ADAPTIVE RESPONSES AND INVASION: THE ROLE OF PLASTICITY AND EVOLUTION IN SNAIL SHELL MORPHOLOGY

By

ERICA JEAN KISTNER

A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN ZOOLOGY

WASHINGTON STATE UNIVERSITY
School of Biological Sciences

May 2009

To the faculty of Washington State University:

The members of the Committee appointed to examine the thesis of ERICA JEAN KISTNER find it satisfactory and recommend that it be accepted.

_____
Mark F. Dybdahl, Ph.D., Chair

_____
Patrick A. Carter, PH.D.

_____
Richard S. Gomulkiewicz, Ph.D.

ACKNOWLEDGEMENT

I thank my parents, Janet and Edward, for supporting me in all my scholarly endeavors. Their love and guidance has propelled me to overcome many challenges and continue to do so. My brother, Chris, whose sense of humor and optimism has helped me become not just a stronger person but a better scientist. My lab mates, Devin, Leslie and Sarah, for giving me great feedback on my research. William Clark and the Orma J. Smith Museum of Natural History who generously loaned me the snail specimens used in my cross species analysis. Mark Evans, my statistician consultant, who provided great insight in both experimental design and data analysis. Lastly, I would like to thank my academic advisor Mark Dybdhal, who has contributed the most to my development as a biologist at WSU. Under his guidance, I developed a strong intellectual foundation in biology that will continue to benefit me in future.

ADAPTIVE RESPONSES AND INVASION: THE ROLE OF PLASTICITY AND EVOLUTION IN SNAIL SHELL MORPHOLOGY

Abstract

by ERICA JEAN KISTNER, M.S.
Washington State University
May 2009

Chair: Mark F. Dybdahl

Only a select few non-native species achieve high abundance and inhabit broad ranges outside of their native habitat. Success of invasive species can be attributed to two contrasting mechanisms: phenotypic plasticity and adaptive evolution. Two studies involving the New Zealand mud snail *Potamopyrgus antipodarum* examined the role that plasticity and evolution play in a successful invader. In the first study, variation in shell morphology was compared between *P. antipodarum* with sympatric populations of *Pyrgulopsis robusta*, a native snail, along the Snake River, Idaho, USA. The Analysis of Variance (ANOVA) found the effect of site on shell morphology to be significant, indicating morphological variation across the sample sites. The Canonical Variate Analysis (CVA) revealed parallel adaptive responses in the shell shape of both species consistent with water flow variation across the sample sites, but whether these responses are evolved or plastic remains unclear.

In a common garden experiment, responses in shell shape morphology of three geographically distinct invasive populations of the New Zealand mud snail *Potamopyrgus antipodarum* were compared to determine the presence of plastic and evolved responses. CVA and traditional length measurements revealed significant differences between F_1 and maternal lineages, suggesting a plastic response. However, offspring maintained among-populations

differences in both shell shape and life history traits. Furthermore, broad sense heritability estimates for shell traits were high, indicating a genetic component. The generational reduction in shell size was attributed to a reduction in water flow in the common garden environment, indicating an adaptive shift in shell shape. A significant population by generation interaction suggests that plasticity and evolution are not mutually exclusive explanations for the differences in shell shape among populations.

Variation in shell morphology of *P. antipodarum* suggests both phenotypic plasticity and adaptive evolution play an important role in invasion success. Invasive *P. antipodarum* matched the adaptive morph of its native counterpart and was able to alter its shell morph within a generation when grown in a common environment, suggesting that invasive populations exhibit adaptive responses to new environmental parameters. The results suggest that plasticity initiates phenotypic change, followed by genetic changes in the direction of the plastic response.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INVASION SUCCESS MECHANISMS

Biological invaders are not only a major concern for conservation, but also provide an opportunity to study ecological and evolutionary processes (Mack et al. 2000). Understanding the mechanisms of invasion success is crucial to their management because only a small percentage of introduced species succeed in establishing themselves. In addition, only a handful of those initial colonizers become widespread, high-density pest species recognized for their ecological impact (Smith et al. 1999). A central focus in invasion ecology is to determine how adaptive responses promote local abundance and geographical spread of biological invaders, either through rapid adaptive evolution or phenotypic plasticity.

Phenotypic plasticity was the first of two proposed invasion success mechanisms. This concept has a long rich history originating from the General-Purpose Genotype hypothesis (Baker 1965). Phenotypic plasticity promotes success across environmental gradients through the production of environmentally-induced phenotypes by a given genotype. (Stearns 1989). Evidence of plasticity is ubiquitous among invasive plants and some animals (Agrawal 2001). On the other hand, adaptive evolution has only been intensively researched over the last decade as an alternative invasion success mechanism. The adaptive evolution hypothesis states that invaders can rapidly evolve specialized genotypes adapted to specific habits, thereby allowing success across broad ranges (Lee 2002). Recent studies have found that many invasive species are founded by genetically diverse populations, presenting the opportunity for adaptive evolution (Prentis et al. 2008). Despite their differences, both mechanisms are thought to lead to invasion success due to an adaptive response to new environments, hence, adaptive responses may be the result of two distinct mechanisms.

The synergy of phenotypic plasticity and adaptive evolution provide a greater understanding of invasion ecology then the two mechanisms could independently. Plasticity may slow evolution by allowing organisms to rapidly adapt phenotypically to new conditions without genetic divergence (Crispo 2008, Fordyce 2006). On the other hand, plasticity may facilitate adaptive evolution if the trait value resulting from plasticity is close to the novel environment's phenotypic optimum (Ghalambor et al. 2007). The sections below present the two aforementioned invasion success mechanisms and their interactions.

Adaptive Evolution

Natural selection can lead to adaptation to novel environments and promote invasion. Modern genetic studies found that adaptation to modern environments can occur within twenty generations or less, suggesting that evolutionary processes may play a key role in successful invasions (Prentis et al. 2008). While most of the evidence for the rapid evolutionary potential of invasive species has been documented for plants, a growing number of invasive invertebrate cases have been found (Prentis et al. 2008, Muller-Scharer et al. 2004, Reznick and Ghalambor 2001, Lee et al. 2003).

Rapid adaptation may act on standing genetic variation or through new mutations. Genetic variation in founding populations has been documented in many plant and invertebrate invasions allowing a rapid response to selection of favorable alleles (Lee et al. 2002, Therriault et. al 2005, Prentis et al. 2008). Standing genetic variation may be particularly important during range expansion, promoting rapid evolution across environment gradients. For example, invasion into fresh water by invasive copepod, *E. affinis*, has been attributed to multiple rapid evolutionary events (Lee et al. 2003). Furthermore, novel environments that invasive species

encounter may promote an increased frequency of previously neutral or deleterious alleles that now infer a fitness benefit in the new range. The fire ant invasion of the southeastern United States is linked to the gene, Gp-9, which regulates self-recognition in worker ants allowing the formation of large multi-queen colonies (Krieger and Ross 2002). On the other hand, mutations may be more important to genetically uniform invaders including clonal taxa. Butin et al. (2005) found that the clonal invasive hemlock woolly adelgid, *Adelges tsugae*, was able to evolve greater cold resistance through mutation, indicating that clones have adaptive potential. At high densities, parthenogens may overcome genetic uniformity through the generation of mutations in the genome. For invasive populations to achieve such evolutionary potential, they must reach a point of mutation saturation, at which there is at least one mutation at each base pair in the genome within each generation (Butin et al. 2005).

Different types of evolutionary change may promote rapid evolution and range expansion of invaders. Genetic bottlenecks commonly experienced by invaders may actually promote adaptive evolution by converting epistatic to additive variance (Prentis et al. 2008). Hybridization in invasive plants may generate novel gene combinations which allows for selection of a phenotype better suited for surviving novel ranges (Lee et al. 2002, Prentis et al. 2008). Genomic modification can lead to important adaptations across environmental gradients and have been documented in both invasive plants and invertebrates. This stress-induced change in the genome may be epigenetic, inherited, or due to transposons rearranging the genome (Agrawal 2001, Lee et al. 2002, and Prentis et al. 2008). Environmental disturbance in the form of fluctuating environments may impact the evolution of invasive species by promoting either organismal flexibility or evolvability (Lee and Gelembuik 2008).

Phenotypic Plasticity

The concept that phenotypic plasticity is a key mechanism for invasive success first appeared in the General-Purpose Genotype (GPG) hypothesis. This hypothesis states that invasiveness is the result of generalism, leading to success over a broad range of environments via phenotypic plasticity (Baker 1965, Vrijenhoek 1998). Given that invaders often lack genetic diversity and some invasive species are clonal, plasticity has been implicated as the driving force in many invasions (Richards et al. 2006, Crispo 2008).

While there is evidence that plasticity is present in invasive species, the effect of plasticity on the overall fitness of the invader is not always well defined (Richards et al. 2006). Modern ecologists and evolutionary biologists have embraced the idea that plasticity can be adaptive and recognized that plasticity itself has a genetic basis (Agrawal 2001, Crispo 2008). The adaptive plasticity hypothesis, which states that phenotypic plasticity evolves to maximize fitness in variable environments, represents the modern view of plasticity (Dudley and Schmitt, 1996). Several hypotheses present alternative explanations for how plasticity may drive invasion. Invasive species may simply be more plastic than their new competitors across novel ranges as exhibited by the greater morphological plasticity in *Lonicera japonica* compared to a related native species (McDowell 2002, Schweitzer and Larson 1999). On the other hand, invaders may evolve plasticity to cope with novel environments (Yeh and Price, 2004). Lastly, incomplete plasticity may allow a species to expand into a new novel environment, moving them from one adaptive peak to another, and potentially leading to adaptive evolution (Ghalambor et al. 2007).

Phenotypic plasticity may also take on a variety of forms in contributing to a successful invasion. From the GPG hypothesis, Richards et al. 2006 proposed three plastic strategies that would benefit a potential invader under either: (1) the Jack-of-All-Trades situation, where

plasticity allows the invader to maintain fitness across a large ecological breadth; (2) the Master-of-Some situation, in which plasticity confirms a greater fitness benefit and potentially high population densities under favorable environments, or (3) the Jack-and-Master case that combines abilities of both strategies. Studies comparing native and invasive plants have found evidence suggesting the existence of these strategies but very few studies have examined them in animal invaders.

Synergy of Evolution and Plasticity

Understanding the role of phenotypic plasticity in evolutionary processes may reveal new insights about invasion ecology. Depending on environmental circumstances, plasticity may shield genotypes from selection, or promote adaptive evolution (Ghalambor et al. 2007, Fordyce 2006, Agrawal 2001). Gene flow may also be influenced by plasticity leading to a decrease in genetic divergence (Crispo 2008). The interactions of these evolutionary processes in conjunction with plasticity may explain why taxa with genomes ranging from uniform to diverse are all able to become successful invaders.

Ghalambor et al. 2007 describes the role that plasticity plays in adaptation to new habitats using Fisher's adaptive peaks. A novel environment presents a new optimal phenotype. Ideal adaptive plasticity would allow an individual to shift its phenotype perfectly, thus matching this new optimum. However, phenotypic plasticity may not always lead to an adaptive response, moving individuals away from the new optimal phenotype resulting in maladaptive plasticity. Incomplete plasticity is also adaptive but places individuals outside the optimum, potentially leading to adaptive evolution. Adaptive or incomplete plasticity may be especially important for genetically uniform invading populations by facilitating movement to a new adaptive peak

(Richards et al. 2006, Dybdahl and Kane 2005). Thus, plasticity can play a role in invasion success but in some cases it may only be the first step in adaptation.

Phenotypic plasticity should be selected over genetic adaptation when environments are spatially or temporary heterogeneous. Furthermore, the plastic response to predictable environmental changes must be quick. (Alpert and Simms 2002). Phenotypic plasticity has a genetic component and has shown to be heritable in some cases (Agrawal 2001, Crispo 2008). Hence, invaders may already be plastic or may evolve plasticity over the course of the invasion (Richards et al. 2006). Phenotypic plasticity also has the potential to increase gene flow between environmental gradients (Crispo 2008). Plastic genotypes adapted to alternative environments hinder local adaptation resulting in a decrease in adaptive divergence. High gene flow between selective environments should favor plasticity over local adaptive evolution especially under a Jack of All Trades strategy (Richards et al. 2006, Crispo 2008). Given that plasticity could allow individuals to adapt to new conditions within a few generations; plasticity should increase in a population under environmental variability (Alpert and Simms 2002). Thus, plasticity would be most advantageous to invaders expanding their range over novel environmental gradients.

Another body of evidence suggests that an initial plastic response may be followed by genetic changes in the direction of the adaptive phenotype (Ghalambor et al. 2007). This process may result in genetic assimilation and reduction of plasticity. Admas and Huningtonfod 2004 found evidence that initial plasticity in Arctic charr morphology was leading to genetic changes in the direction of the plastic response. Plasticity may also be lost due to genetic drift especially if plasticity is no longer beneficial and the environment is homogeneous (Crispo 2008). Adaptive evolution would also be favored over plasticity if it were costly to maintain. The costs associated with the maintenance of mechanisms for sensing changes in the environment, production of

alternate phenotypes, and epistatic effects would result in selection favoring adaptive evolution over plasticity (Agrawal 2001, Crispo 2008). As an invasive species reaches equilibrium in their new range, local adaptation should be favored over plasticity if local habitats are homogenous and if there are heavy costs associated with being plastic.

Study System

The worldwide invasive aquatic snail *Potamopyrgus antipodarum* is an excellent model for testing invasion success mechanisms. *P. antipodarum* are native to lakes and rivers of New Zealand. This species is unique in that both sexuals and clonal females coexist in their native range. Past research suggests that native clones are specialists resulting from adaptive evolution. High clonal diversity in New Zealand arises locally from coexisting sexual ancestors. These clones are endemic to specific lakes and are not widespread in New Zealand (Dybdahl and Lively 1995). Individual clonal genotypes are commonly restricted to specific lake depths so that each depth zone is occupied by a different clonal genotype (Fox et al. 1996, Jokela et al. 1999, Negovetitch and Jokela 2001). However, clonal populations of *P. antipodarum* are worldwide invaders with single clonal lineages becoming geographically widespread in Europe, USA, Japan, and Australia. The invasive distribution patterns of *P. antipodarum* allow for an examination of how adaptive responses, either plastic or evolved, play a role in invasion success.

Rapid expansion of invasive clonal genotypes across environmental gradients suggests that at least some clones are plastic. A single clonal genotype, US 1, has spread rapidly in the western United States since 1987, suggesting an adaptive response to new environments (Keran et al. 2005, Hall et al. 2006). Plasticity should be favored over evolution during the range expansion phases of an invasion (Cripso 2008). Invasive clonal lineages exhibit plastic responses

to salinity but not temperature, indicating that some invasive lineages may be plastic while others are not (Jacobsen and Forbes 1997, Dybdahl and Kane 2005).

Adaptive evolution could also be playing an important role in *P. antipodarum's* success as an invader. Given large population sizes at small spatial scales, clonal lineages of *P. antipodarum* could undergo local adaptation fueled by mutational variation (Butin et al. 2005, Hall et al. 2006). Recent research suggests that plasticity may lead to genetic changes in the direction of the plastic response (Ghalamobor et al. 2007, Admas and Huningtonfod 2004). Plasticity may allow invasive *P. antipodarum* to expand its range but evolution could infer a greater fitness benefit through local adaptation in a homogenous environment.

An examination of shell morphology in *P. antipodarum* may reveal the role plasticity and evolution play during an invasion. The shell is an important reflection of the overall fitness of a snail. It provides protection from both predators and environmental factors such as deification, water depth, temperature, salinity, and water velocity (Verimeiji, 1995, Rolan-Alvarez et.al. 1997, Struhsaker 1968, Janson and Sundberg 1983). Studies have found that snail shells exhibit considerable plasticity (Kemp and Bertness 1984). Invasive clonal lineages may have developed a wide variety of shell morphs specifically adapted to different parts of environmental gradients. Previous studies show that native populations of *P. antipodarum* exhibit variation in shell morphology through both biotic and abiotic factors (Holomuzki and Hasse 2003, Negovetic and Jokela 2001). However, morphological variation in invasive populations has yet to be examined. The invaded range of US 1 genotype is varied in water flow, temperature and salinity (Keran et al. 2005, Hall et al. 2006) providing an excellent opportunity to study the role adaptive responses in shell morphology play in the invasion process.

Two studies explored the roles of phenotypic plasticity and adaptive evolution in *P. antipodarum* invasions. First, a Canonical Variate Analysis (CVA) tested for adaptive responses by determining whether sympatric populations of *P. antipodarum* and a related native snail, *Pyrgulopsis robusta*, exhibited parallel shell shape variation along the Snake River in the western United States. Next, a common garden experiment compared responses in shell shape morphology of three geographically distinct invasive populations of *P. antipodarum*. This experiment also tested for differences in life history traits among offspring lineages as well traditional length measurements and heritability estimates. Thus, the roles of phenotypic plasticity and adaptive evolution are explored, providing insights into the success of *P. antipodarum* invasions.

CHAPTER 2: PARALLEL RESPONSES IN THE SNAKE RIVER

ABSTRACT

An adaptive response, either plastic or evolved, is required for successful invasion of novel environments. Shell morphology is linked with overall fitness and is affected by both abiotic and biotic factors. A Canonical Variate Analysis (CVA) tested for adaptive responses in the invasive snail *P. antipodarum* by determining whether sympatric populations of *P. antipodarum* and native snail *P. robusta* exhibited parallel shell shape variation in the Snake River. Adult snails were sampled from each location. Shell shape was analyzed using geometric morphometric techniques. The CVA assignment test grouped 73% to 93% of both snail species to their rightful sample site and the CVA plot displayed a great deal of overlap between species within sites indicating parallel shell shape. The ANOVA found the effect of site on shell morphology to be significant. CJ Strike Reservoir snails and snails sampled along the Snake River itself exhibited differences in spire and aperture shape consistent with variation in water velocity across the four sample sites. Despite some minor differences between species, *P. antipodarum* exhibited parallel adaptive responses in shell shape with the sympatric *P. robusta* across environmental gradients. However, it remains unclear whether these adaptive responses are plastic or genetic in nature. Adaptive responses in shell shape might enhance the invasion success of *P. antipodarum*.

INTRODUCTION

The two contrasting invasive success mechanisms, phenotypic plasticity and adaptive evolution, have one common key requirement: an adaptive response to the environment. Spatially heterogeneous environments promote adaptation whether those changes are phenotypic

or genotypic in nature (Richards et al. 2006, Lee 2002). Studies have found evidence of both plastic and genetic responses to environmental gradients in invasive species. Morphological changes in the invasive weed *Verbascum thapsus* across an elevation gradient has been attributed to phenotypic plasticity (Parker et al. 2003). On the other hand, morphological adaptations due to rapid evolution have allowed the invasive hemlock woolly adelgid, *Adelges tsuga*, to expand across a latitudinal gradient (Butin et al. 2005).

While the importance of adaptation in morphology of invasive species has been repeatedly demonstrated, little is known about how variation in shell morphology affects the success of the invasive aquatic snail *P. antipodarum*. Rapid morphological adaptations could allow an invader to inhabit a wide range of novel environments. The New Zealand mud snail is a parthenogenetic invader (Dybdahl and Kane 2005).A single invasive *P. antipodarum* genotype has spread across environmental gradients in the Western United States. To spread across such a large geographic range, *P. antipodarum* must have undergone adaptive changes to the varying environmental changes. The presence of an adaptive response may be detected by examining variation in shell morphology. Snail shells are an important aspect of the snail's overall fitness and are known for exhibiting considerable plasticity (Kemp and Bertness 1984). Environmental gradients may favor specific shell morphology according to environmental forces such as current velocity, temperature, and predator abundance (Vermeij 1995, Rolan-Alvarez et.al. 1997, Struhsaker 1968, Janson and Sundberg 1983). While past research has shown that *P. antipodarum* exhibits variation in shell morphology in their native range (Holomuzki and Hasse 2003, Negovetic and Jokela 2001), no research is known concerning morphological variation in invasive populations.

Despite the lack of genetic diversity, this single clonal genotype has spread rapidly across the western United States (http://www.esg.montana.edu/aim/mollusca/nzms/img/nzmsmap.gif). Since 1987, *P. antipodarum* has spread rapidly across the Snake River (Kerans et al. 2005, Hall et al. 2006) which has considerable abiotic spatial variation in water chemistry, temperature, and especially water velocity. Such an environmental gradient provides the ideal setting to examine the role of shell morphology in adaptive responses of an invasive aquatic snail.

Invasive *P. antipodarum* coexists across some of its range with a related native snail genus *Pyrgulopsis* (USFWS, 2005). *P. antipodarum* coexist with *P. robusta* along environmentally distinct sites along the Snake River providing an ideal opportunity to test for the presence of an adaptive response. *P. robusta* has a long evolutionary history within the Snake River dating back hundreds of thousands of years (Hershler and Liu, 2004). On the other hand, *P. antipodarum* has only been in this region for about twenty years but has spread rapidly sometimes reaching great abundance (Kerans et al. 2005, Hall et al. 2006).One indication of an adaptive response would be parallel patterns of shell shape variation in two species (Young et al. 2009). An examination of variation within the same morphological traits of both species may reveal parallel responses, either plastic or evolved, where the two species are sympatric. Such parallel responses suggest a similar adaptive response to environmental variation.

Water velocity is a potential abiotic influence on shell morphology along the Snake River. Variation in shell morphology has been linked to fitness in marine snails along costal shore gradients which vary greatly in water flow (Rolan-Alvarez et.al. 1997). Reciprocal transplant studies have found that snail survival rates were highest in each shell morph's home range indicating the importance of adaptation to wave exposure (Rolan-Alvarez et.al. 1997, Struhsaker 1968, Janson and Sundberg 1983).Much like wave exposure; water flow can have

dramatic effects on shell morphology and may affect the overall fitness of a snail. Lift, drag, and acceleration affect snails in high velocity environments. Shells with low spires and small overall size help confer stability under strong flow. A large aperture allows for a larger foot providing further stability in these unstable conditions (Vermeij 1995). Hence, snail populations in areas with high water velocity should have large apertures and small spires while snails in low water low areas should have larger spires and smaller apertures.

A common method to analyze shape is geometric morphometrics. Morphometrics is a quantitative method of addressing shape comparisons using digitized landmark points (Zelditch et al. 2004). This process is more powerful than older methods of measuring height and width since it measures the overall shape of the entire organism.

To test for the presence of parallel responses in shell morphology, a Canoncial Variate Analysis (CVA) was conducted on 18 digitized landmark points placed *P. antipodarum* and *P. robusta* snails from four sympatric sites along the Snake River (Sheets 2004). A CVA mathematically optimizes between-group differences relative to within-group variation (Zelditch et al. 2004). In other words, the CVA will delineate the differences that vary most between sites and the least within sites indicating which morphological features are shared by the two species at each site.

METHODS

Study System

P. robusta, formally known as *P. idahoensis*, coexists with *P. antipodarum* along the middle ranges of the Snake River in Idaho. *P. idahoensis* was listed as an endangered species in 1992 and was recently delisted due to genetic evidence suggesting that *P. idahoensis* is a

subgroup of *P. robusta* (Hershler and Lui 2004, USFWS 2007). Hence *P. idahoensis* is now categorized as the Snake River population of *P. robusta*. Snake River populations have declined due to deteriorating water quality and fragmentation of river habitats caused by dams and other human disturbances. There is evidence that competition with the invasive *P. antipodarum* may also negatively impact Snake River populations of *P. robusta* (USFWS 2005). *P. robusta* encounters a large variety of flow conditions along the Snake River occurring in both the main stem river and in reservoirs. The springsnail originally evolved in the prehistoric Lake Idaho, which drained hundreds of thousands of years ago allowing ample time for adaptation to river environments (Taylor 1985).

Study Sites

The four sites for the sympatric species comparison were located in the Western United States (Table 2.1). Both adult *P. robusta* and *P. antipodarum* were collected from field sites.

C J Strike Reservoir is a man-made lake created by the impoundment of the Snake River and Bruneau River near Grand View, ID. Specimens from CJ Strike were collected in April 2008 by Mark Dybdahl. Three of the four sites were located along the southwestern section of the Snake River. Snake River specimens were loaned to Washington State University by Orma J. Smith Museum of Natural History in June 2007. Snake River specimens were collected in July 2000 by Stephenson Foster.

Shell Photography

Shells were scrubbed clean of algae, dried, and mounted on museum gel to prevent shadows below a Canon Powershot A620 digital camera on a stable stand attached to a

dissecting microscope. Shells were oriented with the axis of coiling horizontal, and the aperture face up. A millimeter ruler was mounted in the plane of aperture focus. Consistent orientation of the specimen is critical to minimize random error in morphometric analysis. An error series of repeated photos of the same shell were taken to quantify orientation errors. This process was repeated until the error rate was minimal.

Choice of Landmarks

Morphometric landmarks were chosen that are likely to present homologous points on the shell. Homologous points are defined by two criteria: distinctness from other locations and recognizable in all specimens (Zelditch et al. 2004). These were placed on spiral cords, apex, and points around the aperture (Papadopolous et al. 2004). Eighteen homologous landmarks were found on *P. antipodarum* and *P. robusta* (Figure 2.1).

Geometric Morphometric Analysis

The 18 landmark points were digitized from photos using TPSDig Version 2 (Rohlf 1997). Geometric morphometric analyses were conducted using these digitized landmarks. The file of digitized coordinates was opened in CoordGen6 (Sheets 2004), which was used to scale digitized landmarks to unit centroid size, and rotate to minimize the summed squared distances between homologous landmarks. This standard alignment known as Procrustes alignment removes size differences among specimens while retaining allometric relationships, making it possible to analyze shape independent of size (Zelditch et al. 2004). Thus, the effects of non-shape information (position, orientation, and scale) were mathematically eliminated from these landmark configurations using a generalized Procrustes analysis.

Canonical Variate Analysis

A Canonical Variate Analysis (CVA) was conducted across the four sample sites to determine the presence of morphological similarities between the two species and morphological differences among sites. All Canonical Variate Analyses were performed in CVAGen6j (Sheets 2004) and edited using Minitab 15 (Scatter plot, Minitab Version 15, Minitab Inc., State College, Pennsylvania, USA).

A CVA finds the axes that optimize between-group differences relative to within-group variation using partial warp scores. Partial warp scores are computed to a common reference, then a MANOVA is conducted followed by the CVA. This determines the number of distinct CVA axes present in the data at p=0.05 significance, and computes the canonical variate scores of all the specimens in the data set. To determine the number of significant CVs, Bartlett's test (1947) is employed to test for differences in Wilk's lambda (λ) value. Wilk's λ is the sum of squares within groups divided by the total sum of squares within and between groups:

$$\lambda = \det(\mathbf{W})/\det(\mathbf{T}) = \det(\mathbf{W})/\det(\mathbf{W}+\mathbf{B})$$

where det is the determinant of the matrix. Bartlett's test uses the following formula:

$$\mathbf{X}^2 = -(\mathbf{W} - (\mathbf{P} - \mathbf{B} + 1)/2)\ln \lambda$$

where $\mathbf{X}^2$ has an approximately chi-squared distribution, **W** is the degrees of freedom for the within-group sum of squares, **B** is the degrees of freedom for the between-group sum of squares and **P** is the number of variables to determine if there are **G** = **B** + 1 distinct groups. The degrees of freedom within is **W**= **N** – **B**, where **N** is the total number of samples and **G** is the number of groups (Zelditch et al. 2004, Sheets 2004). The CVA also conducts a group assessment test in which specimens were assigned into groups based on their morphological variability. This

assessment test is based on Mahalanobis distances, which are the distances in the space defined by the significant CVA axes. All Canonical Variate Analyses were performed in CVAGen6j (Sheets 2004).

A CVA was also conducted on both species across the four sites (Figure 2.2). CV1 comprised 61.6% of the total variation and separated the snails by species differences. CV2 only explains 16.4% of variation and separated snails by site differences. However these sites differences were very difficult to interpret. Since this study is trying to determine patterns not differences driven by site between the two species; the CVA was rerun by combining the two species across the sites looking for the patterns that would be similar between the two species.

To visualize shell shape differences, landmark vectors plots were generated along the first and second CV. These plots are based on partial warp scores generated by Canonical Variate Analyses (Bookstein 1989). The landmark vectors plots indicate the changes in the relative position of the landmarks as the score on the CV increases (Zelditch et al. 2004). Mean landmark plots were also generated to visualize the general shape differences between the different groups. Mean landmark plots display the mean location of each of the eighteen landmarks for each group analyzed in the CVA. All plots were generating using PCAGen6 and CVAGen6j (Sheets 2004).

Statistical Analysis

The design is a simple completely randomized design with a one-way treatment structure. Site is a fixed effect in this sympatric species comparison. The linear model is:

$$Y_{ij} = \mu + S_i + \epsilon_{ij}$$

where μ is the overall mean shell morphology, S_i is the effect of the i^{th} site , and ϵ_{ij} is the error term. Morphological differences between sites were analyzed using a univariate ANOVA (Proc

GLM, Type III Sum of Squares, SAS Version 9.1, SAS Institute, Cary, North Carolina, USA). If *P. antipodarum* snails differ in shell shape across the sample sites there should be a significant site effect. Additionally, the assignment test should group the majority of both species to their correct sample site indicating parallel shell morphology across the two species.

RESULTS

The CVA found two significant canonical axes (Figure 2.3). Canonical Variate 1 (CV1) was significant ($p < .0001$) and comprised 55.6% of the total variation. Canonical Variate 2 (CV2) was also significant ($p < .0001$) and comprised 34.8% of the total variation. Despite distinct morphological differences between species (Figure 2.1), the assignment test grouped 72.5% to 94% of the snails to the correct site (Table 2.2). There was a lot of overlap between the species within each site; however, there is some clustering of species within sites indicating differences between the species within sites (Figure 2.4).

The ANOVA found significant differences in overall shell morphology among sites (Table 2.3). The effect of site on shell morphology was significant along both CV1 and CV2 (Tables 2.4 and 2.6). A pair-wise comparison of CV1 means among sites found all sample sites to be significantly different except for CJ strike and Snake RM 538 (Table 2.5). A pair-wise comparison of CV2 means among sites found all sample sites to be significantly different (Table 2.7).

CV1 is the most effective discriminator of morphological variation across sites. CV1 Landmark Vectors reveal three structures on the shell that illustrate shape differentiation among the four sites – the apex, body whorl, and aperture (Figure 2.5). The five points at the tip of the apex indicated a trend of decreasing height at the apex. Aperture width and height exhibits an

overall increase in length. The body whorl also exhibits changes in shape, leading to an overall increase in width. To summarize, as the score of CV1 increases (x axis of the CVA plot), the tip of the apex shortens, the body whorl increases in width, and aperture width and height increase.

CV2 is the second most effective discriminator of morphological variation across sites. CV2 further distinguished the snails through differences among sites. CV2 Landmark Vectors also found the apex, body whorl, and aperture to be the most important structures in shape differentiation among the four sites (Figure 2.6). The five points at the tip of the apex indicated a trend of increasing height at the apex. Aperture width and height exhibits an overall decrease in length. The body whorl also exhibits changes in shape, leading to an overall decrease in width and height. To summarize, as the score of CV2 increases (y axis of the CVA plot), the tip of the apex elongates, the overall size of the body whorl is reduced, and aperture width and height increase.

Differences in the three key shape structures among the four sites can be seen in the Mean Landmark plot (Figures 2.7). Site differences reflect the trends seen in the Landmark Vector plots and CV Means. Snails from CJ Strike had the longest apex, the smallest body whorl, and the smallest aperture. Snake River Mile 538 snails had the second longest apex, the second smallest body whorl, and the second smallest aperture. Snake River Mile 545 snails had the third longest apex, the third smallest body whorl, and the third smallest aperture. Finally, snails from Snake River Mile 537 had the shortest apex, the largest body whorl, and the largest aperture. Both *P. robusta* and *P. antipodarum* showed the same trends in shell shape variation across all four sites.

DISCUSSION

This two species Canonical Variate Analysis sought to determine whether an invasive species would exhibit parallel morphological variation in shell shape with a sympatric native species across environmental gradients. If so, then the evidence would suggest an adaptive response in the invader, either plastic or evolved. While there are distinctive among species differences in shape (Figure 2.1 and Figure 2.4), the CVA assignment test grouped 73% to 93% of both snail species to the correct sample site (Table 3.2). The CVA plotted four distinct sites and displayed a great deal of overlap between species within sites indicating parallel shell shape (Figure 2.3 and Figure 2.4). The ANOVA found the effect of site on shell morphology to be significant (Table 2.3). Furthermore, the ANOVA found shell morphology among sites to be significantly different (Table 2.5 and Table 2.7) with the exception of CJ strike and Snake RM 538 along CV1.

P. robusta and *P. antipodarum* exhibited parallel shell shape variation across the Snake River, suggesting parallel responses to environmental gradients. These parallel patterns in shell shape suggest the invasive snail exhibited a similar response compared to its long established native counterpart to environmental variation. Thus, environmentally induced responses indicated by parallel variation in shell shape of two sympatric species is supported in the Snake River sites.

Reservoir snails are predicted to differ from the snail inhabiting sites along the river, especially in spire height and overall aperture shape. CJ Strike Reservoir provides a stable lentic environment while snails located along the Snake River experience higher flow rates. CJ Strike snails had the longest spire, smallest body whorl, and smallest aperture (Figure 2.6). These

extreme morphological features are attributable to the CJ Strike's quiescent water velocity. Longer spires and smaller apertures are favorable in environments with low water flow. However, these same features would be disadvantageous to snails in high velocity environments (Vermeij 1995).

Snake River snails had shorter spires, larger body whorls, and larger apertures than the CJ strike snails (Figure 2.7). A larger aperture results in a larger body whorl allowing for a larger foot which provides greater protection against strong currents. Snails from Snake River Mile 538 and 545 were intermediate in these features. Snails from Snake River Mile 537 had the shortest spire, the largest body whorl, and the largest aperture, indicating a high velocity environment. Both snail species exhibited shell morphs consistent with the water flow rates of their distinct habitats.

While there is evidence that an adaptive response has resulted in parallel shell morphology in *P. robusta* and *P. antipodarum*, whether that response is plastic or evolved remains unclear. It is possible that plasticity has allowed *P. antipodarum* to rapidly adapt its shell shape to a similar morph seen in *P. robusta*. In their native range, there is evidence that *P. antipodarum* exhibits plasticity in shell shape along a water flow gradient (Hasse 2003). Furthermore, *P. antipodarum* may be able to adapt more quickly to recent environment changes via plasticity in shell shape than its native counterpart *P. robusta*. Adaptive evolution is another possible catalyst for the adaptive variation in shell morphs seen in *P. antipodarum*. Clonal populations reach such high density in the invaded range that genetic variation is possible via rapid accumulation of mutations (Butin et al. 2005).

While parallel shell shape between the two snails is present, there is variation in shell shape within sites, suggesting differences between species. Each site and species within that site

exhibits wide variation around each site mean (Figures 2.3 and 2.4). There is also a lot of overlap between CJ strike, Snake RM 538 and Snake RM 537. This is partly due to similarities in shell shape among the sites but incorrect placement of snails to the wrong site could also contribute to this overlap. Fifteen snails from Snake RM 537 were incorrectly assigned to RM 538 (Table 2.2). The assignment test had an error rate ranging from 6% to 27%. This error rate is likely due to within-site variation due to between-species differences.

While the species do exhibit similar shell morphology across sites, they are not complete mirror images of one another in terms of shell shape. Figure 2.4 indicates a significant amount of overlap between species within each site. However, there are large isolated clusters of *P. antipodarum* from RM 545 and *P. robusta* from RM 537 indicating between-species differences. Furthermore, within-site variation differs between species (Figure 2.4). This may be due to incomplete phenotypic plasticity, defined as an incomplete adaptive response to the new fitness optimum in *P. antipodarum* (Ghalambor et al. 2007).

This comparison of shell morphology of two sympatric species showed that the invasive taxa exhibited parallel responses in shell shape with the native taxa across four sample sites. Environmental differences among sites had a significant effect on shell morphology and all sites were found to be significantly different (Tables 2.3, 2.5, and 2.7). Reservoir snails and snails inhabiting the Snake River itself exhibited predicted differences in spire and aperture shape in conjunction with changes in water velocity (Figure 2.6). An adaptive response may be responsible for the parallel shell shape variation among the invasive and native taxa but the mechanism whether plasticity or evolution remains unclear. The two species experience different within site variation, (Figure 2.3) indicating each species may experience different adaptive responses. These between species differences may be due to incomplete adaptive plasticity or

plastic responses to more recent environment changes in *P. antipodarum*. While the two species do experience some differences in shell variation within sites, their overall response to water flow variation across the sites is the same. Thus the hypothesized adaptive response, either plastic or evolved, is supported.

Table 2.1 Collection information for sympatric *P. antipodarum* and *P. robusta* sites

Collection Location	State, Nearest City	Latitude	Longitude	UMT Coordinates (X,Y, Zone)
Snake River (495) Arm of C.J. Reservoir	Grand View, ID	42.9478°N	115.9452°W	586046 , 4755559, 11
Snake River, River mile 545	King Hill, ID	42.9921°N	115.2295°W	644331 , 4761457, 11
Snake River, River mile 538	Glenns Ferry, ID	42.9366°N	115.3106°W	637842 , 4755162, 11
Snake River, River mile 537	Glenns Ferry, ID	42.9388°N	115.3355°W	635807 , 4755362, 11

Table 2.2 Group assignment from CVA-Distance Based
Original Groups along rows, CVA Groups along columns

	C.J. Reservoir (RM 495)	Snake River, River mile 545	Snake River, River mile 538	Snake River, River mile 537
C.J. Reservoir (RM 495)	73	1	6	0
Snake River, River mile 545	1	75	1	3
Snake River, River mile 538	5	1	33	1
Snake River, River mile 537	5	2	15	58

Table 2.3 ANOVA results for the effect of sites in CV1 and CV2

CVA	Source	df	SS	MS	F	p
1	Site	3	0.01593828	0.00531276	200.67	<0.0001
	Error	276	0.00730713	0.00002648		
2	Site	3	0.00641559	0.00213853	125.43	<0.0001
	Error	276	0.00470583	0.00001705		

Table 2.4 ANOVA results for CVA 1 Means

Site	CVA1 LSMEAN	Standard Error	p
CJ Strike	-0.00637615	0.00057527	<0.0001
Snake RM 545	-0.01170769	0.00057527	<0.0001
Snake RM 538	-0.00539936	0.00081356	<0.0001
Snake RM 537	-0.00263186	0.00057527	<0.0001

Table 2.5 ANOVA results for CVA 1 pair wise comparisons between sites

	CJ Strike RM 495	Snake RM 545	Snake RM 538	Snake RM 537
CJ Strike RM 495	-	<0.0001	0.3278	<0.0001
Snake RM 545	<0.0001	-	<0.0001	<0.0001
Snake RM 538	0.3278	<0.0001	-	0.0059
Snake RM 537	<0.0001	<0.0001	0.0059	-

Table 2.6 ANOVA results for CVA 2 Means

Site	CVA2 LSMEAN	Standard Error	p
CJ Strike	0.00571147	0.00046166	<0.0001
Snake RM 545	0.00141442	0.00046166	0.0024
Snake RM 538	-0.00079730	0.00065288	0.2230
Snake RM 537	-0.00672723	0.00046166	<0.0001

Table 2.7 ANOVA results for CVA 2 pair wise comparisons between populations

	CJ Strike RM 495	Snake RM 545	Snake RM 538	Snake RM 537
CJ Strike RM 495	-	<0.0001	<0.0001	<0.0001
Snake RM 545	<0.0001	-	0.0061	<0.0001
Snake RM 538	<0.0001	0.0061	-	<0.0001
Snake RM 537	<0.0001	<0.0001	<0.0001	-

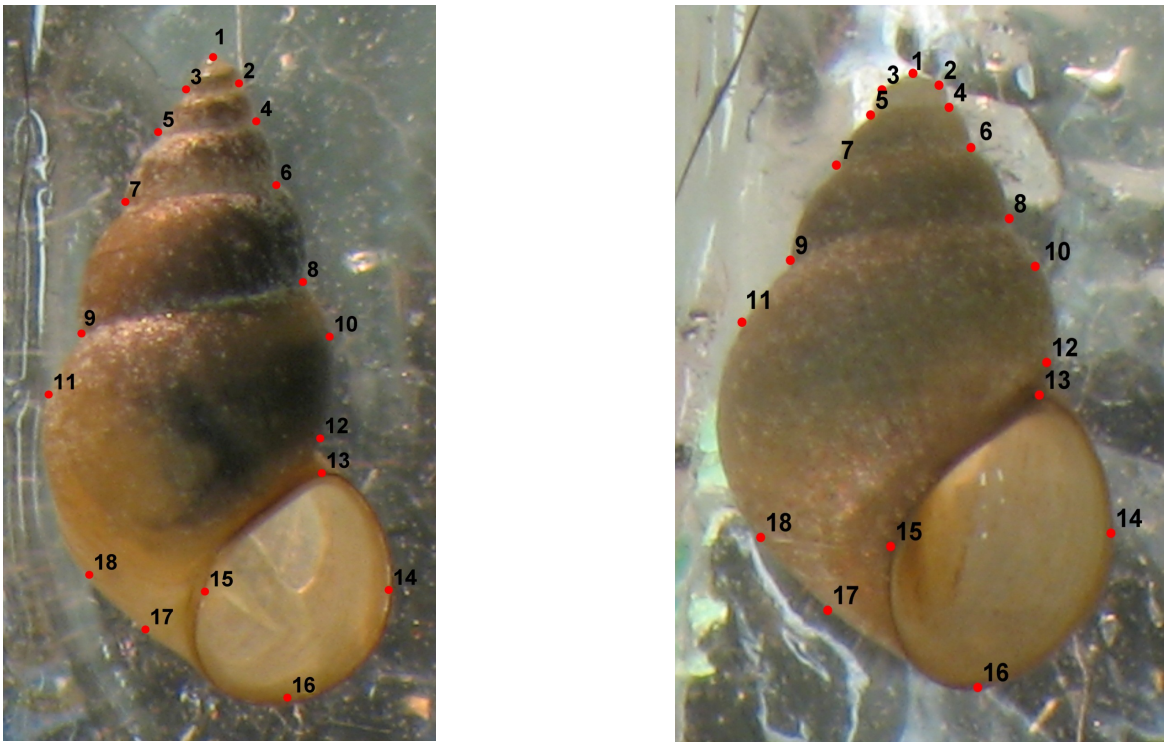

Figure 2.1 Eighteen anatomical landmarks used in morphometric analysis. (Left) 18 points shown on *P. antipodarum* also used on *P. robusta* (Right).

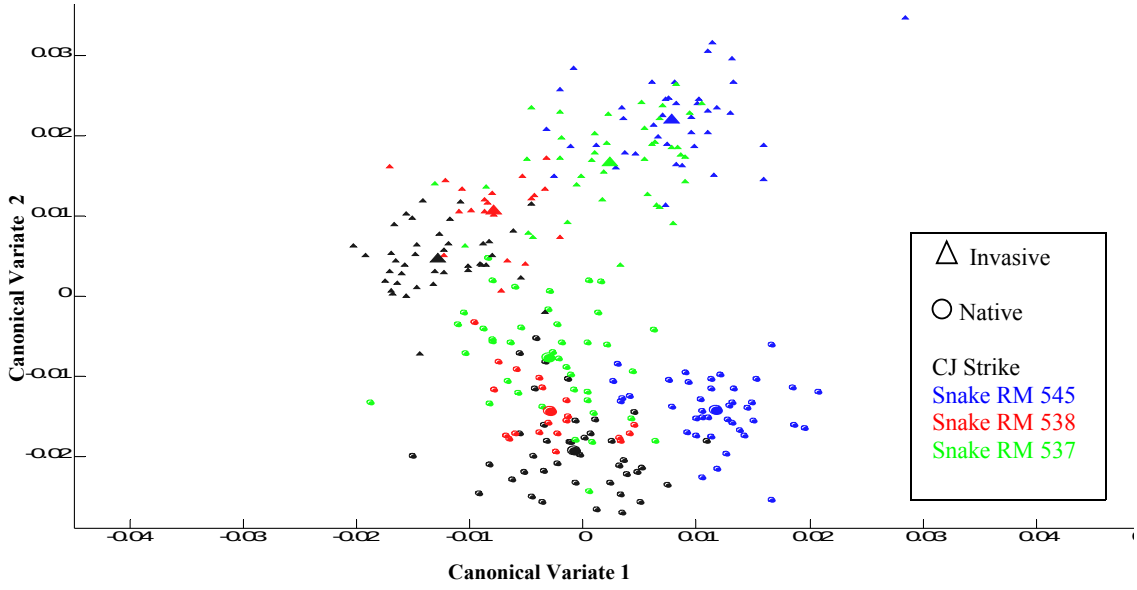

Figure 2.2 Canonical Variate Analysis plot showing the two snail species *P. antipodarum* and *P. robusta* across four separate sites. Canonical Variate 1 was significant ($p < .0001$) and comprised 61.6% of the total variation. Canonical Variate 2 was also significant ($p < .0001$) and comprised 16.4% of the total variation.

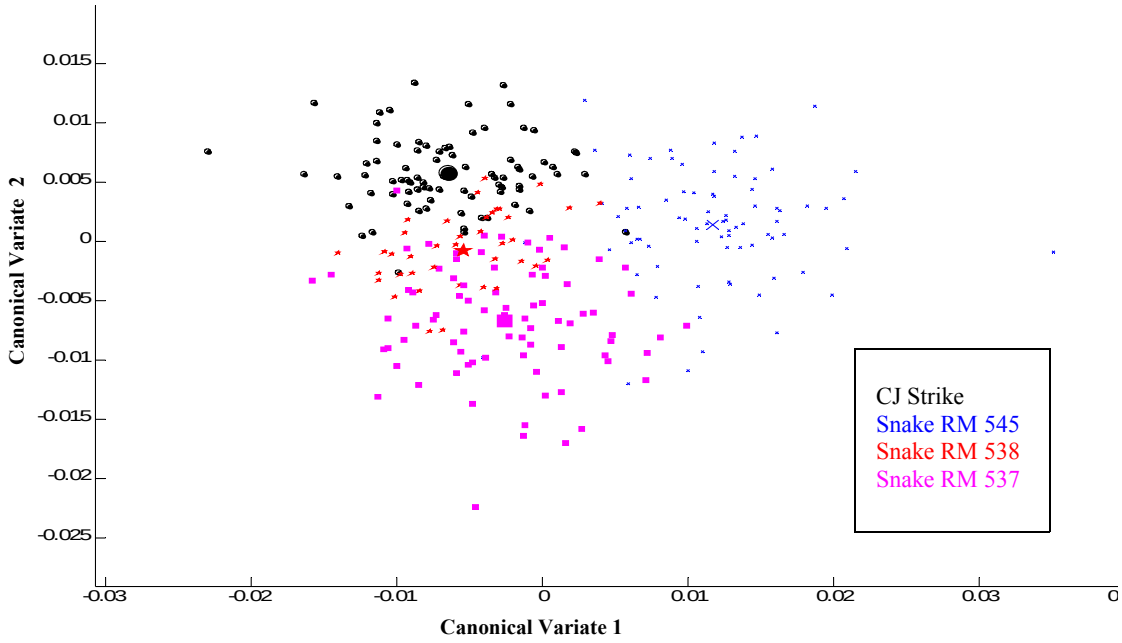

Figure 2.3 Canonical Variate Analysis plot showing four separate sites comprised of both *P. antipodarum* and *P. robusta*. Canonical Variate 1 was significant ($p < .0001$) and comprised 55.6% of the total variation. Canonical Variate 2 was also significant ($p < .0001$) and comprised 34.8% of the total variation.

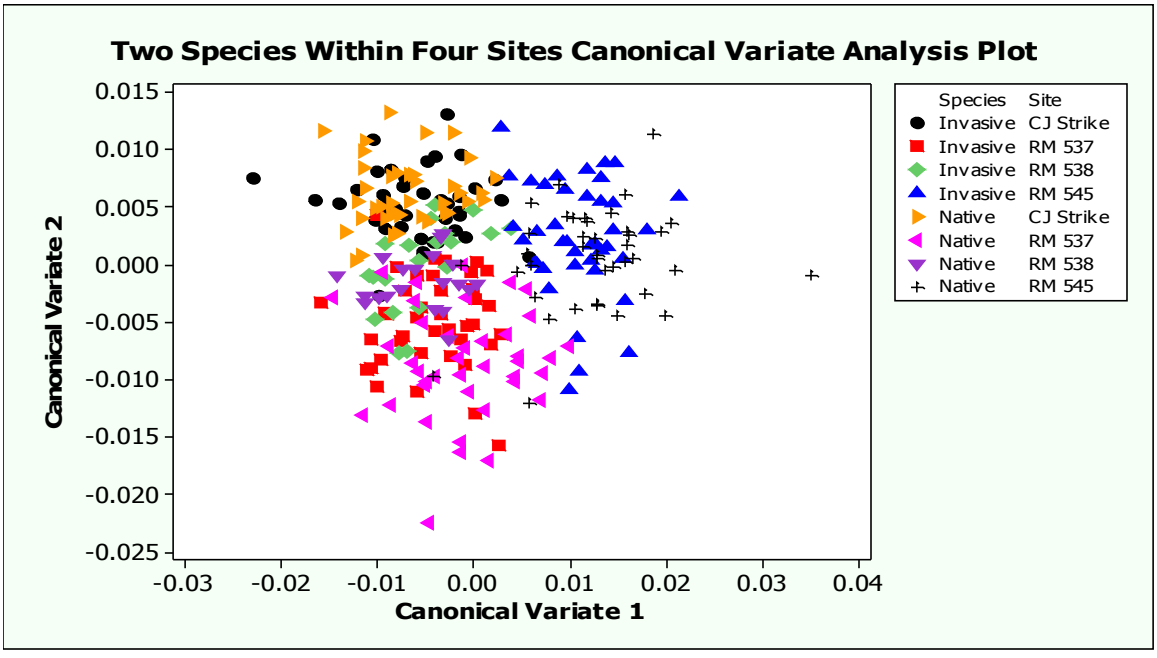

Figure 2.4 Canonical Variate Analysis plot showing the two species within each of the four sites.

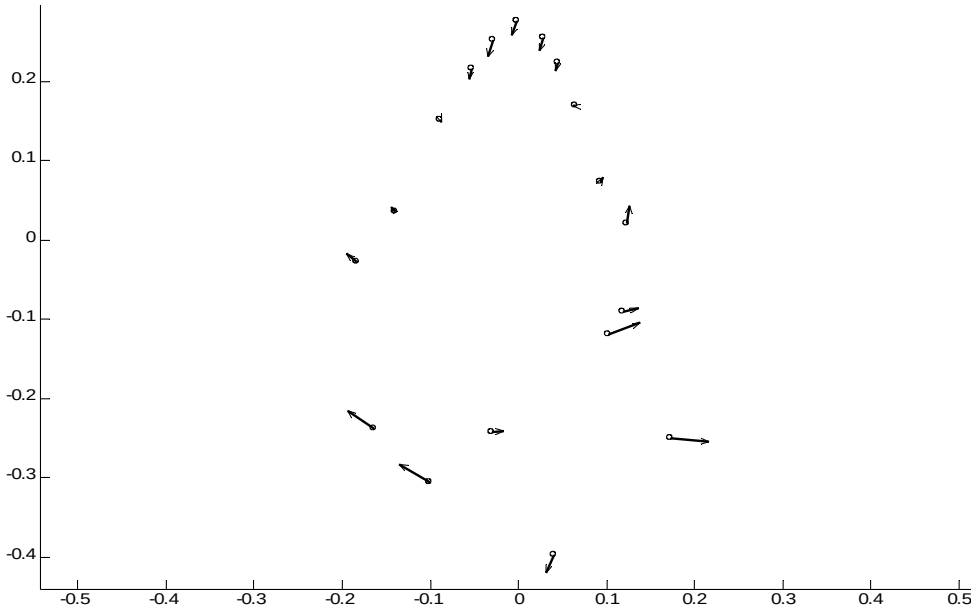

Figure 2.5 Landmark Vectors Procrustes CV1
Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV1 increases.

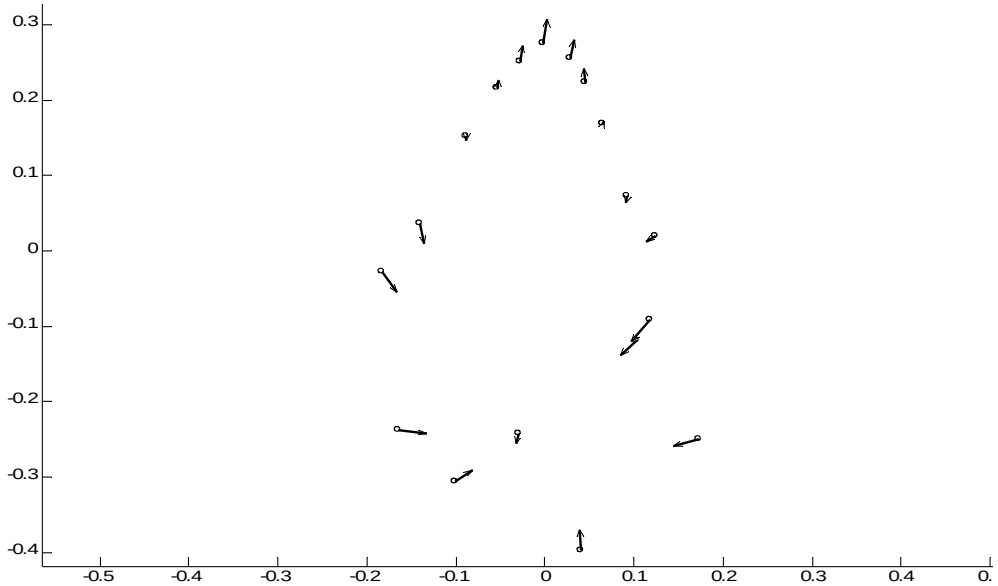

Figure 2.6 Landmark Vectors Procrustes CV2
Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV2 increases.

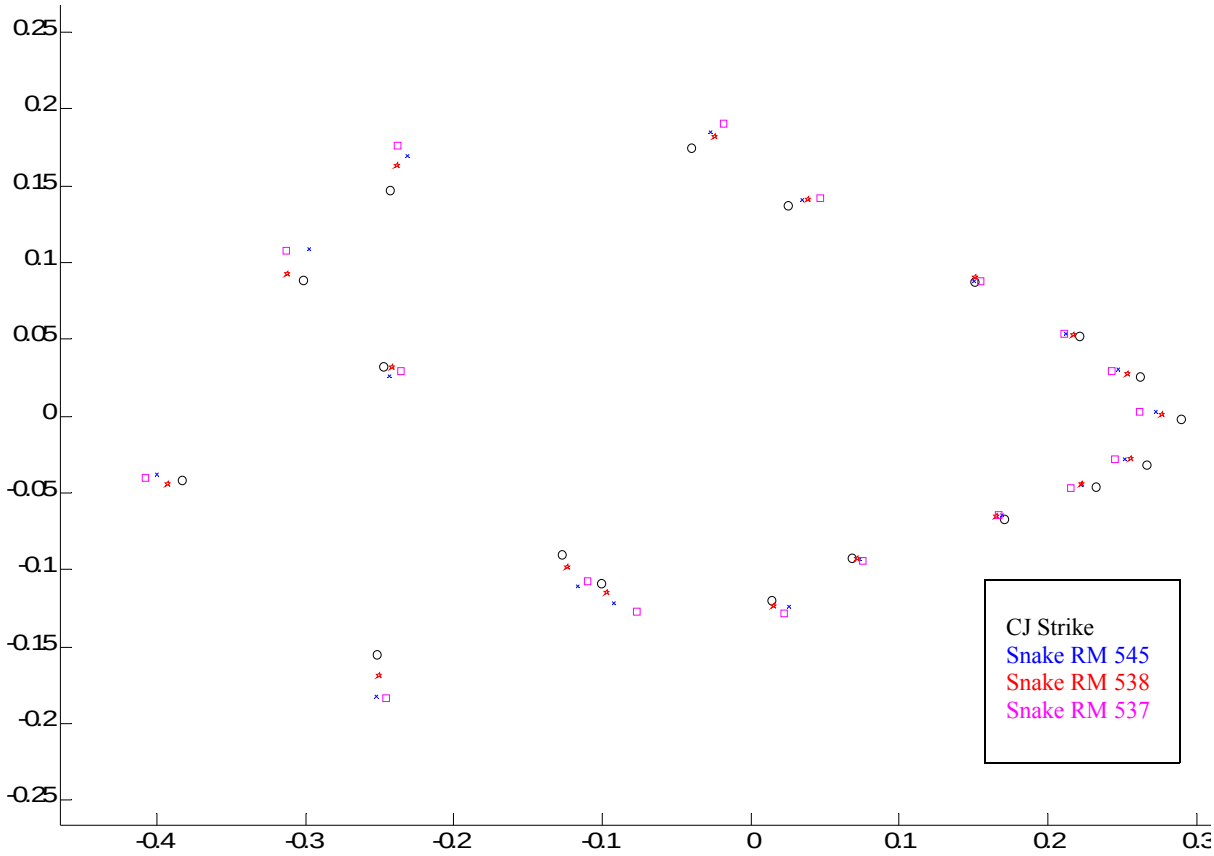

Figure 2.7 Landmark Mean Plot for four sites of *P. antipodarum* and *P. robusta*. Each point represents the mean location for each of the eighteen landmarks across all four populations.

CHAPTER 3: INVASION SUCCESS MECHANISMS

ABSTRACT

Only a small percentage of introduced species succeed in establishing themselves in novel ranges, leading to a central question in invasion ecology: What role does phenotypic plasticity and adaptive evolution play in a species' ecological success? In a common garden experiment, responses in shell shape morphology of three geographically distinct invasive populations of the New Zealand mud snail *Potamopyrgus antipodarum* were compared. Canonical Variate Analyses (CVA) were used to depict overall differences in shell shape. In terms of overall shell shape, the F_1 lineages were significantly different from the maternal lineages indicating a plastic response. However, the F_1 lineages maintained among-population shell shape differences, indicating a genetic response although this variation appeared to be reduced in the F_1 lineages. An analysis of life history traits revealed significant variation in asymptotic length, reproductive rate, and age at first reproduction among F_1 lineages. Traditional shell length measurements found significant differences in the five length measurements between the maternal and F_1 lineages with the exception of Polecat Creek. F_1 lineages maintained some among-population differences but did not vary in aperture height and lower body whorl width consistent with the CVA results. Estimates of heritability were high for both CV values and traditional length measurements. The population by generation interaction effect was significant indicating that plasticity and evolution are not mutually exclusive. Changes in shell shape appear to be adaptive given that the F_1 lineages exhibited a smaller shell morph more suited to a low flow environment within a single generation. This study found evidence for both plastic and evolved responses in the F_1 lineages. Plasticity in conjunction with evolution may play an important role in invasion success.

INTRODUCTION

Biological invaders have been widely recognized as a major conservation issue; however, only a small fraction of non-native taxa successfully establishes and become widespread (Mack et al. 2000). This enigma leads to a key question in invasion ecology: what factors determine a species' ecological success? Two contrasting mechanisms have been recognized as possible means of facilitating the success of invaders over a broad range of environments by producing adaptive responses. First, phenotypic plasticity, which is environmentally sensitive production of alternative phenotypes by given genotypes, facilitates success over a broad range of environments (Stearns 1989). Plasticity may explain the success of clonal or genetically uniform populations as successful invaders. Second, adaptive evolution, leading to genotypes specialized for different local environments, also facilitates success across an environmental gradient (Lee 1999). Both these mechanisms require an adaptive response to the environment - either plastic or evolved.

A common garden experiment examined the importance of plastic and evolved responses in shell morphology and life history traits of three populations of invasive *P. antipodarum* across three sites in the western USA: Bear River in Idaho, Polecat Creek in Wyoming, and Green River in Utah. Geometric Morphometric analyses were conducted on forty adult snails from Idaho, forty specimens from Wyoming and twenty specimens from Utah. The CVA found significant variation in shell morphology among the three populations (Figure 3.1 and Figure 3.2). The shell morphology of invasive snails from these locations was significantly different, thus providing a suitable template to examine the significance of plasticity and evolution in shell shape. Although invasive populations of *P. antipodarum* are clonal, adaptive evolution acting on

mutational variation is possible since invasive populations reproduce rapidly and reach high population densities (Lusshai et al. 2003, Wares et al. 2005). On the other hand, clonal populations of *P. antipodarum* are worldwide invaders with single clonal lineages becoming geographically widespread in Europe, US, Japan, and Australia (Dybdahl and Kane 2005). The pattern of these clonal invasions suggests that at least some clones are plastic.

Invasive populations of *P. antipodarum* exhibit variation in shell morphology across the western USA. If among-population differences in shell morphology disappear within a single generation in a common garden then the variation must be environmentally based plasticity. On the other hand if among-population differences should persist then the among-population variation is genetically based. Consequently, offspring of field-collected snails should produce different shell morphs compared to their maternal ancestors, and among-population variation in shell morphs should be reduced. In addition, these two hypotheses are mutually compatible, both might be true.

An examination of shell morphology under a common environment should provide incite into invasion success mechanisms. Shell shape has a direct affect on fitness through predation defenses and protection against physical or physiological stress (Vermeiji 1995, Rolan-Alvarez et.al. 1997, Struhsaker 1968, Janson and Sundberg 1983). Thus, snail shells provide an excellent template to test the importance of plasticity and adaptive evolution. The section below examines shell morphology as a framework to examine adaptive responses, followed by a brief overview of adaptive evolution and phenotypic plasticity.

Shell Morphology

The shell is a key characteristic of any aquatic snail and plays an important role in overall fitness. Shell morphology has a direct affect on snail fitness on several levels. First, protection against predation is one of the most important functions of the shells. The expression of defensive morphology such as increased shell thickness or spines is often correlated with predator abundance or diversity over a spatial or temporal range (Trussel and Smith 2000). Second, physical stress from the environment such as water velocity and sun or wave exposure produces selection on shell morphology (Verimeiji 1995, Rolan-Alvarez et.al. 1997, Struhsaker 1968, Janson and Sundberg 1983). The magnitude of drag and lift forces acting on shells are affected by properties of the shell shape including convexity, elongation, and surface roughness (Denny and Blanchette 2000). Hence, the shape and size of the shell is a product of both abiotic and biotic forces.

Past research has shown that snail shells exhibit considerable plasticity (Kemp and Bertness 1984). Phenotypic plasticity in shell morphology may have contributed to the success of invader *Potamopyrgus antipodarum* by enabling the snail to produce the optimal shell phenotype across an environmental gradient. Water depth (Jokela et al. 1999), temperature (Dybdahl and Kane 2005), water velocity (Hasse 2003), and predation/parasitism (Levri et al. 2005, Holomuzki and Biggs 2006) have been shown to affect shell morphology in native populations of *P. antipodarum*.

Adaptive Evolution

Adaptive evolution has recently been recognized as an important component of invasive species success. Natural selection could favorably alter the genetic structure of invading

populations making them more fit over time. This rapid evolution could allow specialized invasive genotypes to dominate over native species. Genetic variation exists for an evolutionary response to environments in some invasive populations (Lee 2002, Kolbe et al. 2004, Legar and Rice 2003, Dybdahl and Kane 2005). Environmental disturbance in the form of fluctuating environments may impact the evolution of invasive species by promoting evolvability which can increase an organism's capacity to adapt to changing conditions (Lee and Gelembuik 2008). There is strong evidence that adaptive evolution is the driving force behind many plant invasions (Muller-Scharer et. al 2004, Reznick and Ghalambor 2001) and some animal invasions (Lee et al. 2003).

Adaptive evolution is also possible for invasive clonal populations. Butin et al. (2005) demonstrated that dense invasive clonal populations should have high evolutionary potential over small geographic scales. Normally, parthenogens lack the adaptive potential of sexuals; however, at high population densities mutations occur more rapidly, creating sufficient genetic variation upon which selection may act. Invasive populations of *P. antipodarum* should have great evolutionary potential given that they occupy thousands of linear river kilometers at high densities in the Western United States (Hall et al. 2003).

Phenotypic Plasticity

Phenotypic plasticity has long been thought to be an explanation for the spread of invasive species (Richards et al. 2006, Yeh and Price 2004, Agrawal 2001). There are many means by which plasticity may facilitate successful invasions. First, invasive species may be more plastic than related native species (McDowell 2002). For example, morphological plasticity in an invasive honeysuckle may give fitness advantages over native honeysuckles in the invaded

range (Schweitzer and Larson 1999). Second, invasive species in novel ranges may evolve greater plasticity over time allowing them to out-compete less plastic native counterparts (Yeh and Price 2004). Third, adaptive and incomplete plasticity may allow invaders to survive novel environments by placing individuals within the optimum adaptive peak (Ghalambor et al. 2007).

While plasticity has mostly been documented in invasive plants, there is some evidence that it also occurs in invasive animals (Agrawal 2001). The induced morphological plasticity of water flea, *Daphnia lumholtzi*, has been implicated as the key to its successful invasion of North America (Agrawal 2001). Plasticity in the social structure of the invasive argentine ant may confer significant advantages in variable and novel environments (Ingram 2002).

Plasticity as well as evolution could play an important role in *P. antipodarum*'s invasion success. However, it is still unclear whether invasive *P. antipodarum* lineages are undergoing phenotypic plasticity or adaptive evolution. Studies have shown that invasive clonal lineages exhibit plastic responses to salinity but not in temperature (Jacobsen and Forbes 1997, Dybdahl and Kane 2005). This suggests that some invasive lineages may be plastic while others are not. Although there is some evidence that *P. antipodarum* exhibits morphological plasticity in their native range (Holomuzki and Hasse 2003, Negovetic and Jokela 2001), little is know about morphological variation in invasive populations.

METHODS

Study System

Potamopyrus antipodarum is a fresh water snail native to the lakes and rivers of New Zealand. Native populations are comprised of a mixture of sexual and parthenogenetic individuals, with clonal lineages having arisen from the sympatric sexual population (Dybdahl

and Lively 1995). A rich variety of clonal genotypes occurs in the native range. However, invasive populations in Europe and the USA lack diversity as measured by genetic markers. A single clonal genotype, US 1, has spread rapidly in the Western United States since 1987, and sometimes reaches great abundance (Keran et al. 2005, Hall et al. 2006). In the native range of *P. antipodarum*, variation in shell morphology reflects adaptive responses to abiotic and biotic factors (Holomuzki and Hasse 2003, Negovetic and Jokela 2001). However, little is known about how variation in shell morphology affects the success of invasive populations across broad environmental gradients.

For this common garden experiment maternal lineages were isolated from three geographically distinct populations of invasive US 1 genotype. These populations included Bear River in Idaho, Polecat Creek in Wyoming, and Green River in Utah.

Study Sites and Collection Methods

The three populations used in the common garden experiment were located in the western United States (Table 3.1). Polecat Creek is a geothermal tributary of the Snake River near Flagg Ranch, WY. The Bear River runs through Black Canyon near Soda Springs, ID. The Green River, a chief tributary of the Colorado River, runs through Little Hole near Manila, UT. On average, the three sites differ by temperature, vegetation, and water flow. Water flow is relatively stable at Polecat Creek while Bear River and Green River experience regular flow fluctuations. The Bear River sample site is located downstream from Grace Dam while the Green River sample site is located downstream from Flaming Gorge Dam.

Adult snails were collected from field sites during August 2007 by sifting aquatic vegetation and substrate using wire sieves. Snails were put into plastic bags containing moist

paper towels, placed in a cooler with ice, and transported to a lab at Washington State University, Pullman, WA.

Common Garden

This laboratory common garden experiment determined the level of genetically versus environmentally determined shell morphology among three morphologically distinct populations of *P. antipodarum*. A common garden experiment allows the expression of phenotypes from different populations under uniform environmental parameters.

Snails collected from the three sites were maintained to initiate 30 maternal lineages from each site. The maternal lineages from lab stocks were isolated in 5 oz plastic cups on September 1, 2007. All initial maternal lineages from which experimental offspring, F_1 generation, were obtained were fed 0.24 mg of Spirulena and the water was changed on three alternating days per week. Each week, all offspring from a mother were placed in a separate cup and maintained for two weeks, at which point they were placed in separate cups initiating the F_1 generation. Five offspring replicates from a single mother were randomly assigned to the experimental F_1 lineage.

All snails in the common garden experiment were fed on three alternating days per week and kept at a constant temperature of 18˚C in a 12L:12D cycle. The water in the cups was changed on three alternating days per week. Experimental offspring feeding regiment increased as the snails grew older. Experimental snails were fed 0.02 mg Spirulena until individuals reached a length of 0.8 mm. Snails with a length of 0.8 mm to 1.6 mm were fed 0.04 mg Spirulena. Once snails reached 1.6 mm of length they were fed 0.24 mg Spirulena until the end of the experiment.

Shell length was measured every two weeks until a length of 2.5 mm was obtained; thereafter, length was measured weekly in order to better reveal the shape of the growth curve at age of maturity. Cups were also checked for offspring weekly when individuals reached 2.5 mm. When offspring were found, offspring production was measured for four subsequent weeks resulting in four total reproductive measurements. All offspring were discarded each week. Snails were removed from the experiment after four reproductive measurements were taken.

Shell Photography

Shells were scrubbed clean of algae, dried, and mounted on museum gel to prevent shadows below a Canon Powershot A620 digital camera on a stable stand attached to a dissecting microscope. Shells were oriented with the axis of coiling horizontal, and the aperture face up. A millimeter ruler was mounted in plane of aperture focus. An error series of repeated photos of the same shell were taken to quantify orientation errors. This process was repeated until the error rate was minimal.

Choice of Landmarks

Morphometric landmarks were chosen that are likely to present homologous points on the shell (Figure 3.3). Homologous points are defined by two criteria: distinctness from other locations and recognizable in all specimens (Zelditch et al. 2004). These were placed on spiral cords, apex and points around the aperture (Papadopolous et al. 2004). Eighteen homologous landmarks were found on *P. antipodarum* (Figure 3.3).

Geometric Morphometric Analysis

The landmark points were digitized from photos using TPSDig Version 2 (Rohlf 1997). Geometric morphometric analyses were conducted using these digitized landmarks. The file of digitized coordinates was opened in CoordGen6 (Sheets 2004), which was used to scale digitized landmarks to unit centroid size, and rotated to minimize the summed squared distances between homologous landmarks. This standard alignment, known as Procrustes alignment removes size differences among specimens while retaining allometric relationships making it possible to analyze shape independent of size (Zelditch et al. 2004). Thus, the effects of non-shape information (position, orientation, and scale) were mathematically eliminated from these landmark configurations using a generalized Procrustes analysis.

Canonical Variate Analysis

A Canonical Variate Analysis (CVA) was conducted to determine the presence of morphological differences in shell shape between generations and among populations. A total of three separate CVAs were conducted: one among the maternal lineages, a second among the offspring lineages, and a third containing both generations

A CVA finds the axes that optimize between-group differences relative to within-group variation using partial warp scores. Partial warp scores are computed to a common reference, then a MANOVA is conducted followed by the CVA. This determines the number of distinct CVA axes present in the data at $p=0.05$ significance, and computes the canonical variate scores of all the specimens in the data set. To determine the number of significant CVs, Bartlett's test (1947) is employed to test for differences in Wilk's lambda (λ) value. Wilk's λ is the sum of squares within groups divided by the total sum of squares within and between groups:

$$\lambda = \det(\mathbf{W})/\det(\mathbf{T}) = \det(\mathbf{W})/\det(\mathbf{W}+\mathbf{B})$$

where det is the determinant of the matrix. Bartlett's test uses the following formula:

$$\mathbf{X}^2 = -(\mathbf{W} - (\mathbf{P} - \mathbf{B} + 1)/2)\ln \lambda$$

where $\mathbf{X}^2$ has an approximately chi-squared distribution, **W** is the degrees of freedom for the within-group sum of squares, **B** is the degrees of freedom for the between-group sum of squares and **P** is the number of variables, to determine if there are **G** = **B** + 1 distinct groups. The degrees of freedom within is **W**= **N** – **B**, where **N** is the total number of samples and **G** is the number of groups (Zelditch et al. 2004, Sheets 2004).

The canonical variates analysis also conducts a group assessment test in which specimens were assigned into groups based on their morphological variability. This assessment test is based on Mahalanobis distances, which is the distance in the space defined by the significant CVA axes. All canonical variate analyses were performed in CVAGen6j (Sheets 2004).

To visualize shell shape differences, landmark vectors plots were generated along the first and second CV. These plots are based on partial warp scores generated by Canonical Variate Analyses (Bookstein 1989). The landmark vectors plots indicate the changes in the relative position of the landmarks as the score on the CV increases (Zelditch et al. 2004). Mean landmark plots were also generated to visualize the general shape between the different groups. Mean Landmark plots display the mean location of each of the eighteen landmarks for each group analyzed in the CVA.

These plots were generated for several representative group means to further display the differences between them. All plots were generating using PCAGen6 and CVAGen6j (Sheets 2004).

Traditional Length Measurements Analysis

Traditional length measurements were calculated using TmorphGen6 (Sheets 2004). This program generates a set of traditional length measurements from a geometric landmark data set, of paired coordinate measurements. Unlike the canonical variate analysis, these calculations do not use the Procrustes alignment so differences in sizes can be seen in these measurements. Potential problems were minimized by measuring offspring after they reached asymptotic growth. The following length measurements were calculated: shell height between landmarks 1 and 16, upper body whorl width between landmarks 10 and 11, lower body whorl width between landmarks 12 and 18, aperture width between landmarks 14 and 15, and lastly aperture height between landmarks 13 and 16 (Figure 3.18).

Statistical Analysis

The following model was used to compare morphological differences detected by the CVA among the maternal lineages originating from collection sites. The design for the originating mothers was a simple completely randomized design with a one-way treatment structure. Population (i) is a fixed effect. The linear model is:

$$Yij = \mu + Pi + \epsilon_{(i)j}$$

where μ is the overall mean shell morphology, Pi is the effect of the ith population , and $\epsilon_{(i)j}$ is the error term.

The following model was used to compare morphological differences detected by the CVA among the F_1 lineages under a common environment. The design for the F_1 lineages was a two-stage nested design with population, female , and offspring as factors. Both females (j) and offspring (k) are random factors, while population (i) is fixed. The linear model is:

$$y_{ijk} = \mu_i + \mathrm{P}_{(i)} + F_{(i)\mathrm{j}} + \epsilon_{(ij)k}$$

where μ_i is the overall mean shell morphology, $\mathrm{P}_{(i)}$ is the effect of the i^{th} population, $F_{(i)\mathrm{j}}$ is the effect of the j^{th} female nested within the i^{th} population, and $\epsilon_{(ij)k}$ is the error term.

The following model was used to compare morphological differences detected by the CVA between the maternal and the F_1 generations. The design for the maternal and F_1 lineages is a split-plot design with population, female, generation, and the generation*population interaction as factors. The offspring data was averaged out for each mother to account for unequal replication of offspring. Female (j) is a random factor while population (i) and generation (l) are fixed. The linear model is:

$$y_{ijl} = \mu_i + \mathrm{P}_{(i)} + F_{(i)\mathrm{j}} + G_{(l)} + G*P + \epsilon_{(ij)kl}$$

where μ_i is the overall mean shell morphology, $\mathrm{P}_{(i)}$ is the effect of the i^{th} population, $F_{(i)\mathrm{j}}$ is the effect of the j^{th} female nested within the i^{th} population, $G_{(l)}$ is the effect of the l^{th} generation, $G*P$ is the generation by population interaction term, and $\epsilon_{(ij)kl}$ is the error term. This same design was applied when comparing traditional length measurements between the two generations. A univariate ANOVA was used to analyze morphological differences for all three experimental designs (Proc GLM, Type III Sums of Squares, SAS Version 9.1 SAS Institute, Cary, North Carolina, USA).

P. antipodarum individuals exhibit a logistic growth curve (Dybdahl and Kane 2005); therefore, non-linear growth curves (Proc NLIN, Gauss-Newton method, SAS Version 9.1, SAS Institute, Cary, North Carolina, USA) were generated for all individuals. Curves were fitted to the equation $Y = a / 1 + exp(-b * (X – c))$, where Y is the length of the individual at a given age, X is the age of the snail, a is the asymptotic size, b is the growth rate, and c is the inflection point of the curve (the age at which the snail had achieved 50% of its asymptotic length). The three

growth curve parameters of asymptotic length, growth rate constant, and inflection point were extracted from the growth data for each individual using a non-linear least squares regression to estimate parameter values (Dybdahl and Kane 2005, Proc NLIN, SAS Version 9.1 SAS Institute, Cary, North Carolina, USA) The three growth curve parameters, reproductive rate and age of first reproduction were analyzed using univariate ANOVA (Proc GLM, Type III Sums of Squares, SAS Version 9.1 SAS Institute, Cary, North Carolina, USA) to test for the effects of population.

Variance components and the broad sense heritability were estimated for CV1, CV2 and the five traditional length measurements. The genetic component, V_G, is estimated as the variance among maternal clonal lineages within a population, while the environmental component, V_E, is estimated by the variance among offspring within a maternal lineage (Dybdahl and Kane 2005). Variance components were obtained using Proc Mixed (SAS Version 9.1 SAS Institute, Cary, North Carolina, USA). Population was included as a fixed main effect for both maternal and F_1 lineages while female nested within population was included as a main effect for the F_1 lineages to estimate maternal effects. The broad sense heritability, H^2, was calculated using the following formula:

$$H^2 = V_G / (V_G + V_E)$$

The broad sense heritability or clonal repeatability should be regarded as the upper limit to the degree of genetic determination of a given trait (Lynch and Walsh 1998).

A significant effect of generation would mean that shell morphology differs between the maternal and F_1 generations suggesting a plastic response. A significant effect of population in the F_1 generation analysis would be consistent with an evolved genetically-based response. A significant generation by population interaction would indicate a differential expression of shell

morphology of each generation across populations. High heritability values would indicate the presence of adaptive potential in shell morphology. Additionally, traditional length measurements may reveal adaptive responses to experimental conditions in the F_1 lineages.

RESULTS

Maternal Lineages Canonical Variate Analysis

The CVA conducted on the maternal lineages identified two significant canonical axes (Figure 3.4). Canonical Variate 1 (CV1) was significant ($p < .0001$) and comprised 56.1% of the total variation. Canonical Variate 2 (CV2) was also significant ($p < .0001$) and comprised 43.4% of the total variation. The assignment test grouped 95.8% to 100% of the snails to the correct population (Table 3.2).

The ANOVA showed that significant differences in overall shell morphology in the maternal generation were significant (Table 3.3). The effect of population on shell morphology was significant along both CV1 and CV2 (Table 3.3). A pair-wise comparison of CV1 means among populations found all populations to be significantly different (Table 3.5). A pair-wise comparison of CV2 means among populations found all populations to be significantly different except for Bear River and Polecat Creek (Table 3.7).

CV1and CV2 Landmark Vectors reveal three structures on the shell that illustrate shape differentiation between the three populations– the apex, body whorl and aperture (Figures 3.5, 3.6, and 3.7). CV1 was mostly characterized by differentiation in the body whorl, while CV2 displayed changes in the aperture and the apex.

Maternal Lineages Traditional Length Measurements

Pair-wise comparison of the five length measurements revealed significant differences among all maternal lineages consistent with the Landmark Vector plots (Figure 3.6, Figure 3.7). Green River snails exhibited the largest shell morphs followed by Bear River snails, while Polecat Creek snails exhibited the smallest shell morph in terms of the five traditional length measurements (Tables 3.20-3.29).

F_1 Lineages Canonical Variate Analysis

The CVA conducted on the F_1 lineages identified one significant canonical axis (Figure 3.4). Canonical Variate 1 was significant ($p < .0001$) and comprised 84.5% of the total variation. Canonical Variate 2 was not significant ($p = .015$) and comprised 15.3% of the total variation. The assignment test grouped 78.6% to 96.2% of the snails to the correct population (Table 3.7).

The ANOVA showed that differences in overall shell morphology among populations in the F_1 generation were significant (Table 3.8). The effect of population on shell morphology was significant along both CV1 and CV2 (Table 3.8). The effect of female nested within population was not significant along CV1 and CV2 (Table 3.8) indicating that there was no significant maternal effect. A pair wise comparison of CV1 means among populations found all populations to be significantly different (Table 3.10). A pair-wise comparison of CV2 means among populations found all populations to be significantly different except for Bear River and Green River (Table 3.12).

CV1and CV2 Landmark Vectors indicated three structures on the shell that illustrate shape differentiation between the three populations– the apex, body whorl and aperture

(Figures 3.9, 3.10, and 3.11). The overall shell shape appeared to be differentiating mostly by body whorl and aperture, and less so for the apex.

F_1 Lineages Traditional Length Measurements

Pair-wise comparisons of the five length measurements revealed that among population differences were reduced in the F_1 lineages consistent with the Landmark Vector plots (Figure 3.10 and 3.11). For shell height, aperture width, and upper body whorl width, the only F_1 lineages that were significantly different from each other were Green River and Polecat Creek (Tables 3.21, 3.23, and 3.27). There were no significant differences in aperture height and lower body whorl width among the F_1 lineages (Tables 3.25 and 3.29). Like their maternal ancestors, Green River snails were the largest, Bear River snails were intermediate and Polecat Creek snails were the smallest in terms of shell height, aperture width, and upper body whorl width (Tables 3.20-3.29).

F_1 Lineages Growth Rate Parameters and Reproductive Traits

There were no significant differences in growth rate and inflection point among the F_1 lineages (Table 3.13). Asymptotic length was significant (p=.0046, Figure 3 19, Table 3.13), with Polecat Creek being the lowest; Green River and Bear River were not significantly different.

For reproductive traits, age at first reproduction was significant (p<.0001, Figure 20, Table 3.13) with Bear River reproducing the earliest; Green River and Polecat Creek were not significantly different. The effect of population on reproductive rate was significant (p=.0088,

Figure 3.20, Table 3.13) with Bear River and Green River being the only significantly different pair.

Maternal and F_1 Lineages Canonical Variate Analysis

The CVA conducted on both maternal and F_1 lineages identified two significant canonical axes (Figures 3.12 and 3.13). Canonical Variate 1 was significant ($p < .0001$) and comprised 61.7% of the total variation. Canonical Variate 2 was also significant ($p < .0001$) and comprised 11.9% of the total variation. The assignment test grouped 73.6% to 100% of the snails to the correct population (Table 3.14).

The ANOVA showed that differences in overall shell morphology among the maternal and F_1 lineages were significant (Table 3.15). The effect of population on shell morphology was significant along CV1 and CV2 (Table 3.15). The effect of female nested within population was not significant along CV1 and CV2 indicating that there was no significant maternal effect. The effect of generation was significant along CV1 but not CV2 (Table 3.15). A pair-wise comparison of CV1 means between maternal and F_1 lineages found all offspring to be significantly different from their ancestral mothers (Table 3.17). A pair-wise comparison of CV2 means between maternal and F_1 lineages also found all offspring to be significantly different from their ancestral mothers (Table 3.19).

There was a significant population by generation effect for CV1 and CV2 (Table 3.15), where the CV means for the F_1 lineages were significantly higher than those of the maternal lineages (Figure 3.17). The F_1 lineages exhibit parallel higher mean CV1 values than the maternal lineages while along CV2 F_1 lineages exhibit lower CV2 means with the exception of Polecat Creek where a crossover occurs (Figure 3.17).

CV1and CV2 Landmark Vectors reveal three structures on the shell that illustrate shape differentiation between the maternal and F_1 lineages – the apex, body whorl, and aperture (Figures 3.14, 3.15, and 3.16). CV1 was mostly characterized by differentiation in the body whorl and aperture, while CV2 displayed changes in the apex and the body whorl.

Maternal and F_1 Lineages Traditional Length Measurements Comparisons

The effect of population, generation, and population by generation interaction was significant for all five traditional length measurements (Table 3.15). The effect of female nested within population was not significant for all five traditional length measurements (Table 3.15) indicating that there was no significant maternal effect. Pair-wise comparisons of the five length measurements found some significant differences between all maternal and F_1 lineages consistent with the Landmark Vector plots (Figure 3.15 and 3.16). Green River and Bear River F_1 lineages were significantly different from their maternal lineages for all five length measurements while the Polecat Creek F_1 lineage did not significantly differ from its maternal lineage in any of the five length measurements (Tables 3.20-3.29).

Broad Sense Heritability

Variance component estimations varied between the canonical variates and the traditional length measurements, but yielded high estimates of heritability (<.394) for all traits (Table 3.32). Traditional length measurements had significantly higher H^2 estimates than the overall shape differences depicted by CV1 and CV2 means (Table 3.32).

DISCUSSION

This common garden experiment sought to determine the importance of phenotypic plasticity and adaptive evolution in shell morphology and life history traits of *P. antipodarum.* The generational CVA and traditional length measurement comparisons found morphological differences the between maternal and F_1 generations suggesting a plastic response (Figure 3.12, Table 3.15). However, among F_1 lineages CVA, traditional length measurements, and life history trait comparisons in conjunction with heritability estimates indicate a genetic component (Figure 3.8, Tables 3.13 and 3.15). Both plasticity and evolution seem to be driving shifts in shell morphology. The three maternal populations exhibited shell morphs consistent with the water velocity of their corresponding environments while the F_1 generation exhibited shell morphs more suited to a low flow environment. These results suggest that the observed morphological changes are adaptive (Tables 3.20-3.29).

Since *P. antipodarum* appear to exhibit morphological plasticity in their native range (Holomuzki and Hasse 2003, Negovetic and Jokela 2001), invasive genotypes are predicted to exhibit some plasticity in shell shape as well. Differences between the maternal and F_1 generations as well as the disappearance of among-population differences would indicate that variation among populations in shell shape was due to a plastic response. The F_1 generation was significantly different from the maternal generation in overall shape and some traditional shape measurements. CV1 and CV2 were significantly different between the maternal and F_1 generation (Figure 3.12, Tables 3.16-3.19) indicating a difference in overall shell shape between the generations. Green River and Bear River F_1 lineages were significantly smaller in all five traditional length measurements while Polecat Creek exhibited no significant differences between their ancestral mothers (Tables 3.20-3.29). Among-populations differences were

reduced in F_1 generation but were statistically significant (Figure 3.8, Tables 3.8, 3.20-3.29). The reduction of among-population differences may be evidence of an incomplete adaptive response to the new optimum or incomplete plasticity (Ghalambor et al. 2007). Induced phenotypic changes in morphology may take more than one generation to occur and may even become canalized after the interaction is over (Agrawal 2001). This conclusion that the variation between the maternal and F_1 generations is entirely due to plasticity is weak since only the experiment lasted for only one generation and only one environment was used for the common garden experiment (Crispo 2008).

Significant among-population differences in the F_1 generation indicated genetically based differences in shell shape responses to the environment (Figure 3.8, Tables 3.8, 3.20-3.29). Shell shape variation in the F_1 generation paralleled that of their ancestral lineages. Green River F_1 lineage exhibited the longest shell height, aperture width, and upper body whorl width while Polecat Creek F_1 lineage exhibited the shortest lengths in these traits; this same pattern of variation was seen in the maternal lineages (Tables 3.8, 3.20-3.29). The analysis of life history and reproductive traits revealed further significant among-population differences in asymptotic length, age of first reproduction and reproductive rate (Figures 3.19-3.20, Table 3.13). High estimates of heritability (<.394) for all CVs as well as the traditional length measurements provides further evidence of genetic variation among maternal lineages (Table 3.32). The F_1 lineages maintained among populations differences seen in the maternal lineages suggesting that these morphological differences are inherited. However, despite high estimates of heritability, Green River and Bear River F_1 lineages were always significantly different from their maternal ancestors in all traits suggesting the plasticity may be the initial instigator of adaptive change.

Although in the past adaptive evolution and phenotypic plasticity were considered independent events, recent mounting evidence argues that these two mechanisms are not mutually exclusive (Crispo 2008). To determine whether these two mechanisms are independent, the population*generation effect was examined. A significant population*generation effect was found for CV1 and CV2 and well as all five traditional length measurements (Table 3.15). The population by generation interaction for CV1, explained 61.7% of the total variation in overall shell shape, revealed parallel lines between the two generations indicating the retention of genetic variation while the positive shift in the F_1 generation provides evidence of a plastic response (Figure 3.17). The F_1 lineages appear to be a product of both plastic and evolved adaptive responses, but the question remains: how are the two mechanisms related? This study is consistent with the idea that a plastic response may promote evolved adaptive divergence (Crispo 2008). These results are consistent with a similar study that raised sympatric morphs of Arctic char in a common environment (Adams and Huntingford 2004). Much like the snails from this study, Adams and Huntingford also found significant variation in morphology between the wild and lab-raised fish that was attributable to the environment while genetic differences were found between morphs raised in a common environment. These studies suggest that plasticity may drive adaptive phenotypic change, followed by genetic changes in the direction of the plastic response. Unfortunately, these time-constrained experiments cannot distinguish which response occurred first: plastic or genetic. A long term common experiment of more recently established invasive populations may better test this hypothesis.

Whether or not plasticity drives evolution, these responses do appear to be adaptive. The shell morphs in the maternal populations reflect their natural habitat's water velocity. The fitness of costal marine snails has been linked to shell morphs adapted to different levels of wave

exposure (Rolan-Alvarez et.al. 1997, Struhsaker 1968, Janson and Sundberg 1983). In their native range *P. antipodarum* have been shown to exhibit larger shell morphs in higher flow streams (Hasse 2003). Larger and wider snail's foot results in a greater attachment area that can withstand stronger currents (Dussart 1987) despite the increased effects of lift and drag forces associated with larger surface areas (Statzner and Holm 1989). Green River snails had the largest overall size in shell height, aperture height, aperture width, and body whorl width followed by Bear River (Tables 3.20-3.29). Both the Green River and Bear River sample sites were located downstream from dams suggesting that these populations may experience periods of high flow rates (Vanicek, 1970). Bear River is subject to very strong currents in the summer (http://www.pacificorp.com/hydro_hiws/BelowGraceDamFlow.html). On the other hand, Polecat Creek has low water velocity and its flow rates are relatively consistent throughout the year (Hall and Tank 2003). Polecat Creek snails were the smallest in overall size (Tables 3.20-3.29) much like snails in the native range inhabiting low flow sections of streams (Hasse 2003).

The F_1 generation's smaller shell morphs suggest an adaptive shift to a low flow environment. Green River and Bear River F_1 lineages experience an overall decrease in size that can be attributed to the rearing environment. Green River and Bear River F_1 lineages are significantly smaller then their maternal lineages in all five traditional length measurements (Tables 3.20-3.29). On the other hand, the Polecat Creek F_1 lineage is not significantly different from their maternal lineage in any of the traditional length measurements, but do differ in overall shell shape (Figure 3.12, Tables 3.16-3.29).

This lack of change in shell traits associated with water flow may be due to the similarity in flow rate between Polecat Creek and the common environment. The reduction of among-population differences in the F_1 generation can also be attributed to the rearing environment.

There were no significant differences in any of the traditional length measurements between Green River and Bear River F_1 lineages. The Green River F_1 lineage exhibited significantly longer shell height, aperture width and upper body whorl width than the Polecat Creek F_1 lineage suggesting that some among-population differences were being maintained. In conclusion, the larger, high flow adaptive shell morphs shifted to a smaller shell morph more suited to a low flow environment within a single generation. This ability to rapidly shift shell shape may have contributed to *P. antipodarum's* success across wide environmental gradients in the Western United States over a short span of about two decades (Kerans et al. 2005, Hall et al. 2006).

This common garden experiment showed that both plasticity and evolution influence shell morphology in invasive population of *P. antipodarum*. Significant differences in shell shape between the maternal and F_1 lineages suggest a plastic response, while among offspring differences in shell shape, life history, and reproductive traits as well as high heritability estimates indicate a genetic component. A significant population by generation effect (Figure 3.17) indicates that the two mechanisms are not mutually exclusive; however, it is unclear how plasticity and evolution in shell morphology interact. These plastic and evolved responses in shell morphology appear to be adaptive in both maternal and F_1 lineages. Within a single generation, the larger high flow adaptive shell morphs shifted to a smaller shell morph more suited to a low flow environment, suggesting that invasive populations can quickly adapt to new environmental parameters. While the results of this experiment suggest that plasticity is driving evolution, it is impossible to confirm this hypothesis in a single generation of experimentation (Crispo 2008).

Table 3.1: Collection Information for *P. antipodarum* Common Garden populations

Collection Location	State, Nearest City	Latitude	Longitude	UMT Coordinates (X,Y, Zone)
Polecat Creek	WY, Flagg Ranch	44.1077°N	110.6836°W	525321 , 4883884, 12
Bear River at Black Canyon	ID, Soda Springs	42.32580°N	111.47905°W	434596 , 4709987, 12
Green River at Little Hole	UT, Manila	40.54721°N	109.18936°W	653319 , 4490070, 12

Table 3.2 Group assignment of maternal lineages from CVA-Distance
Original Groups along rows, CVA groups along columns

	Bear River	Green River	Polecat Creek
Bear River	23	1	0
Green River	0	20	0
Polecat Creek	0	0	14

Table 3.3 ANOVA results for the effect of population among maternal lineages in CV1 and CV2

CVA	Source	df	SS	MS	F	p
1	Population	2	0.00076880	0.00038440	116.83	<0.0001
	Error	55	0.00018096	0.00000329		
2	Population	2	0.00064655	0.00032327	101	<0.0001
	Error	55	0.00017605	0.00000320		

Table 3.4 ANOVA results for CV1 Means among maternal lineages

Population	CVA1 LSMEAN	Standard Error	p
Bear River	0.00407957	0.00037823	<0.0001
Green River	-0.00098335	0.00040560	0.0186
Polecat Creek	-0.00494420	0.00046835	<0.0001

Table 3.5 ANOVA results for CV1 pair-wise comparisons between maternal lineages

	Bear River	Green River	Polecat Creek
Bear River	-	<0.0001	<0.0001
Green River	<0.0001	-	<0.0001
Polecat Creek	<0.0001	<0.0001	-

Table 3.6 ANOVA results for CV2 Means among maternal lineages

Population	CVA2 LSMEAN	Standard Error	p
Bear River	0.00193188	0.00037305	<0.0001
Green River	-0.00455683	0.00040005	<0.0001
Polecat Creek	0.00311356	0.00046194	<0.0001

Table 3.7 ANOVA results for CV2 pair-wise comparisons between maternal populations

	Bear River	Green River	Polecat Creek
Bear River	-	<0.0001	0.0516
Green River	<0.0001	-	<0.0001
Polecat Creek	0.0516	<0.0001	-

Table 3.8 Group assignment of F_1 lineages from CVA-Distance Based Original Groups along rows, CVA groups along columns

	Bear River	Green River	Polecat Creek
Bear River	55	2	9
Green River	0	51	2
Polecat Creek	2	4	22

Table 3.9 ANOVA results for the effects of Population and Female (Population) among F_1 lineages in CV1 and CV2

CVA	Source	df	SS	MS	F	p
1	Population	2	0.00190451	0.00095225	149.93	<0.0001
	Female(Population)	54	0.00034298	0.00000635	0.63	0.9683
	Error	90	0.00091400	0.00001016		
2	Population	2	0.00095129	0.00047564	30.31	<0.0001
	Female(Population)	54	0.00084739	0.00001569	1.01	0.4786
	Error	90	0.00140087	0.00001557		

Table 3.10 ANOVA results for CV1 Means among F_1 lineages

Population	CVA1 LSMEAN	Standard Error	p
Bear River	0.00362558	0.00035142	<0.0001
Green River	-0.00567923	0.00041761	<0.0001
Polecat Creek	0.00166124	0.00056242	.0046

Table 3.11 ANOVA results for CV1 pair-wise comparisons between F_1 lineages

	Bear River	Green River	Polecat Creek
Bear River	-	<0.0001	0.0045
Green River	<0.0001	-	<0.0001
Polecat Creek	0.0045	<0.0001	-

Table 3.12 ANOVA results for CV2 Means among F_1 lineages

Population	CVA2 LSMEAN	Standard Error	p
Bear River	0.00236519	0.00055239	<0.0001
Green River	0.00074077	0.00065642	0.2641
Polecat Creek	-0.00570847	0.00088404	<0.0001

Table 3.13 ANOVA results for CV2 pair-wise comparisons between F_1 lineages

	Bear River	Green River	Polecat Creek
Bear River	-	0.0637	<0.0001
Green River	0.0637	-	<0.0001
Polecat Creek	<0.0001	<0.0001	-

Table 3.14 ANOVA results for the effects of population on F_1 life history traits

Trait	Source	df	SS	MS	F	p
Asymptote	Population	2	6.23277155	3.11638578	5.58	0.0046
	Error	144	80.43496448	0.55857614		
Growth Rate	Population	2	0.00003400	0.00001700	0.70	0.4995
	Error	144	0.00350992	0.00002437		
Inflection point	Population	2	1801.599779	900.799889	0.51	0.5989
	Error	144	252152.4605	1751.0588		
Age of First Reproduction	Population	2	43108.24017	21554.12009	24.50	<.0001
	Error	144	127546.8409	879.6334		
Reproductive Rate	Population	2	12.55728943	6.27864472	4.77	0.0088
	Error	589	774.8058862	1.3154599		

Table 3.15 Group assignment of maternal and F_1 lineages from CVA-Distance Based Original Groups along rows, CVA groups along columns

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	21	0	1	2	0	0
Bear River F_1	0	52	0	0	1	13
Green River M	1	0	18	0	1	0
Green River F_1	7	1	2	39	2	2
Polecat Creek M	0	0	0	0	14	0
Polecat Creek F_1	0	2	0	3	1	22

Table 3.16 ANOVA results for the effects of Population, Female (Population), Generation, and Population*Generation for CV1, CV2 and traditional length measurements (mm)

Trait	Source	df	SS	MS	F	p
CV1	Population	2	0.00129179	0.00064590	52.38	<.0001
	Female(Population)	55	0.00071685	0.00001303	1.06	0.4190
	Generation	1	0.00388179	0.00388179	314.80	<.0001
	Population*Generation	2	0.00044691	0.00022345	18.12	<.0001
	Error	55	0.00067820	0.00001233		
CV2	Population	2	0.00049401	0.00024700	28.38	<.0001
	Female(Population)	55	0.00038994	0.00000709	0.81	0.7754
	Generation	1	0.00000894	0.00000894	1.03	0.3152
	Population*Generation	2	0.00046122	0.00023061	26.49	<.0001
	Error	55	0.00047875	0.00000870		
Shell Height	Population	2	11.61244275	5.80622137	37.11	<.0001
	Female(Population)	55	6.78707813	0.12340142	0.79	0.8094
	Generation	1	6.28066251	6.28066251	40.14	<.0001
	Population*Generation	2	3.19299183	1.59649592	10.20	0.0002
	Error	55	8.60625535	0.15647737		
Aperture Width	Population	2	0.94797801	0.47398900	42.38	<.0001
	Female(Population)	55	0.28761938	0.00522944	0.47	0.9972
	Generation	1	0.72694551	0.72694551	64.99	<.0001
	Population*Generation	2	0.26109001	0.13054500	11.67	<.0001
	Error	55	0.61519694	0.01118540		
Aperture Height	Population	2	0.84293996	0.42146998	36.58	<.0001
	Female(Population)	55	0.56082157	0.01019676	0.88	0.6740
	Generation	1	0.29803574	0.29803574	25.87	<.0001
	Population*Generation	2	0.60167300	0.30083650	26.11	<.0001
	Error	55	0.63370891	0.01152198		
Upper Body Whorl Width	Population	2	2.23038632	1.11519316	50.42	<.0001
	Female(Population)	55	0.85622523	0.01556773	0.70	0.9020
	Generation	1	0.90572389	0.90572389	40.95	<.0001
	Population*Generation	2	0.84636116	0.42318058	19.13	<.0001
	Error	55	1.21644729	0.02211722		
Lower Body Whorl Width	Population	2	1.36487791	0.68243896	44.07	<.0001
	Female(Population)	55	0.76428557	0.01389610	0.90	0.6552
	Generation	1	0.51271934	0.51271934	33.11	<.0001
	Population*Generation	2	0.81835942	0.40917971	26.42	<.0001
	Error	55	0.85166221	0.01548477		

Table 3.17 ANOVA results for CV1 Means across Populations and Generations

Population	Generation	CVA1 LSMEAN	Standard Error	p
Bear River	Maternal	-0.00691401	0.00071679	<.0001
Bear River	Offspring	0.00776869	0.00071679	<.0001
Green River	Maternal	-0.01029925	0.00078520	<.0001
Green River	Offspring	-0.00393023	0.00078520	<.0001
Polecat Creek	Maternal	-0.00872180	0.00093850	<.0001
Polecat Creek	Offspring	0.00581021	0.00093850	<.0001

Table 3.18 ANOVA results for CV1 pair-wise comparisons between Generations and Populations

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	<.0001	0.0024	0.0069	0.1315	<.0001
Bear River F_1	<.0001	-	<.0001	<.0001	<.0001	0.1029
Green River M	0.0024	<.0001	-	<.0001	0.2027	<.0001
Green River F_1	0.0069	<.0001	<.0001	-	0.0003	<.0001
Polecat Creek M	0.1315	<.0001	0.2027	0.0003	-	<.0001
Polecat Creek F_1	<.0001	0.1029	<.0001	<.0001	<.0001	-

Table 3.19 ANOVA results for CV2 Means across Populations and Generations

Population	Generation	CVA2 LSMEAN	Standard Error	p
Bear River	Maternal	0.00290631	0.00060224	<.0001
Bear River	Offspring	0.00058563	0.00060224	0.3351
Green River	Maternal	0.00362558	0.00065972	<.0001
Green River	Offspring	-0.00123993	0.00065972	0.0655
Polecat Creek	Maternal	-0.00603437	0.00078852	<.0001
Polecat Creek	Offspring	-0.00055624	0.00078852	0.4835

Table 3.20 ANOVA results for CV2 pair-wise comparisons between Generations and Populations

Maternal Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	0.0086	0.4242	<.0001	<.0001	0.0010
Bear River F_1	0.0086	-	0.0012	0.0458	<.0001	0.2548
Green River M	0.4242	0.0012	-	<.0001	<.0001	0.0002
Green River F_1	<.0001	0.0458	<.0001	-	<.0001	0.5088
Polecat Creek M	<.0001	<.0001	<.0001	<.0001	-	<.0001
Polecat Creek F_1	0.0010	0.2548	0.0002	0.5088	<.0001	-

Table 3.21 ANOVA results for Shell Height (mm) Means across Populations and Generations

Population	Generation	Shell Height LSMEAN	Standard Error	p
Bear River	Maternal	4.99428583	0.08074584	<.0001
Bear River	Offspring	4.46043641	0.08074584	<.0001
Green River	Maternal	5.53015650	0.08845263	<.0001
Green River	Offspring	4.64114961	0.08845263	<.0001
Polecat Creek	Maternal	4.25038214	0.10572112	<.0001
Polecat Creek	Offspring	4.24191310	0.10572112	<.0001

Table 3.22 ANOVA results for Shell Height (mm) pair-wise comparisons between Generations and Populations

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	<.0001	<.0001	0.0047	<.0001	<.0001
Bear River F_1	<.0001	-	<.0001	0.1370	0.1201	0.1062
Green River M	<.0001	<.0001	-	<.0001	<.0001	<.0001
Green River F_1	0.0047	0.1370	<.0001	-	0.0064	0.0064
Polecat Creek M	<.0001	0.1201	<.0001	0.0064	-	0.9550
Polecat Creek F_1	<.0001	0.1062	<.0001	0.0064	0.9550	-

Table 3.23 ANOVA results for Aperture Width (mm) Means across Populations and Generations

Population	Generation	Aperture Width LSMEAN	Standard Error	p
Bear River	Maternal	1.32039500	0.02158838	<.0001
Bear River	Offspring	1.14964053	0.02158838	<.0001
Green River	Maternal	1.48986150	0.02364889	<.0001
Green River	Offspring	1.20601252	0.02364889	<.0001
Polecat Creek	Maternal	1.12494714	0.02826583	<.0001
Polecat Creek	Offspring	1.09259813	0.02826583	<.0001

Table 3.24 ANOVA results for Aperture Width (mm) pair-wise comparisons between Generations and Populations

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	<.0001	<.0001	0.0007	<.0001	<.0001
Bear River F_1	<.0001	-	<.0001	0.0839	0.4904	0.1145
Green River M	<.0001	<.0001	-	<.0001	<.0001	<.0001
Green River F_1	0.0007	0.0839	<.0001	-	0.0321	0.0033
Polecat Creek M	<.0001	0.4904	<.0001	0.0321	-	0.4219
Polecat Creek F_1	<.0001	0.1145	<.0001	0.0033	0.4219	-

Table 3.25 ANOVA results for Aperture Height (mm) Means across Populations and Generations

Population	Generation	Aperture Height LSMEAN	Standard Error	p
Bear River	Maternal	1.76921708	0.02191079	<.0001
Bear River	Offspring	1.65489100	0.02191079	<.0001
Green River	Maternal	1.92357250	0.02400206	<.0001
Green River	Offspring	1.63407448	0.02400206	<.0001
Polecat Creek	Maternal	1.50902714	0.02868795	<.0001
Polecat Creek	Offspring	1.60105577	0.02868795	<.0001

Table 3.26 ANOVA results for Aperture Height (mm) pair-wise comparisons between Generations and Populations

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	0.0005	<.0001	0.0001	<.0001	<.0001
Bear River F_1	0.0005	-	<.0001	0.5245	0.0002	0.1416
Green River M	<.0001	<.0001	-	<.0001	<.0001	<.0001
Green River F_1	0.0001	0.5245	<.0001	-	0.0015	0.3812
Polecat Creek M	<.0001	0.0002	<.0001	0.0015	-	0.0273
Polecat Creek F_1	<.0001	0.1416	<.0001	0.3812	0.0273	-

Table 3.27 ANOVA results for Upper Body Whorl Width (mm) Means across Populations and Generations

		Upper Body Whorl Width		
Population	Generation	LSMEAN	Standard Error	p
Bear River	Maternal	2.14325000	0.03035706	<.0001
Bear River	Offspring	1.92076540	0.03035706	<.0001
Green River	Maternal	2.34961850	0.03325449	<.0001
Green River	Offspring	1.96286203	0.03325449	<.0001
Polecat Creek	Maternal	1.75704929	0.03974672	<.0001
Polecat Creek	Offspring	1.82274780	0.03974672	<.0001

Table 3.28 ANOVA results for Upper Body Whorl Width (mm) pair-wise comparisons between Generations and Populations

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	<.0001	<.0001	0.0002	<.0001	<.0001
Bear River F_1	<.0001	-	<.0001	0.3539	0.0018	0.0551
Green River M	<.0001	<.0001	-	<.0001	<.0001	<.0001
Green River F_1	0.0002	0.3539	<.0001	-	0.0002	0.0091
Polecat Creek M	<.0001	0.0018	<.0001	0.0002	-	0.2475
Polecat Creek F_1	<.0001	0.0551	<.0001	0.0091	0.2475	-

Table 3.29 ANOVA results for Lower Body Whorl Width (mm) Means across Populations and Generations

Population	Generation	Lower Body Whorl Width LSMEAN	Standard Error	p
Bear River	Maternal	2.04397792	0.02540076	<.0001
Bear River	Offspring	1.87808065	0.02540076	<.0001
Green River	Maternal	2.21671600	0.02782514	<.0001
Green River	Offspring	1.87230765	0.02782514	<.0001
Polecat Creek	Maternal	1.70935071	0.03325740	<.0001
Polecat Creek	Offspring	1.81070137	0.03325740	<.0001

Table 3.30 ANOVA results for Lower Body Whorl Width (mm) pair-wise comparisons between Generations and Populations

Population	Bear River M	Bear River F_1	Green River M	Green River F_1	Polecat Creek M	Polecat Creek F_1
Bear River M	-	<.0001	<.0001	<.0001	<.0001	<.0001
Bear River F_1	<.0001	-	<.0001	0.8788	0.0002	0.1131
Green River M	<.0001	<.0001	-	<.0001	<.0001	<.0001
Green River F_1	<.0001	0.8788	<.0001	-	0.0004	0.1610
Polecat Creek M	<.0001	0.0002	<.0001	0.0004	-	0.0356
Polecat Creek F_1	<.0001	0.1131	<.0001	0.1610	0.0356	-

Table 3.31 Estimates of Variance Components for CV1, CV2 and traditional length measurements (mm) among maternal lineages

Measurement	Variance Component	Estimate	Percentage
CV1	Population V_P	0.000020	85.9%
	Residual: Maternal V_M	3.29E-6	14.1%
CV2	Population V_P	0.000017	84.2%
	Residual: Maternal V_M	3.201E-6	15.8%
Shell Height	Population V_P	0.4049	77.4%
	Residual: Maternal V_M	0.1180	22.6%
Aperture Width	Population V_P	0.03279	80.7%
	Residual: Maternal V_M	0.007841	19.3%
Aperture Height	Population V_P	0.04304	78%
	Residual: Maternal V_M	0.01216	22%
Upper Whorl Width	Population V_P	0.08912	82.3%
	Residual: Maternal V_M	0.01918	17.7%
Lower whorl width	Population V_P	0.06527	78.5%
	Residual: Maternal V_M	0.01790	21.5%

Table 3.32 Estimates of Variance Components for CV1, CV2 and traditional length measurements (mm) among F_1 lineages

Measurement	Variance Component	Estimate	Percentage
CV1	Population V_P	0.000024	73.3%
	Female(population) V_M	6.517E-8	0.2%
	Residual: Offspring V_O	8.666E-6	26.5%
CV2	Population V_P	0.000015	48.3%
	Female(population) V_M	6.58E-8	0.2%
	Residual: Offspring V_O	0.000016	51.5%
Shell Height	Population V_P	0	0%
	Female(population) V_M	0.04637	19.8%
	Residual: Offspring V_O	0.1882	80.2%
Aperture Width	Population V_P	0.001532	11.4%
	Female(population) V_M	6.23E-22	0%
	Residual: Offspring V_O	0.01185	88.6%
Aperture Height	Population V_P	0.003339	24.1%
	Female(population) V_M	3.2E-21	0%
	Residual: Offspring V_O	0.01054	75.9%
Upper Whorl Width	Population V_P	0.004292	14.4%
	Female(population) V_M	0	0%
	Residual: Offspring V_O	0.02561	85.6%
Lower Whorl Width	Population V_P	0.004459	30%
	Female(population) V_M	0	0%
	Residual: Offspring V_O	0.01042	70%

Table 3.33 Estimates of Variance Components (V_G and V_E) and the broad sense heritability for CV1, CV2 Means and traditional length measurements

Measurement	V_G	V_E	H^2
CV1	2.329E-5	3.273E-5	.416
CV2	2.020E-5	3.107E-5	.394
Shell Height	.523	.235	.69
Aperture Width	.041	.013	.759
Aperture Height	.055	.014	.797
Upper Whorl Width	.108	.03	.783
Lower Whorl Width	.083	.015	.847

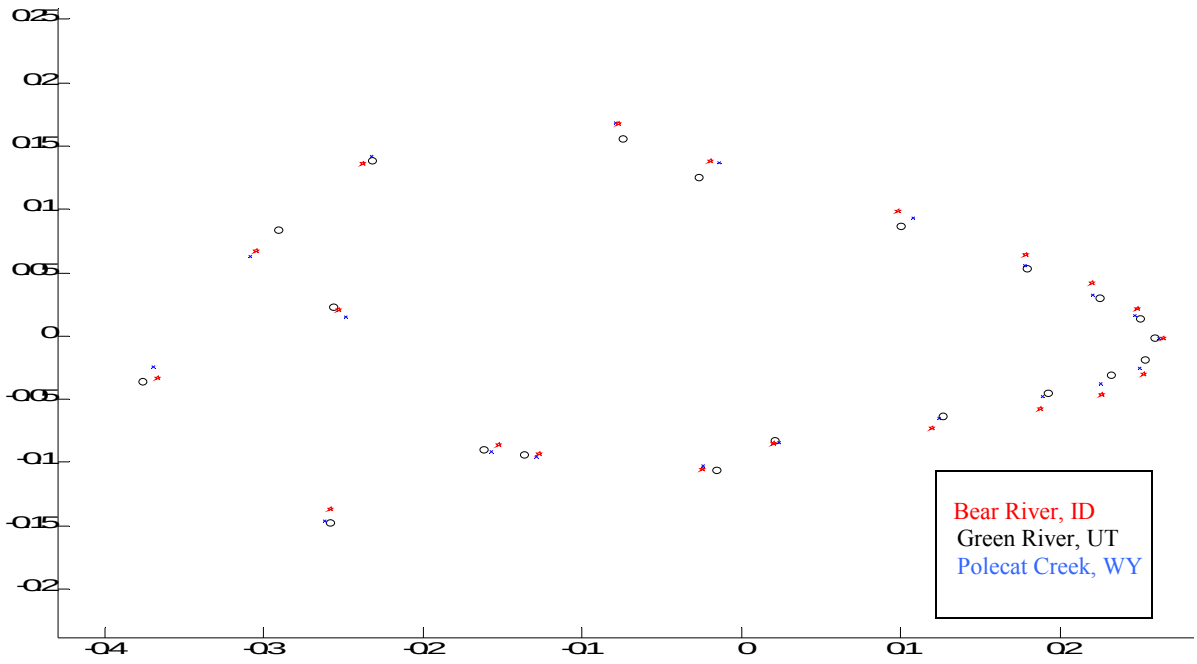

Figure 3.1 Landmark Mean plot for three populations of *P. antipodarum*. Each point represents the mean location for each of the twenty landmarks across all three populations.

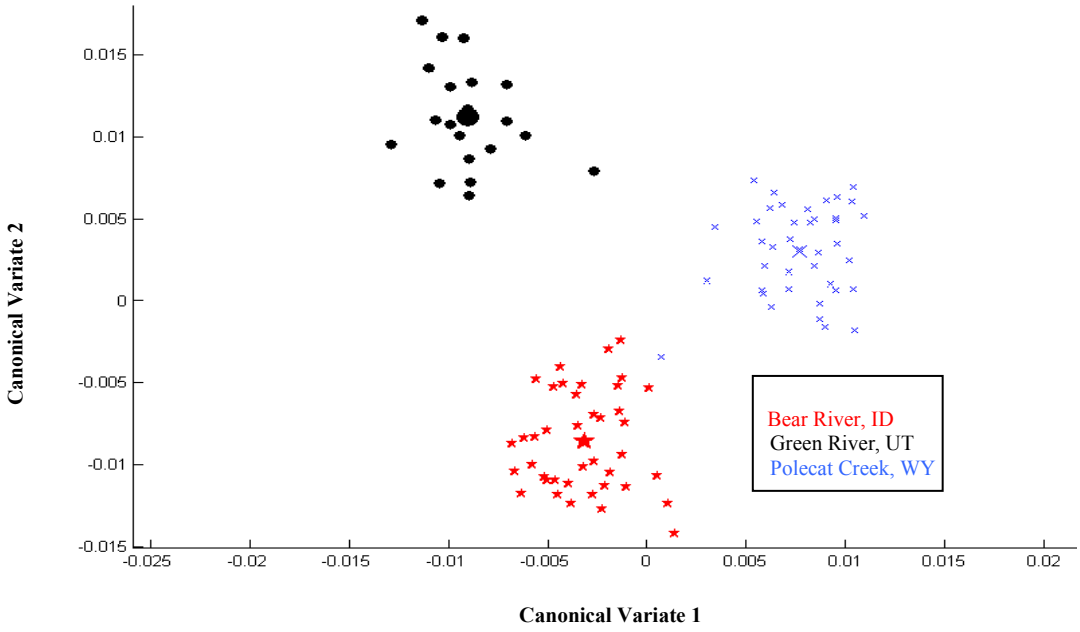

Figure 3.2 Canonical Variate Analysis plot showing three populations along two distinct canonical variate axes. Canonical Variate 1 was significant ($p < .0001$) and distinguished the snails by spiral height comprising 57.5% of the total variation. Canonical Variate 2 was significant ($p < .0001$) and distinguished the snails by aperture width comprising 42.5% of the total variation.

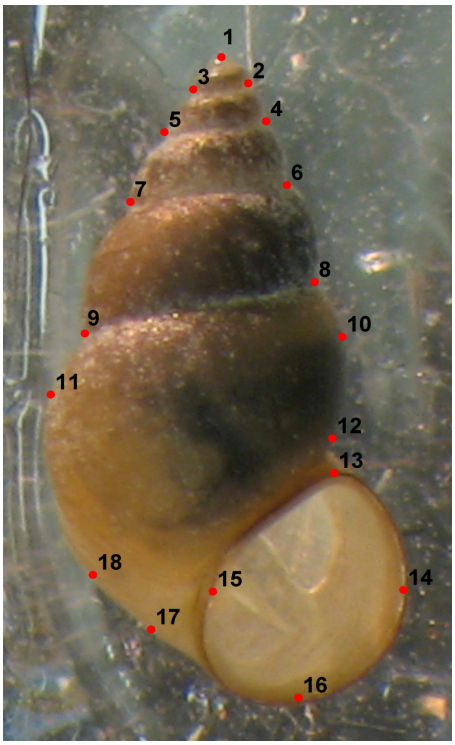

Figure 3.3 Eighteen anatomical landmarks used in morphometric analysis.

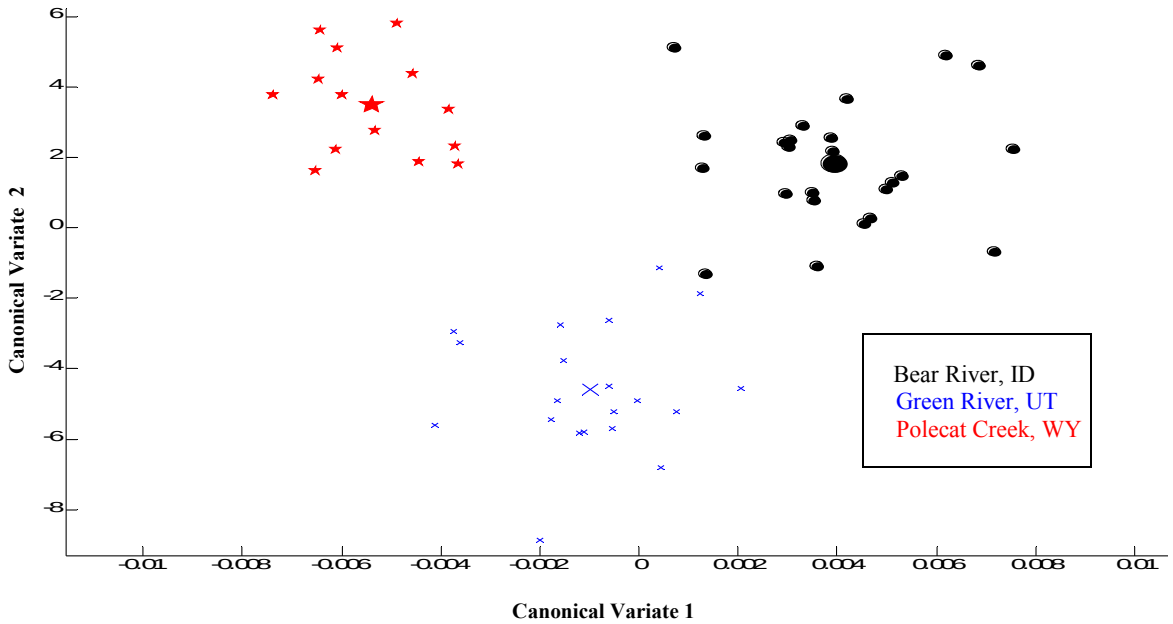

Figure 3.4 Canonical Variate Analysis plot showing three maternal lineages. Canonical Variate 1 was significant ($p < .0001$) and comprised 56.1% of the total variation. Canonical Variate 2 was also significant ($p < .0001$) and comprised 43.4% of the total variation.

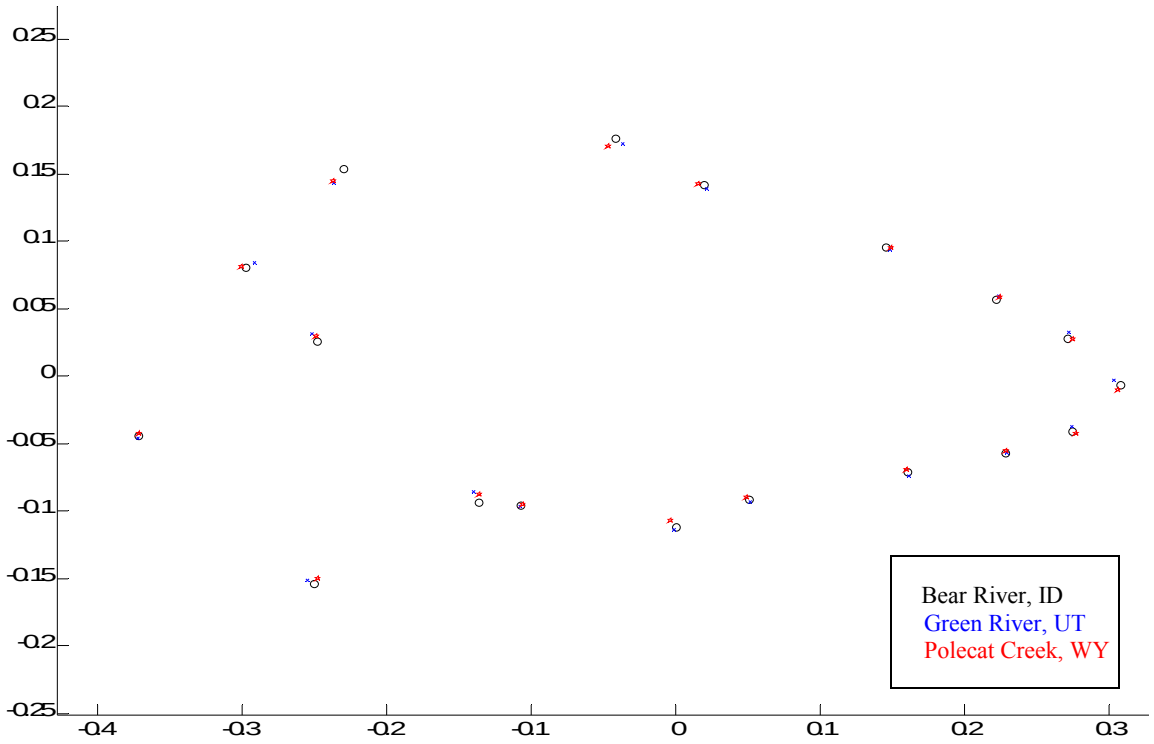

Figure 3.5 Landmark Mean Plot for three maternal lineages. Each point represents the mean location for each of the eighteen landmarks across all three populations.

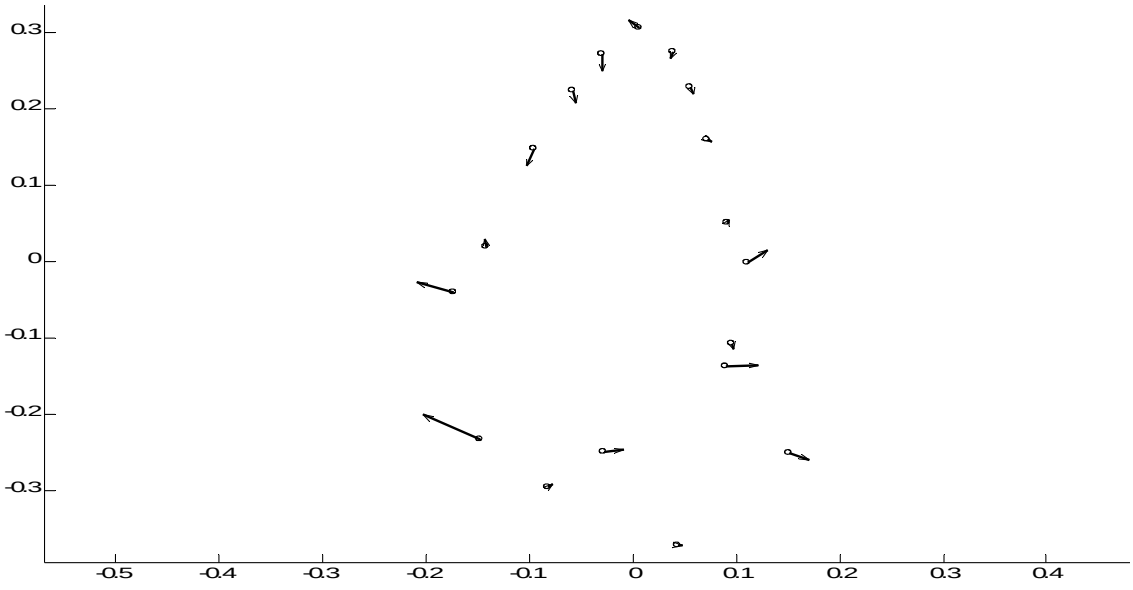

Figure 3.6 Landmark Vectors Procrustes CV1 of maternal lineages
Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV1 increases.

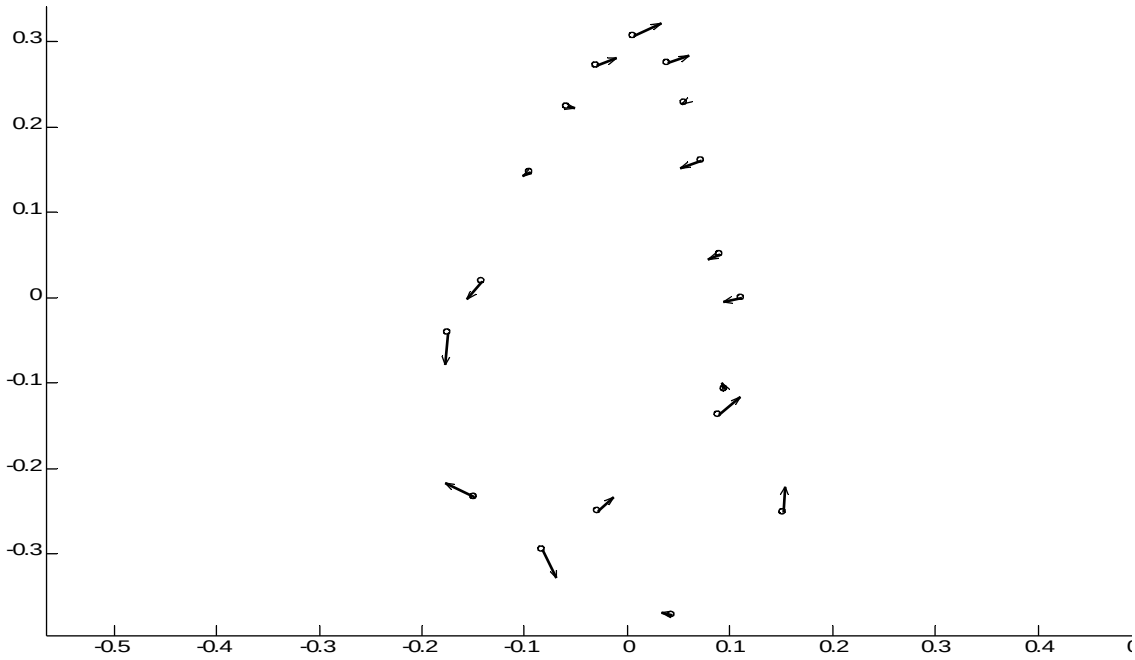

Figure 3.7 Landmark Vectors Procrustes CV2 of maternal lineages
Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV2 increases.

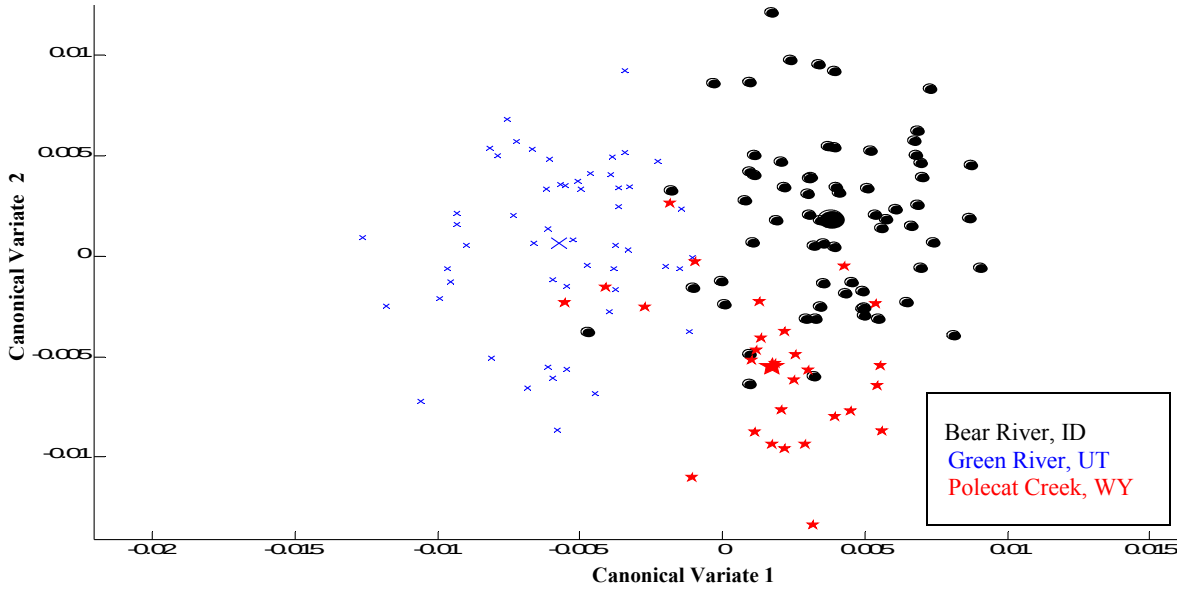

Figure 3.8 Canonical Variate Analysis plot showing three F_1 lineages. Canonical Variate 1 was significant ($p < .0001$) and comprised 84.5% of the total variation. Canonical Variate 2 was not significant ($p = .015$) and comprised 15.3% of the total variation.

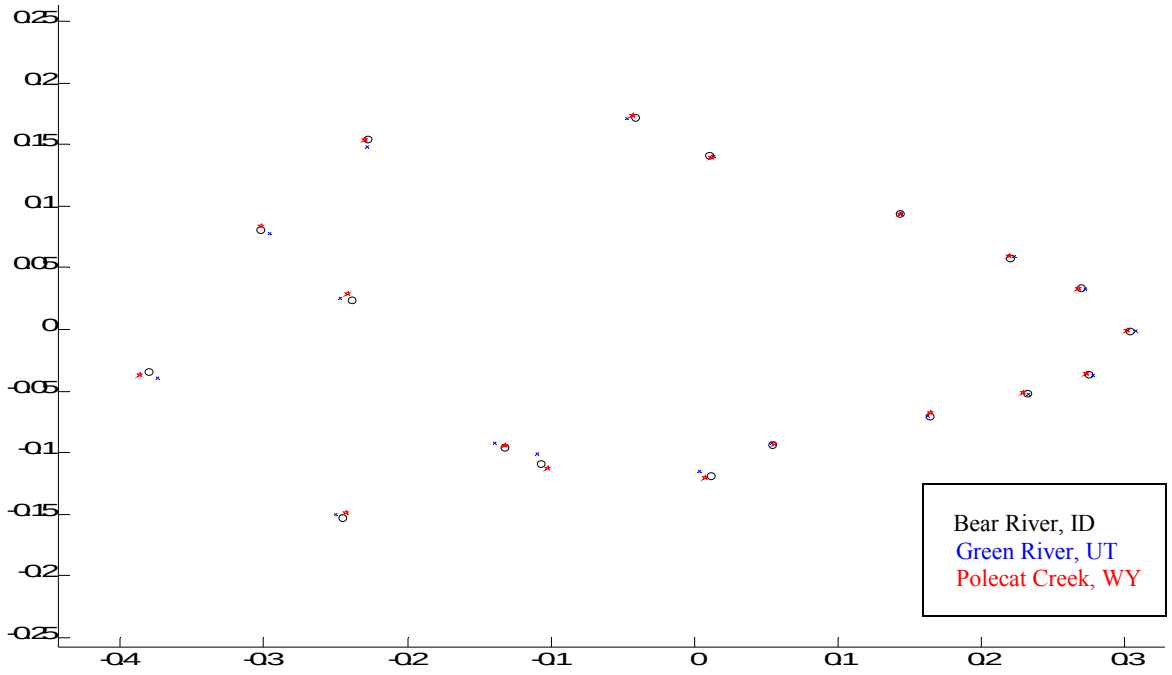

Figure 3.9 Landmark Mean Plot for three F_1 Offspring Lineages. Each point represents the mean location for each of the eighteen landmarks across all three populations.

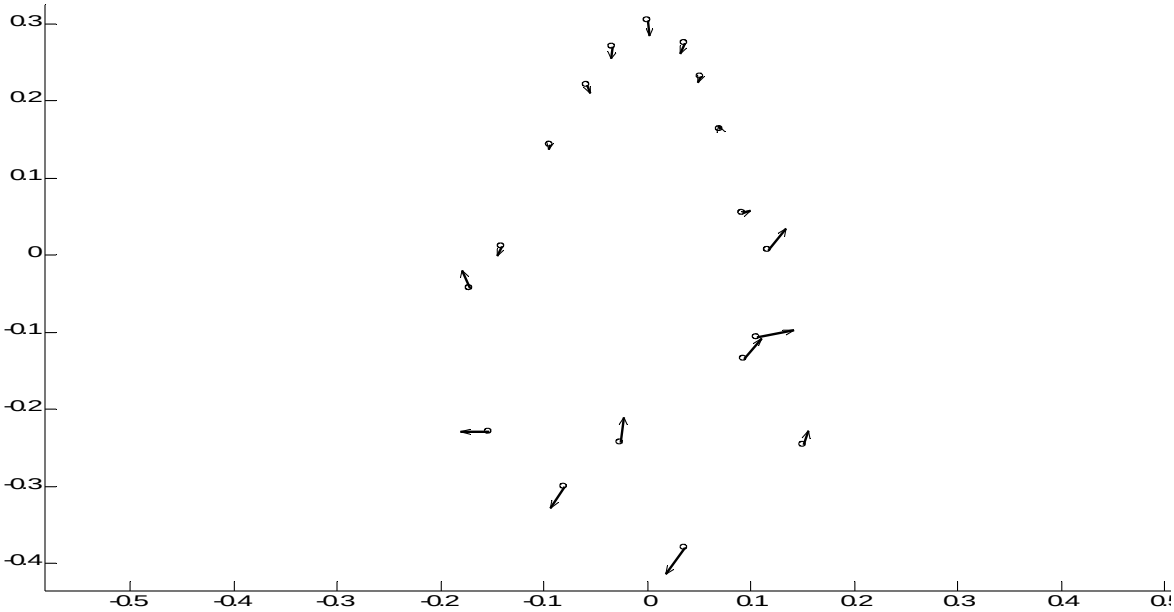

Figure 3.10 Landmark Vectors Procrustes CV1 of three F_1 lineages. Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV1 increases.

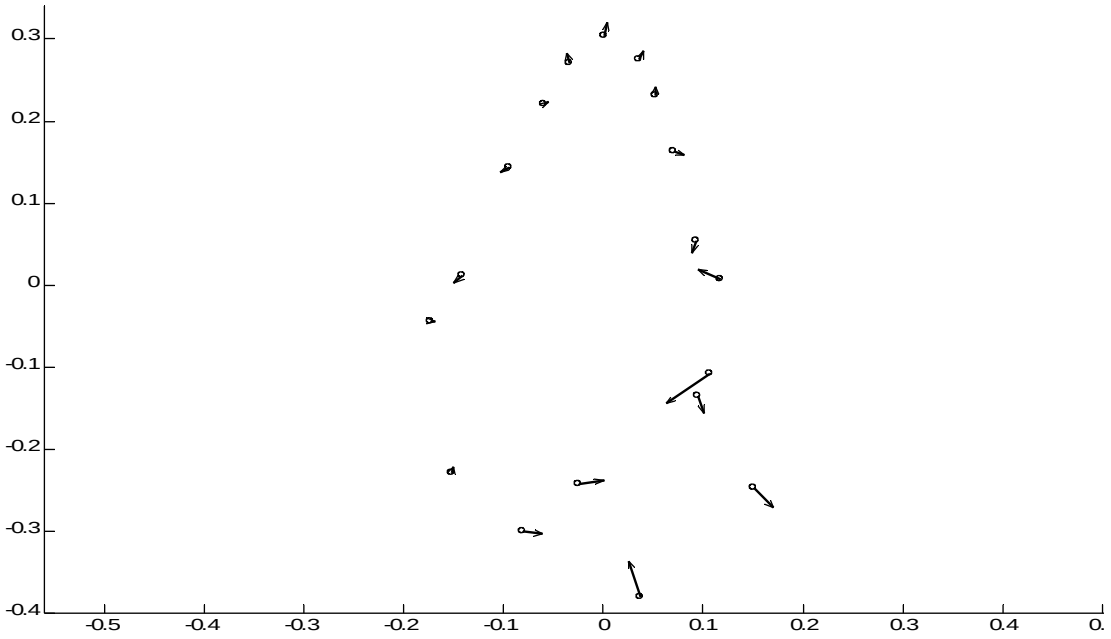

Figure 3.11 Landmark Vectors Procrustes CV2 of three F_1 lineages.
Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV2 increases.

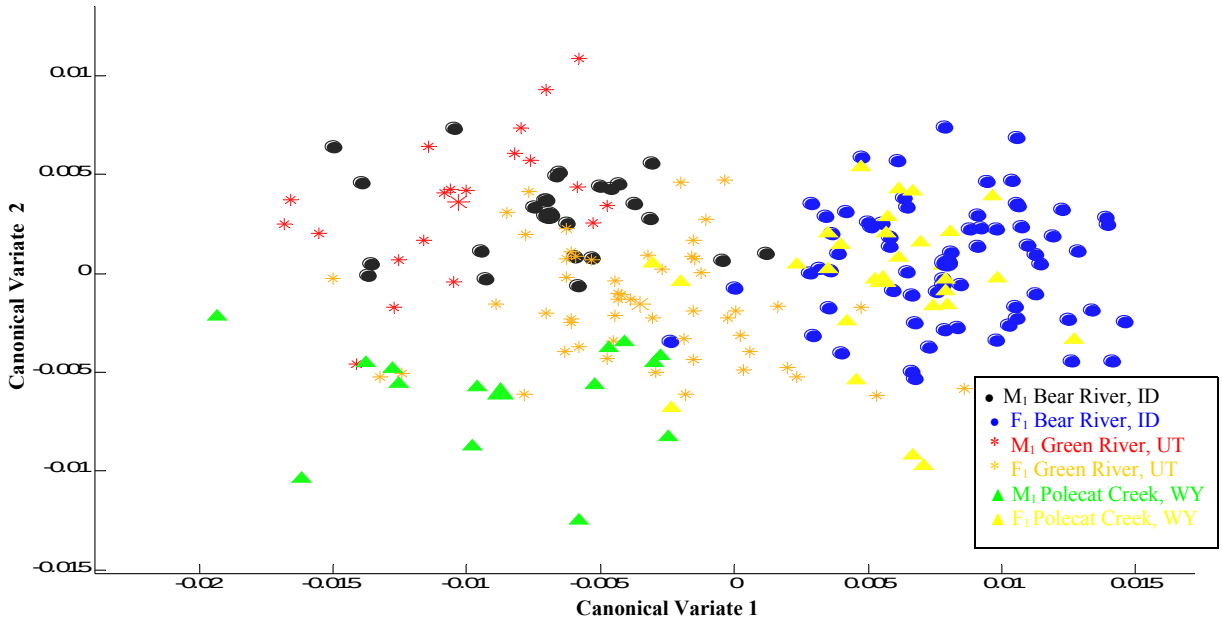

Figure 3.12 Canonical Variate Analysis plot showing three maternal and three F_1 lineages.
Canonical Variate 1 was significant ($p < .0001$) and comprised 61.7% of the total variation.
Canonical Variate 2 was also significant ($p < .0001$) and comprised 11.9% of the total variation.

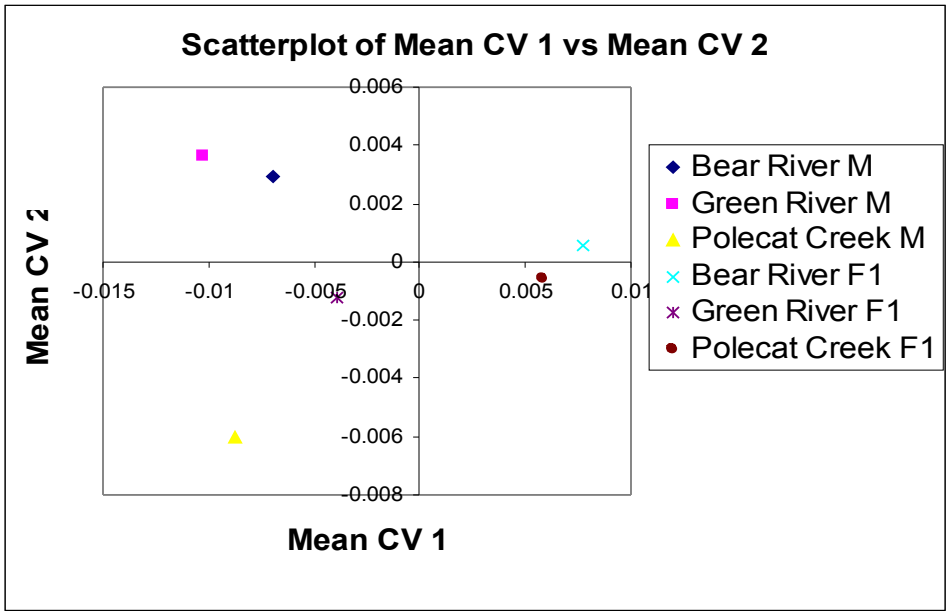

Figure 3.13 CV1 and CV2 Means from Figure 3.12 plotted on a scatterplot. All offspring were significantly different from their ancestral mothers.

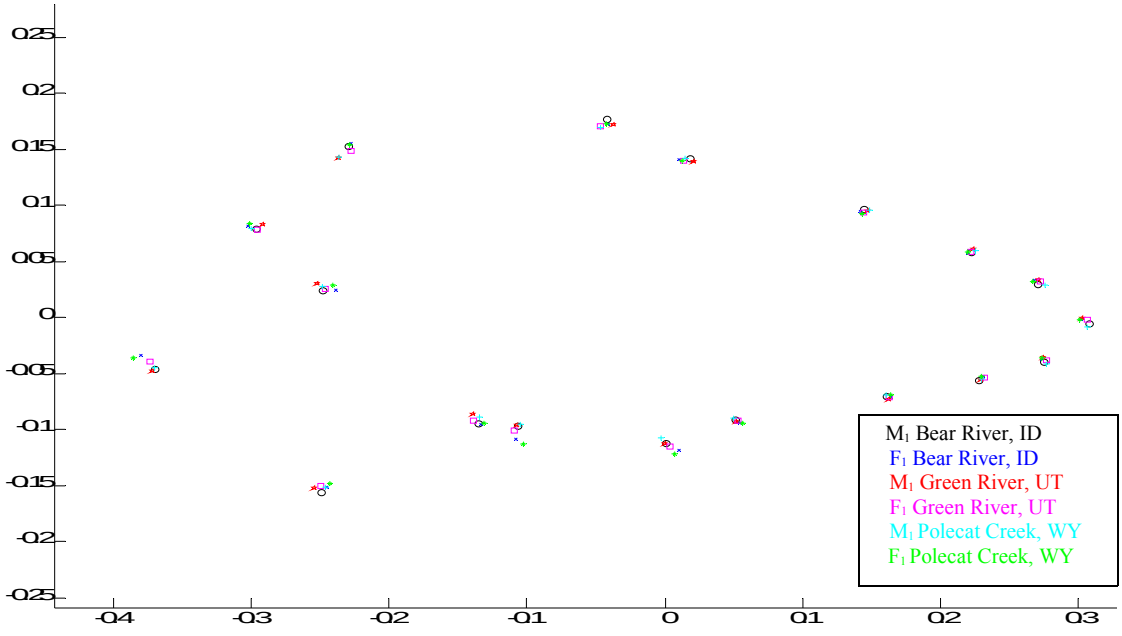

Figure 3.14 Landmark Mean Plot for three maternal and F_1 lineages. Each point represents the mean location for each of the eighteen landmarks across all six groups.

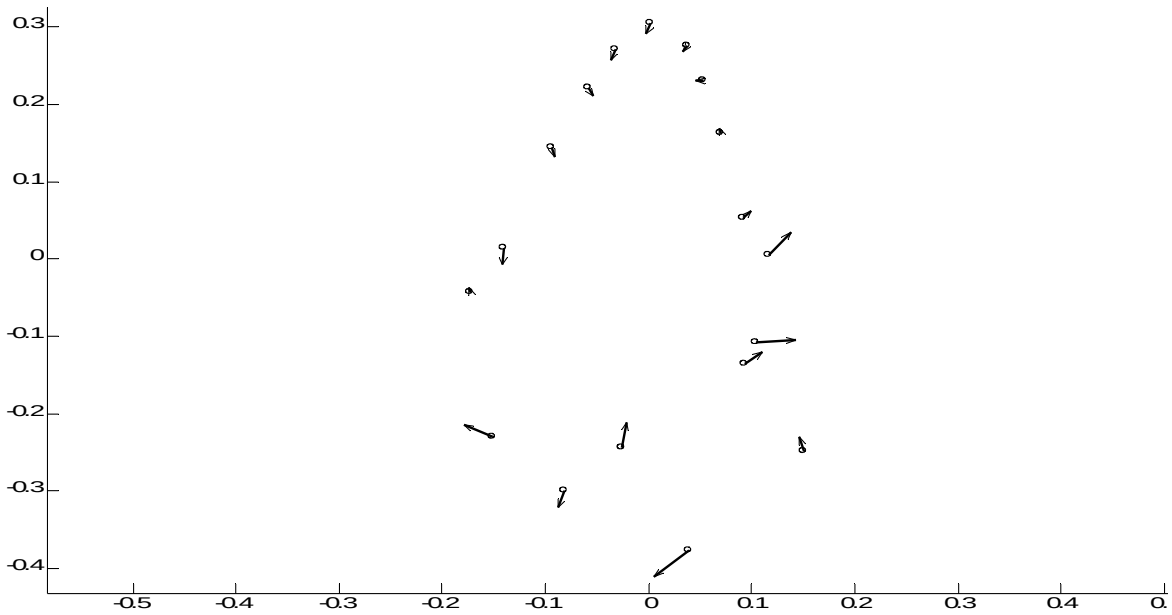

Figure 3.15 Landmark Vectors Procrustes CV1 of maternal and F_1 lineages. Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV1 increases.

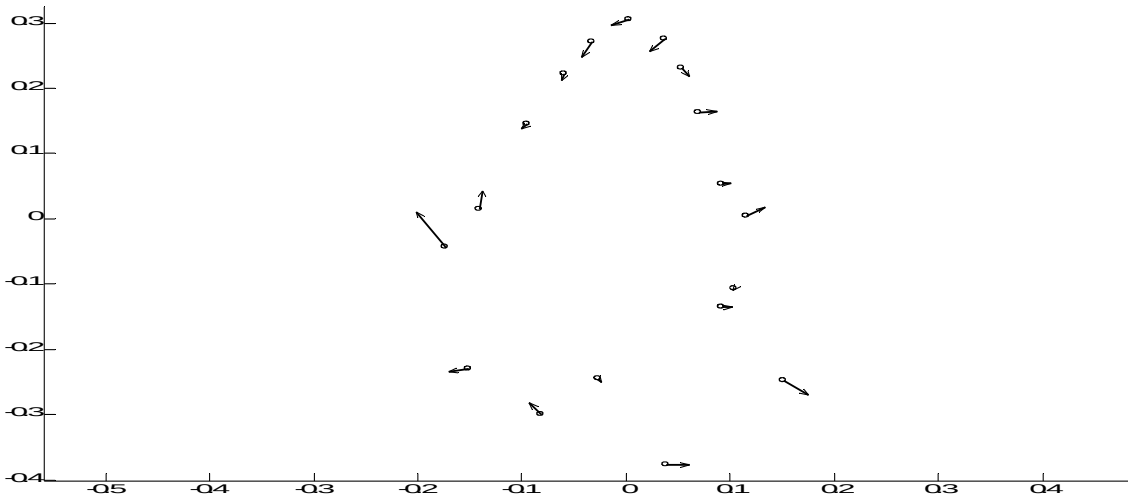

Figure 3.16 Landmark Vectors Procrustes CV2 of Maternal and F_1 lineages. Circles indicate the location of the landmarks in the mean shape of the entire data set. The arrows indicate the changes in the relative position of the landmarks as the score on CV2 increases.

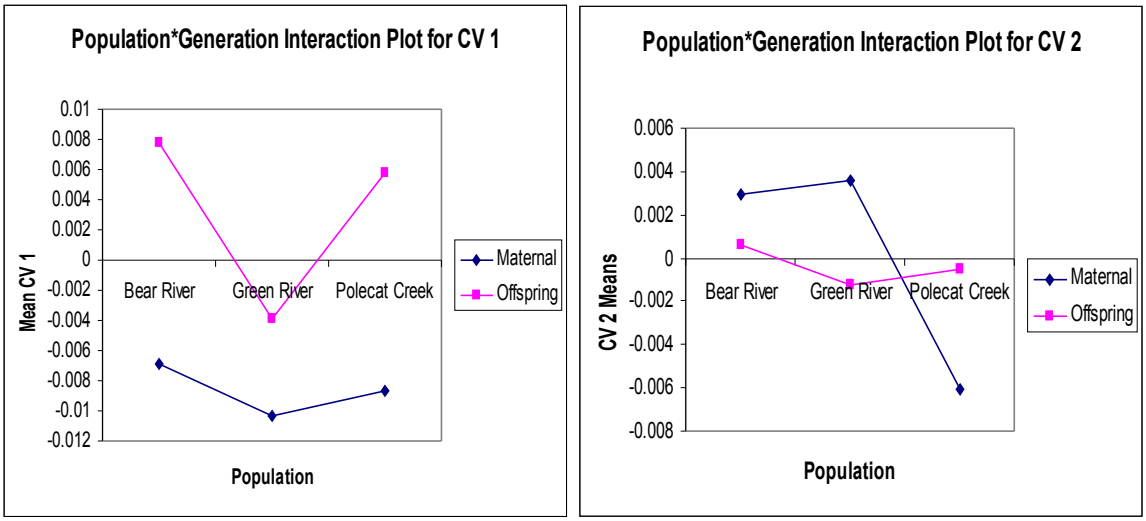

Figure 3.17 Population*Generation Interaction Plot for CV1 and CV2 means. The interaction is significant for both CV1 and CV2.

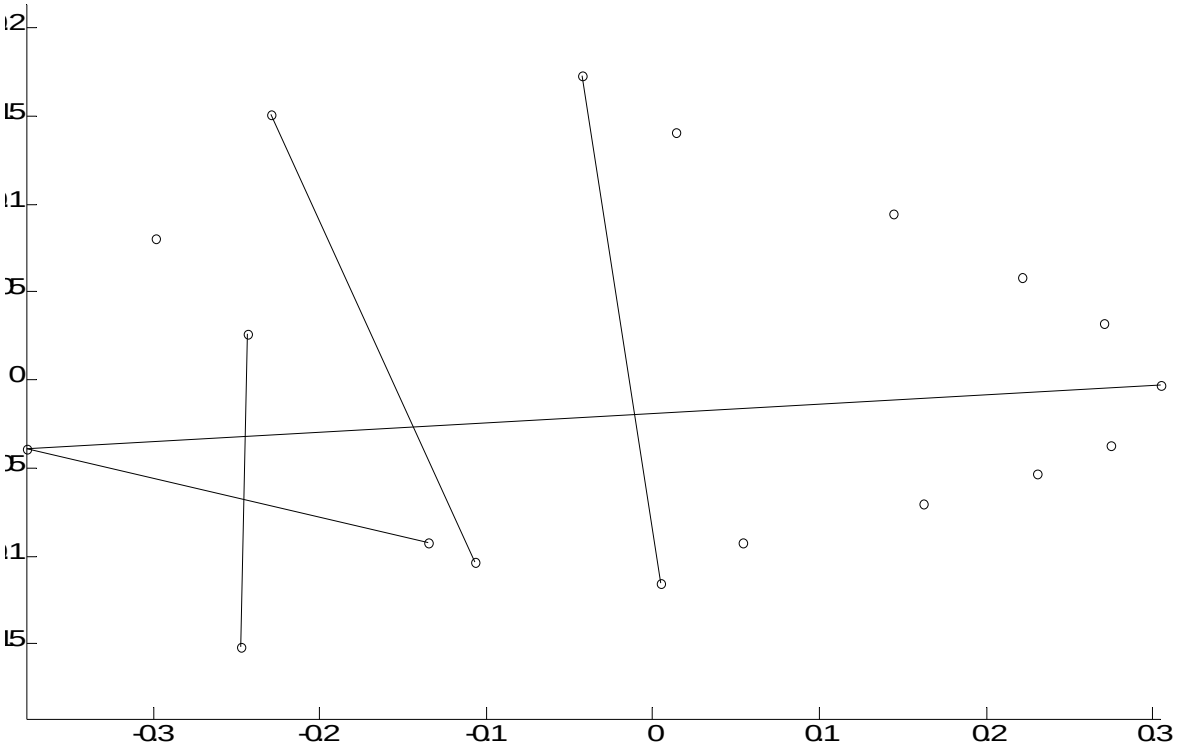

Figure 3.18 Interlandmark Distances. This figure shows a set of interlandmark distances used to calculate traditional length measurements on the shell.

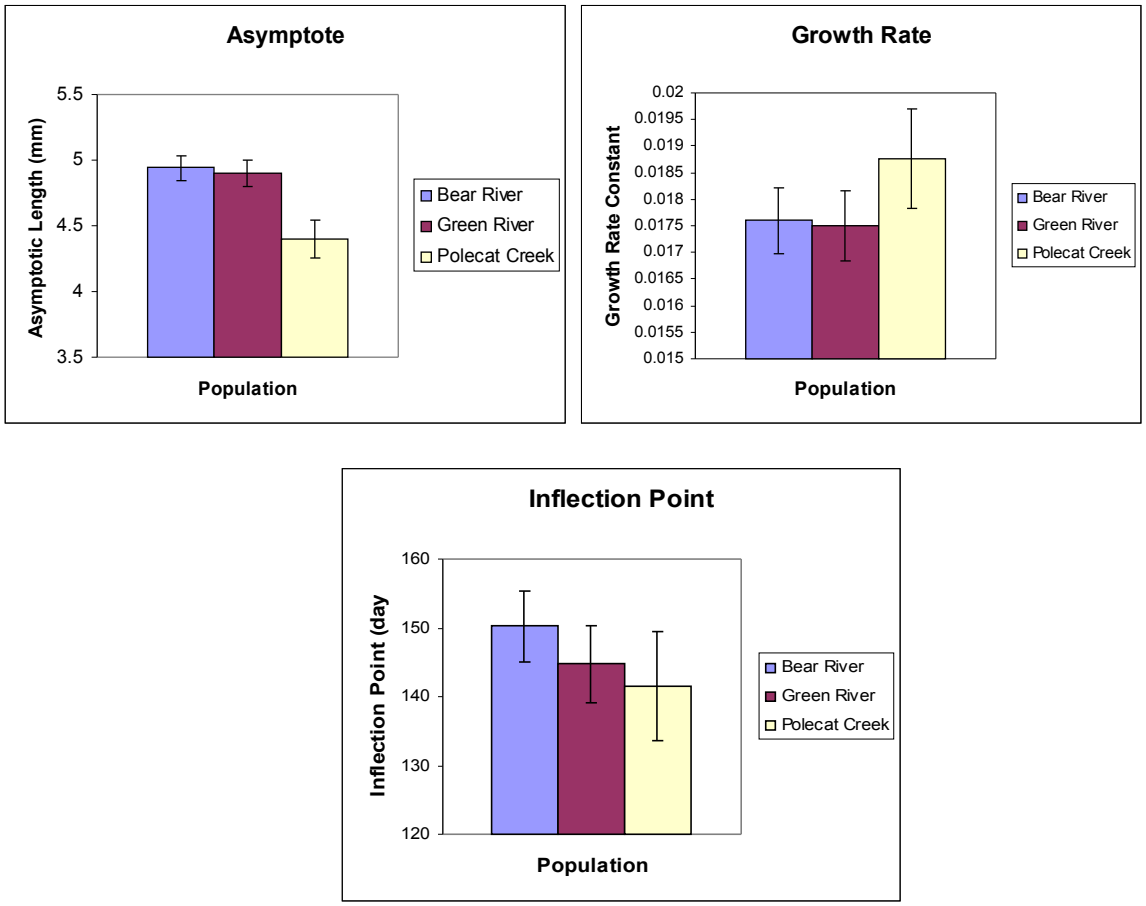

Figure 3.19 Effect of population on growth curve parameters (± se). The effect of population was significantly different for asymptotic length but not inflection point or growth rate.

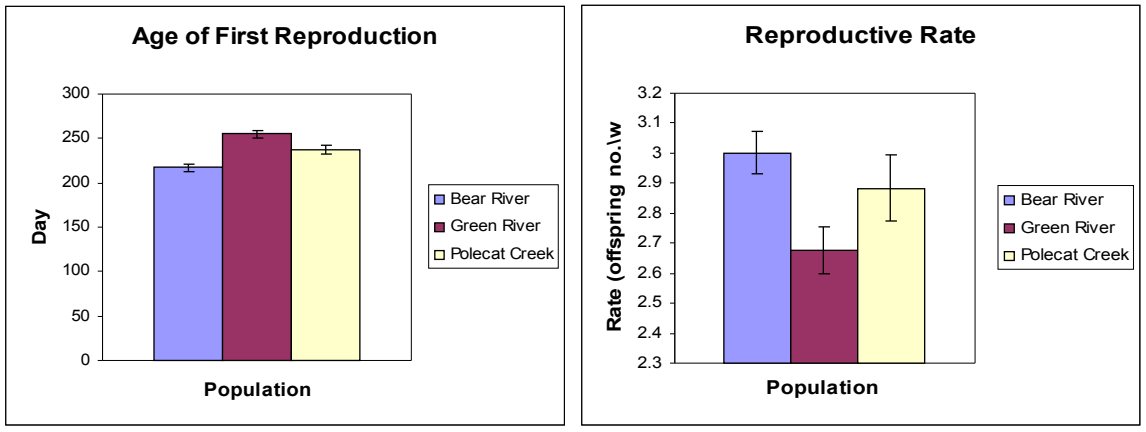

Figure 3.20 Effect of population on reproduction (± se). The effect of population was significant for each trait.

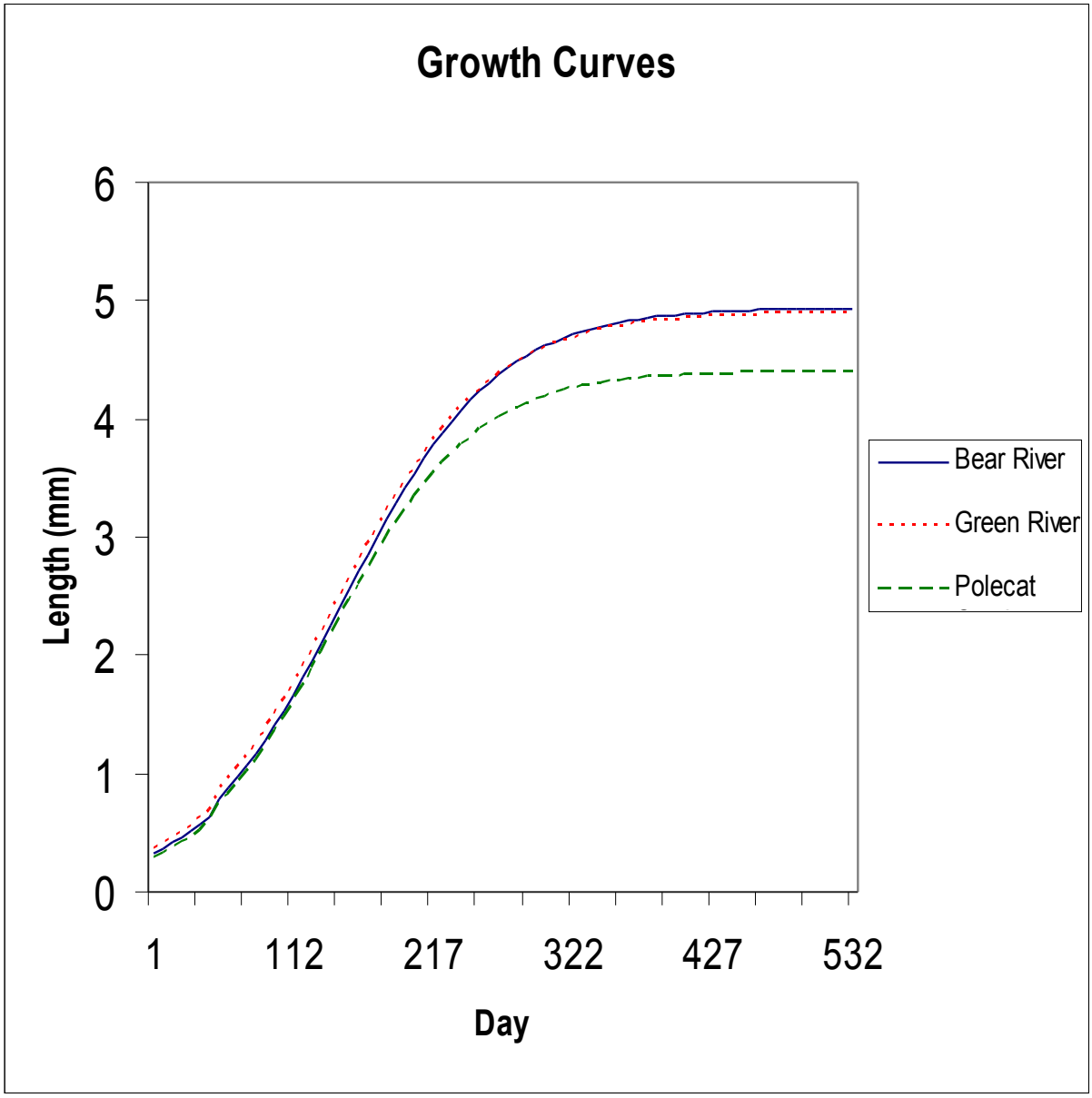

Figure 3.21 Growth curves for each population under a common garden environment.

REFERENCES

Agrawal, A. A. 2001. Phenotypic Plasticity in the Interactions and Evolution of Species. Science 294: 321- 326.

Alpert, P. and E. L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evol. Ecol. 16: 285-297.

Baker, H. G. 1965. Characteristics and modes of origin of weeds. Pages 147-168 in H. G. Baker and G. L. Stebbins, editors. The genetics of colonizing species. Academic Press, New York, New York, USA.

Barlett, M. S. 1947. Multivariate analysis. Journal of the Royal Statistical Society, Series B, 8: 176-197.

Bookstein, F. L. 1989. Principal warps: thin-plate spLineages and the decomposition of deformations. IEEE Transactions on Pattern Analysis and Machine Intelligence 11: 567-585.

Butin E., A. H. Porter, and J. Elkington. 2005. Adaptation during biological invasions and the case of Adelges tsuga. Evolutionary Ecology Research 7: 887-900.

Crispo E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. J. Evol. Biol. 21: 1460-1469.

Denny, M. W. and C. A. Blanchette. 2000. Hydrodynamics, shell shape, behavior, and survivorship in the owl limpet *Lottia gigantean*. Journal of Experimental Biology 203: 2623-2639.

Dewitt, T. J., Robinson B. W. and W. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research **2:** 129-148.

Dudley, S. A. and J. Schmitt. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens Capensis*. The American Naturalist 147(3): 445-465.

Dussart, G. J. 1987. Effects of water flow on the detachment of some aquatic pulmonate gastropods. American Malacological Bulletin 5: 65-72.

Dybdahl, M. F. and C. M. Lively. 1995. Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail (*Potamopyrgus antipodarum*). Journal of Evolutionary Biology 8: 385-398.

Dybdahl, M. F., and S. L. Kane. 2005. Adaptation vs. Phenotypic plasticity in the success of a clonal invader. Ecology 86 (6):1592-1601.

Dybdahl, M. F. 2007. Review: "Best available Biological Information document for Four Petitioned Springsnails in Idaho, Oregon, Washington and Wyoming." Unpublished
Fordyce, J. A. 2006. Review: The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. The Journal of Experimental Biology 209: 2377-2383.

Ghalambor C. K., McKay J. K., S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21: 394-407.

Hall, R. O., Dydahl M. F., and M. C. Vanerloop. 2006. Extremely high secondary production of introduced snails in rivers. Ecological Applications **16**(3): 1121-1131

Hall, R. O. and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnology and Oceanography 48:1120-1128.

Hasse, Martin. 2003. Clinal variation in shell morphology of the freshwater gastropod *Potamopyrgus antipodarum* along two hill-country streams in New Zealand. Journal of the Royal Society of New Zealand 33(2): 549-560.

Hershler, Robert. 1998. A systematic review of the hydrobidd Snails (Gastropoda: Rissooidea) of the Great Basin, Western United States. Part I. Genus Pyrgulopsis. The Veliger 41 (1): 1-132.

Hershler, R. and H. Liu. 2004. Taxonomic reappraisal of species assigned to the north American freshwater gastropod subgenus Natricola (Rissooidea: Hydrobiidae). The Veligar 47: 66-81.

Holomuzki, J. R., and B. J. F. Biggs. 2006. Habitat-specific variation and performance trade-offs in shell armature of New Zealand Mudsnails. Ecology 87(4): 1038-1047.

Ingram, K. K. 2002. Plasticity in Queen Number and Social Structure in the Invasive Argentine Ant (*Lineagepithema Humile*). Evolution 56(10): 2008-2016.

Janson, K. and P. Sundberg. 1983. Multivariate morphometric analysis of two varieties of *Littorina saxatilis* from the Swedish west coast. Mar. Biol. 32: 9-15.

Jokela, J., M. F. Dybdahl, and C. M. Lively. 1999. Habitat-specific variation in life-history traits, clonal population structure, and parasitism in a freshwater snail (*Potamopyrgus antipodarum*). Evolution 51: 1120-1129.

Kemp, P., and M. D. Bertness. 1984. Snail shapes and growth rates: Evidence for plastic shell allometry in *Littorina littorea*. Proc. Natl. Acad. Sci. USA. 81: 811-813.

Kerans B. L., M.F. Dybdahl, M. M. Gangloff, and J. E. Jannot. 2005. Macroinvertebrate assemblages and the New Zealand mud snail, a recent invader to streams of the Greater Yellowstone Ecosystem. Journal of the North American Benthological Society. 24:123-138.

Kolbe, J., R. E. Glor, L. R. Schettino, A. C. Lara, A. Larson, and J. B. Losos. 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431: 177-181.

Kreiger, M. B., and K. G. Ross. 2002. Identification of a major gene regulating complex social behavior. Science 295: 328-332.

Lee, C. E., and G. W. Gelembiuk. 2008. Evolutionary origins of invasive populations. Evolutionary Applications ISSN: 1752-4571.

Lee, C. E. , J. L. Remfert, and G. W. Gelembiuk.2003. Evolution of physiological tolerance and performance during freshwater invasion events. Integrative and Comparative Biology. 43:439-449.

Lee C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology and Evolution 117(8): 386-391.

Legar, E. A., and K. J. Rice. 2003. Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. Ecology Letters 6: 257-264.

Levri, E. P., J. Dillard, and T. Martin. 2005. Trematode infection correlates with shell shape and defence morphology in a freshwater snail. Parasitology 130: 699-708.

Lushai, G., H. D. Loxdale, and J. A. Allen. 2003. The dynamic clonal genome and its adaptive potential. Biological Journal of the Linnean Society 79:193-208.

Lynch, M. 1984. Destabilizing hybridization, general purpose genotypes and geographic parethnogenesis. Quarterly Review of Biology 59:257-290.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic Invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10: 689-710.

McDowell, S. and C. Lee. (2002). Photosynthetic characteristics of invasive and non-invasive species of Rubus (Rosaceae). American Journal of Botany 89: 1431-1438.

Minitab Version 15, Minitab Inc., State College, Pennsylvania, USA.

Muller-Scharer H., U. Schaffner, and T. Steinger. 2004. Evolution in invasive plants: implications for biological control. Trends in Ecology and Evolution 19: 417-422.

Negovetic, S., and J. Jokela. Life history variation, phenotypic plasticity, and subpopulation structure in a freshwater snail. Ecology 82(10): 2805-2815.

Papadopoulos L. N., J. A. Todd, and E. Michel. 2004. Adulthood and phylogenetic analysis in gastropods: character recognition and coding in shells of Lavigeria (Cerithiodea, Thiaridae) from Lake Tanganyika. Zoological Journal of Linnean Society 140: 223-240.

Parker, I. M., J. Rodriguez, and M. E. Loik. 2003. An Evolutionary Approach to Understanding the biology of Invasions: Local Adaptation and General-Purpose Genotypes in the Weed Verbascum thapsus. Conservation Biology 17: 59-72.

Pigliucci, M. 2001. Phenotypic Plasticity: Beyond Nature and Nurture, Johns Hopkins University Press.

Prentis P. J., J.R.U. Wilson, E. E. Dormontt, D. M. Richardson, and A. J. Lowe. 2008. Adaptive evolution in invasive species. Trends in Plant Science 13(6): 288-294.

Reznick, D. N. and C. K. Ghalambor. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112-113: 183-198.

Richards, C. L., Bossdorf, O., Muth, N. Z., Gurevitch, J. and M. Pigliucci. 2006.Jack of all Trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters **9**: 981-993.

Rohlf, F. J. 1997. TPSDIG. for Windows Version 2. Department of Ecology and Evolution, State University of New York, Stony Book (available by ftp from life.Bio.SunnySB.edu/MORPHMET).

Rohlf, F. J. 2000. TpsRegr. Version 1.26. Department of Ecology and Evolution, State University of New York, Stony Brook (available by ftp from life.Bio.SunnySB.edu/MORPHMET).

Rolan-Alvarez E., K. Johannesson, and J. Erlandsson. 1997. Maintenance of a cline in the marine snail Littorina Saxatilis: the role of home site advantage and hybrid fitness. Evolution 51(6): 1838-1847.

SAS Version 9.1, SAS Institute, Cary, North Carolina, USA.

Schweitzer J. A. and K. C. Larson. 1999. Greater morphological plasticity of exotic honeysuckle species may make them better invaders than native species. Journal of the Torrey Botanical Society 126(1): 15-23.

Sheets, D. H. 2004. IMP software. Dept. of Physics, Canisius College, Buffalo, NY, 14208, Dept. of Geology, SUNY at Buffalo, Buffalo NY 14260 (available from http://www.canisius.edu/~sheets/morphosoft.htm)

Smith, C. S., W. M. Lonsdale, and J. Fortune. 1999. When to ignore advice: invasion predictions and decision theory. Biological Invasions 1: 89-96.

Statzner B. and Holm T. F. 1989. Morphological adaptation of shape to flow: microcurrents around lotic macroinvertebrates with known Reynolds numbers at quasi-natural flow condtion. Oecologia 78: 145-157.

Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience 7: 436-445.

Struhsaker J. W. 1968. Selection mechanisms associated with intraspecific snail variation in *Littorina picta* (Prosobranchia: Mesgastropoda). Evolution 22: 459-480.

Talor, D. W. 1985. Evolution of Freshwater drainages and mollusks in Western North America. In. C.J. Smiley (ed.), Late Cenozoic History of the Pacific Northwest. American Association for the Advancement of Science, Pacific Division: San Francisco, CA. 265-321pp.

Trussell, G. C. and D. L. Smith. 2000. Induced defenses in response to an invading crab predator: An explanation of historical and geographic phenotypic change. P. N. A. S. 97(5): 2123-2127.

U. S. Fish and Wildlife Service. 2005. Draft best available information for four petitioned springsnails in Idaho, Oregon, Washington, and Wyoming. Snake River Fish and Wildlife Office. Boise, Idaho. 1-82pp.

U. S. Fish and Wildlife Service. 2007. Service removes Idaho Springsnail from the Endangered Species List. Mountain-Prairie Region Office. Lakewood, Colorado.

Vanicek, C. D. 1970. Distribution of Green River Fish in Utah and Colorado Following Closure of Flaming Gorge Dam. The Southwestern Naturalist 14 (3): 297-315.

Vermeij, G. J. 1995. A Natural History of Shells. Princeton University Press, Princeton, N. J. 232pp.

Vrijenhoek, R. C. 1998. Animal clones and diversity. BioScience 48:617-628.

Wares, J. P., A. R. Hughes, and R. K. Grosberg. 2005. Mechanisms that drive evolutionary change: Insights from species introductions and invasions. Pages 201-228 *in* Sax, D. F., S. D. Gaines, and J. J. Stachowicz, eds. *Species invasions: Insights into ecology, evolution, and biogeography*. Sinauer Associates, Inc. Sunderland, Massachusetts.

Young, K. A., J. Snooeks and O. Seehausen.2009. Morphological Diversity and the Roles of Contingency, Chance, and Determinism in African Cichlid Radiations. PLoS ONE 4(3): e4740.

Yeh P. J. and T. D. Price. 2004. Adaptive plasticity and the successful colonization of a novel environment. The American Naturalist 164(4): 531-542.

Zelditch M. L., Swiderski, Sheets H. D. and W. L. Fink. 2004. Geometric Morphometrics for Biologists: A Primer. Elseiver Academic Press, New York, N. Y. 437.