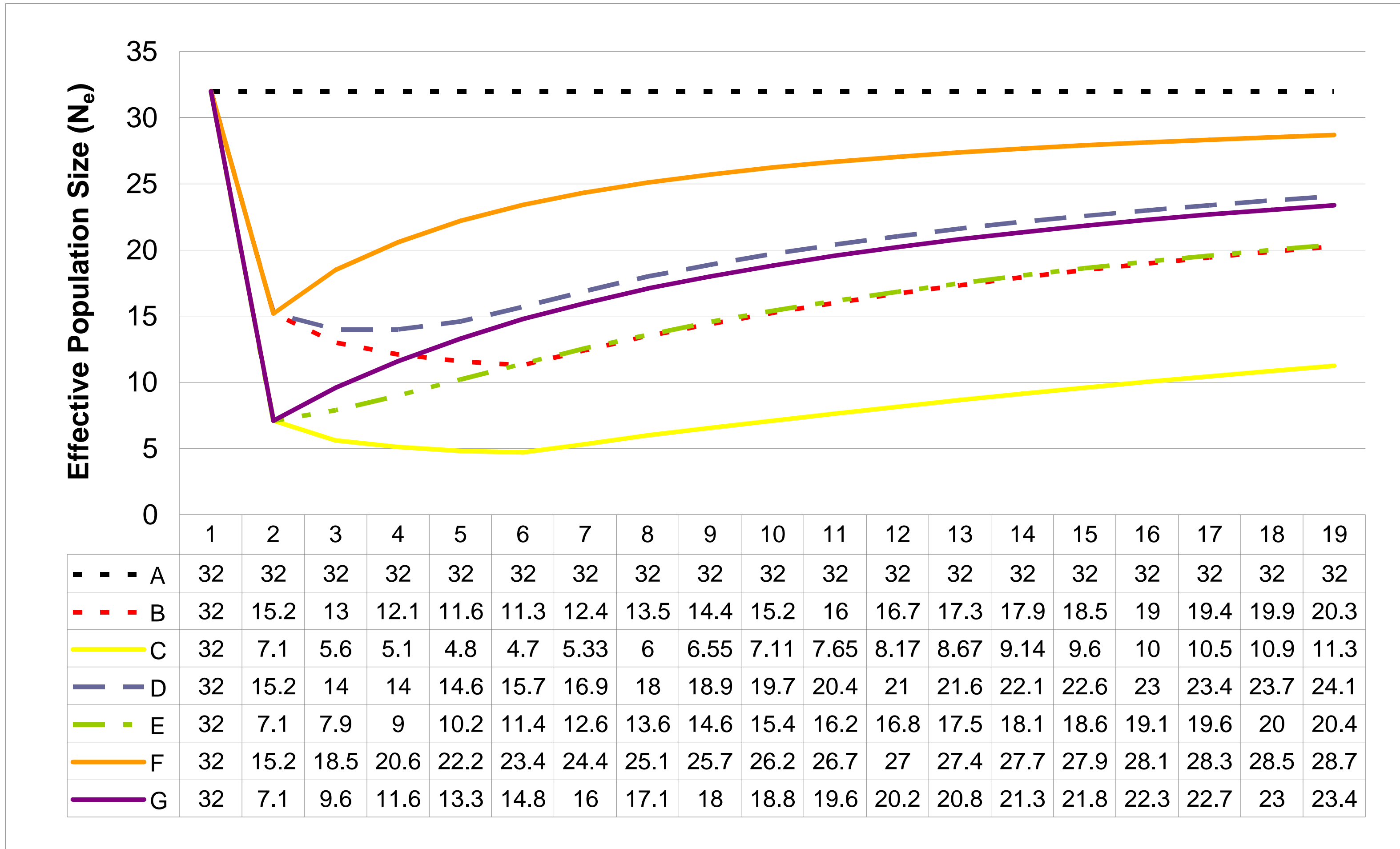
Populations A maintained a constant population size over the seven generations and acted as a 'control' group. Populations B and C simulated demographic bottlenecks that lasted for five generations, but differed in severity, reducing to ten and four individuals, respectively, in generation two. Populations D and E emulated a positive growth rate over five generations after an initial demographic bottleneck, but differed in both initial severity (size ten or four respectively) and growth rate. Populations F and G simulated a single generation demographic bottleneck after which the population size was immediately restored to the original numbers in generation three, varying only in severity (size ten or four respectively).

The progeny used to generate each new generation were selected at random from all pupae in each population at time of selection. Selection was done on individuals in the pupa stage because at this life stage the sex of the individual can be easily determined and all individuals were guaranteed virgin, so there was no potential for overlapping generations.

Calculation of Effective Population Size

The effective population size (cumulative) was calculated for each population using formula [1.3] and these values are presented in Table 2.2. Note that in population A, the effective population size is equal to the census population size because there has been no change in size over all generations. Also, the growth rate for population E was designed to result in an effective population size approximately equal to population B in generation seven and likewise the growth rate for population D was designed to result in an effective population size approximately equal to population G in generation seven. This was done in order to justify meaningful comparisons

Table 2.2 Effective Population Size as a Result of Census Population Size Manipulation



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
B	32	15.2	13	12.1	11.6	11.3	12.4	13.5	14.4	15.2	16	16.7	17.3	17.9	18.5	19	19.4	19.9	20.3
C	32	7.1	5.6	5.1	4.8	4.7	5.33	6	6.55	7.11	7.65	8.17	8.67	9.14	9.6	10	10.5	10.9	11.3
D	32	15.2	14	14	14.6	15.7	16.9	18	18.9	19.7	20.4	21	21.6	22.1	22.6	23	23.4	23.7	24.1
E	32	7.1	7.9	9	10.2	11.4	12.6	13.6	14.6	15.4	16.2	16.8	17.5	18.1	18.6	19.1	19.6	20	20.4
F	32	15.2	18.5	20.6	22.2	23.4	24.4	25.1	25.7	26.2	26.7	27	27.4	27.7	27.9	28.1	28.3	28.5	28.7
G	32	7.1	9.6	11.6	13.3	14.8	16	17.1	18	18.8	19.6	20.2	20.8	21.3	21.8	22.3	22.7	23	23.4

between the populations at this time point. There was no adjustment for sex ratio because all populations were kept at an equal ratio of males to females. The assumption was also made that the variance in family sizes among females was equal to 2, and does not affect the calculation of effective population size.

In order to reduce variation in total progeny numbers among populations due to resource availability, a base amount of medium of four grams was used for the jars containing four individuals and an additional 0.2 grams of medium was added for every individual in excess to four. Thus, all populations of size 32 had 9.6 grams of medium. All jars received fresh medium at the time of selection.

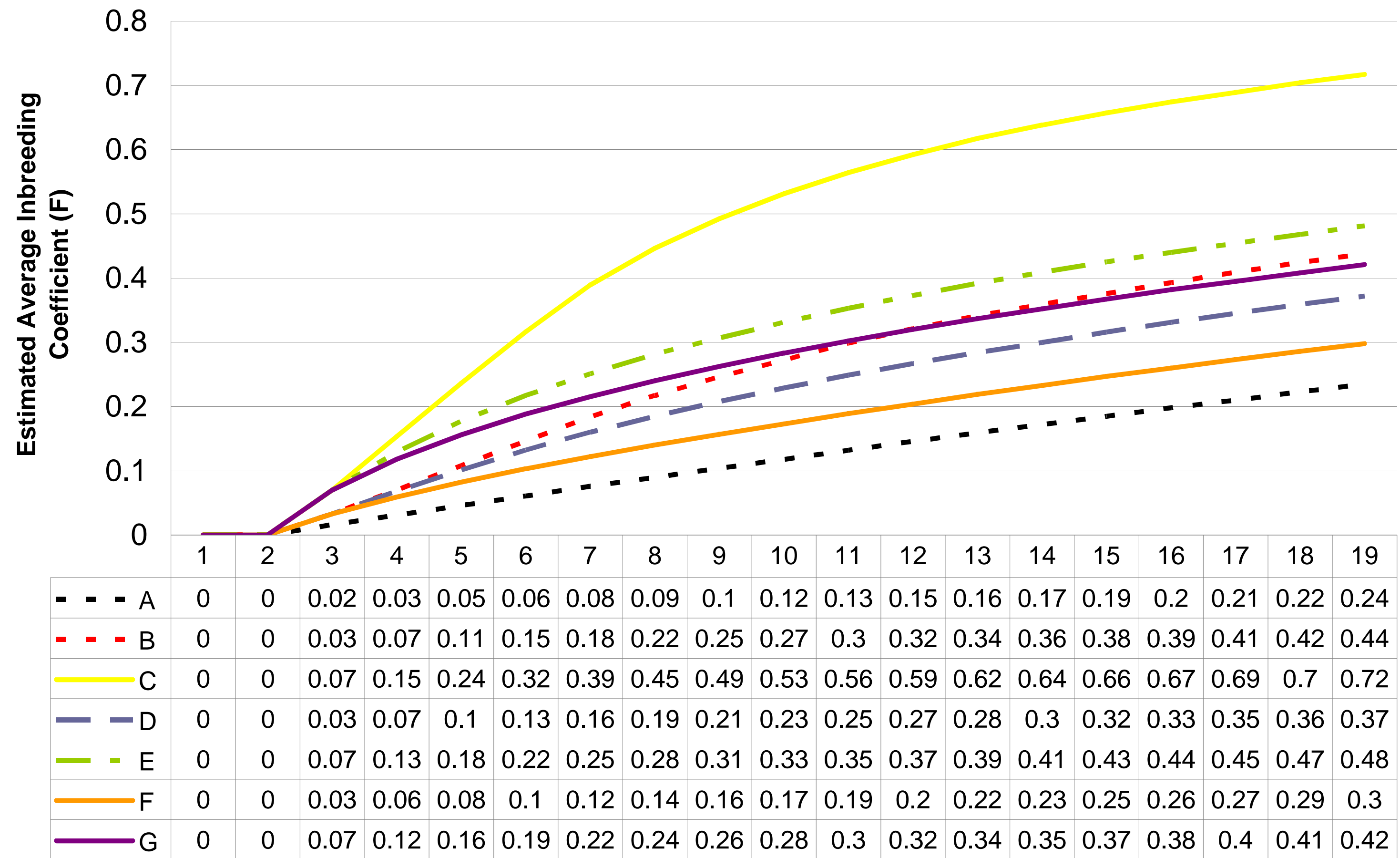
Calculation of Inbreeding Accumulation

Due to random mating within populations, the inbreeding accumulation had to be estimated because pedigree information for individuals is not known. Inbreeding accumulation was estimated using Falconer and Mackay's (1996, p.59) equation:

$$F_t = \frac{1}{2N_e} + \left(1 - \frac{1}{2N_e}\right) \times F_{t-1} \qquad [2.1]$$

where: F_t is the inbreeding coefficient in generation t, F_{t-1} is the inbreeding coefficient in the previous generation and N_e is the effective population size. The estimated inbreeding accumulation for each population in each generation is presented in Table 2.3.

Table 2.3 Estimated Inbreeding Accumulation over 19 Generations



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A	0	0	0.02	0.03	0.05	0.06	0.08	0.09	0.1	0.12	0.13	0.15	0.16	0.17	0.19	0.2	0.21	0.22	0.24
B	0	0	0.03	0.07	0.11	0.15	0.18	0.22	0.25	0.27	0.3	0.32	0.34	0.36	0.38	0.39	0.41	0.42	0.44
C	0	0	0.07	0.15	0.24	0.32	0.39	0.45	0.49	0.53	0.56	0.59	0.62	0.64	0.66	0.67	0.69	0.7	0.72
D	0	0	0.03	0.07	0.1	0.13	0.16	0.19	0.21	0.23	0.25	0.27	0.28	0.3	0.32	0.33	0.35	0.36	0.37
E	0	0	0.07	0.13	0.18	0.22	0.25	0.28	0.31	0.33	0.35	0.37	0.39	0.41	0.43	0.44	0.45	0.47	0.48
F	0	0	0.03	0.06	0.08	0.1	0.12	0.14	0.16	0.17	0.19	0.2	0.22	0.23	0.25	0.26	0.27	0.29	0.3
G	0	0	0.07	0.12	0.16	0.19	0.22	0.24	0.26	0.28	0.3	0.32	0.34	0.35	0.37	0.38	0.4	0.41	0.42

Phenotypic Data Collection

To measure average fitness of the different populations, pupa weights, adult weights, and mean number of progeny per female were recorded. Additionally, fecundity and fertility were evaluated by a protocol established to measure mean three-day egg lay and four-day egg hatchability. It was assumed that a greater average pupa weight, adult weight, progeny numbers, fecundity and fertility would be associated with higher population fitness. One uncontrolled effect that may have had an influence on the data was cannibalism. Cannibalism occurs most often by larva eating eggs and adults eating pupae (Wade 1976). It is expected that there will be a higher rate of cannibalism in the jars with a greater amount of individuals, but there is no easy way to control for this, and it actually appears to increase the fitness of surviving individuals (Mertz and Robertson 1970).

Pupa Weight

Pupa weight was measured on every individual at the initial set-up of all the populations (generation one) and after random selection of individuals in each subsequent generation through generation 11. Only individuals selected to keep from each generation were weighed. Measurements were taken on a Mettler Toledo XP26 analytical balance with precision of 0.002mg.

Adult Weight

After each population was sorted, adult individuals were placed in a Fisher Scientific Isotemp freezer at -20ºC. Adults were removed and thawed at a later time (varies by individual) for DNA extraction; post-thaw weights were recorded for generations one and seven.

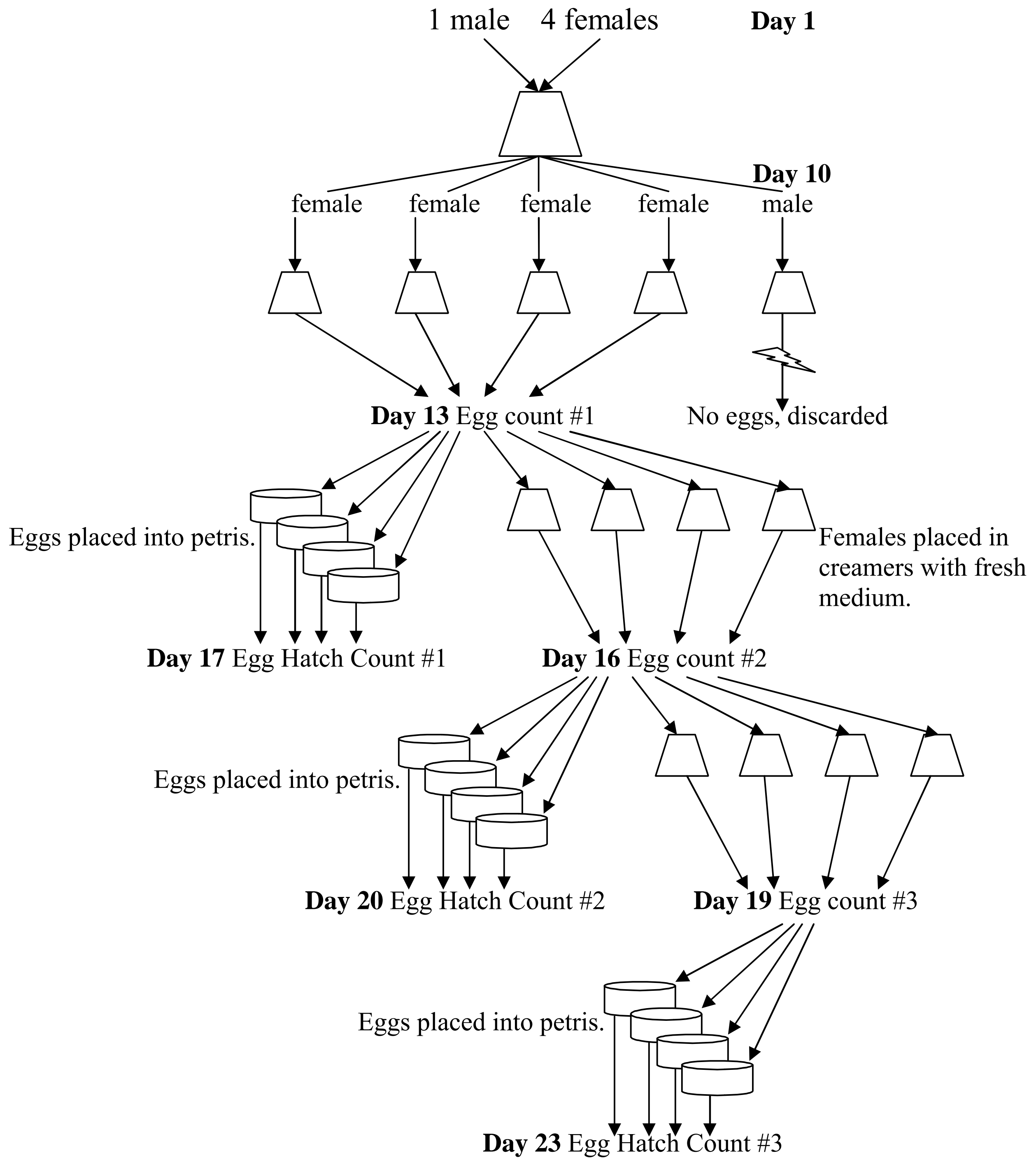
Progeny Counts

At the time of sorting and random selection, all larvae, pupae and young adults were counted in generations one through eight. Eggs were not counted and were not included in the total progeny number reported for each population. Eggs were excluded because it was expected there would be few eggs present in the jars at this time due to cannibalism by larvae. Time available for processing of the populations was also insufficient to ensure good accuracy.

Fecundity and Fertility

Assays for fecundity and fertility of female beetles in generation 19 were performed by first setting up five families each for populations A and C, replication 3 only, with one male and four female individuals chosen randomly from their respective populations. These families were allowed to mate for ten days prior to separation. On day ten, all individuals were placed in separate creamers containing 1/4tsp of the medium. After three days, the number of eggs laid was recorded for each female. This was repeated two more times to get three records per female for three-day egg lay and the mean three-day egg lay was used as a measure of fecundity. All of the eggs recovered per egg count were placed in a single petri dish without medium and four days post-egg count the number of larvae present was recorded. This produced three measurements of the proportion of eggs hatching, per female. The mean proportion of eggs hatching, per female, was used as a measure of fertility, subsequently referred to as hatchability (Figure 2b).

Figure 2b. Fecundity and Fertility Assay: Set-up for One Family



Measuring Genetic Variation through Heritability Estimation

Heritability of pupa weight was first estimated in generation two using a full-sibling covariance analysis. Two single-pair matings were set up in each population (using excess progeny from generation one that were not included in the maintenance of treatment lines; refer to Figure 2c), for a total of 42 pairs (2 pairs/population x 7 populations/replicate x 3 replicates). The individuals used for each mating were assumed to be unrelated. Each pair of pupae was weighed before being placed in a creamer with approximately ten grams of medium. After 25 days, eight pupa progeny (four males and four females) from each pair were weighed[2]. A total of 288 progeny were weighed (8 progeny/pair x 36 pairs); six mating pairs failed to produce offspring. Data from all replications was pooled for each population because there should be no difference in heritability between populations or replications in generation two.

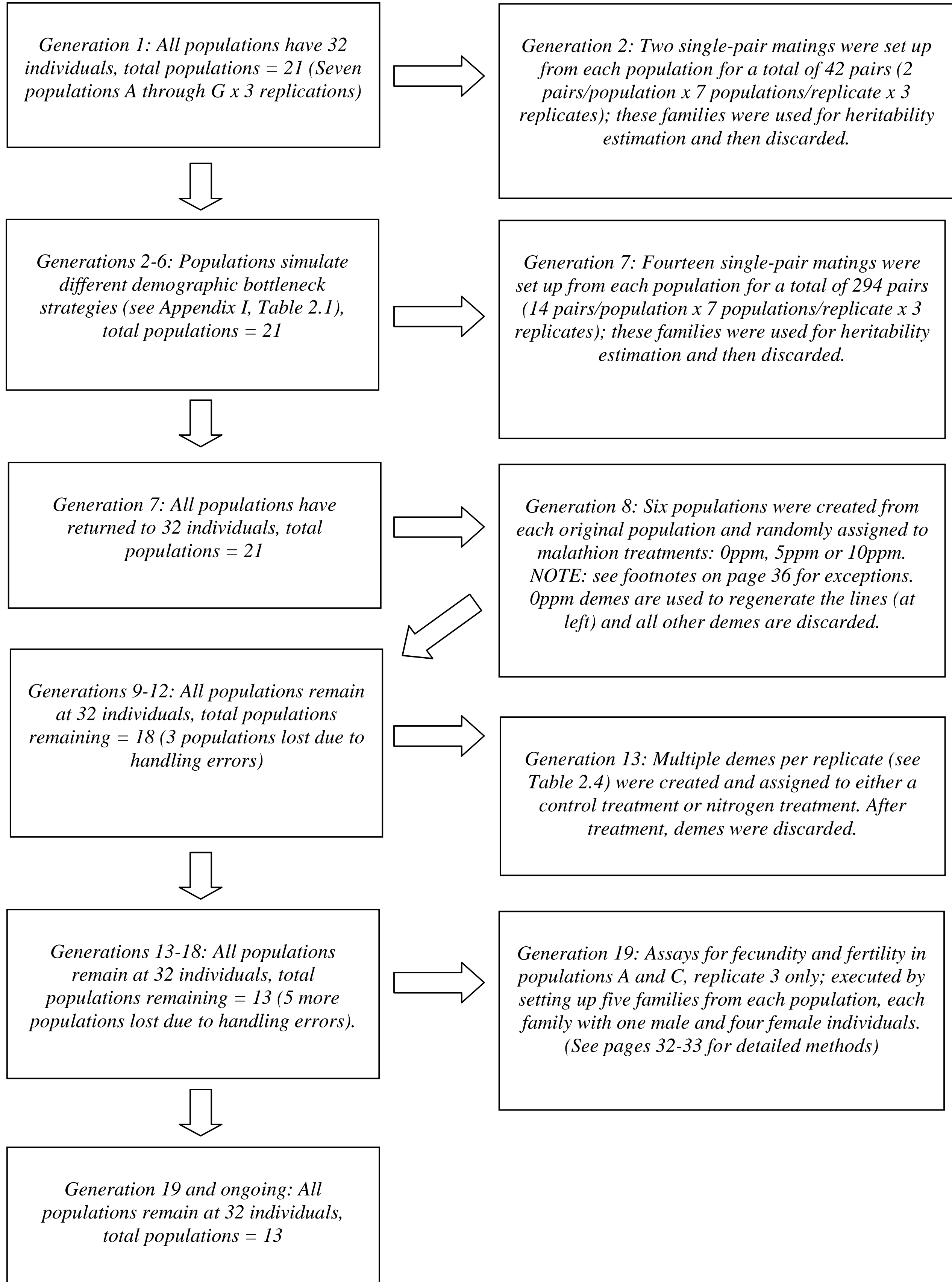
Heritability of pupa weight was again calculated for generation seven, using excess progeny. Fourteen male-female pairs from each of the populations, A through G, per replication, were put into creamers. Each pair was weighed before being placed into the creamers. In replication one, after 25 days, six pupa progeny (three males and three females) from each pair were weighed.[3] In replications two and three, after 25 days, four pupa progeny (two males and two females) from each pair were weighed.[4] A total of 1248 progeny were weighed (6 progeny/pair x 92 pairs, 4 progeny/pair x 169 pairs, + 20 other individuals); twenty-four mating

[2] One pair (Pair #2, Population D, Replication 1) had only 5 female and 3 male pupae total, so these eight individuals were weighed.

[3] Pair #3, Population C and Pair #13, Population D had only 3 pupae, so these were weighed. Pair #2, Population F had only 4 male and 2 female pupae total, so these six pupae were weighed. Pair #10, Population F had only 4 pupae, so these were weighed.

[4] Pair #1, Population C, Replication 2 had only 4 pupae, so these were weighed. Pair #3, Pair #5, and Pair #6, Population C, Replication 2 and Pair #8, Population C, Replication 2 had only one pupa, so this pupae was weighed alone. Pair #12, Population C, Replication 2 and Pair #11, Population F, Replication 2 had only 3 pupae, so these were weighed.

Figure 2c. Complete Experiment Flow Chart



pairs failed to produce pupae offspring by 25 days. Data for each population (all replications) was pooled for analysis.

Predictions for the change in heritability were calculated based on equation [1.5].

Environmental Stress Experiments

After the initial seven generations of varying population size were completed and all populations had returned to 32 individuals, the populations of beetles were challenged with environmental stressors in two instances. In generation eight, the populations were exposed to malathion insecticide. In generation thirteen, the level of atmospheric oxygen available to the beetles was restricted (Figure 2c).

Malathion Treatment

In generation eight, the pupae available at the time of sorting were used to set up the malathion experiment. In each replication, six populations were created from each original population[5]. The six populations were randomly assigned to one of three different malathion treatments: 0ppm, 5ppm or 10ppm. These concentrations were chosen based on previous work published by Dunbrack et. al (1995) which showed a significant decrease in population growth at 5ppm and 10ppm malathion compared to untreated populations (0ppm). The malathion was applied to the jars prior to the placement of any individuals in the jars. It was applied in water droplets (1% malathion concentration) onto the surface of the flour and then the flour was stirred to promote the dispersion of the insecticide within the jar. Jars assigned to the 0ppm of malathion treatment still received water droplets without any insecticide added.

[5] Within replication 2, there was insufficient progeny to create duplicate jars for 5ppm and 10ppm for populations B and C. Within replication 3, only the control and 10ppm treatments were duplicated.

Sixteen male and sixteen female pupae were placed on top of the medium mixture. The individuals entering one of the control (0ppm malathion) jars, per replication, per population were weighed using the Mettler Toledo XP26 analytical balance. These individuals later became the parents of all populations in generation nine.

After 25 days, all 0ppm, 5ppm and 10ppm malathion treated populations were sorted. All adults, young adults, pupa and larva were counted. The percent mortality for generation eight individuals was calculated based on the number of live adults remaining out of the original thirty-two. The total number of progeny produced, surviving to the day of sort, was also recorded.

Atmospheric Oxygen Restriction Experiment

Generation 13 pupae were used to create multiple demes, per replicate (Table 2.4), which were then randomly assigned to either the control treatment or the nitrogen treatment. Each deme contained sixteen male and sixteen female adult beetles in 9.6g of medium. For this experiment, a split-plot design was used. Demes designated as controls were placed in a 33ºC, high humidity VWR Scientific 2250 model, CO_2 incubator for 72 hours. Demes assigned to the nitrogen treatment were placed in a second 33ºC, high humidity VWR Scientific 2250 model, CO_2 incubator with a continuous flow of nitrogen gas at 300ccm (25psi) being pumped into the chamber. All replicate one jars were incubated simultaneously at time 1, replicate two jars were incubated at time 2, and replicate three jars were incubated at time 3. After the 72hr incubation, the jars were removed and allowed to sit and dry out overnight. After drying, the jars were sorted and the number of live and dead adult beetles was counted and recorded.

Table 2.4 Atmospheric Oxygen Restriction Experiment

Population	Treatment (# of demes)					
	Control			Nitrogen		
	Replication 1	Replication 2	Replication 3	Replication 1	Replication 2	Replication 3
A	1	3	2	2	3	3
B	2	0	3	3	0	3
C	2	0	1	3	0	2
D	2	3	4	2	3	2
E	3	0	3	3	0	3
F	3	3	2	3	3	3
G	1	3	2	2	3	3

During replication 3, the incubator doors were sealed with tape in order to block air from passing outside the incubator. The decision was made to alter the experiment in this way when the results from the first two replicates did not resemble the expected results based on a pilot experiment, the cause likely owing to an insufficient air-tight seal; in the pilot experiment, six out of the seven populations (A through G) had fewer adults surviving after 56 hours in the oxygen restricted incubator when compared to the control incubator.

Statistical Analysis

All analyses were done using SAS software (version 9.1). Most variables measured in this study were analyzed using an analysis of variance through PROC MIXED. In general, the models included population, generation, replication and treatment (where applicable) as fixed effects. An analysis of covariance was required to analyze the adult weight data because there

was a significant effect of days frozen on the post-thaw weight; 'days in freezer' was used as the covariate in the model. Specific details on each model used are given in Chapter 3.

A set of non-orthogonal contrasts was used to make meaningful comparisons between specific linear combinations of populations. These contrasts were used for most variables measured. To examine the effect of severity, the H_0: Populations B + F = Populations C + G (severity of 10 vs. severity of 4) was tested. To examine the effect of bottleneck duration, the H_0: Populations B + C = Populations F + G (duration of five generations vs. one) was tested. To examine the effect of population growth rate post-bottleneck, the H_0: Populations D + E = Populations F + G (slow growth vs. fast growth) was evaluated. The Bonferroni correction was used to determine significant differences between population combinations.

The PROC VARCOMP procedure (REML method) was used to estimate the additive genetic and phenotypic variance components from our full-sib mating data. This required the full-sib matings to be nested within replication to allow pooling of the data from all replications to obtain a more precise estimate of heritability.

The PROC FACTOR procedure (principal component method) was used to create single factors from multiple correlated variables measured in this study. These factors included: the phenotypic factor (based on generation eight pupa weight, generation seven adult weight and total number of progeny in generation seven), the stress factor (based on the proportion of adults surviving and the total number of progeny surviving the 10ppm malathion treatment), the variance factor (based on the additive genetic variance and the phenotypic variance in generation seven), and the inbreeding factor (based on the effective population size in generation eight and the estimated inbreeding accumulation in generation 8). PROC CORR and PROC REG

procedures were then used to investigate linear and polynomial associations, respectively, between the factors.

Type I error rate was set at $\alpha=0.05$ as the threshold for significance for all hypothesis tests. Lack of significant differences imply that a P-value higher than 0.05 would result if the null hypothesis were to be rejected.

Chapter 3: Results and Data Analysis

Phenotypic Data

Pupa Weight

Individual pupa weights were recorded and the least squares means were quantified for generations one through eleven for each population (Tables 3.1 – 3.11). Population means were compared within each generation. The statistical model used was $Y_{ijk} = \mu + P_i + R_j + \varepsilon_{ijk}$, with population and replication as fixed effects in the model. There were no significant differences in mean pupae weight between populations in any generation when using the Tukey HSD adjustment for all pairwise comparisons.

A set of orthogonal contrasts was used to compare mean pupae weight in generation eight to generation one for each population (Table 3.12). The statistical model used in the ANOVA was $Y_{ijk} = \mu + G_i + R_j + \varepsilon_{ijk}$, with generation and replication as fixed effects in the model. There was no significant difference in mean pupae weight between these generations for any population. Also, there were no significant differences between bottleneck severity, duration or growth rate of populations.

Adult Weight

Adult weights were collected on individuals in generation one and generation seven. The adults were weighed post-thaw and since not all adults were in the freezer for an equal number of days, ‘days in freezer’ had to be added as a covariable to the ANOVA. The analysis was done

Table 3.1 Generation One Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	1.923	1.922	1.860	1.902 ± 0.0207
B	1.944	1.906	1.901	1.917 ± 0.0136
C	1.900	1.901	1.896	1.899 ± 0.0016
D	1.911	1.864	1.780	1.852 ± 0.0385
E	1.953	1.857	1.856	1.889 ± 0.0323
F	1.867	1.880	1.813	1.853 ± 0.0204
G	1.922	1.884	1.819	1.875 ± 0.0301

Table 3.2 Generation Two Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	2.033	2.042	2.002	2.026 ± 0.0123
B	2.131	2.184	1.932	2.082 ± 0.0767
C	1.953	2.060	2.053	2.022 ± 0.0347
D	2.056	2.054	2.032	2.047 ± 0.0077
E	2.097	2.085	1.946	2.043 ± 0.0484
F	1.906	2.063	1.948	1.972 ± 0.0471
G	1.895	2.016	2.113	2.008 ± 0.0628

Table 3.3 Generation Three Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	2.124	2.082	1.966	2.057 ± 0.0472
B	1.974	1.987	1.949	1.970 ± 0.0110
C	2.223	2.222	1.997	2.147 ± 0.0753
D	1.993	2.065	1.997	2.019 ± 0.0233
E	2.289	2.084	1.992	2.122 ± 0.0879
F	1.823	2.063	1.998	1.961 ± 0.0715
G	2.02	2.019	1.877	1.972 ± 0.0477

Table 3.4 Generation Four Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	2.040	2.008	1.964	2.004 ± 0.0220
B	2.022	2.054	2.073	2.050 ± 0.0147
C	1.932	2.043	1.980	1.985 ± 0.0322
D	1.977	2.164	2.038	2.060 ± 0.0551
E	2.173	2.102	1.927	2.067 ± 0.0732
F	1.958	2.046	1.967	1.990 ± 0.0280
G	2.030	2.013	1.894	1.979 ± 0.0428

Table 3.5 Generation Five Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	2.099	2.005	1.899	2.001 ± 0.0577
B	2.149	2.164	2.109	2.141 ± 0.0166
C	1.960	1.991	1.710	1.887 ± 0.0890
D	1.915	2.181	1.924	2.007 ± 0.0872
E	2.302	2.122	1.956	2.127 ± 0.0998
F	1.930	2.163	1.967	2.020 ± 0.0722
G	2.017	2.045	1.905	1.989 ± 0.0427

Table 3.6 Generation Six Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	1.994	2.036	1.992	2.007 ± 0.0142
B	2.123	2.216	1.847	2.062 ± 0.1108
C	1.991	1.853	1.768	1.870 ± 0.0649
D	1.889	2.087	1.949	1.975 ± 0.0584
E	2.190	1.974	1.924	2.029 ± 0.0819
F	2.016	2.123	2.027	2.056 ± 0.0340
G	1.935	2.071	1.911	1.972 ± 0.0498

Table 3.7 Generation Seven Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	1.888	2.108	1.857	1.951 ± 0.0789
B	2.093	2.109	1.953	2.052 ± 0.0496
C	2.008	1.797	1.804	1.870 ± 0.0691
D	1.830	2.074	1.862	1.922 ± 0.0764
E	2.272	2.029	1.916	2.072 ± 0.1050
F	2.001	2.084	2.001	2.028 ± 0.0277
G	1.879	2.086	1.940	1.968 ± 0.0614

Table 3.8 Generation Eight Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	1.939	2.03	1.898	1.955 ± 0.0390
B	2.037	2.073	1.829	1.980 ± 0.0762
C	1.990	1.720	1.939	1.883 ± 0.0827
D	1.872	2.037	1.824	1.911 ± 0.0645
E	2.146	2.055	1.830	2.010 ± 0.0939
F	1.995	1.984	1.860	1.947 ± 0.0432
G	1.920	1.910	1.906	1.912 ± 0.0043

Table 3.9 Generation Nine Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	1.975	1.966	1.866	1.936 ± 0.0351
B	2.028	1.866	1.864	1.919 ± 0.0542
C	1.868	1.802	1.824	1.831 ± 0.0193
D	1.835	1.968	1.767	1.857 ± 0.0589
E	2.111	1.916	1.670	1.899 ± 0.1275
F	1.991	1.913	1.846	1.917 ± 0.0420
G	1.835	1.918	1.809	1.854 ± 0.0330

Table 3.10 Generation Ten Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	2.001	1.975	1.92	1.965 ± 0.0238
B	2.021	.	2.053	2.037 ± 0.0158
C	1.875	.	1.672	1.773 ± 0.1014
D	1.799	1.987	1.917	1.901 ± 0.0547
E	2.179	.	1.642	1.911 ± 0.2682
F	1.969	1.985	1.818	1.924 ± 0.0533
G	1.873	2.069	1.742	1.895 ± 0.0948

Table 3.11 Generation Eleven Mean Pupa Weight

Population	Mean Pupae Weight (mg)			Pooled Mean Pupae Weight (mg ± SE)
	Replication 1	Replication 2	Replication 3	
A	1.966	2.076	1.796	1.946 ± 0.0816
B	1.927	.	2.019	1.973 ± 0.0457
C	1.935	.	1.841	1.888 ± 0.0473
D	1.823	2.073	1.956	1.951 ± 0.0724
E	2.234	.	1.821	2.028 ± 0.2062
F	2.008	2.125	1.957	2.030 ± 0.0497
G	1.883	1.984	1.971	1.946 ± 0.0315

Table 3.12 Generation One and Generation Eight Mean Pupa Weight

Population	Pooled Mean Pupae Weight (mg ± SE)	
	Generation 1	Generation 8
A	1.902 ± 0.0207	1.955 ± 0.0390
B	1.917 ± 0.0136	1.980 ± 0.0762
C	1.899 ± 0.0016	1.883 ± 0.0827
D	1.852 ± 0.0385	1.911 ± 0.0645
E	1.889 ± 0.0323	2.010 ± 0.0939
F	1.853 ± 0.0204	1.947 ± 0.0432
G	1.875 ± 0.0301	1.912 ± 0.0043

separately for generations one and seven, so the least squares means were calculated around the mean 'days in freezer' for each generation (Table 3.13). The statistical model used was $Y_{ijk} = \mu + P_i + R_j + \beta_1(x_{ijk} - \bar{x}) + \varepsilon_{ijk}$; population and replication are fixed effects in the model, and 'days in freezer' is the covariable whose effect is estimated by the regression coefficient β_1.

Table 3.13 Adult Weight Means Post-Thaw in Generations One and Seven; Corrected for Days in Freezer

Population	**Pooled Mean Adult Weight (mg)**			
	Generation 1	Tukey HSD Grouping*	Generation 7	Tukey HSD Grouping*
A	1.120	a	0.986	c
B	1.227	a	1.162	b
C	1.203	a	1.050	c
D	1.182	a	1.152	b
E	1.193	a	1.288	a
F	1.174	a	1.243	ab
G	1.086	b	1.250	ab

*Tukey HSD Groupings significant at P<0.05

Adult weight means were compared using Tukey's HSD test within each generation (see Table 3.13). Population G had a significantly lower mean adult weight compared to all other populations, P<0.05, in generation one. Populations A through F were not significantly different from one another in generation one. Populations were not expected to be significantly different at this point, so this difference was likely due to chance alone.

In generation seven, there were four different Tukey groupings. Populations E, F and G fell into the heaviest group and populations A and C fell into the lightest, and these two groups were mutually exclusive. The groupings were significant at P<0.05. There were no significant differences between populations with approximately equal effective population sizes.

When performing contrasts to measure the differences between bottleneck severity, duration or growth rate, there was a significant difference observed between populations that endured a five generation long bottleneck (populations B and C) and only a single generation bottleneck (populations F and G). Single generation bottleneck populations were 0.140mg heavier on average, compared to five generation bottleneck populations, $P<0.05$. There was a significant difference between populations of comparable effective population size, B and E, $P<0.05$. Even though the initial bottleneck severity in population E was greater, these adults were heavier than those from population B, which endured a five generation long bottleneck event.

Progeny Counts

The total number of progeny per population was calculated and compared between populations in generation one and generation seven (Table 3.14). The statistical model used was $Y_{ijk} = \mu + P_i + R_j + \varepsilon_{ijk}$, with population and replication as fixed effects in the model. There were no significant differences in total progeny among populations in generation one or generation seven when using the Tukey HSD adjustment for all pairwise comparisons. A difference of 188 total progeny would have been needed to declare populations significantly different; the largest difference observed was 123 progeny between population F (351 total progeny) and C (228 total progeny).

A set of one-tailed orthogonal contrasts was used to compare the total number of progeny in generation one to generation seven (H_A: total progeny in generation seven of population i < total progeny in generation one of population i). The statistical model used in the ANOVA was $Y_{ijk} = \mu + PG_i + R_j + \varepsilon_{ijk}$. Population and generation were combined to create a single factor, and thus one fixed effect in the model and replication was also a fixed effect in the model. A

Table 3.14 Mean Total Progeny in Generation One and Generation Seven

Population	Total Number of Progeny			
	Generation 1		Generation 7	
	Mean	Standard Error	Mean	Standard Error
A	314	± 10.7	266	± 33.9
B	327	± 12.4	268	± 34.8
C	315	± 44.8	228*	± 31.6
D	318	± 19.3	287	± 47.1
E	322	± 6.60	328	± 34.8
F	313	± 14.4	351	± 8.30
G	338	± 13.0	335	± 44.4

* Significant decrease in mean total progeny compared to generation 1, P=0.0253

significant decrease in the total number of progeny in generation seven when compared to generation one was seen only in population C (228 and 315 total progeny respectively), P=0.0253.

There were no significant differences found between bottleneck severities, durations, or growth rates. There were also no significant differences between populations of comparable effective population size.

Fecundity and Fertility

Fecundity was measured on individual female beetles as their average three-day egg lay based on three observations. These values were then averaged over females coming from a single population (Table 3.15). Only populations A and C from replication three in generation 19 were used in this experiment. These populations were chosen because they represent the highest (C) and lowest (A) predicted levels of inbreeding accumulation. The statistical model used to analyze

this data was $Y_{ij} = \mu + P_i + \varepsilon_{ij}$, with population as a fixed effect in the model. Population A laid a significantly greater number of eggs (42.62 eggs per female) than population C (31.65 eggs per female), P=0.0146.

Fertility was measured as the mean proportion of eggs laid that successfully produced a larva by the fourth day after the egg counts were obtained (or hatchability). Differences in fertility of the males used in the matings could influence fertility measures in the females. This effect was accounted for by including the random effect of male, designated as S_j in the statistical model (Figure 2b). The model used to analyze this data was $Y_{ijk} = \mu + P_i + S_j(P_i) + \varepsilon_{ijk}$, where P represents the population, a fixed effect in the model and S_j represents the male (nested within each population) to which a group of four females was mated. There was no significant difference in hatchability between population A and population C (Table 3.15).

Table 3.15 Fecundity and Fertility Assay:
Populations A and C, Replication 3 Only

	Population A	**Population C**
Fecundity*:		
Mean three day egg lay (± SE)	42.62 ± 3.045	31.65 ± 3.012
Fertility:		
Mean proportion of eggs hatching by day four (± SE)	0.600 ± 0.0305	0.565 ± 0.0340

* Significant difference between populations, P=0.0146

Pupa Weight Heritability

To estimate pupa weight heritability in generations two and seven, single pairs of individuals were randomly chosen from the parent populations and placed together to mate in creamers. Under this mating design, the covariance between full-siblings estimates one half of the additive genetic variance (Table 3.16). The ratio of the additive genetic variance over the phenotypic variance estimates the heritability of pupa weight. The statistical model used to obtain the variance component estimates in generation two for each population was $Y_{ij} = \mu + M_i + \varepsilon_{ij}$; where the mating pair M_i, is a random effect. This model pools all the data from each population, regardless of which replication the parents originated from; there should be no effect of replication on heritability in generation two because all individuals are assumed to have zero inbreeding accumulation (coancestry is assumed to be zero for all individuals in generation one). Population B and population D had too many unsuccessful breeding pairs in generation two to allow adequate estimation of variance components.

Table 3.16 Analysis of Variance Table for Generation Two and Seven Pupa Weight Heritability Estimates (Adapted from Becker 1984)

Source of Variation	df	SS	MS	EMS
Between matings	m-1	SS_m	MS_m	$\sigma_w^2 + k\sigma_m^2$ [1]
Between progeny, within matings	n-m	SS_w	MS_w	σ_w^2

[1] σ_m^2 is equivalent to the covariance of full sibs

For generation seven, a nested model was used to estimate the variance components because matings originating from a common replication are more likely to resemble one another

due to coancestry. In this case, the statistical model used was $Y_{ijk} = \mu + R_i + M_j(R_i) + \varepsilon_{ijk}$; replication was a fixed effect and the single-pair matings were nested within replication as a random effect in the model. Additive genetic variance, phenotypic variance, and heritability were estimated for each population and the values are presented in Table 3.17.

Table 3.17 Variance Component Estimates Based on Full-Sib Covariance Analysis

Population	**Additive Genetic Variance ($\hat{\sigma}_A^2$)**		**Total Phenotypic Variance ($\hat{\sigma}_P^2$)**		**Heritability ($\hat{h}^2$)**		**Coefficient of Additive Variance (CV_a x 100)**	
	Generation		Generation		Generation		Generation	
	2	7	2	7	2	7	2	7
A	0.04026	0.011310*	0.04969	0.043905	0.81	0.26	9.2	5.1
B	.	0.019045	.	0.037133	.	0.51	.	6.4
C	0.08488	0.024400*	0.08935	0.051340**	0.95	0.47	13.2	8.5
D	.	0.008233	.	0.030506	.	0.27	.	4.6
E	0.05116	0.011406**	0.05827	0.046323	0.89	0.25	10.7	4.9
F	0.02412	0.007150*	0.04759	0.041945	0.51	0.17	7.1	4.0
G	0.08126	0.022340*	0.09874	0.052710**	0.82	0.42	13.6	7.3

* Significant decrease in variance at P < 0.05 compared to generation 2
** Significant decrease in variance at P < 0.01 compared to generation 2

Both variance components, $\hat{\sigma}_A^2$ and $\hat{\sigma}_P^2$, were compared between generation two and seven within each population, excluding populations B and D. An F-test (based on the ratio of variances) was used to determine significance. All populations examined showed a significant decrease in additive genetic variance in generation seven when compared to generation two when $\alpha=0.05$. Population E still showed a more extreme decrease in additive genetic variance, at $\alpha=0.01$ (Table 3.17). However, only populations C and G showed a significant decrease in total phenotypic variance in generation seven when compared to generation two ($P<0.01$). Regardless, all the populations showed a decrease in the estimated heritability for pupa weight in generation seven when compared to generation two, as predicted.

Equation [1.5] was used to calculate the expected heritability in generation seven based on the predicted level of inbreeding accumulation. Expected heritabilities are reported in Table 3.18. In each case, the actual heritability estimated was less than that predicted using Falconer and Mackay's equation.

Table 3.18 Predicted Generation Seven Pupa Weight Heritability and Actual Estimates

Population	Generation 2 Heritability ($\hat{h}_0^2$)	Inbreeding Accumulation In Generation Seven	Generation 7 Heritability ($\hat{h}_t^2$)	
			Predicted	Actual
A	0.81	0.076	0.64	0.26
B	.	0.184	.	0.51
C	0.95	0.389	0.85	0.47
D	.	0.16	.	0.27
E	0.89	0.251	0.74	0.25
F	0.51	0.122	0.24	0.17
G	0.82	0.215	0.62	0.42

Environmental Stress Experiments

Malathion Insecticide Treatment

The total number of adult beetles remaining after the malathion treatment was counted and the mean proportion (%) of adults surviving was calculated for each population, at each concentration: 0ppm, 5ppm and 10ppm (Table 3.19). The statistical model used to analyze this data was $Y_{ijk} = \mu + P_i + T_j + P_i \times T_j + \varepsilon_{ijk}$; P_i is the fixed effect for the population, T_j is the fixed effect for the malathion concentration and the population by treatment interaction is also included as a fixed effect in the model. The interaction between population and treatment was found to be non-significant. There were also no significant differences in adult survival found between populations. There was a significant effect of malathion concentration on adult survival

Table 3.19 Malathion Stress Experiment:
Mean Adult Survival and Mean Total Progeny

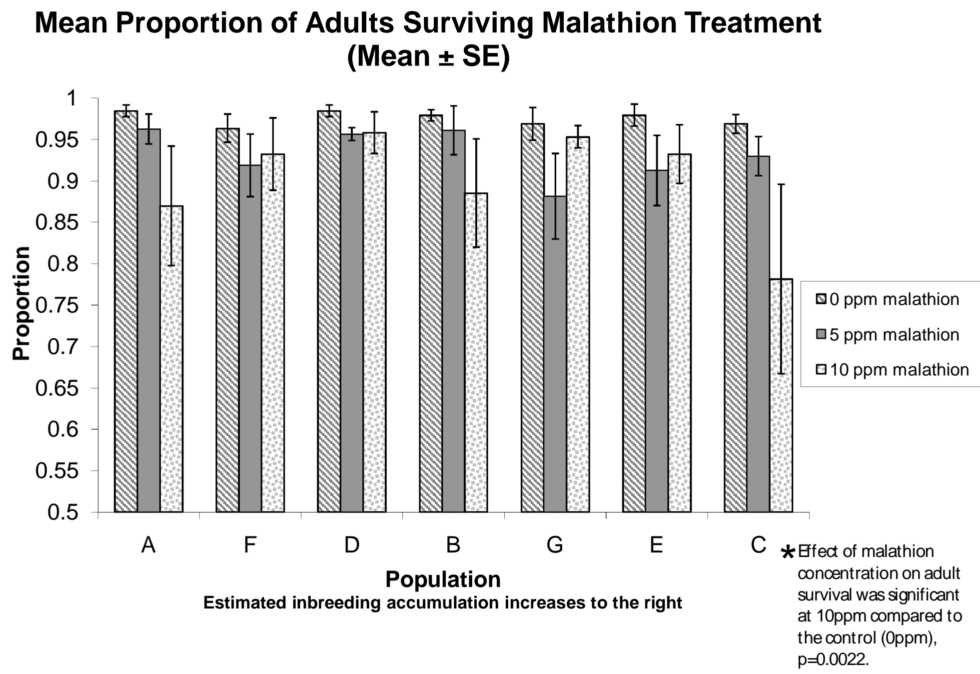
Population	Mean Proportion of Adults Surviving Malathion Treatment (%)			Mean Total Number of Progeny Surviving Malathion Treatment			
	0ppm	5ppm	10ppm*	0ppm	5ppm	10ppm	Tukey HSD Groupings***
A	98.4	96.3	87.0	262.5	237.0	255.5	a
B	97.9	96.1	88.5	272.7	279.8	251.8	a
C	96.9	93.0	78.1	227.2	226.3	116.7**	b
D	98.4	95.6	95.8	297.3	268.0	306.2	a
E	97.9	91.3	93.2	301.5	275.8	271.2	a
F	96.4	91.9	93.2	307.8	305.0	298.5	a
G	96.9	88.1	95.3	311.8	284.2	312.7	a

*Indicates a significant decrease when compared to the control group (0ppm), P=0.0022.
**Indicates a significant decrease when compared to the control group (0ppm), P=0.047.
***Tukey HSD groupings at 10ppm, significant at P<0.05.

in this experiment, P=0.0047; Dunnett's test was used to compared the means for 5ppm and 10ppm to the 0ppm control. There was no significant difference between 0ppm and 5ppm, but there was a decrease in adult survival at 10ppm compared to the control, p=0.0022 (data summarized in Figure 3a). There were no significant differences between bottleneck severity, duration or post-bottleneck population growth rate.

The mean number of progeny surviving the malathion treatment was also calculated for each population (Table 3.19). This data was used to compare populations within each level of the malathion treatment, and also compare the effect of malathion concentration within each population. This approach was used because visual inspection of the data suggested a significant interaction between population and malathion concentration which was not detected when running the same model used above for adult survivorship, likely due to insufficient power for the statistical test.

Figure 3a. Mean Proportion of Adults Surviving Malathion Treatment



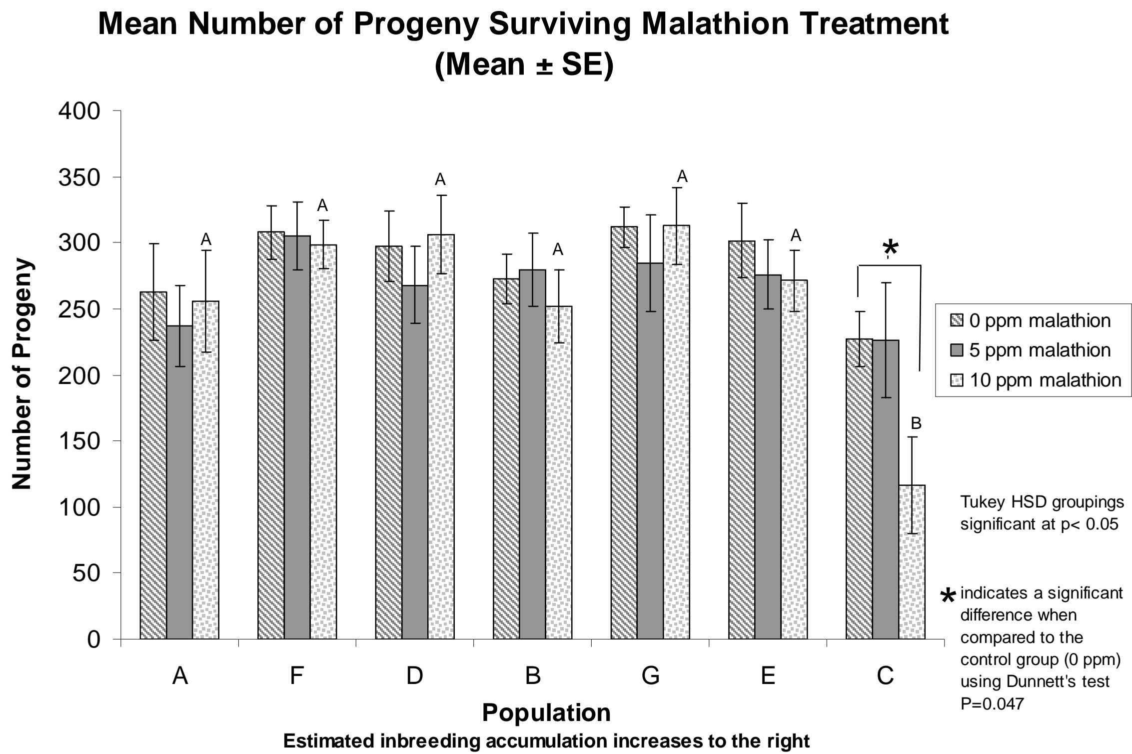
The statistical models used to analyze this data were $Y_{ik} = \mu + P_i + \varepsilon_{ik}$ and $Y_{jk} = \mu + T_j + \varepsilon_{jk}$, to compare populations within each level of malathion concentration and to compare the effect of malathion concentration within each population, respectively. There were no significant differences in mean progeny between populations at the 0ppm and 5ppm levels of the malathion treatment. However, at 10ppm malathion, population C had significantly fewer progeny than all other populations, P<0.05, using the Tukey HSD test for all pairwise comparisons. Similarly, when the effect of malathion concentration was analyzed within each population, using Dunnett's test to compare all treatments to the control, population C showed a significant decrease in the total number of progeny at 10ppm malathion when compared to the 0ppm control (P=0.047). A significant effect of the malathion treatment on the total number of progeny was not found within any other population (data summarized in Figure 3b).

A significant difference was found between populations that differed in bottleneck duration. Populations that only has a single generation bottleneck (F and G) had significantly more progeny than populations that endured a five generation long bottleneck (B and C), P<0.05.

Atmospheric Oxygen Restriction Experiment

The total number of adult beetles remaining after the 3 day nitrogen treatment was counted and the mean proportion of adults surviving was calculated for each population, for both the control and nitrogen exposed demes. The statistical model initially used to analyze the data was $Y_{ijkl} = \mu + P_i + T_j + R_k + P_i \times T_j + R_k \times T_j + \varepsilon_{ijkl}$; including population, treatment, replication and the interactions between treatment and population, and replication and treatment, all as fixed effects. Both the interactions between treatment and population, and replication and treatment,

Figure 3b. Mean Number of Progeny Surviving Malathion Treatment



were significant at P=0.0298 and P<0.0001 respectively. Therefore, data was analyzed using the model $Y_{ijk} = \mu + P_i + T_j + P_i \times T_j + \varepsilon_{ijk}$ within each replication. There was no significant effect of population, treatment or the interaction in replication one, so the populations were not compared. There was a significant effect of the nitrogen treatment in replication two (P=0.0117), but no difference in the effect between populations and no significant interaction. There was a significant effect of both treatment (P=0.0014), population (P<0.0001), and a significant interaction (P=0.0029) in replication three, so using Fisher's LSD (due to small sample size and lack of statistical power), the effect of treatment was compared within each population and the populations were compared, pairwise, within the nitrogen treatment (see Table 3.20).

Table 3.20 Atmospheric Oxygen Restriction Stress Experiment: Replication 3, Mean Adult Survival

Population	**Mean Proportion (%) of Adults Surviving Nitrogen Stress**		
	Control	Nitrogen	Groupings**
A	100	36.5*	d
B	100	61.5*	bc
C	96.9	26.6*	d
D	100	65.6*	abc
E	100	51.0*	c
F	100	77.1*	a
G	100	72.9*	ab

*Indicates a significant difference when compared to the control, P<0.01
**Groupings based on pairwise contrasts for nitrogen treatment, significant at P<0.05.

In replication three, there was a significant decrease in the mean proportion of adults surviving in the nitrogen treatment when compared to the control group in all populations, P<0.01. There were also significant differences between populations within the nitrogen treatment. Populations were grouped based on the pairwise contrasts where P<0.05. Populations

D, F and G had the highest proportion of adults surviving the nitrogen treatment, while populations A and C had the lowest and these two groups were mutually exclusive.

Significant differences were also found between populations that had bottleneck events differing in severity (B and F vs. C and G), and duration (B and C vs. F and G), $P<0.05$. There was greater adult survival in populations with less severe bottleneck events and shorter durations of the event. Replication three results likely differed from the previous two replicates because tape was added to seal the incubator to prevent nitrogen from escaping.

Factor Analysis

Phenotypic Factor

The "phenotypic factor" represents the underlying influence on the collective phenotype of the populations post-bottleneck and was created based on adult weight and total number of progeny in generation seven, and pupa weight in generation eight. Only the first factor created in PROC FACTOR was retained and named the "phenotypic factor". This factor explains 55.76% of the variation in these three phenotypic variables. The factor pattern (or loadings), which is equivalent to the correlation between each variable and the phenotypic factor is displayed in Table 3.21.

Stress Factor

The "stress factor" represents the underlying influence on the phenotype of the populations under environmental stress (aka. malathion insecticide) based on the proportion of adults and total progeny surviving the 10ppm malathion treatment. Only one factor created in

PROC FACTOR was retained and named the “stress factor”. This factor explains 83.66% of the variation in both of these variables. The factor pattern is displayed in Table 3.21.

Table 3.21 Factor Patterns for Phenotypic, Stress, Variance Component and Inbreeding Factors

Variable	**Factor Pattern**			
	Phenotypic Factor	**Stress Factor**	**Variance Component Factor**	**Inbreeding Factor**
Generation 8: Pupa Weight	0.69630	-	-	-
Generation 7: Adult Weight	0.82104	-	-	-
Generation 7: Total Progeny	0.71681	-	-	-
Adult Survival in 10ppm malathion treatment	-	0.91466	-	-
Total Progeny Surviving in 10ppm malathion treatment	-	0.91466	-	-
Generation 7: Additive Genetic Variance	-	-	0.90092	-
Generation 7: Phenotypic Variance	-	-	0.90092	-
Average Inbreeding Accumulation In Generation 8	-	-	-	0.97946
Effective Population Size In Generation 8	-	-	-	-0.97946
Mean Communality Estimate (like R^2)	0.5576	0.8366	0.8116	0.9593

Variance Component Factor

The "variance component factor" represents the common influence on the additive genetic variance and the phenotypic variance estimates calculated post-bottleneck (generation 7). Only one factor created in PROC FACTOR was retained and named the "variance component factor". This factor explains 81.16% of the variation in both of these measurements. The factor pattern is displayed in Table 3.21.

Inbreeding Factor

The "inbreeding factor" represents the common variation between the effective population size and the estimated inbreeding accumulation in generation eight. This factor was created because the relationship between these two parameters is not linear; this factor provides a regressor that encompasses the majority of the variation for each of the variables and for which each variable was equally important in its creation. Only one factor created in PROC FACTOR was retained and named the "inbreeding factor". This factor explains 95.93% of the variation in both of these measurements. The factor pattern is displayed in Table 3.21.

Correlation and Regression Analyses

Correlation of Factors

It was expected that the phenotypic factor and the stress factor would be correlated because they essentially measure the influence of the same underlying factor, the difference depending on how that influence changes under environmental stress. It was found that the phenotypic factor and the stress factor had a significant positive correlation, P=0.0151. Therefore, these two factors are correlated as expected; as the values of the factor scores for one

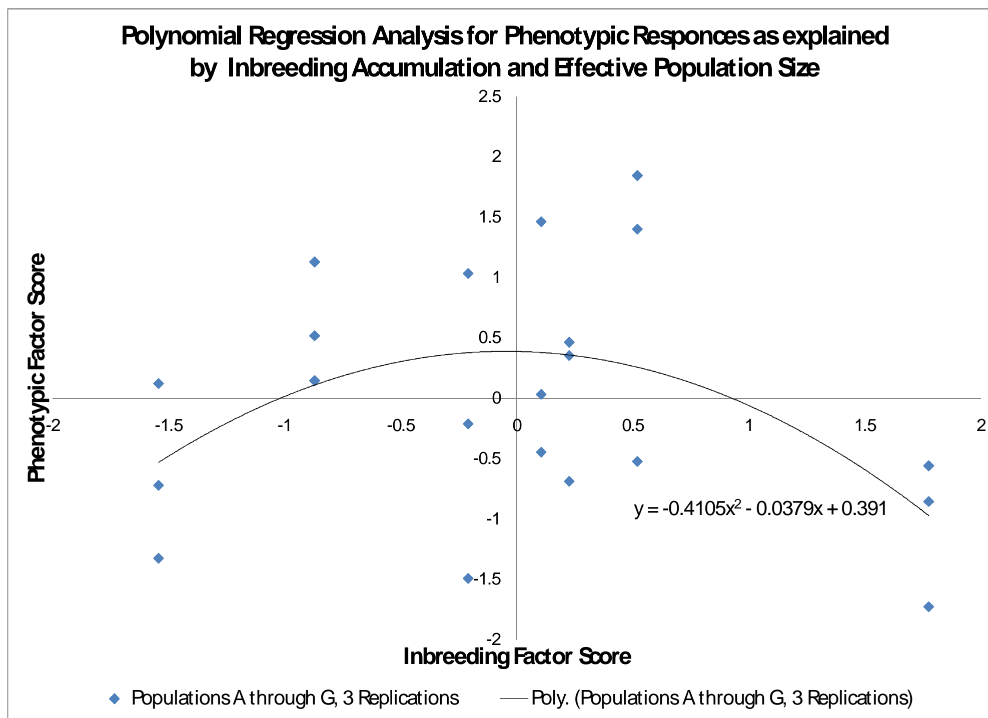
increase, the factor scores for the other also increase. The variance component factor was not significantly correlated with either the phenotypic factor or the stress factor.

Of particular interest were the relationships between the inbreeding factor and the three other factors. There was no significant linear correlation between the inbreeding factor and the variance component factor, or the phenotypic factor. There was a borderline significant negative linear correlation between the inbreeding factor and the stress factor, P=0.0597. Therefore, as the inbreeding factor scores increase (inbreeding increases and effective population size decreases) the stress factor scores decrease (proportion of adult survival and total progeny numbers both decrease).

Polynomial Regression on the Inbreeding Factor

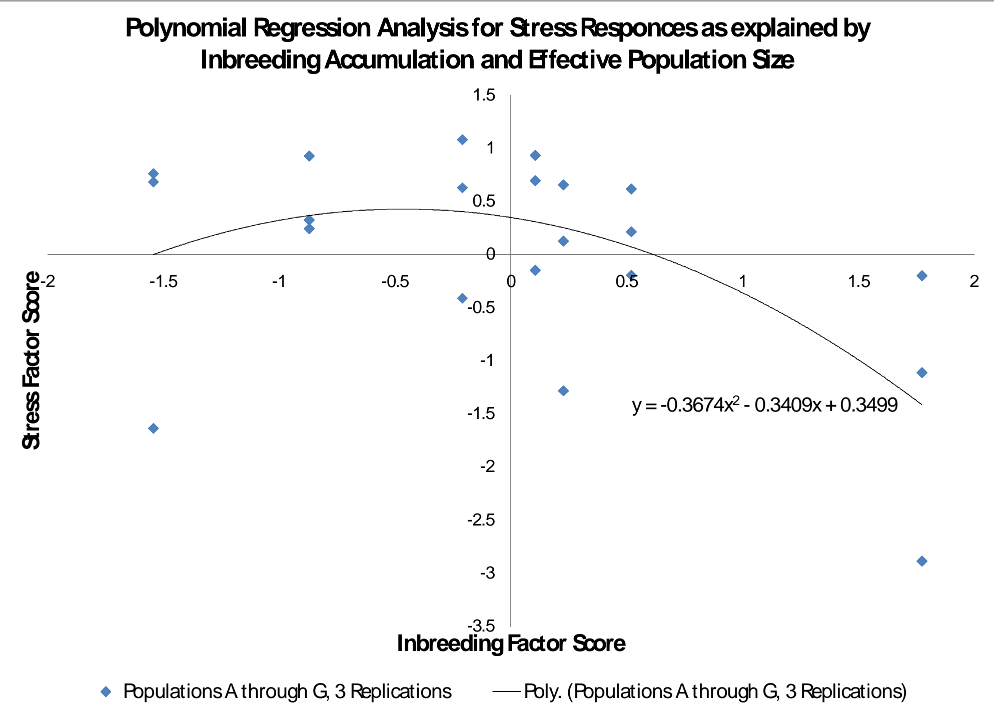
After plotting the phenotypic factor scores and the stress factor scores against the inbreeding factor scores, it appeared there may be a non-linear relationship between the factors that was not detected by the linear correlation analysis. A polynomial regression analysis was run for each factor using the inbreeding factor as the independent variable: $Y_{ik} = \beta_0 + \beta_1 x_i + \beta_2 x_i^2 + \varepsilon_{ik}$. In each case, the two variables included in the regression model were the inbreeding factor score and the square of the inbreeding factor score. This type of regression model did not explain a significant amount of variation in the variance component factor scores. The regression model for the phenotypic factor was borderline significant, P=0.0680, but was a better model than the simple linear correlation (Figure 3c).

Figure 3c. Polynomial Regression of the Phenotypic Factor on the Inbreeding Factor



The non-linear regression model for the stress factor on the inbreeding factor was significant, P=0.0159. The peak of the function, where populations perform the best under environmental stress was around an inbreeding factor score of -0.5. The factor scores are centered at zero, therefore an inbreeding factor score of -0.5 is less inbreeding than the average population in generation eight of this study. Therefore, populations with even less inbreeding (population A) did not perform as well as those with modest levels (just below average) of inbreeding, and populations with much more inbreeding accumulation (population C) performed the worst (Figure 3d).

Figure 3d. Polynomial Regression of the Stress Factor on the Inbreeding Factor



Chapter 4: Discussion

Genetic Variance and Fitness

Quantitative methods were used to estimate genetic variation in our populations in generations two and seven. Using single-pair matings, we were able to estimate the additive genetic variance as twice the covariance between full siblings. The additive genetic variance in generation two was not the same for all seven populations, therefore to gauge the loss of genetic variance over time, the additive genetic variance was compared within each population between generations two and seven. All populations (excluding B and D due to missing values), had a significant decrease in additive genetic variance after the bottleneck event compared to generation two ($P<0.05$). This could be the result of losing rare alleles from the gene pool, because the effect of genetic drift is magnified in small populations. It is also likely that with such small population sizes, the accumulation of inbreeding led to higher levels of homozygosity within individuals and the populations.

Because phenotypic variance did not significantly decrease concurrently with additive genetic variance in most populations, the estimates for pupa weight heritability also decreased. Therefore, even though most populations shown the same variance in their phenotype (pupa weight) post-bottleneck as they had previously, the proportion of that variability that is heritable has decreased. This could have consequences on the ability of the populations to adapt and evolve under changing environmental conditions, potentially reducing its ability to persist long-term.

The actual heritability estimated in generation seven was less than the predicted values, and this may indicate that our populations may actually be more inbred than we originally

predicted based on effective population size. This also has major implications for conservation because there is likely to be inadequate management of threatened populations if the level of inbreeding and the limits for adaptive potential for these populations is underestimated.

Many previous studies have shown an increase in the genetic variance in a population following a reduction in population size (Bryant et al. 1986, Carson and Wisotzkey 1989, Cheverud and Routman 1996, Lopez-Fanjul et al. 1999, Van Buskirk and Willi 2006, Wade et al. 1996). These events may be due to chance alone, through genetic drift or they are likely the result of a change in frequency for genes with usually non-additive effects (Cheverud and Routman 1996, Lopez-Fanjul et al. 1999, Van Buskirk and Willi 2006). It is events such as this that support the theory of speciation from a founder group of individuals. The populations will regain genetic diversity as their size increases and new mutations occur. This type of increase in genetic variance may not occur in larger populations because these may be mutations that occur regularly, therefore the alleles are already present in a large population, but are not present in a small population. Also, if an allele is found in low frequency, but is favored by natural selection, the allele will become more frequent over time, increasing the frequency of heterozygotes and increasing the population mean for that trait. A large population is more likely to be in a steadier state of allele frequency, at some point of optimum, until changes in the environment occur.

It has also been shown that a population that recovers rapidly from small population size will suffer only a small reduction in the average heterozygosity (Nei et al. 1975). In this context, it can then be argued that these populations are able to adapt to a new environment through evolution and speciation regardless of a demographic bottleneck event. Similarly, when populations are small, gene frequencies are determined by drift rather than natural selection and

many alleles are lost during a bottleneck which may be advantageous to rapid adaptation if these alleles produced a disadvantageous phenotype.

There is also empirical support that low genetic variation in a population is not necessarily the result of a recent demographic bottleneck and is perhaps not a good indication of the level of endangerment of a population (Zhang et al. 2002). The same authors examined data from different animal species and found that many populations show no evidence for a historical bottleneck, yet they have very low genetic variation and have the ability to expand when resources are available. However, disregarding genetic information when estimating extinction risk and forming recovery plans for threatened populations could be risky (Frankham 2005). Zhang et al.'s (2002) examples may be an exception to the more common result experienced by populations with low genetic diversity: inbreeding depression becoming detrimental to population survival.

The favorable correlation between heterozygosity and fitness has been reviewed by many including Mitton and Grant (1984) and David (1998) and in many cases supported, establishing the theory that an increase in heterozygosity across the genome favorably affects fitness-related traits (Gaffney et al. 1990, Hoffman et al. 2004, Koehn et al. 1988, Tiira et al. 2006). Therefore, an increase in inbreeding and a decrease in genetic diversity have been associated with a decrease in population fitness. However, the heterozygosity-fitness correlations (HFC) were weak and the sample sizes generally used to establish them have been insufficient to detect true significance (David 1998). The same author also found the existence of a HFC does not appear to be universal.

When a population has a large number of alleles at a locus, it is expected that most individuals will be heterozygotes if the conditions for Hardy-Weinberg equilibrium are met:

large, random mating population with no migration, mutation, or selection. The more alleles that are lost from the population, the fewer heterozygotes the population could possibly have. Because heterozygosity has not been measured directly in this study, it had to be assumed that over time, as the predicted inbreeding accumulation increased in the experimental populations, heterozygosity decreased. During this time, the populations were small in size and could be highly affected by genetic drift and were at great risk to lose allelic diversity. The effect expected in this study was a decrease in the mean of fitness related traits with an increase in inbreeding accumulation.

There was no significant difference between mean pupa weights observed in our *T. castaneum* populations, even after the bottleneck. This could be the result of stabilizing selection favoring an intermediate phenotype as suggested by Rich et. al. (1984), who observed drift variances for *T. castaneum* pupa weight and progeny numbers and found the variance of the means to be significantly less than expected based on the estimates of additive genetic variance and effective population size. Also, populations that had more inbreeding accumulation, particularly B and C, required more days for the larvae to mature into pupae (personal observation) suggesting there may be an optimum weight for individuals to reach and by some compensatory mechanism, individuals will not pupate until they are within this range. Therefore, a measure on days to pupation may be a more accurate assessment for population fitness. Populations with a shorter average time until pupation would have a reproductive advantage over other populations, however, they would also utilize resources in their environment more quickly, which could lead to a faster extinction rate. This may be one explanation for stabilizing selection of pupa weight in *Tribolium*.

There were significant differences observed among the adult weights of the seven different populations in generation seven ($P<0.05$). The most inbred population C, had a significantly lower adult weight than all other populations except A (for $\alpha=0.05$). It was expected to see population C in the lightest group while population A was hypothesized to have a heavier mean adult weight. Adult weight has been found to be positively correlated with fitness (Pray 1997). It is possible we see the populations with an intermediate level of inbreeding have a higher mean for fitness related traits because previous studies have shown that less severe bottleneck events actually provide a fitness benefit (Theodorou and Couvet 2006). Bottleneck duration was also an important factor effecting adult weight in this study.

When counting the total number of progeny at time of sorting in each of the populations, population C was the only group to show a significant decrease in progeny counts after the bottleneck compared to the original counts in generation one. Population C was the most highly inbred population following the bottleneck because the census size decreased to four individuals and thus remained for an additional four generations, only returning to 32 individuals in generation seven. It is possible we only see a change in the mean value for this trait in population C because it is so highly inbred ($F=0.49$ by generation 8). To not see a steady decrease in the mean phenotypic value of the trait as inbreeding accumulation increases, suggests that the effect of genetic variance on these traits may not be purely additive, and that epistasis can "rescue" the phenotype until inbreeding reaches a critical point where fitness begins to rapidly decline.

When data for pupa weight, adult weight and total progeny counts were combined into a single factor, the "phenotypic factor", there was no significant linear correlation between this factor and the inbreeding factor. However, when a polynomial regression model was used, the result was borderline significant ($P=0.0680$). Therefore, it was a better model than a linear

relationship. The polynomial regression in Figure 3c illustrates how populations with intermediate levels of inbreeding have higher phenotypic factor scores (corresponding to higher means for the fitness related traits) than populations with little or a lot of inbreeding accumulation, again suggesting a fitness benefit for a modest demographic bottleneck.

Long-Term Effects on Fitness

Population bottleneck events have led to decreased fecundity in the Montana pronghorn population in the season following the stochastic event (Dunn and Byers 2008), but in this study the effect of such an event was investigated in populations after twelve generations of random mating had passed since returning to a census size of 32 individuals. Fecundity and fertility were only assayed on populations A and C in generation 19. These populations were chosen because they represent the extremes: the least amount of inbreeding accumulation (F=0.235) and the most (F=0.717) in this generation. Despite the time since the historical bottleneck in population C, fitness consequences were still observed for the population. Population C had a significantly lower fecundity, 31.65, measured as the mean three-day egg lay in females (P=0.0146), compared to population A, with 42.62. This further supports the decrease in mean of fitness related traits with an increase in inbreeding. There was no significant difference in the fertility of females between the populations. Also, no differences in the physical appearance of eggs were detected (personal observation).

Effects of Environmental Stress

Consistent with the previous results, in the malathion experiment the most inbred line, population C, had a reduction in the proportion of adults surviving in 10ppm compared to the other levels of the insecticide, although there was not enough statistical power to declare a significance. Population C did show a significant decrease in the total number of progeny remaining after the malathion treatment at 10ppm ($P=0.047$) when compared to the control; all other populations had a non-significant change. Population C also had significantly less progeny at 10ppm when compared to all other populations ($P<0.05$). It was also found that the duration of the bottleneck event had a significant effect on the number of progeny surviving under this stress; when the bottleneck lasted more generations, there was a greater reduction in the number of progeny surviving. This data suggests a relationship between the level of inbreeding accumulation in the populations and population fitness, measured as the survival rate of individuals.

When data for adult survival and progeny survival in 10ppm were combined into a single factor, the "stress factor", there was a borderline significant linear correlation between this factor and the inbreeding factor, $P=0.0597$. When a polynomial regression model was used, the result was significant, $P=0.0159$. The polynomial regression in Figure 3d illustrates the relationship between the stress factor scores and the inbreeding factor scores for all 21 populations. The near significant trend we saw with the phenotypic factor becomes more pronounced and significant when these populations are under environmental stress. The effect of the stress factor should have been like that of the phenotypic factor if stress did not have an effect on the phenotypic expression of traits. Audo and Diehl (1995) also found a stronger correlation between heterozygosity and fitness when environmental conditions were considered moderately stressful,

however they did not see the same result when populations were thought to be under the most stress.

In the atmospheric oxygen restriction experiment, there was a significant effect of limiting oxygen on adult survival in all populations ($P<0.01$), however the severity of the effect differed between populations. Populations A, C and E showed the greatest reduction in adult survival (the most and least inbred populations), and populations D, F and G showed the least (intermediate levels of inbreeding). Bottleneck severity and duration had a significant effect on adult survival under this stress. More severe and longer bottleneck events result in decreased adult survival. This result is similar to the results obtained for adult weight. There may be a small advantage for less severe bottleneck events, but more severe inbreeding accumulation becomes detrimental.

Chapter 5: Conclusions

There was a significant loss of additive genetic variance in all populations in generation seven regardless of the severity or duration of the demographic bottleneck or the population growth rate afterwards. Not all populations had a decrease in phenotypic variance, but the pupa weight heritability estimates for each were lower in generation seven as predicted by Falconer and Mackay (1996). A decrease in the heritable variation in traits could have a detrimental effect on a populations' potential for evolution.

The most inbred population, C, showed a significant decrease in the mean value of fitness related traits, such as adult weight, total progeny, fecundity and survivorship. Populations with inbreeding accumulation less than the average (in this study) had the highest values for fitness related traits. The relationship between inbreeding and fitness in these populations was better explained using quadratic models. This suggests that dominance and epistatic gene effects are playing a role in the phenotypic expression of these traits. The relationship between inbreeding and fitness becomes even more significant when the populations are under environmental stress.

This study has also shown that the effects of a demographic bottleneck can extend many generations past population size recovery. Population C has significantly lower fecundity, even after twelve generations post-bottleneck.

Bottleneck duration appeared to be the most important effect compared to severity and post-bottleneck population growth rate. This has important conservation implications because it suggests that populations should recover in size as quickly as possible to prevent the deleterious effects of inbreeding accumulation on fitness. This should be taken into consideration in recovery strategies for endangered and threatened species' population management.

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