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An Investigation of Postzygotic Reproductive Isolation and Phenotypic Divergence in the Bark Beetle *Dendroctonus Ponderosae*

Ryan R. Bracewell
Utah State University

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AN INVESTIGATION OF POSTZYGOTIC REPRODUCTIVE ISOLATION AND
PHENOTYPIC DIVERGENCE IN THE BARK BEETLE

DENDROCTONUS PONDEROSAE

by

Ryan R. Bracewell

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

of

MASTER OF SCIENCE

in

Ecology

Approved:

Dr. Karen E. Mock
Major Professor

Dr. Michael E. Pfrender
Committee Member

Dr. Barbara J. Bentz
Committee Member

Dr. Byron R. Burnham
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2009

ABSTRACT

An Investigation of Postzygotic Reproductive Isolation and Phenotypic
Divergence in the Bark Beetle *Dendroctonus ponderosae*

by

Ryan R. Bracewell, Master of Science

Utah State University, 2009

Major Professor: Dr. Karen E. Mock
Department: Wildland Resources

Understanding reproductive isolation and divergence is the focus of speciation research. Recent evidence suggested that some *Dendroctonus ponderosae* populations produced hybrids with reproductive incompatibilities, a reproductive boundary undetected by phylogeographic analyses using molecular markers. Additionally, the unique bifurcated distribution of *D. ponderosae* and the proposed isolation-by-distance gene flow pattern around the Great Basin Desert provided a unique opportunity to investigate the evolution of postmating (postzygotic) isolation while also understanding phenotypic divergence along latitudinal (climatic) gradients. First, I characterized the strength, biological pattern, and geographic pattern of postzygotic isolation in *D. ponderosae* by crossing increasingly divergent populations in a common garden environment. There was little evidence of hybrid inviability in these crosses, yet geographically distant crosses produced sterile males, consistent with expectations under Haldane's rule. Hybrid male sterility appeared at a threshold among increasingly

divergent populations, was bidirectional (reciprocal crosses were affected), and less geographically distant crosses did not show significant gender-specific decreases in fitness. Second, a separate investigation of two critical phenotypic traits (body size and development time) was conducted on intrapopulation F_2 generation offspring from a common garden experiment. Genetic differences contributing to phenotypic variance were interpreted within the context of the previously described reproductive incompatibilities, gene flow patterns, and latitudinal gradients. Genetic differences in development time were striking between faster developing and more synchronized northern populations and slower developing, less synchronized southern populations. Differences in development time were not detected between populations at similar latitudes. Body size, although more variable than developmental time, generally conformed to expectations, with northern populations being smaller than southern populations. Average adult size was found to be quite different between many populations and did vary between populations at similar latitudes, yet relative sexual size dimorphism was rather consistent. There was no evidence of correspondence between phenotypic traits (body size and development time) and either reproductive boundaries or gene flow patterns. The results suggest that latitudinally imposed climatic differences are likely driving phenotypic divergence between populations.

(89 pages)

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CHAPTER 1

INTRODUCTION

Most organisms fall into distinct biological groups which we define as species, and understanding why these exist and how they form is the focus of speciation research (Coyne and Orr 2004). In accepting the biological species concept (Dobzhansky 1935; Mayr 1942) as the most widely agreed upon definition of a species, which simply states, “Species are groups of interbreeding natural populations that are reproductively isolated from other such groups” (Mayr 1995), it is clear that speciation research attempts to determine *how* groups become reproductively isolated (Coyne and Orr 2004).

Because of the long time scales over which species typically form, understanding the evolution of reproductive isolation is difficult since it typically can not be observed from start to finish. Therefore, our understanding of speciation comes from studies that are conducted on recently diverged sibling species (e.g., Naisbit et al. 2002; Ramsey et al. 2003; Reed and Markow 2004) within species complexes (e.g., Zeng and Singh 1993) or across divergent host races of a single species (e.g., Feder et al. 1994; Via 1999; Via et al. 2000). The premating (prezygotic) and/or postmating (postzygotic) barriers identified, the underlying genetic mechanisms investigated, and the potential cause of reduced gene flow determined. All of this information can then be used to infer what might have lead to, or could lead to, reproductively isolated groups. Unfortunately, there are difficulties in both retrospective and prospective approaches. Diverging populations within a species are not guaranteed to become separate species, and species that are currently separate have likely amassed multiple

pre-mating and post-mating barriers thereby obscuring past evolutionary history (Coyne and Orr 2004). Therefore, there is continued interest in identifying incipient speciation events (i.e., populations with reproductive barriers that have arisen recently) so that critical information regarding the initial barriers that restrict gene flow and the initial genetic mechanisms causing isolation can be identified (Noor and Feder 2006).

Dendroctonus ponderosae Hopkins (Coleoptera: Curculionidae, Scolytinae), also known by its common name, the mountain pine beetle, is a native insect that is broadly distributed and found throughout many western North American coniferous forests (Wood 1982). A recent study investigating the heritability of body size and development time fortuitously crossed two geographically distinct populations (southern California and central Idaho) and uncovered an apparent postzygotic reproductive barrier in which the resulting hybrid offspring were unfit and largely incapable of reproduction (Bentz et al. unpublished). Interestingly, a concurrent phylogeographic analysis (utilizing geographically similar populations as Bentz et al. unpublished) did not detect this isolation and described gene flow occurring in an isolation-by-distance pattern bounding the Great Basin Desert in a horseshoe shape (Mock et al. 2007). The results from Bentz et al. (unpublished) were quite unexpected and suggested an incipient speciation event given the lack of distinct population genetic structure consistent with reproductive isolation (Mock et al. 2007). Furthermore, preliminary evidence suggested that it was the hybrid males that were effectively sterile in the population crosses (Bentz et al. unpublished) in which case the incompatibilities conformed to one of the best known and earliest forms of postzygotic isolation, known as Haldane's rule (Haldane 1922).

Haldane's rule was originally described from a literature review of crosses between animal species including mostly mammals, birds, and Lepidoptera (Haldane 1922; Laurie 1997). Haldane (1922) found that "When in the F₁ offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex" (Haldane 1922). Hybrid sterility conforming to Haldane's rule is widely considered the first postzygotic isolating barrier to emerge between diverging taxa (Coyne and Orr 1989, 1997) and evidence of its occurrence is widespread (Laurie 1997). In most bark beetles, and *D. ponderosae* in particular, males are known to be the heterogametic sex (Lanier and Wood 1968; Lanier 1981).

Dendroctonus ponderosae was initially described as two separate species (*D. ponderosae* and *D. monticolae*, Hopkins 1909) yet the hybrid breakdown observed in Bentz et al. (unpublished) was not consistent with the previously described species boundary (Hopkins 1909; Hay 1956). Studies establishing the current synonymized status involved extensive population crossing experiments encompassing most of *D. ponderosae*'s range (Hay 1956; Lanier and Wood 1968). However, an effective analysis of fertility of both offspring sexes from many population hybrids was not undertaken (Hay 1956; Lanier and Wood 1968). Furthermore, few broad scale investigations of multiple phenotypic traits have been undertaken which could highlight divergence and isolation within *D. ponderosae* (Bentz et al. 2001).

In considering *D. ponderosae*'s bifurcated distribution along latitudinal (climatic) gradients (Wood 1982), the proposed gene flow patterns (Mock et al. 2007), and potential reproductive isolation (Bentz et al. unpublished) an extremely unique system emerges in which to also study phenotypic divergence. Insect body size and

development time are known to vary in latitudinally distributed species (Mousseau and Roff 1989; Blanckenhorn and Fairbairn 1995; Mousseau 1997) and more northern populations of *D. ponderosae* have been found to be genetically smaller with faster development (Bentz et al. 2001). It is unclear if genetic differences in body size and development time occur repeatedly within *D. ponderosae* along the two latitudinal gradients, if any differences are associated with reproductively isolated populations, or if phenotypic divergence relates to gene flow and genetic divergence estimates utilizing neutral molecular markers (Mock et al. 2007).

A great deal is known about *D. ponderosae* distribution (Wood 1982), life history (reviewed in Amman and Cole 1983), and phylogeography (Mock et al. 2007), all of which provide an extensive knowledge base for research. Although *D. ponderosae* is by most standards not considered a “model” organism in the sense of, e.g., *Drosophila*, the quantity of studies that have been conducted because of its economic importance far exceeds most other insects and propels *D. ponderosae* into an emerging model system. However, it is also because *D. ponderosae* is not a traditional model organism that the research conducted herein is unique. Intense investigation into speciation and the evolution of postzygotic isolation has been undertaken across entire groups (*Drosophila*: Coyne and Orr 1989, 1997; Lepidoptera: Presgraves 2002) and in well known species (e.g., Jiggins et al. 2001; Christianson et al. 2005; Kopp and Frank 2005; Demuth and Wade 2007; Good et al. 2008) and these studies have provided invaluable insight. However, it is not known whether the conclusions drawn are representative of the processes driving diversification and species formation in the vast majority of other organisms.

The overarching goal of my thesis research was to investigate and characterize postzygotic isolation and phenotypic divergence in *D. ponderosae*. Specifically, my objectives include confirming the postzygotic reproductive isolation observed by Bentz et al. (unpublished) and determining whether postzygotic isolation is also present between southern California populations and other populations. I also set out to determine whether there is increasing postzygotic isolation between increasingly divergent populations or if isolation occurs at a threshold within the range of *D. ponderosae*. Additionally, I tested whether *Wolbachia* bacteria (a known postzygotic isolating mechanism in insects (Werren 1997; Stouthmaer et al. 1999) are detectable and potentially contributing to reproductive incompatibilities.

In characterizing phenotypic divergence within *D. ponderosae*, my objectives were to investigate body size (a sexually dimorphic trait) and development time across the widely distributed populations used to investigate postzygotic isolation. I set out to determine if genetic differences would be consistent with latitudinal adaptations, whereby southern U.S. populations would, on average, be larger and have slower development times than populations from more northern latitudes. Further, I wanted to determine if any detected differences might coincide with reproductive boundaries (Bentz et al. unpublished) and genetic divergence and gene flow patterns described from the recent phylogeographic analysis (Mock et al. 2007).

In total, these findings help improve our understanding of *D. ponderosae* reproductive isolation and phenotypic divergence. Not only is this information critical to bark beetle researchers who work relentlessly to understand this important insect but

also provides critical insight into the earliest stages of postzygotic isolation and species formation.

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CHAPTER 2

THE EVOLUTION OF POSTZYGOTIC ISOLATION AND HYBRID MALE
STERILITY IN *DENDROCTONUS PONDEROSAE***Abstract**

To study the evolution of reproductive isolation is to study speciation, and a clear understanding of the earliest stages of divergence is crucial to our understanding of the speciation process. Recent evidence suggests that some *Dendroctonus ponderosae* populations produce hybrids with reproductive problems; a reproductive boundary undetected by phylogeographic analyses. Additionally, *D. ponderosae*'s unique distribution and proposed isolation-by-distance gene flow pattern provides an opportunity to investigate the evolution of postzygotic isolation. I sought to characterize the strength, biological pattern, and geographic pattern of reproductive isolation, in *D. ponderosae*. Multiple populations were crossed in a common garden environment and investigated for hybrid inviability and hybrid sterility. While there was little evidence of hybrid inviability, geographically distant crosses produced sterile males, consistent with expectations under Haldane's rule. Hybrid male sterility appeared at a threshold among increasingly divergent populations, was bidirectional (reciprocal crosses were affected) and less geographically distant crosses did not show significant sex specific decreases in the fitness variables analyzed. Furthermore, there was no evidence of unidirectional male sterility in less geographically distant crosses. It therefore appears that reproductive isolation in the form of hybrid male sterility is occurring within *D. ponderosae* and it is likely quite recent.

INTRODUCTION

One of the fundamental questions in biology is what causes a single interbreeding species to, through time, diverge into two reproductively isolated species. An integral component of speciation is the formation of barriers that impede and eliminate reproduction. These barriers can be classified as prezygotic (e.g., spatial, temporal, and behavioral isolation) or postzygotic (e.g., hybrid inviability and hybrid sterility) (Dobzhansky 1951; Coyne and Orr 2004) and identifying these barriers and determining their strength is crucial to our understanding of species formation. However, an ongoing difficulty in speciation research is the identification of the initial barrier(s) facilitating divergence, since multiple barriers can accumulate and be replaced during the complete speciation process (Ramsey et al. 2003; Coyne and Orr 2004). The barrier(s) that initiate divergence and trigger species formation could indeed be quite different from the barriers that exist between the end products of the speciation process.

Postzygotic isolating barriers in particular have received considerable attention, likely because postzygotic isolation is largely considered irreversible. Comparative meta-analyses have characterized the evolution of postzygotic isolation in several groups (Frogs: Sasa et al. 1998; Birds: Price and Bouvier 2002), including two studies specifically in insects (*Drosophila*: Coyne and Orr 1989, 1997; Lepidoptera: Presgraves 2002). In insects, incompatibilities tend to gradually accumulate over long periods of time (i.e., hundreds of thousands to millions of years), progressing from hybrid sterility to hybrid inviability between increasingly genetically divergent taxa (Coyne and Orr 1989, 1997; Presgraves 2002). Coyne and Orr (1989, 1997) and Presgraves (2002) also

provide overwhelming support for one of the most established rules in evolutionary biology, Haldane's rule (Haldane 1922), which states that "When in the F1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous [heterogametic] sex" (i.e., the male in XY taxa (*Drosophila*) and the female in ZW taxa (Lepidoptera)). Although multiple genetic mechanisms have been suggested to contribute to Haldane's rule (reviewed in Laurie 1997; Coyne and Orr 2004), hybrid sterility of the heterogametic sex is widely considered the first postzygotic barrier to form in nascent species (Coyne and Orr 2004).

Hybrid male sterility does appear to be the first postzygotic barrier to form, but it has also been found to be polygenic and epistatically complex in animals (Davis and Wu 1996; Orr and Irving 2001; Good et al. 2008) and is most often observed in taxa that have previously been recognized as separate species or subspecies (Laurie 1997). For example, one of the best studied cases of hybrid male sterility occurs in crosses between two lineages, recognized as species, (*Drosophila pseudobscura* and *D. persimilis*) which are thought to have diverged ~ 1 mya (Wang and Hey 1996). Therefore, although hybrid male sterility is typically the first postzygotic barrier to arise, selection and drift have been operating in different ways on multiple traits for long periods of time, to the point that different biological lineages (i.e., species, subspecies, etc.) are clearly discernable. Because of the clear differentiation of most organisms prior to the expression of hybrid sterility, some argue that most postzygotic isolation likely arises well after the prezygotic barriers that initiated divergence (Mallet 2006). Additionally, most studies of hybrid sterility are disconnected from ecological

processes that might lead to speciation since the study organisms inhabit different environments, and are often allopatric (Coyne and Orr 2004).

Recent evidence in a non-model species of a broadly distributed phytophagous insect, the mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), suggests that postzygotic isolation could be quite rapid and precede prezygotic barriers. *Dendroctonus ponderosae* is a native bark beetle in western North American forests that feeds and reproduces in the phloem layer of 11 species of pine (Wood 1982). It has a widespread distribution (Figure 2-1) and is a species of great interest because outbreaks are often landscape level events, causing considerable tree mortality (Cole and Amman 1980; Westfall and Ebata 2007). In a recent study, severe hybrid breakdown was observed, with little to no offspring production, in crosses between mountain pine beetle populations from southern California and central Idaho (Bentz et al. unpublished). This study, however, was not designed to elucidate the specific hybrid sex(s) affected and was geographically limited. Additionally, infection with *Wolbachia*, a bacterial manipulator of insect reproduction (Werren 1997; Stouthmaer et al. 1999) that has been implicated in rapid postzygotic isolation in insects (Hoffmann et al. 1986; Turelli and Hoffmann 1991) and has never been assessed in this species. The findings of severe hybrid breakdown were quite unexpected given previous crossing experiments and karyological studies (Hay 1956; Lanier and Wood 1968) suggesting that geographically distinct *D. ponderosae* comprise a single species. Moreover, a recent rangewide phylogeographic analysis of *D. ponderosae* failed to detect pronounced genetic divergence between populations from these same areas in California and Idaho, using both nuclear and mitochondrial markers

(Mock et al. 2007). This was all the more odd given that even *Wolbachia* infections have been shown to leave a molecular genetic signal suggesting reproductive isolation (Reordhanz and Levine 2007).

Joint consideration of the findings of Bentz et al. (unpublished) and Mock et al. (2007) leaves us with a paradox: there appears to be severe hybrid breakdown occurring in some interpopulation crosses, yet neutral molecular markers failed to detect divergence or a spatially abrupt decrease in gene flow between these same populations. Mock et al. (2007) did find clinal variation in gene flow between populations from southern California and Idaho following an isolation-by-distance pattern around the Great Basin and Mojave Deserts, which could reflect increasing reproductive isolation via the gradual accumulation of postzygotic incompatibilities (*sensu* Edmands 1999). Therefore, I was provided a unique opportunity to investigate postzygotic isolation within a clinally distributed species and determine whether reproductive isolation occurs gradually or at a threshold in population crosses.

I hypothesized that the hybrid breakdown observed when populations from southern California and central Idaho were crossed (Bentz et al. unpublished) was due to hybrid male sterility (conforming to Haldane's rule) and that I would find a positive clinal relationship between the degree of postzygotic isolation and the geographic divergence between populations. Specifically, the objectives of this study were to (1) confirm the postzygotic reproductive isolation observed by Bentz et al. (unpublished) between populations of *D. ponderosae* in southern California and central Idaho, (2) determine whether postzygotic isolation is also present between southern California populations and other populations, (3) determine whether postzygotic isolation

increases in a linear fashion between increasingly divergent populations or if it occurs at a threshold within the range of *D. ponderosae* and (4) determine whether *Wolbachia* bacteria are detectable and a potential mechanism contributing to reproductive isolation.

MATERIALS AND METHODS

Study organism: Because of the economic impact of *Dendroctonus ponderosae*, a great deal of research has been undertaken to understand its reproduction (Reid 1958, 1962a, 1962b; Amman 1972). A male/female pair constructs a tunnel (gallery) under the bark of a tree while traveling upward in the phloem layer, with alternating pockets of eggs deposited in niches on opposite sides of the gallery. After egg hatch, the larvae feed laterally, pupate, and emerge from underneath the bark. Laboratory rearing protocols are well established (e.g., Lanier and Wood 1968; Langor 1990; Bentz et al. 2001), and beetles are easily propagated in freshly cut tree sections. Stringent rearing in the lab produces ~ 98-99% virgin females (Reid 1958; McCambridge 1969a, 1969b) and male virginity is not required since males are capable of multiple matings (Bentz, unpublished data). Gender of adult *D. ponderosae* is easily determined using morphological differences on the 7th abdominal tergite (Lyon 1958). Generation time in a laboratory setting at 21° C varies among populations, ranging from 60-110 days depending on the geographic location of source populations (Bentz et al. 2001). Reproductive output from individual *D. ponderosae* matings can be quantified by peeling off the outer layer of bark and counting eggs and signs of egg hatch (larvae and larval mines). Intraspecific matings in the lab have shown that egg “hatchability” (larvae/egg niche) is not affected by rearing tree species (Lanier and Wood 1968).

Crossing experiments and population selection rationale: To characterize postzygotic isolation within *D. ponderosae*, a line-cross analysis was performed to assess hybrid viability and fitness among F₁ offspring. Eight populations, located around the Great Basin Desert and representing a large portion of the geographic species range, were selected for sampling (Table 2-1) (Figure 2-1). Because a pattern of increasing genetic isolation-by-distance was found to exist around the Great Basin Desert in *D. ponderosae* (Mock et al. 2007), I assumed that by selecting increasingly geographically divergent populations I would thereby select increasingly genetically divergent populations. The southernmost population, CA, was chosen as a common source population included in all crosses due its geographic isolation, genetic divergence from other populations (Mock et al. 2007), apparent reproductive incompatibility with at least one other population (ID) (Bentz et al. unpublished), and presence in an atypical host tree species (*Pinus monophylla*). A population sympatric with CA but infesting a different host tree species (*P. lambertiana*) was also sampled (CA1). All other locations were selected in an attempt to span and exceed the geographic distance between CA and ID while sampling around the proposed gene flow barrier, the Great Basin Desert (Figure 2-1).

Field collection and laboratory propagation: *Dendroctonus ponderosae* were field-collected by felling larvae-infested trees in the spring of 2007 and cutting each tree into 14-16 inch sections. In the laboratory, infested tree sections were stored at ~3° C. Once all populations were collected, tree sections were placed in rearing containers and maintained at ambient room temperature (~21° C) until development was complete. Rearing containers consisted of garbage cans with a glass collecting jar

fixed to the outside. *Dendroctonus ponderosae* exhibits positive phototaxis and after emerging into the darkness of the container they quickly migrate to the jar. The emerging adults were collected daily, placed in petri dishes lined with filter paper moistened with distilled water, and stored up to 20 days at $\sim 3^{\circ}$ C. Emerging adults were randomly selected from the peak of emergence for each population (~ 15 days of petri dishes with the most beetles), and used for crossing experiments. Individuals were selected from the peak emergence to obtain beetles with average development time characteristics and to decrease the probability of collecting re-emerging, reproductively exhausted parents.

All laboratory crosses were performed in a common garden environment ($\sim 21^{\circ}$ C, photoperiod $\sim 9L:15D$) and were achieved by placing a female, and then a male, in a pre-drilled hole in the phloem layer of fresh uninfested field-collected tree sections. Slight differences in infesting protocols were used for each generation and are described under each assay subheading. All propagation was performed in a common host tree species, lodgepole pine (*Pinus contorta* var. *latifolia*) using bolts ($\sim 16''$ tree sections) acquired from Wasatch-Cache National Forest, Utah on two separate occasions. Bolts were sealed with paraffin wax prior to beetle infesting to reduce desiccation. Placing a male and female into a pre-drilled hole in the phloem (hereafter termed a pair) does not guarantee mating, which is defined by copulation and sperm transfer. Mating was not directly determined for any pairing but assumed if offspring were produced. Female behavior in the absence of males was determined (described under Hybrid Fitness subheading) in an attempt to uncover differences that could suggest mating had

occurred in sterile crosses and establish that laboratory protocols were indeed producing unfertilized females.

Hybrid inviability assay: Hybrid inviability occurs when “hybrids suffer developmental difficulties causing full or partial lethality” (Coyne and Orr 2004), and can be accompanied by an extreme distortion in sex ratio or loss of one of the hybrid sexes, often conforming to Haldane’s rule. To assess hybrid inviability within and between populations, I conducted a) reciprocal F_1 crosses ($CA \text{ ♂} \times P_x \text{ ♀}$ and $CA \text{ ♀} \times P_x \text{ ♂}$; Figure 2-2) between the CA population and each of the remaining seven populations (P_x) and b) F_1 crosses within each population ($CA \text{ ♂} \times CA \text{ ♀}$ and $P_x \text{ ♂} \times P_x \text{ ♀}$; Figure 2-2). For each of the 14 reciprocal interpopulation F_1 crosses and 8 intrapopulation F_1 crosses, male/female pairs were manually inserted into a randomly selected bolt from one of two lodgepole pine cut just prior to the start of the assay. Ten pairs were set up in this manner for interpopulation F_1 crosses and 20 pairs for intrapopulation F_1 crosses. Larger quantities of intrapopulation F_1 crosses were performed to decrease inbreeding and ensure sufficient progeny for backcrossing experiments (see below). Growing space was standardized by spacing each male/female pair 1.2 inches from its neighbor around the circumference of each bolt. A 1 in.² portion of screen was fixed over the entrance hole to prevent immediate escape of adults. Each infested bolt was placed in a separate rearing container and maintained at ambient room temperature ($\sim 21^\circ \text{C}$).

Each bolt contained offspring from multiple pairs from a specific cross (i.e., a cohort). F_1 progeny from interpopulation (hybrid) and intrapopulation crosses were collected daily, placed in petri dishes lined with filter paper moistened with distilled water, and stored at $\sim 3^\circ \text{C}$ for further analysis. Due to the cryptic nature of *D.*

ponderosae reproduction and the need to collect offspring, mating success was determined after offspring emergence was completed, by removing the bark layer and inspecting galleries for larval mines leading to pupal chambers and adult exit holes.

Offspring sex ratio, the total number emerged adults, and the number of successful galleries were tabulated. A pair of individuals was considered able to produce viable offspring if there were ≥ 5 pupal chambers with adult emergence holes, and the cohort included both males and females. *Dendroctonus ponderosae* is known to produce female skewed sex ratios (e.g., Amman and Cole 1983; Cerezke 1995), so if males and females were produced and appeared in roughly the same ratio as the source populations, even if slightly skewed, this would suggest no inviability.

Hybrid fitness assay: Hybridization may affect progeny fitness in a variety of ways, including outbreeding vigor (heterosis), outbreeding depression, and complete hybrid sterility. Hybrid sterility may be physiological or behavioral, e.g., when “hybrids suffer problems in the development of the reproductive system or gametes” or “hybrids suffer neurological or physiological lesions that render them incapable of successful courtship” (Coyne and Orr 2004). To assess the fitness of F_1 hybrid progeny, F_2 backcrosses (crosses between F_1 progeny from interpopulation crosses and F_1 progeny from intrapopulation crosses) were performed (Figure 2-2). F_2 backcrosses allow direct assessment of sterility and reproductive fitness of F_1 hybrids.

The hybrid fitness assay was performed using 16 in. bolts from two live lodgepole pines which were collected just prior to the hybrid fitness assay. Propagation was performed as described above, except that bark strips were removed between pre-drilled holes, so that each male/female pairing was confined to one longitudinal 2.4 in.

wide strip of bark and phloem. There was a maximum of 14 different hybrid cohorts available from the reciprocal hybrid inviability assay. Hybrid cohorts (F_1 progeny from interpopulation crosses) were reciprocally backcrossed to both source populations, resulting in a total of 56 possible F_2 backcross combinations (14 hybrid cohorts x 2 sexes x 2 source populations). Each F_2 backcross combination was replicated with 10 pairings in a randomly selected bolt. Intrapopulation F_2 crosses were also performed and replicated with 20 pairings per population, in two randomly selected bolts. Additionally, 27 females were randomly selected from among the F_1 progeny and inserted singly into bolts to determine if the rearing methods were indeed producing virgin females, and for a comparison with potentially sterile pairings.

Dendroctonus ponderosae is highly fecund, and a female can easily oviposit more than 100 eggs (Amman 1972), which would be nearly impossible to count in a timely manner given the rapid desiccation of inviable eggs and the size of the experiment. Therefore, all pairs (and female only infestations) were allowed to proceed for 26 days before reproduction was halted through refrigeration. The remaining bark and phloem were then stripped from the bolt so that reproductive output could be tabulated. Any unhatched eggs within the first 15 cm of the gallery were considered inviable, based on known egg hatch and gallery extension rates at room temperature (Logan and Amman 1986; Bentz et al. 1991). For each pair, number of eggs and larvae within the first 15 cm, total gallery length, and total number of larvae in the entire gallery were recorded.

Four fitness measures were used in the analysis: number of eggs laid (15cm), proportion of viable eggs (15cm) (number of larvae/number eggs laid), total gallery

length and total egg hatch. F_2 intrapopulation progeny were analyzed separately to determine if population level variation in these fitness measures was present. Female-only infestations were found to be dramatically different than infestations using pairs and only means are reported (see Results). For F_2 hybrid backcrosses, fitness measures were standardized by dividing each observation by its midparent mean, producing a value of 1 when fitness is equal to the midparent mean, >1 when heterosis is present, and <1 when fitness is decreased (Edmands 1999). This standardization accounts for population-level differences in mean fitness that could influence cross population comparisons. All analyses were done using the standardized values, with the implicit assumption that genetic variation in fitness traits is additive.

Based on previous studies, there was an expectation that some pairings would fail to lay eggs, and/or the adults would prematurely emerge from bolts and fail to construct a gallery (Lanier and Wood 1968). Increases in the failure to lay eggs (oviposit) or failure to construct a gallery in F_2 backcrosses, compared to the F_2 intrapopulation crosses, could be interpreted as behavioral sterility (F_1 hybrids with intermediate phenotypes have courtship or communication difficulties). Oviposition and early adult emergence from a bolt were tallied as binary variables. If one or more eggs were laid, oviposition was considered successful (1), compared to no oviposition (0). Similarly, if a pair failed to construct a 15 cm gallery, gallery failure was tallied as 1 for that pair.

Significant differences in fitness measures from the F_2 backcrosses and the F_2 intrapopulation crosses were tested using GLIMMIX (SAS Institute, Cary, North Carolina, USA, version 9.1.3). GLIMMIX is an approach that models both fixed and

random effects and handles nonnormal response distributions. The proportion of viable eggs was modeled using a binomial distribution. Total egg hatch and the number of eggs laid were count data and Poisson distributed, and total gallery length was normally distributed (Gaussian distribution). Pairwise differences between F_2 intrapopulation crosses were tested using a Tukey-Kramer HSD multiple comparison test.

F_2 intrapopulation crosses were analyzed for the four fitness measures using the source population as the main fixed effect. Standardized F_2 backcross data were modeled using four factors that were treated as fixed effects, including: 1) distance from the CA population (geographic distance, measured as the cumulative linear distance in miles around the Great Basin Desert from CA, Figure 2-1), 2) CA parent (F_0) sex (reciprocal), 3) sex of the F_1 hybrid individual used in backcross (hybrid sex), and 4) source population to which the hybrid was backcrossed (backcross population). Oviposition and gallery failure were modeled using a binomial distribution. A single fixed effect, cross type (e.g., F_2 backcross or F_2 intrapopulation cross), was used in these two models.

***Wolbachia* detection:** 85 DNA extractions from five populations (17 individuals per population), were analyzed for *Wolbachia* infection using a PCR based assay. DNA extractions used in the analysis were a subset of those in Mock et al. (2007) and included populations collected from the same general areas as CA and ID used in this study, as well as populations near La Grande, OR, Flagstaff, AZ and Klamath, OR (specific localities provided in Mock et al. 2007). *Wolbachia*-specific primers that amplify the *wsp* gene were used along with a slightly modified PCR protocol from Jeyaprakash and Hoy (2000), a study that positively amplified both A and

B strains from 47 arthropod species. PCR was performed in a 50 µl volume containing 50 mM Tris (pH 9.2), 16mM ammonium sulphate, 0.25 mM of each dNTP, 1.75mM MgCl₂, 0.5 µM of both Wsp-F forward and Wsp-R reverse primers, and 1 unit of Taq polymerase. A linked cycle profile for Long PCR (Jeyaprakash and Hoy 2000) was used. *Wolbachia* positive controls included a high and low titre from *Drosophila simulans* (obtained from Christian Stauffer, Institute of Forest Entomology, Forest Pathology and Forest Protection, BOKU—University of Natural Resources) and an infected bark beetle, *Hypothenemus hampei* (Vega et al. 2002). PCR products were visualized on a 1.4% agarose gel under UV illumination with ethidium bromide. Similar methods have been used to detect infections in other bark beetles (Stauffer et al. 1997).

RESULTS

Hybrid inviability: Difficulty in rearing beetles out of field-collected trees reduced the number of pairings for AZ and CA1 F₁ intrapopulation crosses, from 20 to 10 pairs each. Additionally, the AZ population generally appeared emaciated and lethargic and displayed atypical behavior during rearing which included extensive tunneling underneath the bark by adults prior to emergence. This has been suggested to slightly increase inbreeding (McCambridge 1969a, 1969b). Therefore, due to concerns about AZ and possible mating under the bark prior to emergence, hybrid inviability results involving AZ crosses should be interpreted with care. Results of crosses with the AZ population are provided only in tables to demonstrate that it is likely that

hybrids were produced. This population was not used in the hybrid fitness assay (see below).

All interpopulation (hybrid) F₁ crosses produced hybrid male and female offspring (Table 2-2). The male:female sex ratio of each of the F₁ hybrid cohorts varied from 1:0.85 to 1:2.57, and were nearly entirely within the range of F₁ intrapopulation crosses (1:0.85 to 1:2.33) when excluding AZ. The overall percentage of fertile pairs was very high for all F₁ intrapopulation crosses ($\geq 95\%$) except AZ. Most F₁ hybrid crosses also showed high fertility ($\geq 90\%$ in 11 of 12 F₁ hybrid crosses). In the CA ♀ x OR ♂ F₁ cross, 80% of pairs produced offspring, while only 30% of pairs in the AZ ♀ x CA ♂ cross produced offspring (Table 2-2). Of the few F₁ crosses that did not produce offspring (both intrapopulation and interpopulation), I observed that it was usually due to the premature death of one of the pair.

Hybrid fitness: *behavioral sterility*: All F₁ crosses (intrapopulation and interpopulation) produced ample quantities of progeny, therefore, a total of 480 F₂ backcross pairings and 140 F₂ intrapopulation pairings were conducted in the hybrid fitness analysis. A total of 66 F₂ backcross pairings (14%) failed to reach the 15 cm gallery mark and 63 (13%) failed to oviposit. These percentages were similar to that observed in the F₂ intrapopulation pairings (23/140, ~16%, and 14/140, ~10%, respectively). Cross type (i.e., F₂ backcross or F₂ intrapopulation cross) was not a significant predictor of oviposition (df=1, 618, F=0.97, P=0.3258) or the failure to produce a >15 cm gallery (df=1, 618, F=0.63, P=0.4273). These results suggest that there was no increased number of failed galleries or lack of oviposition in F₂ backcrosses, relative to results from F₂ intrapopulation crosses. Such behavior in some

pairs is probably influenced by factors outside the control of this experiment.

Subsequent analyses were conducted only on pairs with egg deposition, regardless of gallery length.

Hybrid fitness: F_2 intrapopulation crosses: Significant differences among F_2 intrapopulation crosses were observed in the four fitness characteristics measured (Table 2-3). The proportion of viable eggs was significantly different among F_2 intrapopulation crosses ($df=6, 119, F=32.33, P<.0001$), with proportions ranging from 0.50 in CA to 0.91 in ID (Table 2-3). Significant differences among F_2 intrapopulation crosses were also detected in the number of eggs laid ($df=6, 119, F=5.63, P<.0001$), total gallery length ($df=6, 119, F=6.54, P<.0001$) and total egg hatch ($df=6, 119, F=39.57, P<.0001$) (Table 2-3). Tukey-Kramer probabilities for pairwise comparisons between populations are summarized in Table 2-4. Differences across populations grouped by geographic location or prior host use were not apparent. Comparisons between the sympatric CA and CA1 populations from different host tree species were significantly different in only one of four fitness measures; proportion of viable eggs (Table 2-4).

Hybrid fitness: *females only*: Of the 27 females randomly selected from F_1 progeny and introduced into bolts, 9 females laid eggs. The average number of eggs laid was low (mean = 1.5 ± 0.76), and of those, no egg hatch was observed. Total gallery length was also low (mean = 12.44 ± 1.27) and it was observed that most unmated females emerged prematurely from the bolt. Only 3 of 27 females mined >15 cm of gallery.

Hybrid fitness: backcrosses, fitness measure proportion of viable eggs:

Geographic distance from CA ($P=0.0101$), hybrid sex ($P=0.0183$), backcross population ($P=0.0271$) and the interaction of geographic distance x hybrid sex ($P=0.0016$), were significant in explaining differences in proportion of viable eggs among F_2 backcrosses (Table 2-5). Backcrosses that utilized hybrid males from the most geographically distant crosses (CA x ID and CA x UT) resulted in low proportion of viable eggs (Figure 2-3). Backcrosses using hybrid females did not show a decrease in proportion viable eggs, and were either above or similar to the midparent means (Figure 2-3). The three way interaction, geographic distance x hybrid sex x reciprocal, was not significant (Table 2-5), suggesting that low egg hatch in F_2 backcrosses utilizing hybrid males from the most distant crosses was not directional (i.e., both CA mothers and CA fathers produce sterile individuals in crosses with ID and UT).

Fitness measure total egg hatch: Geographic distance from CA ($P<.0001$), hybrid sex ($P=0.0213$), and the interaction of geographic distance x hybrid sex ($P<.0001$) were significant in explaining differences in total egg hatch among F_2 backcrosses (Table 2-6). An increase in total egg hatch in F_2 backcrosses using hybrid males from the geographically proximal CA2 population suggests some heterosis (Figure 2-4). However, total egg hatch in F_2 backcrosses using hybrid males declined as geographic distance from CA increased, with almost no egg hatch when the two most geographically distant populations were crossed (Figure 2-4). Total egg hatch from F_2 backcrosses using hybrid females were similar to the midparent mean in all crosses except ID, where a 40% increase was observed.

Fitness measure eggs laid: No significant differences among F₂ backcrosses in number of eggs laid were found for any main effect or all possible interactions, although the interaction of geographic distance x hybrid sex approached significance (P=0.0508, Table 2-5). The lowest numbers of eggs laid were observed in F₂ backcrosses that utilized hybrid males from the most distant crosses (CA x ID, CA x UT) (Figure 2-5); the same crosses identified as showing low egg viability and highly reduced total egg hatch.

Fitness measure total gallery length: Multiple main effects and their interactions were significant in explaining differences in total gallery length, including the interaction of geographic distance x hybrid sex (Table 2-6). F₂ backcrosses using F₁ hybrid males from more geographically distant crosses (CA x OR, CA x ID, CA x UT) resulted in reduced total gallery length relative to midparent means (Figure 2-6).

Hybrid males from the two most distant crosses, (CA x ID and CA x UT), showed a drastic decrease in fertility, when considering both egg viability and total egg hatch (Table 2-7). Only 6 of 72 pairs had any egg hatch in contrast to female hybrids from those same crosses that were found to have high fertility rates (63 of 66) (Table 2-7). I consider this evidence of incomplete sterility, since most F₂ backcrosses utilizing hybrid males had at least one pair with egg hatch (Table 2-7). There is some evidence of increasing severity and near complete sterility in one direction of the cross; hybrid males from CA mothers had only 1 of 34 galleries with egg hatch (with merely 3 eggs that hatched), while hybrid males from CA fathers had 5 of 38 galleries with egg hatch. This was not significant in the models for total egg hatch or proportion of viable eggs (geographic distance x reciprocal x hybrid sex, Tables 2-5 and 2-6) but might be

difficult to detect given the low numbers of fertile pairings. F₂ backcrosses utilizing hybrid males from CA x ID and CA x UT crosses did produce galleries of substantial length (unstandardized mean = 36.28 ± 1.45), far greater than what was seen in female-only infestations (mean = 12.44 ± 1.27), yet still significantly shorter than the backcrosses utilizing the female hybrids (unstandardized mean = 41.09 ± 1.51) (df=1, 136, F=4.88, P=0.0288).

My results suggest hybrid male sterility occurs at a threshold within the *D. ponderosae* populations used in this study, rather than in a linear fashion between increasingly divergent populations (Figure 2-7). However, hybrid males and females from many population crosses appear to follow different fitness trajectories in F₂ backcrosses and fluctuated markedly at some intermediate distances (e.g., Figure 2-4). In an attempt to determine whether there were any significant effects on hybrid fitness in population crosses that were not producing sterile males, post-hoc analyses of total egg hatch and the proportion of viable eggs in F₂ backcrosses were undertaken, excluding all ID and UT hybrids. Only geographic distance approached significance in this analysis (P=0.0503) (Table 2-8). However, the geographic distance x hybrid sex interaction was not significant, suggesting that sex of the hybrid did not significantly influence the relationship (Table 2-8). No main or interactive effects were significant in explaining differences in the proportion of viable eggs. These results suggest there is no increase in the number of sterile individuals produced when crossing increasingly distant populations, (when excluding ID and UT), nor a significant sex-specific decrease in the proportion of viable eggs or total egg hatch in backcrosses utilizing increasingly geographically divergent hybrids.

Wolbachia: *Wolbachia* was detected in 2 of the 85 beetle samples tested (2.3%). One individual was from La Grande, OR, and the other was from Klamath, OR. There were no detections among individuals from CA and ID. All positive controls amplified and showed bands, although the *D. simulans* low titre was usually quite faint. All positives were consistent with the expected ~0.6-kb fragment. Negative controls did not amplify.

DISCUSSION

Postzygotic isolation: I present evidence that hybrid inviability, the more advanced stage of postzygotic isolation, is not present within the *D. ponderosae* populations analyzed. My results are consistent with the multiple crossing studies utilizing various populations from throughout this species range that have all successfully produced male and female hybrids (Hay 1956; Lanier and Wood 1968; Bentz et al. unpublished). Furthermore, all of the reciprocal population crosses produced both male and female offspring in ratios similar to source populations and published estimates (Amman and Cole 1983; Cerezke 1995).

I did, however, find evidence of extremely reduced egg hatch conforming to Haldane's rule in F₂ crosses using reciprocal CA x ID hybrids and CA x UT hybrids (Tables 2-5, 2-6, and 2-7). I interpret this egg hatch reduction as being due to hybrid male sterility. My results suggest that this hybrid male sterility is incomplete since some backcrosses using hybrid males from CA x ID and CA x UT were capable of producing offspring (Table 2-7). Intraspecific variation in the degree of male sterility is

not uncommon, as has been observed in multiple species of *Drosophila* (Reed and Markow 2004; Kopp and Frank 2005) and is thought to appear primarily in incipient species.

Hybrid male sterility did not appear to be associated with host tree differences. Although host and geographic location are confounded in this experiment, because all crosses were with the CA population from *Pinus monophylla*, the CA population readily produced fertile hybrids of both sexes with a sympatric population from *Pinus lambertiana*, as well as with 3 of the 5 populations from *Pinus contorta* (CA2, CA3, OR). Moreover, previous molecular genetic comparisons found no differentiation between subsamples of California populations from different host species (*P. contorta*, *P. lambertiana*) (Mock et al. 2007). One could speculate that although all crosses were made only with CA, the results might have been similar if a sympatric population in *P. contorta* had been used in all crosses.

Mating was not directly observed in sterile backcrosses, although it is suspected to have occurred. Matings using sterile hybrid males did result in fewer eggs laid and also a significant reduction in total gallery length when compared to fertile females from those same crosses (Table 2-7). However, females inserted without a male exhibited a far more drastic reduction in gallery length, laid almost no eggs and were observed to emerge prematurely from bolts. I also found that the cross type (F₂ backcross or F₂ intrapopulation cross) was not a significant predictor of short galleries or a lack of oviposition even though many backcrosses included sterile males. Similar studies investigating *D. ponderosae* and its sympatric sibling species *D. jeffreyi* conclusively demonstrated that even these two separate species mate under laboratory

conditions and show sperm transfer, gallery construction, and egg laying, yet exhibit the more advanced stage of postzygotic isolation and produced inviable hybrids (Lanier and Wood 1968).

My results also demonstrated significant differences in several fitness measures among F_2 intrapopulation crosses. Interestingly, a pattern to these differences was not evident when considering prior host use or geographic proximity of certain populations (Tables 2-3 and 2-4). In general, my results are largely consistent with previous studies within *D. ponderosae* that have detected differences in multiple life-history traits among populations (Bentz and Mullins 1999; Bentz et al. 2001) and further establish regional differences within this species.

Spatial patterns of postzygotic isolation: My results suggest that hybrid male sterility occurs at a threshold, rather than a clinal gradient, among increasingly divergent population crosses within *D. ponderosae* found surrounding the Great Basin Desert (Figure 2-7). Furthermore, when crosses that produced sterile males were excluded, there was little evidence of a sex-specific decrease in the proportion of viable eggs or total egg hatch in F_2 crosses that utilized increasingly divergent F_1 hybrids (Table 2-8). Hybrid males did show a slight decrease in total egg hatch in the most geographically distant cross (822 mi. = CA x OR) (Figure 2-4), although this was not a significant effect in the models (Table 2-8). My results seem consistent with a rather simple genetic basis for hybrid male sterility that occurs at a threshold of divergence. My results seem less consistent with the accumulation of multiple genes of small effect on fitness and sterility since this would likely be expressed as decreased egg viability in the increasingly divergent population crosses or seen as an increase in the percentage of

sterile individuals. Furthermore, although hybrid sterility clearly followed Haldane's rule (affecting the heterogametic sex), hybrid male fitness was not always lower than hybrid female fitness. Many hybrid male cohorts had increased mean total egg hatch and mean proportion of viable eggs when compared to hybrid females from the same cross (Figures 2-3 and 2-4).

The apparent threshold for hybrid male sterility that was observed between hybridizations with OR and ID is perplexing given the close proximity of these populations (163 miles), their use of identical host trees (*P. contorta*), overall morphological and ecological similarity, and shallow genetic distance (Mock et al. 2007). The observed reproductive boundary observed in CA x OR and CA x ID/UT populations suggests that ID and UT have acquired an incompatibility that one could speculate might affect OR x ID/UT hybrid males as well. However, these crosses were not assessed and further research is needed to establish a potential boundary between OR and ID.

Genetic mechanisms causing sterility: There are three genetic mechanisms that could be contributing to postzygotic isolation and hybrid male sterility in *D. ponderosae*: endosymbiont-induced incompatibilities, chromosomal rearrangements and genic incompatibilities (Coyne and Orr 2004). I tested *D. ponderosae* for one of the most well known endosymbionts that causes reproductive isolation (*Wolbachia*) and my results suggest that although detected in two populations, it is likely not causing the observed incompatibilities. Three lines of evidence support this conclusion. 1) *Wolbachia* was detected in only a few beetles (2.3%) and none of the CA and ID individuals tested positive. 2) Incompatibilities are typically manifest as hybrid

inviability, not hybrid sterility (Werren 1997; Stouthmaer et al. 1999) and therefore, infection influencing reproduction would have been seen as a loss of fertility in my reciprocal crosses, which I did not observe (Table 2-2). 3) *Dendroctonus ponderosae* does not show the molecular signature of a “*Wolbachia* sweep”, where haplotypes that are associated with infected females “sweep” through populations and decrease mtDNA diversity (Dean et al. 2003; Jiggins 2003; Narita et al. 2006). *Dendroctonus ponderosae* has actually been shown to have very high haplotypic diversity yet low overall nucleotide polymorphism (Mock et al. 2007). I encountered only one known case of endosymbiont-facilitated hybrid male sterility in the literature and it occurred in *Drosophila* infected with Streptococcal L-forms (Somerson 1984). Therefore, in sum, *Wolbachia* and endosymbiont infections seem an unlikely explanation for the observed reproductive incompatibilities.

Chromosomal rearrangements have historically been implicated in species formation (King 1993), but the likelihood of a rearrangement affecting only hybrid *D. ponderosae* males is questionable. Macro-molecular mutations (fusions and fissions) would likely disrupt meiosis in both gametes, resulting in sterile individuals in both hybrid sexes and micro-molecular mutations such as inversions or translocations would need to be associated with chromosomal regions affecting only hybrid males. Although a rearrangement that affects only one hybrid sex is quite possible, theory predicts that any strongly underdominant mutation would have difficulties getting fixed without strong genetic drift (such as in a very small, isolated population) (Rieseberg 2001, and citations within), and there is no molecular evidence of that scenario in *D. ponderosae* (Mock et al. 2007).

Genic incompatibilities are widely considered to be the most common mode of postzygotic isolation and these incompatibilities occur through the accumulation of divergent genes that have negative epistatic interactions in hybrids (Bateson – Dobzansky – Muller (BDM) incompatibilities) (Bateson 1909; Dobzhansky 1937; Muller 1942). An extension of the BDM model, known as dominance theory (Orr 1993; Turelli and Orr 1995) is most often used to explain Haldane’s rule, suggesting that X-linked recessives are expressed in hemizygous individuals due to negative epistatic interactions between the X and the autosomes. Dominance theory is considered the universal explanation for Haldane’s rule (Coyne and Orr 2004), although individual cases of hybrid male sterility have been associated with many different mechanisms (reviewed in Laurie 1997). Assuming incompatibilities between X-linked loci are responsible for *D. ponderosae* hybrid male sterility, my results suggest that sex-linked incompatibilities have independently accumulated in both the CA and ID/UT populations. Reciprocal crosses between these populations produced sterile males and therefore the males had X’s derived from both CA and ID/UT populations. Therefore, sterility occurred regardless of the maternal origin of the X. Bidirectional incompatibilities appear to be common in cases of hybrid male sterility (Coyne and Orr 1989, 1997). However, given the lack of strong genetic divergence in *D. ponderosae*, one might assume unidirectional incompatibilities to be expressed first or unidirectional expression in less geographically distant crosses, and I did not detect this.

A possible piece in this puzzle is *D. ponderosae*’s chromosomal structure. This species is described as $n = 11 + \text{neo-XY}$ (Lanier and Wood 1968), and is thought to have been derived from an ancestral configuration of 12XY_p by a fusion of the X with

the largest autosomal chromosome, followed by a loss of the ancestral Y_p , resulting in the “new” X homologue becoming the “new” Y (Lanier 1981). Therefore, the largest chromosomes in *D. ponderosae* are the neo X and Y sex chromosomes (Lanier and Wood 1968). Comparative analyses between *Drosophila* species have shown that species with larger X's express hybrid male sterility at lower genetic distances than species with smaller X's (Turrelli and Begon 1997). Turrelli and Begon (1997) argue that this is explained by dominance theory and largely considered a result of the expression of recessive X-linked alleles that would accumulate faster on a larger X's purely because of chromosome size, and are then expressed when in a hemizygous state. Unfortunately, little is known about the Neo XY condition in *D. ponderosae* and how this might contribute to reproductive isolation and the expression of hybrid male sterility. More research is needed into the karyotypes of multiple *D. ponderosae* populations and hybrids.

Hybrid male sterility and a lack of neutral genetic signal: Although the exact genetic mechanism causing hybrid male sterility in *D. ponderosae* remains elusive, what is clear is the failure of neutral molecular markers to identify what seems to be an abrupt decrease in gene flow between populations that produce sterile male hybrids (Mock et al. 2007). Molecular genetics is commonly used to infer species boundaries, population subdivision, and patterns of gene flow and my results suggest that hybrid male sterility may go undetected with an analysis based solely on neutral molecular markers.

There appear to be three possible explanations as to why reproductive isolation was not detected using molecular markers, and they are not exclusive: 1) hybrid male

sterility is an ineffective isolating mechanism since gene flow can still occur via fertile females, 2) the onset of hybrid male sterility is recent and molecular differentiation has yet to occur, and 3) *D. ponderosae* populations are so large that drift is minimal and divergent alleles at neutral markers are slow to divergence and fixation.

Introgression primarily through hybrid females is a possibility, but seems doubtful given that hybridization would result in a substantial decrease in gene flow since nearly all resulting males would not be passing on their genes. Only recently have the influences of heterogametic incompatibilities on gene flow been modeled (Wang 2003; Wang and Zhao 2008). Wang (2003) and Wang and Zhao (2008) do suggest that sterility of hybrid males should effect the genetic structure of the incompatible populations, although the underlying BDM incompatibility (X-autosomal interactions, X-Y interactions, etc.) would effect the strength of the barrier to gene flow. Currently, the mechanism contributing to hybrid male sterility in *D. ponderosae* is unknown although the observed bidirectional incompatibilities should lead to pronounced isolation (Wang and Zhou 2008).

The onset of hybrid male sterility may be quite recent given that mtDNA percent sequence divergence across all *D. ponderosae* populations is rather small (COI and COII, 0.7 %) and there is little geographic structuring of haplotypes (Mock et al. 2007). Additionally, the amount of genetic differentiation (both nuclear and mtDNA) between CA and ID is similar to the amount of differentiation between ID and a population from British Columbia, Canada; a population that is hypothesized to have recently colonized lodgepole pine forests following the northward retreat of Pleistocene glaciers (Mock et al. 2007). Assuming that genetic divergence has occurred at roughly the same rate as

seen between the ID and the northern B.C., Canada population, this could suggest a very recent post-Pleistocene onset of hybrid male sterility within *D. ponderosae*. However, interpretation of the molecular genetic data could be influenced by *D. ponderosae* population size, since analyses based on neutral markers rely on drift to create allele frequency differences. *Dendroctonus ponderosae* population sizes are known to be substantial and can grow quite large during outbreaks. A recent *D. ponderosae* outbreak has affected over 15 million ha in B.C., Canada (www.for.gov.bc.ca) and at this size, drift might be minimal. Similarly, Mock et al. (2007) found that sequence diversity within populations was remarkably high, suggesting a limited effect of drift.

Further research: There was clear evidence of reproductive incompatibilities between populations of *D. ponderosae*, with some populations apparently producing sterile male offspring. However, because of the cryptic nature of *D. ponderosae* reproduction, I was unable to directly observe copulation and sperm transfer in crosses that failed to produce offspring. Furthermore, although one potential mechanism, *Wolbachia* infection, can likely be ruled out, both chromosomal mutations and genic interactions could be contributing to the observed sterility. Determining a mechanism is complicated by the potential contributions of the Neo XY chromosomal configuration in *D. ponderosae*. Further research should focus on determining whether males are indeed transferring sperm and if the sperm are motile (a commonly used approach for detection of male sterility in *Drosophila*), investigating potential karyotypic differences between populations and their hybrids, and establishing if a reproductive barrier does indeed exist between OR and ID populations.

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Table 2-1. Collection location and host for *Dendroctonus ponderosae* used in population crosses.

<i>Identifier</i>	<i>Locality (nearest city)</i>	<i>Elevation (ft.)</i>	<i>Geographic Distance^a</i>	<i>Latitude and Longitude</i>	<i>Host tree</i>
CA	Big Bear Lake, CA	6865	0	34° 15' N, 116° 54' W	<i>Pinus monophylla</i>
CA1	Arrowbear Lake, CA	6656	9	34° 12' N, 117° 03' W	<i>Pinus lambertiana</i>
CA2	Kernville, CA	8932	152	36° 01' N, 118° 15' W	<i>Pinus contorta</i>
CA3	Old Station, CA	4879	518	40° 37' N, 121° 29' W	<i>Pinus contorta</i>
OR	Prairie City, OR	5252	822	44° 17' N, 118° 24' W	<i>Pinus contorta</i>
ID	Stanley, ID	6588	985	44° 17' N, 115° 02' W	<i>Pinus contorta</i>
UT	Garden City, UT	7162	1228	41° 58' N, 111° 31' W	<i>Pinus contorta</i>
AZ	Flagstaff, AZ	9230	1692	35° 19' N, 111° 42' W	<i>Pinus flexilis</i>

^a Measured as the cumulative linear distance in miles around the Great Basin Desert from CA.

Table 2-2. Hybrid inviability assay results and offspring sex ratios. F₁ intrapopulation crosses were conducted using 20 pairs (except CA2 and AZ) and F₁ interpopulation crosses (hybrids) used 10 pairs. Male:Female sex ratio calculated from 50 randomly selected offspring from intrapopulation and interpopulation crosses.

<i>Crosstype</i>	<i>Maternal population</i>	<i>Paternal population</i>	<i>Total offspring</i>	<i>Proportion fertile^a(n)</i>	<i>Male:Female sex ratio</i>
Intrapopulation					
	CA	CA	604	0.95 (20)	1:2.13
	CA1	CA1	260	1.00 (20)	1:2.13
	CA2	CA2	169	1.00 (10)	1:0.85
	CA3	CA3	574	1.00 (20)	1:1.27
	OR	OR	703	0.95 (20)	1:0.92
	ID	ID	348	1.00 (20)	1:2.33
	UT	UT	413	1.00 (20)	1:1.17
	AZ	AZ	103	0.70 (10)	1:3.90
Interpopulation (hybrids)					
	CA1	CA	251	0.90 (10)	1:1.38
	CA	CA1	210	1.00 (10)	1:2.13
	CA2	CA	286	0.90 (10)	1:1.17
	CA	CA2	133	1.00 (10)	1:0.85
	CA3	CA	326	1.00 (10)	1:1.38
	CA	CA3	211	1.00 (10)	1:1.00
	OR	CA	266	1.00 (10)	1:1.17
	CA	OR	231	0.80 (10)	1:1.50
	ID	CA	331	1.00 (10)	1:2.57
	CA	ID	199	1.00 (10)	1:1.00
	UT	CA	94	0.90 (10)	1:1.50
	CA	UT	373	1.00 (10)	1:0.92
	AZ	CA	52	0.30 (10)	1:1.81
	CA	AZ	150	1.00 (10)	1:1.27

^a Pair considered fertile if evidence of ≥ 5 pupal chambers with exit hole, indicating emergence of adult.

Table 2-3. Fitness characteristics of source populations, as assessed in F₂ crosses. Values given as arithmetic means and ± standard error.

<i>Population</i>	<i>Number of crosses</i>	<i>Proportion fertile^a</i>	<i>Eggs laid</i>	<i>Proportion of viable eggs</i>	<i>Total gallery length (cm)</i>	<i>Total eggs hatched</i>
CA	19	0.95	17.84 (± 1.50)	0.50 (± 0.08)	45.84 (± 2.1)	25.95 (± 3.78)
CA1	16	1.00	15.50 (± 1.88)	0.81 (± 0.05)	39.88 (± 3.6)	27.25 (± 5.10)
CA2	18	0.94	26.06 (± 1.97)	0.80 (± 0.06)	31.86 (± 2.5)	28.22 (± 2.83)
CA3	17	0.88	20.82 (± 1.50)	0.68 (± 0.08)	40.97 (± 2.7)	30.94 (± 4.46)
OR	19	0.95	23.31 (± 2.21)	0.76 (± 0.07)	35.79 (± 2.0)	31.36 (± 4.03)
ID	18	0.78	20.61 (± 2.40)	0.67 (± 0.09)	26.91 (± 3.4)	21.67 (± 4.08)
UT	19	1.00	28.32 (± 1.45)	0.91 (± 0.02)	44.45 (± 2.6)	47.32 (± 4.88)

^a Coupling considered fertile if total egg hatch > 0.

Table 2-5. GLIMMIX model results testing for significant differences among F₂ backcrosses in number of eggs laid and proportion of viable eggs.

EFFECT	Eggs laid (15cm)			Proportion of viable eggs (15cm)		
	df	F Value	P Value	df	F Value	P Value
Geographic distance	1, 401	0.84	0.3607	1, 197	6.74	0.0101*
Hybrid sex	1, 401	0.73	0.3944	1, 197	5.66	0.0183*
Reciprocal	1, 401	0.41	0.5214	1, 197	0.01	0.9117
Backcross population	1, 401	0.03	0.8550	1, 197	4.96	0.0271*
Reciprocal x hybrid sex	1, 401	0.03	0.8616	1, 197	0.00	0.9893
Geographic distance x backcross population	1, 401	0.06	0.8096	1, 197	2.12	0.1472
Backcross population x hybrid sex	1, 401	0.02	0.8959	1, 197	1.14	0.2866
Reciprocal x backcross population	1, 401	0.04	0.8340	1, 197	0.02	0.8885
Geographic distance x hybrid sex	1, 401	3.84	0.0508	1, 197	10.24	0.0016*
Geographic distance x reciprocal	1, 401	0.35	0.5561	1, 197	0.18	0.6702
Geographic distance x backcross population x hybrid sex	1, 401	0.74	0.3903	1, 197	0.79	0.3740
Geographic distance x reciprocal x hybrid sex	1, 401	0.02	0.8983	1, 197	0.01	0.9215
Geographic distance x reciprocal x backcross population	1, 401	0.45	0.5024	1, 197	0.04	0.8386
Reciprocal x backcross population x hybrid sex	1, 401	0.09	0.7657	1, 197	0.00	0.9990
Geographic distance x reciprocal x backcross population x hybrid sex	1, 401	0.15	0.6978	1, 197	0.04	0.8348

* Significant at an $\alpha=0.05$

Table 2-6. GLIMMIX model results testing for significant differences among F₂ backcrosses in total egg hatch and total gallery length.

EFFECT	Total egg hatch			Total gallery length		
	df	F Value	P Value	df	F Value	P Value
Geographic distance	1, 401	33.89	<.0001*	1, 401	13.96	0.0002*
Hybrid sex	1, 401	5.34	0.0213*	1, 401	0.20	0.6586
Reciprocal	1, 401	0.03	0.8584	1, 401	0.24	0.6216
Backcross population	1, 401	0.71	0.3990	1, 401	7.35	0.0070*
Reciprocal x hybrid sex	1, 401	0.08	0.7720	1, 401	13.90	0.0002*
Geographic distance x backcross population	1, 401	0.49	0.4848	1, 401	0.84	0.3588
Backcross population x hybrid sex	1, 401	0.74	0.3912	1, 401	5.99	0.0148*
Reciprocal x backcross population	1, 401	0.53	0.4672	1, 401	10.70	0.0012*
Geographic distance x hybrid sex	1, 401	36.87	<.0001*	1, 401	4.07	0.0442*
Geographic distance x reciprocal	1, 401	0.90	0.3442	1, 401	0.93	0.3357
Geographic distance x backcross population x hybrid sex	1, 401	1.05	0.3056	1, 401	2.16	0.1425
Geographic distance x reciprocal x hybrid sex	1, 401	1.31	0.2526	1, 401	5.09	0.0246*
Geographic distance x reciprocal x backcross population	1, 401	0.10	0.7539	1, 401	3.04	0.0822
Reciprocal x backcross population x hybrid sex	1, 401	0.00	0.9873	1, 401	1.93	0.1654
Geographic distance x reciprocal x backcross population x hybrid sex	1, 401	0.00	0.9992	1, 401	0.01	0.9042

* Significant at an $\alpha=0.05$

Table 2-7. Unstandardized values from all F₂ backcrosses utilizing CA x ID and CA x UT hybrids. Response variables (eggs laid, proportion viable eggs, total gallery length, total egg hatch) given as means and standard error.

	<i>Cohort</i>	<i>Backcross population</i>	<i>Number of crosses</i>	<i>Number with egg hatch</i>	<i>Proportion fertile^a</i>	<i>Eggs laid (15cm)</i>	<i>Proportion of viable eggs (15cm)</i>	<i>Total gallery length</i>	<i>Total egg hatch</i>
	(ID ♀ x CA ♂)	ID	10	1	0.10	11.3 (2.07)	0.10 (0.10)	29.65 (4.55)	2.70 (2.70)
	*	CA	10	1	0.10	12.9 (2.22)	0.06 (0.06)	32.50 (3.94)	3.30 (3.30)
Hybrid Males	(CA ♀ x ID ♂)	ID	8	0	0.00	6.25 (1.33)	0	34.00 (3.14)	0
	*	CA	9	0	0.00	24.0 (5.15)	0	40.16 (3.34)	0
	(UT ♀ x CA ♂)	UT	10	1	0.10	12.9 (2.33)	0.10 (0.10)	36.60 (1.35)	3.30 (3.30)
	*	CA	8	2	0.25	7.75 (1.85)	0.05 (0.04)	34.94 (4.76)	1.60 (1.48)
	(CA ♀ x UT ♂)	UT	10	0	0.10	10.70 (2.62)	0	40.05 (4.07)	0
	*	CA	7	1	0.14	14.43 (2.76)	0	44.50 (6.77)	0.43 (0.43)
	(ID ♀ x CA ♂)	ID	8	8	1.00	19.63 (2.35)	0.65 (0.10)	35.75 (5.36)	20.50 (4.04)
	*	CA	9	8	0.89	22.67 (2.67)	0.60 (0.12)	37.33 (3.14)	30.56 (6.22)
Hybrid Females	(CA ♀ x ID ♂)	ID	7	6	0.86	26.14 (6.00)	0.81 (0.14)	40.86 (5.39)	46.00 (13.10)
	*	CA	9	9	1.00	19.66 (2.46)	0.91 (0.07)	34.61 (3.22)	35.11 (6.44)
	(UT ♀ x CA ♂)	UT	10	10	1.00	30.90 (4.14)	0.90 (0.06)	39.15 (2.43)	42.30 (7.30)
	*	CA	5	5	1.00	15.20 (2.06)	0.83 (0.07)	36.30 (2.62)	26.20 (5.29)
	(CA ♀ x UT ♂)	UT	9	8	0.89	22.67 (3.01)	0.77 (0.11)	44.11 (3.56)	35.89 (6.70)
	*	CA	9	9	1.00	20.44 (1.78)	0.57 (0.10)	58.06 (2.43)	38.78 (6.35)

* Same as above

^a Fertile if pairing resulted in any egg hatch

Table 2-8. GLIMMIX model results of post hoc testing for significant differences among F₂ backcrosses in total egg hatch and proportion of viable eggs while excluding all CA x ID and CA x UT hybrids.

EFFECT	<i>Total egg hatch</i>			<i>Proportion of viable eggs (15cm)</i>		
	df	F Value	P Value	df	F Value	P Value
Geographic distance	1, 263	3.87	0.0503	1, 106	0.04	0.8444
Hybrid Sex	1, 263	0.74	0.3904	1, 106	0.03	0.8631
Reciprocal	1, 263	0.38	0.5376	1, 106	0.00	0.9464
Backcross population	1, 263	1.44	0.2308	1, 106	0.49	0.4840
Reciprocal x hybrid sex	1, 263	0.03	0.8524	1, 106	0.16	0.6918
Geographic distance x backcross population	1, 263	2.25	0.1352	1, 106	0.03	0.8562
Backcross population x hybrid sex	1, 263	1.81	0.1793	1, 106	0.05	0.8277
Reciprocal x backcross population	1, 263	0.58	0.4489	1, 106	0.04	0.8415
Geographic distance x hybrid sex	1, 263	0.69	0.4078	1, 106	0.37	0.5460
Geographic distance x reciprocal	1, 263	1.47	0.2262	1, 106	0.32	0.5711
Geographic distance x backcross population x hybrid sex	1, 263	2.87	0.0915	1, 106	0.01	0.9337
Geographic distance x reciprocal x hybrid sex	1, 263	0.34	0.5590	1, 106	0.03	0.8610
Geographic distance x reciprocal x backcross population	1, 263	0.04	0.8449	1, 106	0.03	0.8746
Reciprocal x backcross population x hybrid sex	1, 263	0.01	0.9131	1, 106	0.19	0.6644
Geographic distance x reciprocal x backcross population x hybrid sex	1, 263	0.11	0.7366	1, 106	0.08	0.7727

* Significant at an $\alpha=0.05$

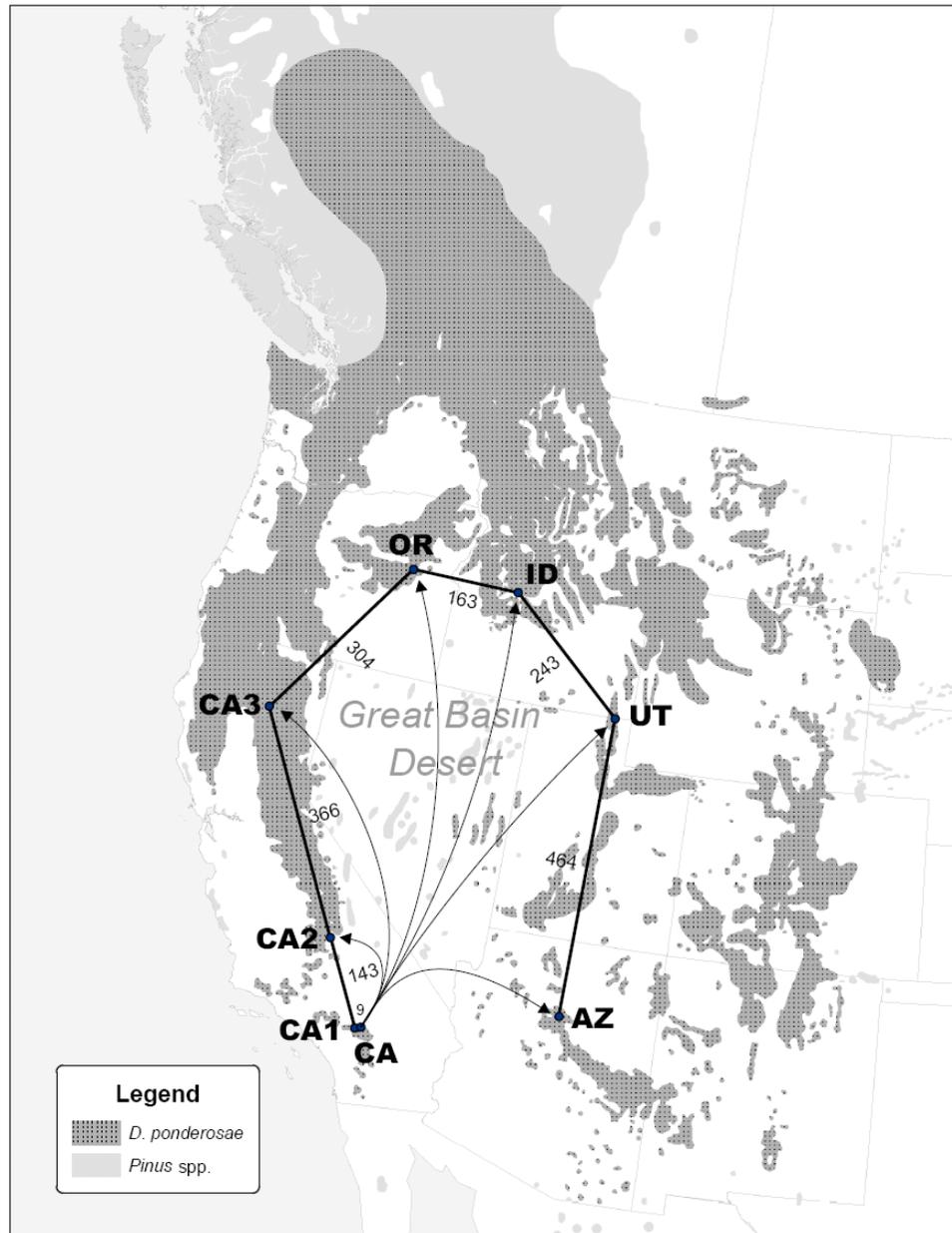


Figure 2-1. Population collection areas and schematic diagram of crossing experiment. Numbers represent the miles between adjacent populations.

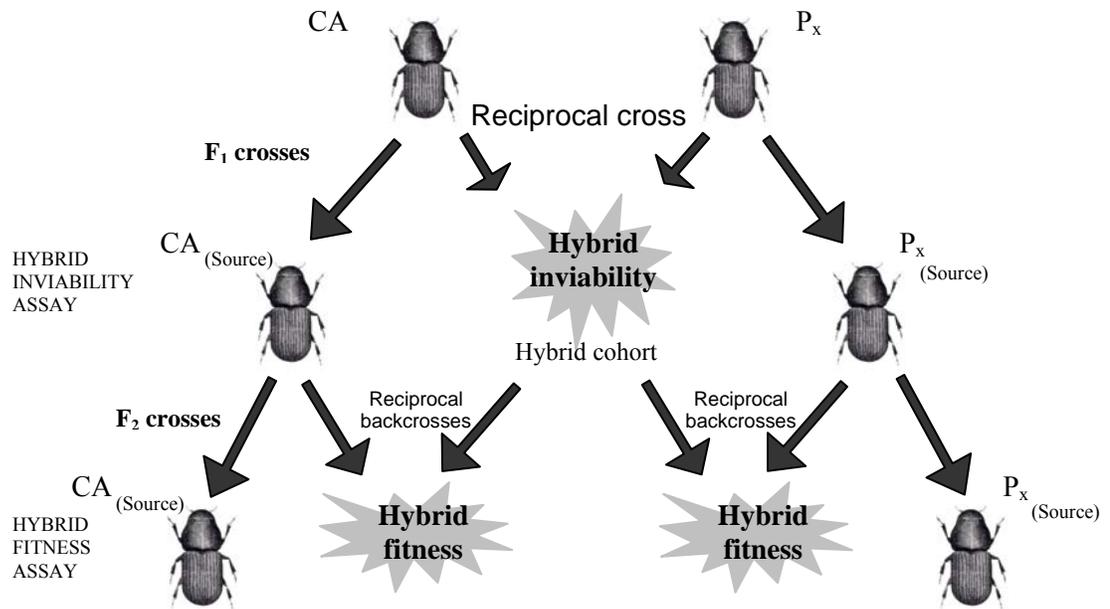


Figure 2-2. Diagram of common garden crossing assays used to investigate postzygotic isolation in *Dendroctonus ponderosae*. Reciprocal crosses were made between field collected CA and P_x populations (where P_x are increasingly divergent populations, CA1-AZ) to determine if hybrids are produced (Hybrid inviability assay). Hybrids that are produced (Hybrid cohorts) are used in reciprocal backcrosses to determine if there is decreased fitness and sterility (Hybrid fitness assay). Parent populations (CA and P_x) are maintained each generation as references (Source populations).

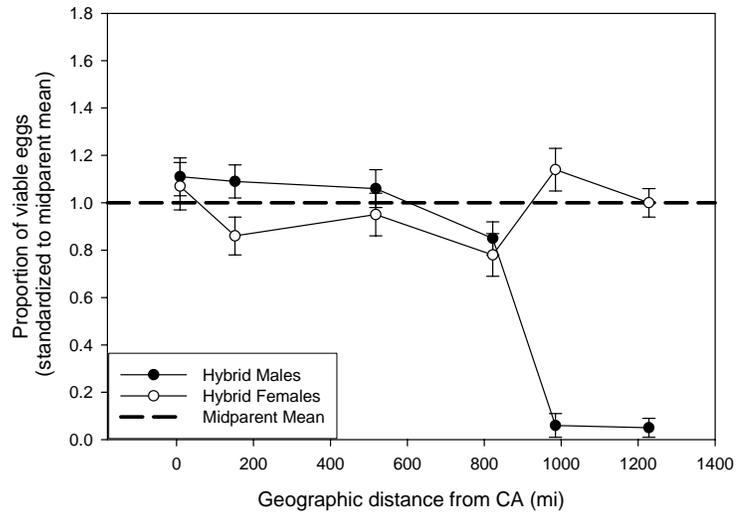


Figure 2-3. Mean and standard error of the proportion of viable eggs (first 15 cm of gallery) in F_2 backcrosses as a function of geographic distance. Geographic distance is the cumulative linear miles around the Great Basin Desert from CA (see Figure 2-1) to the population used in F_1 hybrid crosses.

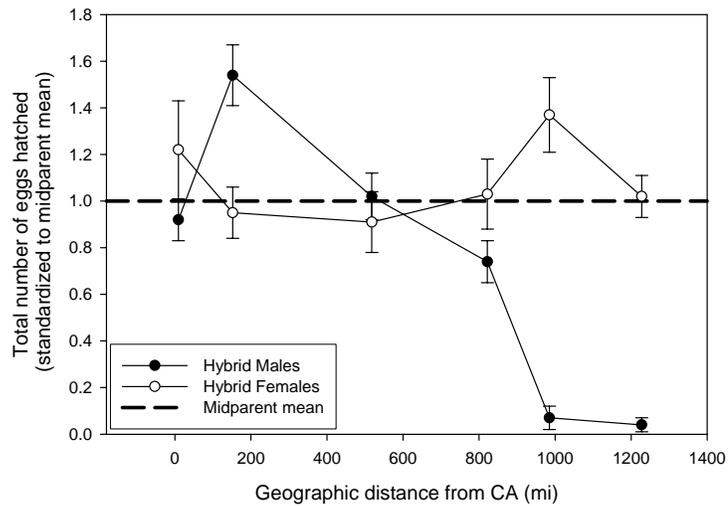


Figure 2-4. Mean and standard error of total egg hatch in F_2 backcrosses as a function of geographic distance.

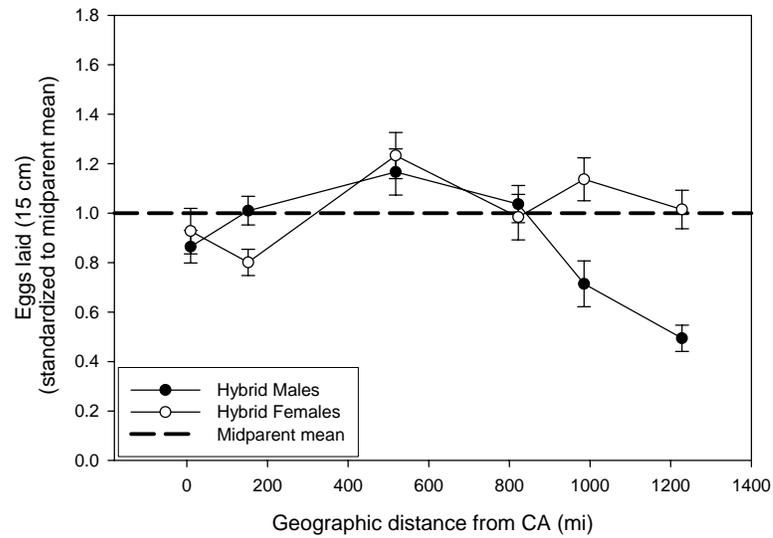


Figure 2-5. Mean and standard error of the number of eggs laid (first 15 cm of gallery) in F_2 backcrosses as a function of geographic distance.

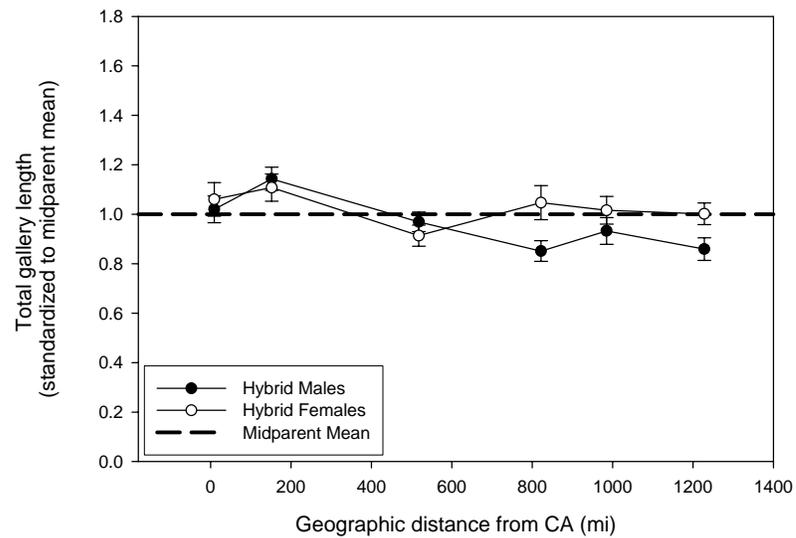


Figure 2-6. Mean and standard error of total gallery length in F_2 backcrosses as a function of geographic distance.

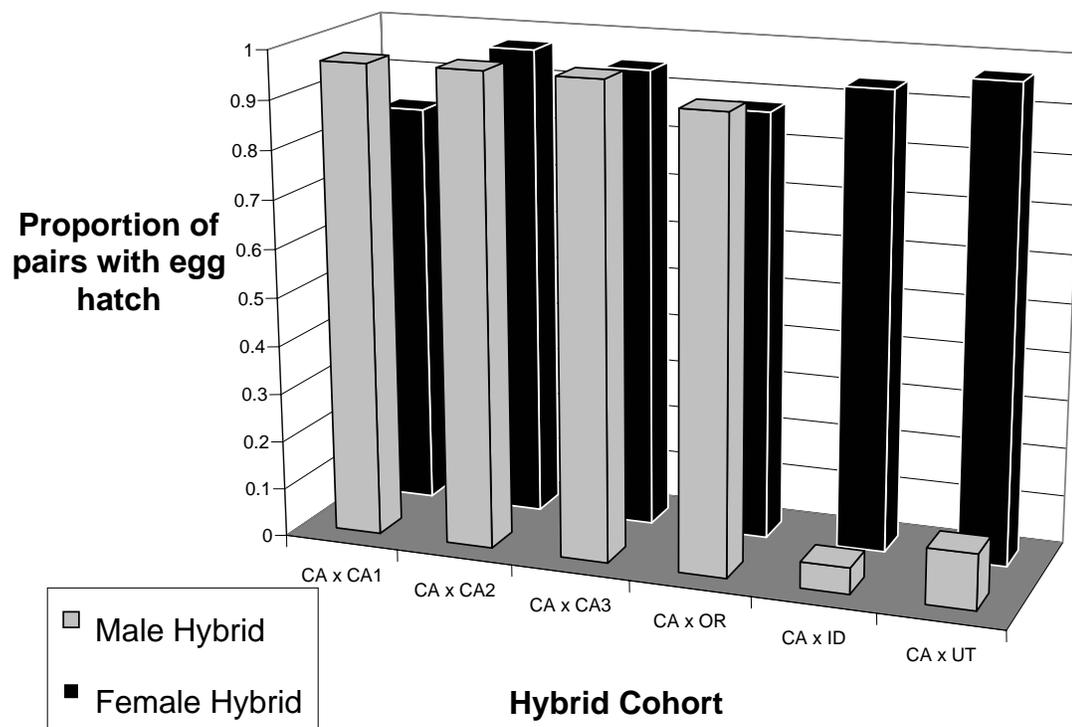


Figure 2-7. Proportion of F₁ offspring that were fertile in backcrosses (fertile if >1 egg hatched). Number of backcrosses from left to right (hybrid male n=34, 35, 34, 40, 37, 35; hybrid female n=30, 36, 37, 33, 33, 33).

CHAPTER 3
INVESTIGATION OF DEVELOPMENT TIME AND BODY SIZE
WITHIN THE CLINALLY DISTRIBUTED
DENDROCTONUS PONDEROSAE

Abstract

Body size and development time are two critical phenotypic traits that are often adaptive in insects. In cooler climates (often imposed by latitude), a species will typically show decreased size (converse Bergmann's rule) and an increased developmental rate. The mountain pine beetle *Dendroctonus ponderosae* presents an interesting opportunity to study this phenomenon since it has a bifurcated distribution along two extensive latitudinal gradients in the western U.S., separated by the Great Basin Desert. Furthermore, there is evidence of some reproductive isolation (hybrid male sterility) and it is unclear if isolated populations are phenotypically divergent. To assess size and developmental rate differences along latitudinal gradients and between isolated populations, I conducted two generations of random mating in a common garden experiment utilizing 7 *D. ponderosae* populations selected from around the Great Basin Desert, and determined body size and development time in the F₂ generation. Genetic differences in development time were striking between faster developing northern populations (from northern California, Oregon, Idaho, and Utah) and slower developing southern populations (from southern California and Arizona). Furthermore, development occurred in a less synchronized fashion in southern populations than in northern populations. Body size, although more variable, generally

conformed to expectations, with individuals in northern populations being smaller than those in southern populations. Differences in development time were not detected between populations at similar latitudes, while differences in body size were found, and are possibly due to elevational differences or other factors such as host tree species. Although average size was different between many populations, relative sexual size dimorphism was found to be rather consistent. My results suggest that latitudinally-imposed climatic differences are likely driving phenotypic divergence between populations, but that other factors are responsible for the maintenance of size differences between sexes.

INTRODUCTION

Phenotypic differences are apparent in most species, subspecies, and even many allopatric populations, resulting from contrasting regimes of genetic drift and/or selection. In insects, body size and development time are two phenotypic traits known to vary within a species and are generally considered to be important environment-specific adaptations (Nylin and Gotthard 1998). In many ectotherms, and particularly insects, body size tends to decrease as latitude increases, a pattern that has been described as the “converse of Bergmann’s rule” (Masaki 1978; Roff 1980; Mousseau 1997). As the amount of thermal input decreases (as seen along clines in both latitude and altitude) a species may adapt by decreasing its body size and also increasing its developmental rate in an attempt to maintain an adaptive life cycle (Dingle and Mousseau 1994; Blanckenhorn and Fairbairn 1995; Berner et al. 2004). This

phenotypic response to environmental differences can be plastic (Nylin and Gotthard 1998) and/or due to genetic variation for those traits (e.g., Mousseau and Roff 1989; Blanckenhorn and Fairbairn 1995; Bentz et al. 2001). Genetic differences in development time could lead to temporal isolation between populations and potentially be a premating mechanism facilitating speciation. Body size is known to be linked to fecundity and potentially to population dynamics in insects; larger females typically produce more offspring (Honek 1993). Therefore, identifying genetic differences in size and development time could help identify divergent and isolated populations and be informative about the evolutionary history and broad scale adaptive patterns of widely distributed species.

Dendroctonus ponderosae Hopkins (Coleoptera: Curculionidae, Scolytinae) is a single species with gene flow occurring in a horseshoe shaped distribution around the Great Basin Desert (Mock et al. 2007) (Figure 3-1). Populations in the southern most reaches of the *D. ponderosae* range are at the ends of the horseshoe (southern California, northern Arizona) and are the most genetically divergent, although they occupy similar climatic regimes. Although isolated populations of *D. ponderosae* can be found in sparse high elevation pine forests throughout Nevada, the main *D. ponderosae* distribution in the western U.S. occurs along two latitudinal clines (Figure 3-1). Recent evidence from population crossings within *D. ponderosae* suggests hybrid male sterility occurs at a threshold in crosses between a population from southern California and populations from Idaho and Utah (Chapter 2), while less geographically distant crosses between southern California and Oregon produce fertile hybrid offspring (Chapter 2). Geographically proximal populations that inhabit similar regimes (Oregon

and Idaho) appear to harbor the threshold for the onset of hybrid male sterility. Interestingly, this apparent reproductive barrier was not detected using molecular markers (Mock et al. 2007), suggesting that the barrier is recent or that it does not significantly impair gene flow among populations. Previous investigations into size and development time in *D. ponderosae* found that beetles from populations in central Idaho and northern Montana are smaller and have faster development times when compared to a population from southern Utah (Bentz et al. 2001). Overall size has also historically been considered a character to distinguish between different *Dendroctonus* species (Hopkins 1909; Lanier and Wood 1968; Wood 1982). This unique situation presents an opportunity to compare the relative inputs of neutral and selective processes on phenotypic divergence across a large landscape scale, assessing whether variation in body size and developmental rates is more consistent with neutral molecular genetic divergence (Mock et al. 2007) or climate gradients (Bentz et al. 2001), and whether the traits differ in populations that produce sterile males (Chapter 2).

Here, I investigated body size (a sexually dimorphic trait in *D. ponderosae*) and development time across multiple populations which span a portion of the species' geographic range in the western U.S. I hypothesized that 1) consistent with the converse of Bergmann's rule and adaptive divergence, populations in the southern part of the range would, on average, be larger and have slower development times than populations from more northern latitudes, and 2) genetic differences in size and/or development time due to genetic drift or local adaptation would be detected along reproductive boundaries previously described in *D. ponderosae*.

MATERIALS AND METHODS

Population Collection: Seven *D. ponderosae* populations were collected from coniferous forests bounding the Great Basin Desert in the spring of 2007 by felling larvae infested trees (Table 3-1) (Figure 3-1). Sections from the bole of each tree (~14-16 in.) were collected and the cut ends were sealed with paraffin wax to reduce desiccation. The sections were then transported to the USDA Forest Service Research Station in Logan, Utah, and placed in refrigeration (~3° C). After all populations were collected, the tree sections were removed from refrigeration and placed in rearing containers at room temperature (~21° C) to allow development to the adult stage. Emerging adults were collected from each population daily and placed in petri dishes with moistened filter paper and then returned to ~3° C for storage. Individuals to be used for continued matings were randomly chosen from the peak emergence period (~15 days with highest total of beetles) of each population. Adult gender was determined using characters on the 7th abdominal tergite (Lyon 1958).

Assessing population level differences: To characterize relative differences in development time and body size across *D. ponderosae* populations, I conducted intrapopulation matings for two generations in a common garden environment. Rearing in a common environment allows for the separation of genetic from environmental effects on phenotypic variation. Multiple generations of matings were conducted to minimize maternal effects due to the original collection environment (e.g., prior host use). The common garden environment consisted of a constant temperature (22.5° C) with constant light (24L:0D), and utilized a single rearing tree species, lodgepole pine

(*Pinus contorta* var. *latifolia*). Similar rearing protocols have been used previously (e.g., Bentz et al. 2001).

Each population was reared through two generations (e.g., F₁, F₂). For laboratory propagation, two randomly selected bolts (~16 in tree sections) cut from a single live uninfested lodgepole pine from the Wasatch-Cache NF, UT, were used for each generation. Cut ends were waxed with paraffin to reduce desiccation and preserve phloem quality. Matings were performed by inserting a female, and then a male (termed a pair) into a pre-drilled hole in the phloem of each bolt. Each pair was spaced 1.2 inches from its neighbor around the circumference of the bolt to homogenize infestation density and brood competition. After inserting each pair, a small piece of screen was fixed over the entrance hole to prevent escape. After all pairs were in place, the infested bolts were individually enclosed in screen so that the resulting offspring could easily be collected and their emergence time monitored.

Infested bolts were placed in two separate temperature-controlled rearing chambers (one bolt per chamber) set at 22.5° C. Twenty four pairs per population (12 pairs per bolt) were used to produce the F₁ generation. For each population, adult beetles from the peak emergence (~15 days with highest total of beetles) from all F₁ bolts were pooled and 20 pairs (10 pairs per bolt) randomly selected to produce the F₂ generation. Total development time (e.g., the time from introduction of male/female pairs to brood adult emergence) of the F₂ generation was determined by tabulating the number of adults emerged from bolts, by population, every other day, until beetles quit emerging (~10 days without an individual). Pronotum width (a proxy for overall size) was measured on up to 50 F₂ beetles per sex per population. Measures were taken from

randomly selected beetles from pooled F₂ collections (pooled from replicate bolts). All size and development time comparisons were conducted exclusively on F₂ generation adults.

Statistical Analysis: Differences among populations in size (pronotum width) and total development time were analyzed using mixed models in SAS (SAS Institute, Cary, North Carolina, USA, version 9.1.3). Prior to the analysis the response variable pronotum width was examined for normality using histograms, symmetry plots and quantile plots. The pronotum data was found to be normally distributed and analyzed using PROC MIXED with population as the main fixed effect. Significant differences in size were found among male and female adults (df=1, 668, F=428.21, P<.0001), and therefore genders were analyzed separately. Development time data were analyzed using a three parameter logistic growth model (Meyer 1994) that incorporates the total number of adults emerged (k), time from 10% to 90% adult emergence (Δt), and median emergence day (t_m). Model parameter estimates were determined using PROC NLMIXED, and plots of predicted values and residuals used to check the model fit. The resulting parameter estimates were analyzed using PROC MIXED. Replicate bolts were placed in temperature chambers, and the development time model included temperature chamber as a random effect to account for expected slight deviations in temperature between chambers. Post hoc pairwise comparisons of development time and size between populations were conducted using Tukey-Kramer HSD tests.

RESULTS

Development time: Significant differences among populations were found in median development time at a constant 22.5° C ($df=6, 6, F=42.05, P<.0001$).

Development time of individuals from the three populations collected from the most southern latitudes (CA, CA1, AZ) were significantly different from the four populations collected from the more northern latitudes (CA3, OR, ID, UT) (Table 3-2). Within these two latitudinal groups, no significant differences were detected (Table 3-2).

Median development time for the three southern populations was nearly double the time observed for individuals from northern populations (Table 3-2, Figure 3-4).

Populations from the southern latitudes also required a significantly greater number of days to progress from 10% to 90% emergence ($df=6, 7, F=30.39, P<.0001$) (Table 3-2) and a plot of the emergence curves shows the longer window of time required for emergence (Figure 3-4). The total number of beetles to emerge was also significantly different between populations ($df=6, 6, F=6.39, P=0.0200$).

Adult Size: Overall size (pronotum width) was found to be significantly different among populations in both males ($df=6, 315, F=35.56, P<.0001$) and females ($df=6, 341, F=31.08, P<.0001$). Males from the AZ population were found to be on average significantly larger than males from all other populations (Table 3-3) (Figure 3-2). UT, CA and CA1 males were of moderate size and not significantly different from one another, yet significantly larger than males from more northern populations, CA3, OR and ID, which were on average the smallest (Table 3-3) (Figure 3-2). In females, patterns were generally similar to those observed in males. Females from the AZ

population were significantly larger than females from all other populations (Table 3-3) (Figure 3-2). The UT population had the second largest individual size on average, and was significantly different from all other populations except CA1. Females from the northern latitude populations, ID, OR, CA3 were the smallest; however, CA and CA1 were somewhat smaller than expected given the size of the males from those same populations (Table 3-4). General trends in decreased size with latitude were observed in clines on both sides of the Great Basin Desert (Figure 3-3), although populations at similar latitudes on opposite sides (e.g., UT and CA3 or AZ and CA) were often significantly different in size (Table 3-3).

Differences in overall size between males and females between populations were sometimes quite pronounced, and the average AZ male, which is typically the smaller of the sexes, was actually larger than the average ID female (Table 3-3). However, post hoc investigation of sexual size dimorphism within populations suggests that the differences in size between the average male and female were rather consistent (Figure 3-5, Table 3-3). Most populations (6 of 7) exhibited ~10-12% difference in size between males and females while the CA population was slightly less dimorphic and showed only a 7% difference (Table 3-3).

DISCUSSION

Genetic differences in *D. ponderosae* development time and adult size were observed among geographically separated populations reared through two generations at a constant temperature (22.5 °C). My findings are consistent with previous studies

describing pronounced local population differences in morphology, susceptibility to cold, and development time (Sturgeon and Mitton 1986; Bentz and Mullins 1999; Bentz et al. 2001). Most striking was the clear biogeographical difference seen in development time. Populations from northern latitudes in the western U.S. (CA3, OR, ID, UT), developed significantly faster and in nearly half the amount of time when compared to populations from the most southern latitudes (CA, CA1, AZ). Furthermore, the timing of development was less synchronized in southern populations, which emerged over a significantly longer window of time than northern populations (Figure 3-4, Table 3-2).

Adult size was variable among populations, and a clear biogeographical break was not evident. Latitudinal trends in size (i.e., decreased size with increased latitude) were pronounced in populations from the east side of the Great Basin (ID, UT, AZ), but less evident in populations from the west side (CA (CA1), CA3, OR). The western populations showed a weak latitudinal trend, but significance varied across the sexes (Figure 3-3) (Table 3-3). For instance, females from different latitudes in the western populations (CA (CA1), CA3 and OR) were not significantly different in size, but in accordance with the converse of Bergmann's rule, males from northern populations (OR and CA3) were significantly smaller than southern populations (CA, CA1). In general, my results are consistent with the broad scale patterns described for many ectotherm species whereby populations from more northern latitudes are both smaller and have faster developmental rates than populations within the same species that are found at more southern latitudes (Dingle and Mousseau 1994; Blanckenhorn and Fairbairn 1995).

The results indicate that there was no significant difference in development time and overall size between the populations on either side of a proposed hybrid sterility threshold (OR and ID) (Chapter 2). Further, the most genetically divergent populations investigated in this study (CA and AZ), based on neutral molecular markers (Mock et al. 2007), were also not significantly different in their development times. In sum, it therefore appears that body size and development time most clearly coincide with latitudinally imposed climatic differences and less with the proposed reproductive boundary (Chapter 2) and patterns of molecular genetic divergence (Mock et al. 2007). These findings indicate that body size and development time variation are strongly influenced by genetically based differences shaped by selection to local climate.

The slower developmental rates in southern populations could be interpreted as an adaptation to the increased thermal input likely encountered in lower latitudes. Although the mechanism is unclear, this could be an adaptation to maintain univoltinism and emergence synchrony (Bentz et al. 2001), which are both considered important to *D. ponderosae* reproductive success (Amman 1973; Safranyik 1978). Such striking differences in developmental timing could potentially lead to temporal reproductive isolation between northern and southern populations if these populations were to ever occur in sympatry. Other evidence suggests that gene flow does occur in an isolation-by-distance pattern between northern and southern populations (Mock et al. 2007) and that fertile offspring are produced when some northern and southern populations are crossed (Chapter 2). Unfortunately, critical information about life history strategies in southern *D. ponderosae* populations is somewhat limited since most investigations have involved northern populations. It seems likely that populations

located between the distinct northern and southern populations might display a gradient of development times, creating a continuum of interbreeding populations. There is some potential evidence of this scenario, as Bentz et al. (2001) found that the F₂ generation of a southern Utah population (geographically intermediate between AZ and UT), reached 50% emergence in ~100-110 days (when reared at ~21° C in two host species). Although unable to make direct comparison because of slight differences in rearing temperature, median development time estimates from Bentz et al. (2001) do appear to fall between the UT population (~80 days) and AZ population (~150 days).

Dendroctonus ponderosae populations used in this study were collected from a variety of latitudes, altitudes, and host species, thereby confounding any one affect (Table 3-1). In addition to the influence of climate, long term selection imposed by different host species may influence morphology in *D. ponderosae* (Sturgeon and Mitton 1986; Langor and Spence 1991). Long term host specificity could be a contributing factor to the variation observed in my results on adult size, but seem less likely a contributing factor in the striking development time differences. The three southern populations were collected from three *Pinus* species (*P. monophylla*, *P. lambertiana*, *P. flexilis*), and in a common garden environment, development time was not significantly different across the three hosts. Differences in adult size were observed across these same populations. However, host alone was not the only factor influencing size in my study. Adults from the UT population, collected from lodgepole pine, were significantly larger than adults from all other populations also collected from lodgepole pine (ID, CA3, OR). Further, sympatric populations CA and CA1 were from two different hosts and were not significantly different in size. Altitude has also been

suggested to influence adult insect size, although the directional patterns are somewhat inconsistent (e.g., Bidau and Marti 2007). In my study I found that the two populations from the highest altitudes (AZ, UT), although from different latitudes, were on average the largest beetles.

There was a remarkably consistent relative difference between males and females with respect to size. This difference did not seem to be affected by latitude, and remained rather constant even as average sex-specific sizes varied among populations, suggesting that the force maintaining this difference is rather constant over populations. Size differences between male and female *D. ponderosae* have previously been described (e.g. Sturgeon and Mitton 1986; Cerezke 1995; Bentz et al. 2001). However, with the exception of Bentz et al. (2001) results from these studies potentially include environmental and maternal effects because measurements were taken on beetles that emerged directly from field collected trees. Environmental influences have been shown to increase variation in size and inflate sexual size dimorphism in many insects (Teder and Tammaru 2005). My findings of sexual size dimorphism and a relatively constant difference between the sexes after multiple generations of random mating in a common garden environment are therefore a unique contribution to our understanding of this species. What maintains these differences is largely unknown, and size assortative mating in *D. ponderosae* has not been found, suggesting that there is no direct sexual selection on size (Pureswaran and Borden 2003). Mate choice in *D. ponderosae* is thought to occur primarily through stridulation (Ryker and Rudinsky 1976) and olfaction (Pureswaran and Borden 2003), yet it is unknown whether sexual selection drives sexual size dimorphism in *D. ponderosae*. Multiple hypotheses have been

proposed to explain the evolution of sexual size dimorphism (Fairbairn 1997) including strong natural selection on female body size, since larger females typically have higher reproductive output (Honek 1993).

In conclusion, I found clear evidence of genetic differences between many populations in development time and body size. These differences did not clearly coincide with previous evidence of restricted gene flow between distant populations around the Great Basin Desert (Mock et al 2007). There was also no apparent phenotypic threshold between populations consistent with the threshold observed in hybrid male sterility (Chapter 2). It therefore appears that adaptive divergence in response to latitudinally-imposed differences in climate is the best explanation for divergence in body size and development time within *D. ponderosae*.

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Table 3-1. Collection location and host tree species of *Dendroctonus ponderosae* populations sampled for body size and development time comparisons.

Population	Locality (nearest city)	Elevation (ft.)	Latitude and Longitude	Host tree
CA	Big Bear Lake, CA	6865	34° 15' N, 116° 54' W	<i>Pinus monophylla</i>
CA1	Arrowbear Lake, CA	6656	34° 12' N, 117° 03' W	<i>Pinus lambertiana</i>
CA3	Old Station, CA	4879	40° 37' N, 121° 29' W	<i>Pinus contorta</i>
OR	Prairie City, OR	5252	44° 17' N, 118° 24' W	<i>Pinus contorta</i>
ID	Stanley, ID	6588	44° 17' N, 115° 02' W	<i>Pinus contorta</i>
UT	Garden City, UT	7162	41° 58' N, 111° 31' W	<i>Pinus contorta</i>
AZ	Flagstaff, AZ	9230	35° 19' N, 111° 42' W	<i>Pinus flexilis</i>

Table 3-2. Parameter estimates of a three parameter logistic growth model fit to emergence data from seven *Dendroctonus ponderosae* populations reared in a common garden environment. Pairwise differences between the populations for each parameter estimate were tested using Tukey's HSD test. Means followed by the same letter within a column are not significantly different.

Population	Total Emerged (K)	Median Development Time (t_m)	10%- 90% Emergence (Δt)
CA	83 (32.63)b	133.95 (6.22)a	74.99 (5.38)a
CA1	55 (32.63)b	154.21 (6.22)a	87.25 (5.38)a
CA3	133 (32.63)ab	73.08 (6.22)b	26.05 (5.38)b
OR	291 (32.63)a	73.01 (6.22)b	27.02 (5.38)b
ID	140 (32.63)ab	69.33 (6.22)b	25.97 (5.38)b
UT	108 (32.63)b	75.99 (6.22)b	27.10 (5.38)b
AZ	98 (32.63)b	149.05 (6.22)a	82.85 (5.38)a

Table 3-3. Mean pronotum width (mm) of *Dendroctonus ponderosae* from seven populations reared in a common garden environment. Pairwise differences in size between populations were tested using a Tukey-Kramer HSD test. Also shown is the percent sexual size dimorphism between male and female adult beetles for each population. Means followed by the same letter within a column are not significantly different.

<i>Population</i>	<i>n</i>	<i>Male pronotum (mm)</i>	<i>n</i>	<i>Female pronotum (mm)</i>	<i>Percentage dimorphism^a (%)</i>
CA	44	1.81 (0.01)b	50	1.95 (0.02)c	7
CA1	29	1.80 (0.02)b	48	1.99 (0.01)bc	10
CA3	50	1.73 (0.01)c	50	1.94 (0.01)cd	11
OR	50	1.70 (0.01)c	50	1.92 (0.02)cd	11
ID	50	1.68 (0.01)c	50	1.88 (0.01)d	11
UT	49	1.81 (0.01)b	50	2.05 (0.01)b	12
AZ	50	1.90 (0.02)a	50	2.14 (0.02)a	11

^a Computed from means, $(F - M)/F * 100$

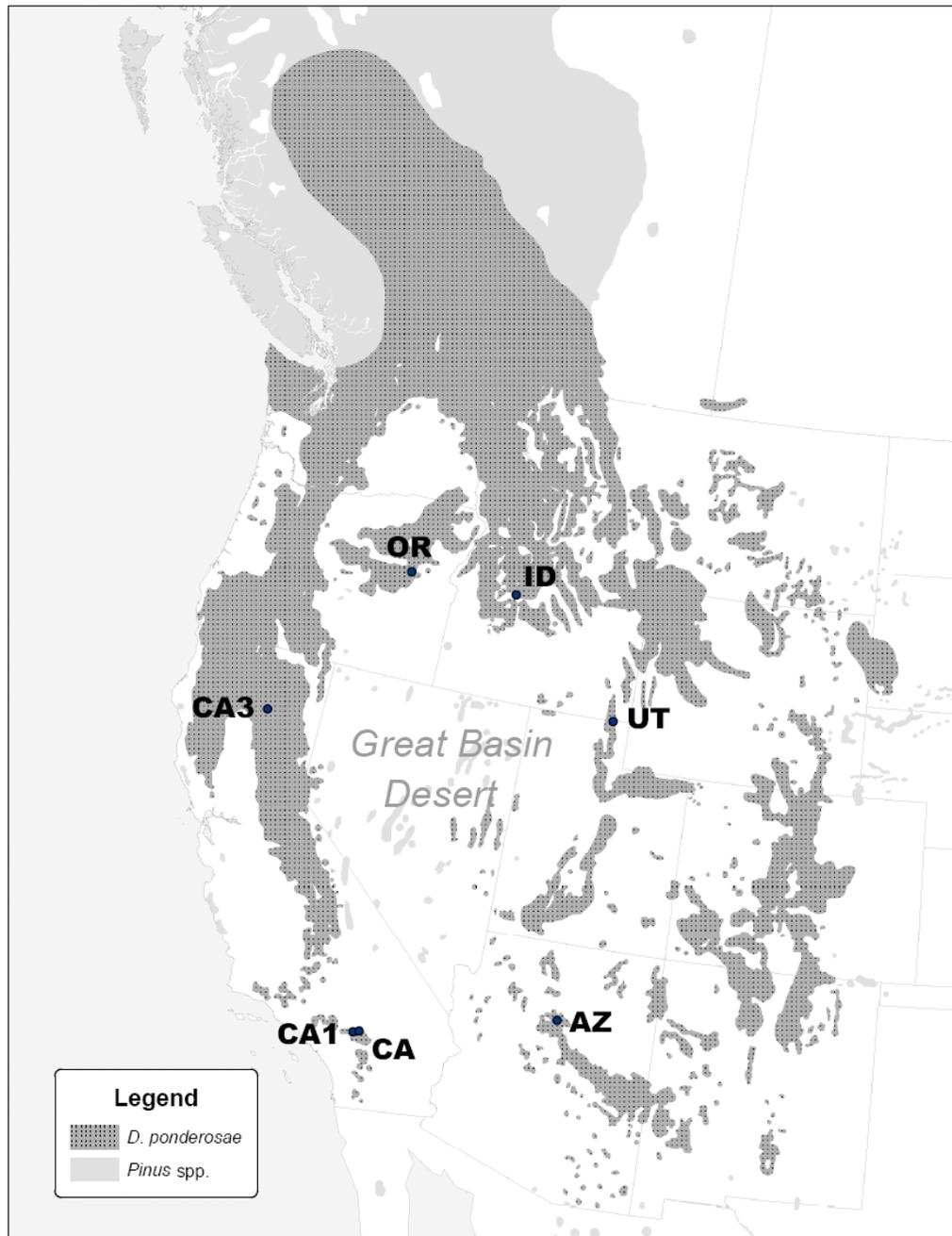


Figure 3-1. Location of *Dendroctonus ponderosae* populations sampled for body size and development time comparisons. Additional details for each population are found in Table 3-1.

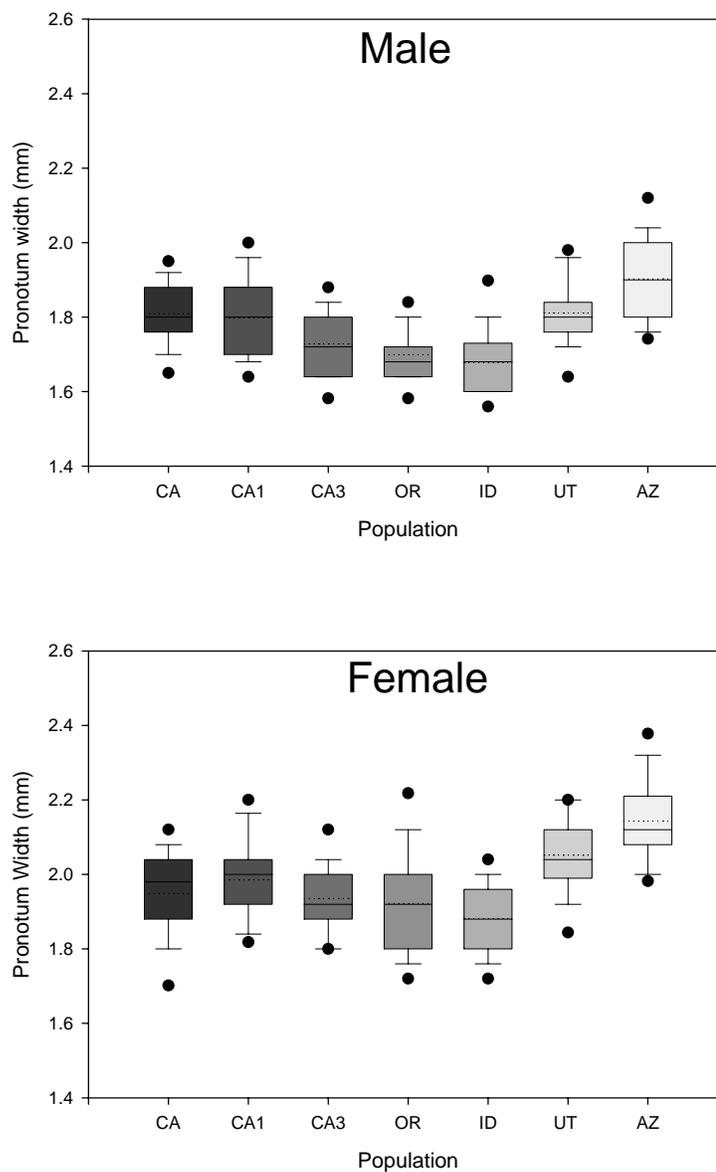


Figure 3-2. Pronotum width (mm) of adult beetles from seven *D. ponderosae* populations after two generations in a common garden environment. The most genetically divergent populations (CA (CA1), and AZ (Mock et al. 2007)) are displayed at the far left and far right. Outliers (●) are of the 5/95th percentile.

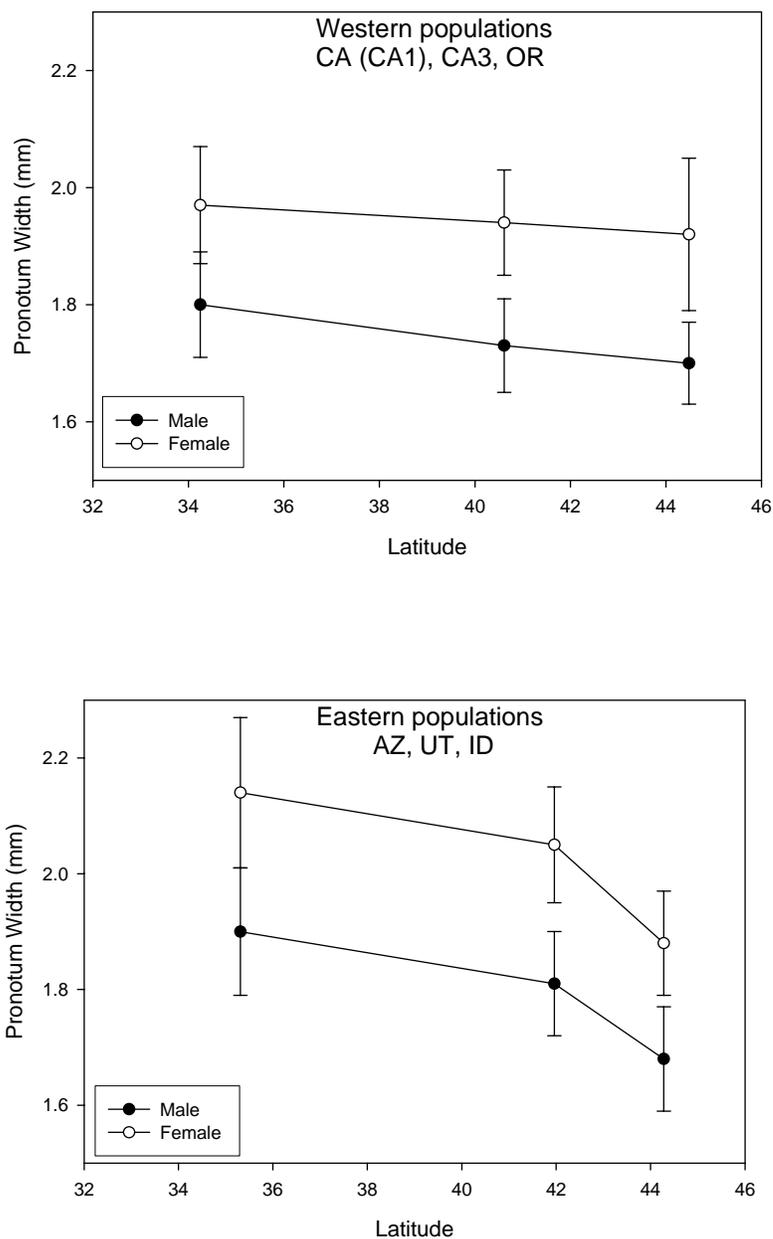


Figure 3-3. Pronotum width (mean and one standard deviation) of adults collected from populations located along latitudinal clines on either side of the Great Basin Desert. Sympatric CA and CA1 populations were pooled together since they were not significantly different from one another (Table 3-3). A general trend of decreasing size with increased latitude is seen, yet stronger in eastern populations.

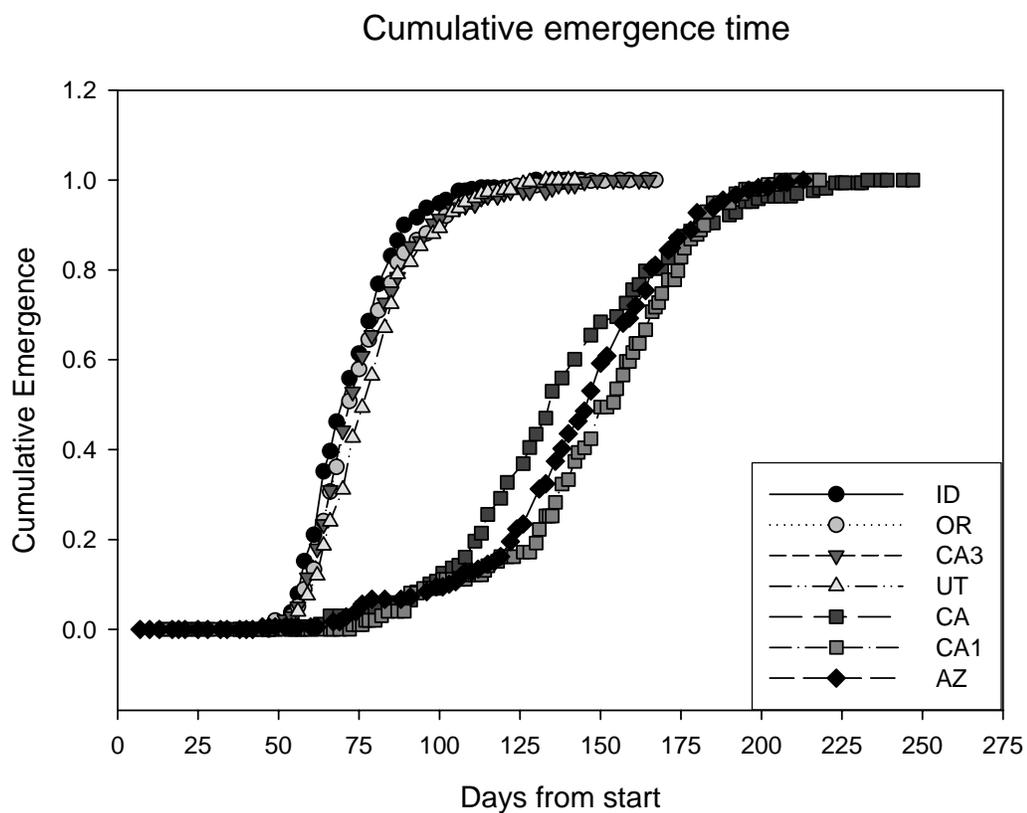


Figure 3-4. Cumulative emergence time of *Dendroctonus ponderosae* from seven populations reared in a common garden environment. Two distinct groups are apparent, and populations from northern latitudes (CA3, OR, ID, UT) developed faster than individuals from southern populations (CA, CA1, AZ).

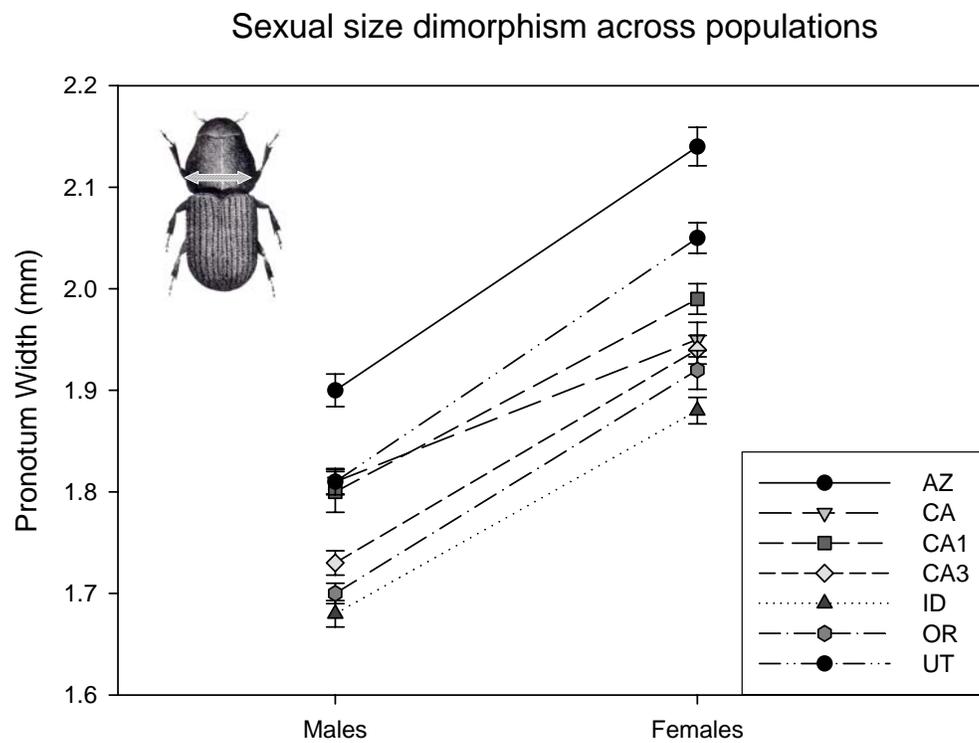


Figure 3-5. Mean and standard error of pronotum width (mm) of males and females from seven *Dendroctonus ponderosae* populations.

CHAPTER 4

SUMMARY

The overarching goal of my thesis research was to investigate and characterize postzygotic isolation and phenotypic divergence in *D. ponderosae* to gain a better understanding of potential mechanisms facilitating species formation. I found clear evidence that of the *Dendroctonus ponderosae* populations used in my study, all crosses produced both male and female hybrid offspring. Therefore, there was no evidence of hybrid inviability. However, hybrid male offspring from the two most geographically distant crosses (CA x ID and CA x UT) appear to be largely incapable of reproduction. I interpret these findings as evidence of hybrid male sterility within what is currently described as *D. ponderosae*, and these results clearly conform to what is thought to be the earliest sign of postzygotic isolation, Haldane's rule (Coyne and Orr 1989, 1997). Furthermore, sterility appears to be incomplete since some hybrid males are still able to produce offspring, suggesting that an incipient speciation event may be underway (Reed and Markow 2004; Kropp and Frank 2005). Surprisingly, the onset of hybrid male sterility appears to occur at a threshold in population crosses and less geographically distant crosses are not adversely affected and appear to have comparable levels of hybrid fitness.

I also found genetic differences between populations in two critical life history traits, development time and body size. Most striking were the latitudinal differences in development time, with the more northern populations developing in nearly half the amount of time required by the more southern populations. Further, I found that

populations typically followed previously described body size trends in insects (Mousseau 1997) with populations in southern latitudes being generally larger than their conspecifics. Interestingly, phenotypic differences between populations appeared to coincide most directly with latitudinal (climatic) adaptations, and less with previously described neutral gene flow patterns and genetic divergence (Mock et al. 2007). Furthermore, there were no phenotypic differences between populations corresponding to the hybrid male sterility threshold (OR and ID populations) described in Chapter 2.

To date, many studies of divergence and speciation in bark beetles, and *D. ponderosae* in particular, have focused on the evolution of host races (e.g., Sturgeon and Mitton 1986; Langor et al. 1990) and less on allopatric speciation, although evidence of geographic isolation facilitating genetic divergence in other bark beetles is common (e.g., Six et al. 1999; Kelley et al. 1999). This focus on sympatric speciation via host race formation seems odd given that allopatric speciation has long been considered the predominant avenue for speciation (Mayr 1963; Coyne and Orr 2004). Future studies of speciation in phytophagous insects and particularly bark beetles should include in-depth investigations of multiple populations from throughout the species range, and integrate multiple techniques including phylogeography, comparative analyses of multiple phenotypic traits, and tests of reproductive compatibility. Any one of these techniques used independently could identify quite different and potentially contrasting mechanisms contributing to speciation. This is clearly evident within *D. ponderosae*, since crossing studies suggest hybrid male sterility occurs at a boundary between two populations (Chapter 2) that do not differ in critical phenotypic traits

(development time and size; Chapter 3) and show no evidence of a gene flow constriction between them (Mock et al. 2007).

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