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CRYPTIC POSTZYGOTIC ISOLATION IN AN ERUPTIVE SPECIES OF BARK BEETLE (*DENDROCTONUS PONDEROSAE*)

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Studies of postzygotic isolation often involve well-differentiated taxa that show a consistent level of incompatibility, thereby limiting our understanding of the initial stages and development of reproductive barriers. *Dendroctonus ponderosae* provides an informative system because recent evidence suggests that distant populations produce hybrids with reproductive incompatibilities. *Dendroctonus ponderosae* shows an isolation-by-distance gene flow pattern allowing us to characterize the evolution of postzygotic isolation (e.g., hybrid inviability, hybrid sterility) by crossing populations along a continuum of geographic/genetic divergence. We found little evidence of hybrid inviability among these crosses. However, crosses between geographically distant populations produced sterile males (consistent with Haldane's rule). This effect was not consistent with the fixation of mutations in an isolation-by-distance pattern, but instead is spatially localized. These reproductive barriers are uncorrelated with a reduction in gene flow suggesting their recent development. Crosses between geographically proximal populations bounding the transition from compatibility to hybrid male sterility showed evidence of unidirectional reduction in hybrid male fecundity. Our study describes significant postzygotic isolation occurring across a narrow and molecularly cryptic geographic zone between the states of Oregon and Idaho. This study provides a view of the early stages of postzygotic isolation in a geographically widespread species.

KEY WORDS: **Hybrid sterility, mountain pine beetle, reproductive incompatibility, speciation.**

A fundamental question in biology is what causes a single interbreeding species to diverge into two reproductively isolated species. An essential component of speciation is the formation of reproductive barriers that impede and eliminate gene flow. 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 characterizing these barriers and determining their strength and development through time is crucial to our understanding of species boundaries and species formation. An ongoing difficulty in speciation research is the identification of the initial

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reproductive barrier(s) facilitating divergence, because multiple barriers can accumulate and be replaced during the complete speciation process (Ramsey et al. 2003; Coyne and Orr 2004; Sobel et al. 2010). The initial barrier(s) that trigger divergence and species formation could be quite different from the barriers that exist between species further along in the speciation process.

Postzygotic isolating barriers have been a focus of many speciation studies, as postzygotic isolation is largely considered irreversible. Comparative meta-analyses have characterized the evolution of postzygotic isolation in several groups, including fruit flies (Coyne and Orr 1989, 1997), frogs (Sasa et al. 1998), butterflies (Presgraves 2002), and birds (Price and Bouvier 2002). In insects, reproductive 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*] or the female in ZW taxa [Lepidoptera]). Hybrid sterility of the heterogametic sex is widely considered the first postzygotic barrier to form in nascent species (Wu et al. 1996; Coyne and Orr 2004).

Hybrid sterility of the heterogametic sex has been found to be polygenic and epistatically complex in animals (Davis and Wu 1996; Orr and Irving 2001; Good et al. 2008; Reed et al. 2008) and is most often observed in taxa that have already been recognized as separate species or subspecies (Laurie 1997). Therefore, although hybrid male sterility is typically the first postzygotic barrier to arise in male heterogametic taxa, by the time it is documented, it is genetically complex and selection and drift have been operating for long periods, 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 onset of hybrid sterility, some argue that postzygotic isolation typically arises well after the prezygotic barriers that initiate the speciation event (Mallet 2006). Additionally, studies of postzygotic isolation are often unable to assess the ecological processes that might facilitate divergence because the study organisms inhabit different environments, and are often allopatric (Sobel et al. 2010).

Recent evidence in a nonmodel species of a broadly distributed insect has potentially uncovered a case of incipient postzygotic isolation. The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae) is a native bark beetle in western North American forests that feeds and reproduces in the phloem layer of at least 11 species of pine (Wood 1982). It has a widespread distribution (Fig. 1) and is a

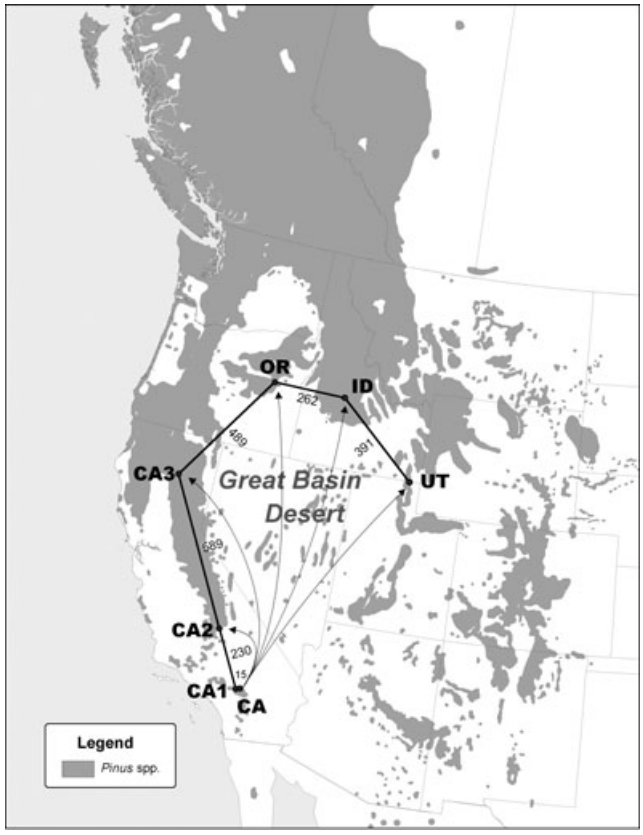

Figure 1. ***Dendroctonus ponderosae*** **host tree distribution, population collection areas and schematic diagram of crossing experiment. The distribution of** ***D. ponderosae*** **generally follows that of its primary host pines (*Pinus albicaulis*, *P. contorta*, *P. flexilis*, *P lambertiana*, *P. monticola*, *P. ponderosa*), extending northward into British Columbia and western Alberta, Canada, and south into Arizona and southern California, United States. The northern and southern boundaries of** ***D. ponderosae*** **are currently limited by climate, not host trees, and are difficult to identify due to low population densities. The CA population was hybridized with increasingly divergent populations collected from around a proposed gene flow barrier, the Great Basin Desert (Mock et al. 2007). Previous research identified severe hybrid breakdown when CA was crossed with ID (B. J. Bentz et al. unpubl. data). Numbers represent kilometers between adjacent populations.**

species of great interest because outbreaks are often landscape-level events, causing considerable tree mortality (Cole and Amman 1980; Safranyik et al. 2010). In a recent study, hybrid breakdown was observed in crosses between beetle populations from southern California and central Idaho (B. J. Bentz et al. unpubl. data). The hybrid breakdown was quite unexpected given previous crossing experiments and karyological studies (Hay 1956; Lanier and Wood 1968) suggesting that geographically distinct *D. ponderosae* populations comprise a single species. Moreover, a recent range-wide phylogeographic analysis of *D. ponderosae* using both nuclear and mitochondrial markers did not find pronounced genetic divergence between populations from the

same areas that showed hybrid breakdown when crossed (Mock et al. 2007).

Joint consideration of the findings of B. J. Bentz et al. (unpubl. data) 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 find substantial divergence or a spatially abrupt decrease in gene flow indicative of reproductive isolation. 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). However, the impact of parental divergence on reproductive compatibility is often hard to predict (Edmands 2002) and postzygotic isolation conforming to Haldane's rule can appear uncorrelated with geographic distance (Demuth and Wade 2007). Therefore, *D. ponderosae* provides a unique opportunity to characterize postzygotic isolation due to its clinal distribution with an embedded reproductive incompatibility. Additionally, by describing the pattern leading to postzygotic isolation we can gain some insight into the underlying genetic complexity of the incompatibility; a gradual, negative correlation between reproductive fitness and crossing distance is suggestive of the accumulation of small, deleterious, genetic effects, whereas a threshold would be suggestive of an abrupt genetic change between differentiated populations. A clear mismatch between patterns of reproductive incompatibility and gene flow patterns described with molecular markers could suggest recent postzygotic isolation, and/or indicate ongoing gene flow in spite of the negative effects of interpopulation mating. Our objective was to describe the geographic pattern of postzygotic reproductive isolation in *D. ponderosae* and determine whether isolation increases in a linear fashion between increasingly geographically/genetically divergent populations.

Materials and Methods

STUDY ORGANISM

Dendroctonus ponderosae is an economically and ecologically important insect with well-characterized life history and reproduction (Reid 1958, 1962a,b; Amman 1972). A male/female pair constructs a tunnel (gallery) under the bark of a tree while traveling vertically in the phloem layer, ovipositing egg clusters in alternating niches on opposite sides of the gallery. After egg hatch, the larvae feed horizontally from the parent gallery, pupate, and emerge from underneath the bark. Laboratory rearing protocols for *D. ponderosae* are well established (e.g., Lanier and Wood 1968; Langor et al. 1990; Bentz et al. 2001), and beetles are easily propagated in freshly cut tree sections. Laboratory-reared females are ∼98–99% virgin (Reid 1958; McCambridge 1969a,b) and males are able to mate multiple times (B. J. Bentz, unpubl. data). Sex of adult *D. ponderosae* is easily determined using morphological differences on the seventh abdominal tergite (Lyon 1958). Males are the heterogametic sex (Lanier and Wood 1968). Generation time (time from parent coupling to brood adult emergence) in a laboratory setting at 21°C varies, ranging from ∼60 to 110 days depending on the original geographic location of the population (Bentz et al. 2001). Reproductive output from *D. ponderosae* matings can be quantified by peeling off the bark and phloem and counting eggs and signs of egg hatch (larvae or larval mines).

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 F_1 offspring. Seven populations, located around the perimeter of the Great Basin Desert and representing a large portion of the species range, were selected for sampling (Table 1, Fig. 1). Because a pattern of genetic isolation-by-distance was found to exist in a horseshoe pattern around the Great Basin Desert in *D. ponderosae* (Mock et al. 2007), we assumed that by selecting increasingly geographically divergent populations we 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), and apparent reproductive incompatibility with at least one other population (ID) (B. J. Bentz et al. unpubl. data). A

Table 1. Collection location and host tree species for *Dendroctonus ponderosae* used in population crosses.

Identifier	Locality (nearest city)	Elevation (m)	Latitude and longitude	Host tree
CA	Big Bear Lake, CA	2092	34° 15′ N, 116° 54′ W	*Pinus monophylla*
CA1	Arrowbear Lake, CA	2029	34° 12′ N, 117° 03′ W	*Pinus lambertiana*
CA2	Kernville, CA	2722	36° 01′ N, 118° 15′ W	*Pinus contorta*
CA3	Old Station, CA	1487	40° 37′ N, 121° 29′ W	*Pinus contorta*
OR	Prairie City, OR	1601	44° 17′ N, 118° 24′ W	*Pinus contorta*
ID	Stanley, ID	2008	44° 17′ N, 115° 02′ W	*Pinus contorta*
UT	Garden City, UT	2183	41° 58′ N, 111° 31′ W	*Pinus contorta*

population sympatric with CA but utilizing a different host tree species (*Pinus lambertiana*) was also sampled (CA1) to test for reproductive isolation arising from host use. The remaining five populations were selected in an attempt to span and exceed the geographic distance between CA and ID while sampling around the Great Basin Desert (Table 1) (Fig. 1).

FIELD COLLECTION AND LABORATORY PROPAGATION

Dendroctonus ponderosae were field-collected by felling infested trees containing multiple larvae and cutting each tree into 35–40 cm 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 adult development was complete. Rearing containers consisted of metal cans with glass collecting jars fixed to the outside. Upon emerging from the tree sections, adult beetles quickly migrate from the dark container to the illuminated jar. Emerging adults were collected daily, placed in Petri dishes lined with filter paper moistened with distilled water, and stored up to 20 days at ~3°C. Adults were randomly selected from the peak of emergence for each population (~15 days of highest cumulative number of beetles), and used for crossing experiments. Individuals were selected from the period of peak emergence to obtain beetles representative of population specific development time characteristics and to decrease the probability of collecting re-emerging, reproductively exhausted parents.

All crosses were performed in a common garden environment (~21°C, photoperiod ~9L:15D) and were achieved by placing a female, and then a male, in a predrilled hole in the phloem layer of fresh uninfested field-collected tree sections. Slight differences in crossing protocols were used for each generation and are described below. All crosses were performed in a common host tree species, lodgepole pine (*Pinus contorta* var. *latifolia*) using bolts (~40 cm long tree sections) acquired from the Wasatch-Cache National Forest, Utah on two separate occasions. All bolts were sealed with paraffin wax to reduce desiccation. Placing a male and female into a predrilled hole in the phloem (hereafter termed a pair) does not guarantee mating, which is defined by copulation and sperm transfer. Mating was not directly verified for any pairing but assumed if oviposition occurred. Unmated females were evaluated for gallery construction and oviposition to check this assumption (described under Hybrid fitness assay, below).

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,

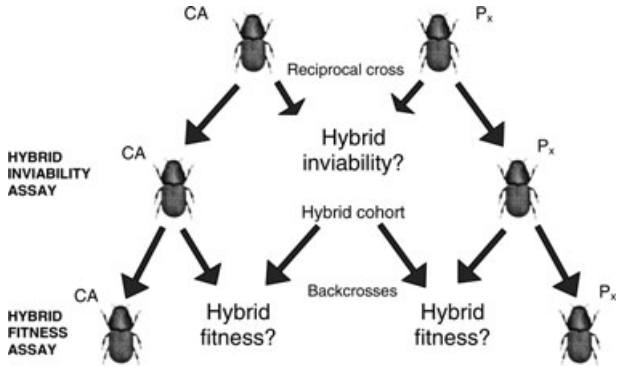

Figure 2. Diagram of common garden crossing experiment used to investigate postzygotic isolation in *Dendroctonus ponderosae*. Reciprocal crosses were made between field collected CA and P_x populations (where P_x are increasingly divergent populations, CA1-UT) to determine if hybrids are produced (Hybrid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

we conducted (1) reciprocal crosses (CA ♂ × P_x ♀ and CA ♀ × P_x ♂; Fig. 2) between the CA population and each of the remaining six populations (P_x) and (2) crosses within each population (CA ♂ × CA ♀ and P_x ♂ × P_x ♀; Fig. 2). For each of the 12 reciprocal interpopulation crosses and seven intrapopulation crosses, male/female pairs were manually inserted into a randomly selected bolt from one of two lodgepole pine cut just prior to the experiment. Ten replicate pairs for each cross were set up in this manner for interpopulation crosses and 20 pairs for intrapopulation crosses. Larger quantities of intrapopulation crosses were performed to ensure sufficient progeny for backcrossing experiments (see below). Growing space was standardized by spacing each male/female pair 3 cm from its neighbor around the circumference of each bolt. A small section of wire screen was fixed over the entrance hole to prevent immediate escape of adults. Each bolt was placed in a separate rearing container and maintained at ambient room temperature (~21°C).

Each bolt contained offspring from multiple pairs from a specific cross (i.e., a cohort). F_1 progeny from interpopulation and intrapopulation crosses were collected daily, placed in Petri dishes lined with filter paper moistened with distilled water, and stored at ~3°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.

The number of fertile pairs was tabulated for each interspecific and intraspecific cross. Offspring sex ratio was calculated from 50 randomly selected individuals to ensure both sexes were being produced, and total number of offspring was tabulated for

each cohort. A pair was considered able to produce viable offspring if there were ≥5 pupal chambers with adult emergence holes. Sex ratios of interpopulation crosses were compared directly to intrapopulation crosses and not the Fisherian 50:50 because *D. ponderosae* populations are known to produce female-skewed sex ratios (e.g., Amman and Cole 1983; Cerezke 1995) and therefore fewer hybrid males were expected.

HYBRID FITNESS ASSAY

Hybridization may affect progeny fitness in a variety of ways, including outbreeding vigor (heterosis), outbreeding depression, and hybrid sterility. Hybrid sterility may be physiological (e.g., compromised reproductive system or gametes) or behavioral (e.g., incapable of successful courtship). To assess the reproductive fitness of F_1 hybrid progeny, backcrosses (crosses between F_1 progeny from interpopulation crosses and F_1 progeny from intrapopulation crosses) were performed (Fig. 2). Backcrosses allow a direct assessment of reproductive fitness and sterility of F_1 hybrids.

The hybrid fitness assay was performed using ∼40 cm long bolts from two live lodgepole pines collected just prior to the experiment. To standardize the resources available for each mating pair, and eliminate gallery overlap, crosses were performed as described above except that strips were removed between predrilled holes, thereby confining each male/female pair to one longitudinal 6.1-cm-wide section of bark and phloem. A maximum of 12 different hybrid cohorts were 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 48 possible backcross combinations (12 hybrid cohorts × 2 sexes × 2 source populations). Each backcross combination was replicated with 10 pairings in a randomly selected bolt. Intrapopulation crosses were performed again to produce an F_2 generation and were 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 individually into bolts to investigate gallery construction and oviposition in putatively unmated females.

Dendroctonus ponderosae is highly fecund, and a female can easily oviposit more than 100 eggs (Amman 1972), which are difficult to accurately score as viable in a timely manner given the rapid desiccation of inviable eggs and the size of the experiment. Therefore, all pairs (and female only insertions) were allowed to proceed for 26 days from pair insertion before reproduction was halted through refrigeration. The remaining bark and phloem were then removed 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 published egg hatch and gallery extension rates at room temperature (Logan and Amman 1986; Bentz et al. 1991). Fifteen centimeters was chosen based on pilot studies to maximize the quantity of eggs and larvae recorded while taking into consideration the difficulty of identifying inviable eggs that desiccate through time. For each pair, number of eggs and larvae within the first 15 cm of gallery, total length of the gallery (cm), and total number of larvae in the entire gallery (total egg hatch) were recorded.

Four fitness measures were used in the analysis: number of eggs oviposited in the first 15 cm, proportion of eggs oviposited in the first 15 cm that were viable (number of larvae/number eggs oviposited), total gallery length, and total egg hatch. F_1 intrapopulation crosses were analyzed separately to determine whether population-level differences in these same fitness measures were present. Based on previous studies, there was an expectation that some pairings would fail to oviposit, and/or the adults would prematurely emerge from bolts and fail to construct a gallery (Lanier and Wood 1968). Increases in the failure to oviposit or failure to construct a gallery in backcrosses, when compared to the F_1 intrapopulation crosses, could suggest prezygotic isolation via behavioral sterility (F_1 hybrids with intermediate phenotypes have courtship or communication difficulties in backcrosses). Oviposition and early parent adult emergence from a bolt were tallied as binary variables. If at least one egg was deposited, oviposition was considered successful (1) whereas no oviposition resulted in a 0. If a pair failed to construct at least 15 cm, gallery failure was tallied as 0 for that pair whereas galleries ≥15 cm received a 1.

Significant differences in fitness measures between backcrosses and F_1 intrapopulation crosses were tested using generalized linear mixed models in the SAS procedure GLIMMIX (SAS Institute, Cary, NC, version 9.1.3). GLIMMIX is a flexible approach that models both fixed and random effects and handles nonnormal response distributions and overdispersed data. In F_1 intrapopulation analyses, each fitness variable was modeled using an error distribution specific to the response type. The proportion of viable eggs was modeled using a binomial distribution, total egg hatch and the number of eggs oviposited were count data and modeled using a Poisson distribution, and total gallery length was modeled using a normal distribution (Gaussian distribution). Pairwise differences between F_1 intrapopulation crosses were tested using a Tukey–Kramer Honestly Significant Differences multiple comparison test. For backcross analyses, fitness measures were standardized to the midparent mean. Standardizing provides a way to indicate the magnitude of the deviation from the additive expectation and allows for comparisons between hybrids produced from populations differing in fitness measures (Edmands 1999). The method for modeling each backcross fitness measure was determined based on iterative model fitting and determining best-fit models based on residual plots. Total gallery length, proportion of viable eggs, and total egg hatch were standardized by dividing each observation by the midparent mean and modeled using a normal (Gaussian) distribution and a dispersion parameter. The

number of eggs oviposited was modeled using a Poisson distribution, with an offset equal to the logarithm of the midparent mean, and a dispersion parameter.

F_1 intrapopulation crosses were analyzed for the four fitness measures using the source population as the main fixed effect. 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 kilometers around the Great Basin Desert from CA, Fig. 1), (2) sex of the CA parent (reciprocal), (3) sex of the F_1 hybrid individual used in the 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 with cross type (e.g., backcross or F_1 intrapopulation cross) used as a fixed effect.

Results

HYBRID INVIABILITY

All interpopulation crosses produced hybrid male and female offspring (Table 2). The male:female sex ratio of each of the F_1 hybrid cohorts varied from 1:0.85 to 1:2.57, and were nearly entirely within the range of intrapopulation crosses (1:0.85 to 1:2.33). Difficulty in rearing beetles out of field-collected trees for the CA2 population reduced the number of pairings for intrapopulation crosses, from 20 to 10 pairs. The overall proportion of fertile pairs was very high for all intrapopulation crosses (0.95). Interpopulation crosses also showed high fertility (≥0.95 in 11 of 12 crosses). In the CA ♀ × OR ♂ cross, there was a slight reduction to 0.80 (Table 2). Of the few crosses that did not produce offspring (both intrapopulation and interpopulation), it was usually due to premature death of one of the pair.

HYBRID FITNESS

All crosses produced ample quantities of F_1 progeny; therefore, a total of 480 backcross pairings and 140 F_1 intrapopulation pairings were performed in the hybrid fitness analysis. A total of 66 backcrosses (14%) failed to construct at least a 15 cm gallery and 63 (13%) failed to oviposit. Similar percentages were observed in F_1 intrapopulation crosses (23/140, ∼16%, and 14/140, ∼10%, respectively). Cross type (i.e., backcross or F_1 intrapopulation cross) was not found to be a significant predictor of oviposition or gallery failure. These results suggest that in our backcrosses there was little evidence of prezygotic isolation; that is, no significant increases in the number of failed galleries or failure to oviposit, relative to the F_1 intrapopulation crosses. Subsequent analyses were conducted only on pairs that oviposited, regardless of gallery length.

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

Crosstype	Maternal population	Paternal population	Total offspring	Proportion fertile[1] (*n*)	Male:Female sex ratio
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
Interpopulation					
	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

[1]Pair considered fertile and able to produce viable offspring if evidence of ≥5 pupal chambers with exit hole, indicating emergence of adult.

Table 3. **Fitness characteristics of *D. ponderosae* populations, as assessed from intrapopulation crosses. Values given as arithmetic means and ±1 standard error. Means followed by the same letter within a column are not significantly different. Eggs oviposited and proportion of viable eggs are based on measurements from the initial 15 cm of gallery.**

Population	Number of crosses	Proportion fertile[1]	Eggs oviposited	Proportion of viable eggs	Total gallery length (cm)	Total eggs hatched
CA	19	0.95	17.84 (±1.50)d,e	0.50 (±0.08)e	45.84 (±2.1)a	25.95 (±3.78)c,d
CA1	16	1.00	15.50 (±1.88)e	0.81 (±0.05)c,b,d	39.88 (±3.6)b,a	27.25 (±5.10)c,b
CA2	18	0.94	26.06 (±1.97)b	0.80 (±0.06)b	31.86 (±2.5)b,c	28.22 (±2.83)c,b
CA3	17	0.88	20.82 (±1.50)d,c	0.68 (±0.08)d	40.97 (±2.7)b,a	30.94 (±4.46)c,b
OR	19	0.95	23.31 (±2.21)b,c	0.76 (±0.07)c,b	35.79 (±2.0)b,a,c	31.36 (±4.03)b
ID	18	0.78	20.61 (±2.40)d,c	0.67 (±0.09)c,d	26.91 (±3.4)c	21.67 (±4.08)d
UT	19	1.00	28.32 (±1.45)a	0.91 (±0.02)a	44.45 (±2.6)a	47.32 (±4.88)a

[1]**Coupling considered fertile if total egg hatch>0.**

F_1 intrapopulation crosses

Significant differences among F_1 intrapopulation crosses were observed in the four fitness characteristics measured (Table 3). The proportion of viable eggs was significantly different among F_1 intrapopulation crosses (df = 6, 119, $F = 32.33$, $P < 0.0001$), with proportions ranging from 0.50 in CA to 0.91 in ID (Table 3). Significant differences among F_1 intrapopulation crosses were also detected in the number of eggs oviposited (df = 6, 119, $F = 16.09$, $P < 0.0001$), total gallery length (df = 6, 119, $F = 6.54$, $P < 0.0001$), and total egg hatch (df = 6, 119, $F = 39.57$, $P < 0.0001$) (Table 3). No clear geographical grouping between populations with significantly different fitness measures was observed (Table 3). 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 3).

Backcrosses

Multiple main effects and interactions were significant in explaining differences in the proportion of viable eggs among backcrosses (Table 4). Geographic distance from CA, hybrid sex, backcross population and the interaction of geographic distance × hybrid sex were found to be highly statistically significant, with particularly high F values (Table 4). Backcrosses that used hybrid males from the most geographically distant crosses (CA × ID and CA × UT) resulted in low proportion of viable eggs (Fig. 3A). Backcrosses using hybrid females from these same hybrid cohorts did not show a decrease in proportion viable eggs, and were either greater than or similar to the midparent means (Fig. 3A).

Similarly, geographic distance from CA, hybrid sex, and the interaction of geographic distance × hybrid sex were significant in explaining differences in total egg hatch among backcrosses (Table 4). An increase in total egg hatch in backcrosses using hybrid males from the geographically proximal CA × CA2 cross suggests some heterosis at intermediate crossing distances (Fig. 3B). However, total egg hatch in 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 (Fig. 3B). Total egg hatch from backcrosses using hybrid females were similar to the midparent mean in all crosses except ID (1585 km from CA), where a 40% increase was observed.

Significant differences among backcrosses in number of eggs oviposited were found for two main effects and several interactions (Table 4). Consistent with total egg hatch and proportion of viable eggs, geographic distance, hybrid sex, and the geographic distance × hybrid sex interaction had particularly high F values and were highly statistically significant (Table 4). The lowest number of eggs oviposited were observed in backcrosses that used hybrid males from the most distant crosses (CA × ID, CA × UT) (Fig. 3C). These were the same crosses with low egg viability and highly reduced total egg hatch.

Total gallery length was highly variable among backcrosses and multiple main effects and their interactions were significant, including the interaction of geographic distance × hybrid sex (Table 4). Backcrosses using F_1 hybrid males from more geographically distant crosses (CA × OR, CA × ID, CA × UT) resulted in reduced total gallery length relative to midparent means (Fig. 3D).

Hybrid males from the two most distant crosses, (CA × ID and CA × UT), showed a drastic decrease in fertility, when considering both egg viability and total egg hatch (Tables 5 and 6). Only six of 72 backcrosses had any egg hatch in contrast to female hybrids originating from the same hybrid cohort that were found to have high fertility rates (63 of 66) (Table 6). We consider this clear evidence of hybrid male sterility, although most backcrosses utilizing hybrid males had at least one pair with some egg hatch (Table 6). We found some evidence of increased sterility in one direction of the cross; hybrid males from CA mothers had only 1 of 34 galleries with egg hatch (only three eggs hatched), whereas

Table 4. GLIMMIX model results testing for significant differences among backcrosses in four fitness characteristics. Eggs oviposited and proportion of viable eggs are based on measurements from the first 15 cm of gallery.

Effect		Eggs oviposited		Proportion of viable eggs		Total egg hatch		Total gallery length	
	df	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value	*F* value	*P* value
Geographic distance	5,369	10.31	<.0001	18.90	<.0001	9.76	<.0001	4.83	0.0003
Hybrid sex	1,369	11.72	0.0007	38.68	<.0001	26.51	<.0001	5.40	0.0207
Reciprocal	1,369	0.22	ns	0.50	ns	0.12	ns	6.99	0.0085
Backcross population	1,369	2.14	ns	19.41	<.0001	0.02	ns	10.36	0.0014
Reciprocal×hybrid sex	1,369	0.76	ns	2.66	ns	3.58	ns	11.64	0.0007
Geographic distance× backcross population	5,369	4.66	0.0004	5.16	0.0001	1.61	ns	0.75	ns
Backcross population× hybrid sex	1,369	5.00	0.0260	0.43	ns	0.00	ns	4.91	0.0272
Reciprocal×backcross population	1,369	3.94	0.0479	0.00	ns	1.02	ns	11.02	0.001
Geographic distance× hybrid sex	5,369	9.25	<.0001	33.08	<.0001	18.01	<.0001	2.49	0.0310
Geographic distance× reciprocal	5,369	1.64	ns	0.96	ns	2.27	0.0472	2.07	ns
Geographic distance× backcross population× hybrid sex	5,369	1.90	ns	0.58	ns	1.26	ns	2.97	0.0121
Geographic distance× reciprocal×hybrid sex	5,369	0.71	ns	6.25	<.0001	0.71	ns	2.29	0.0451
Geographic distance×reciprocal× backcross population	5,369	1.67	ns	2.56	0.0269	1.19	ns	1.86	ns
Reciprocal×backcross population×hybrid sex	1,369	0.31	ns	0.05	ns	0.01	ns	5.18	0.0234
Geographic distance× reciprocal×backcross population×hybrid sex	5,369	3.55	0.0038	0.74	ns	0.58	ns	3.22	0.0074

ns=not significant at α=0.05.

hybrid males from CA fathers had five of 38 galleries with egg hatch (mean = 21 eggs hatched).

Of the 27 putatively unmated females randomly selected from F_1 progeny and introduced into bolts, nine females oviposited, of which no eggs hatched. The average number of eggs was low (mean = 1.5 ± 0.76). Total gallery length was also low (mean = 12.44 ± 1.27) and it was observed that most unmated females emerged prematurely from the bolt with only three of 27 females mining ≥15 cm of gallery.

Our initial results suggest that hybrid male sterility occurs at a geographic threshold within the *D. ponderosae* populations used in this study, rather than in a linear fashion between increasingly divergent populations (Table 5, Fig. 3). To test whether hybrid male sterility was occurring between geographically proximal populations on either side of the detected geographic threshold, we crossed OR and ID populations to determine the fitness effects on hybrids. Similar methodology was used (see subheading Hybrid fitness in Methods), although we increased the quantity of backcrosses and intrapopulation crosses (n = 30 per combination). Based on earlier results, we used only observations with oviposition and eliminated observations with early mortality of one (or both) of the breeding pair (i.e., dead within the first 15 cm). GLIMMIX analyses included only one main fixed effect, the specific cross (e.g., (OR♀ × ID♂)♂ × ID♀, or ID♂ × ID♀) and standardizing the fitness measures in this two-population comparison was unnecessary. The proportional data were modeled with a binomial distribution, the count data (eggs oviposited and total number of eggs hatched) were modeled with a Poisson distribution, and total gallery length was normal. We tested all pairwise combinations using a Tukey–Kramer Honestly

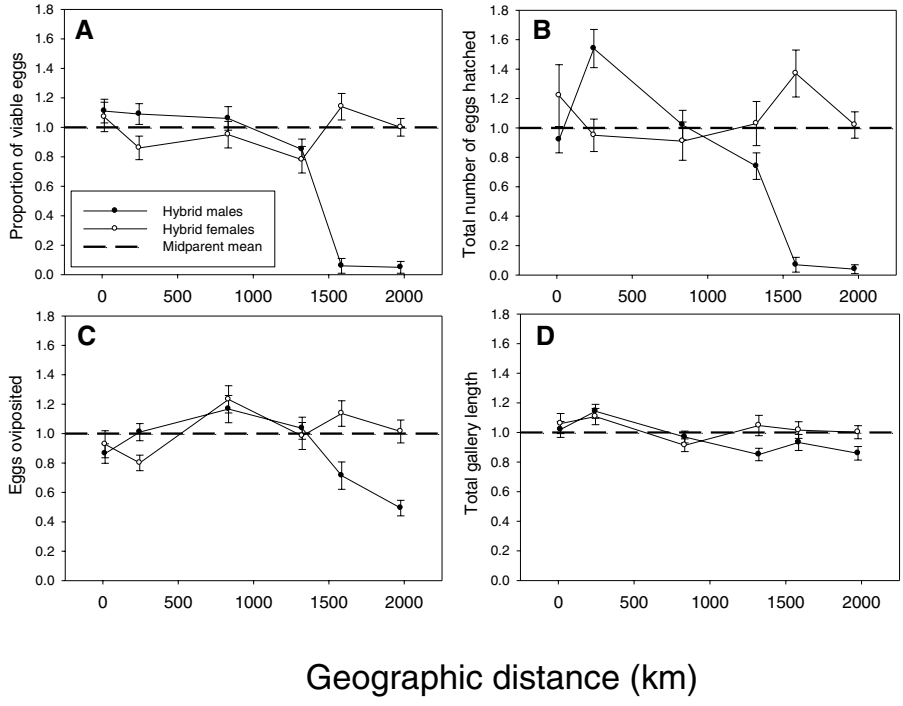

Figure 3. Plots of the four fitness measures collected from backcrosses. Data were standardized by dividing each observation by the midparent mean, producing a value of 1 when the trait value equals the midparent mean (dashed line), >1 when heterosis is present, and <1 when fitness is decreased. Error bars are ±1 standard error. Geographic distance was measured as the cumulative linear distance around the Great Basin Desert from CA to the other population used in the initial cross (see Fig. 1).

Significant Differences multiple comparison test. We found significant differences in crosses with respect to the proportion of viable eggs (df = 9,248, $F = 42.81$, $P < 0.0001$), total number of eggs hatched (df = 9,250, $F = 112.92$, $P < 0.0001$), number of eggs oviposited (df = 9,250, $F = 18.30$, $P < 0.0001$), and total gallery length (df = 9,250, $F = 5.72$, $P < 0.0001$). When compared to both intrapopulation crosses, hybrid males produced from one direction of the cross, ID♀ × OR♂, showed a significant, and substantial, reduction in both the quantity of viable eggs, and total egg hatch (Fig. 4, cross #5, #7). Although not as pronounced, we also detected a significant reduction in total egg hatch in reciprocal (ID♂ × OR♀) hybrid males and females backcrossed to OR (Fig. 4, cross #3, #4). One hybrid male backcross type appeared to be especially detrimental to reproduction. In addition to the previously described reductions, the (ID♀ × OR♂)♂ × ID♀ backcross had significantly reduced gallery length, number of eggs oviposited (Fig. 4), and had the lowest proportion of fertile hybrid males (Table 7). Small proportions of sterile hybrid males were found in three of the four possible hybrid male backcrosses (Table 7).

Discussion

EVIDENCE OF POSTZYGOTIC ISOLATION

We present clear evidence of postzygotic isolation in a species with little molecular genetic signal of reproductive isolation, manifest as reduced egg hatch in backcrosses using reciprocal CA × ID and CA × UT hybrid males. We interpret this extreme egg hatch reduction as being indicative of hybrid male sterility, conforming to Haldane's rule. Our results also suggest that hybrid male sterility between geographically distant populations (CA × ID and CA × UT) is not complete because some backcrosses using

Table 5. Proportion of F_1 offspring from increasingly divergent population crosses that were fertile (≥1 egg hatched) in backcrosses.

	Population cross					
	CA×CA1	CA×CA2	CA×CA3	CA×OR	CA×ID	CA×UT
Male hybrid in backcross	0.97 ($n = 34$)	0.97 (35)	0.97 (34)	0.93 (40)	0.05 (37)	0.11 (35)
Female hybrid in backcross	0.83 (30)	0.97 (36)	0.95 (37)	0.88 (33)	0.94 (33)	0.97 (33)

Table 6. Values from all backcrosses utilizing CA×ID and CA×UT hybrids. Response variables (eggs oviposited, proportion viable eggs, total gallery length, total egg hatch) given as means and standard error.

Cohort	Backcross population	Number of crosses	Number with egg hatch	Proportion fertile[1]	Eggs oviposited (15 cm)	Proportion of viable eggs (15 cm)	Total gallery length	Total egg hatch
Hybrid males								
(ID ♀×CA ♂)	ID	10	1	0.10	11.30 (2.07)	0.10 (0.10)	29.65 (4.55)	2.70 (2.70)
*	CA	10	1	0.10	12.90 (2.22)	0.06 (0.06)	32.50 (3.94)	3.30 (3.30)
(CA ♀×ID ♂)	ID	8	0	0.00	6.25 (1.33)	0	34.00 (3.14)	0
*	CA	9	0	0.00	24.00 (5.15)	0	40.16 (3.34)	0
(UT ♀×CA ♂)	UT	10	1	0.10	12.90 (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 ♀×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)
Hybrid females								
(ID ♀×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)
(CA ♀×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 ♀×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 ♀×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.

[1]Fertile if pairing resulted in any egg hatch.

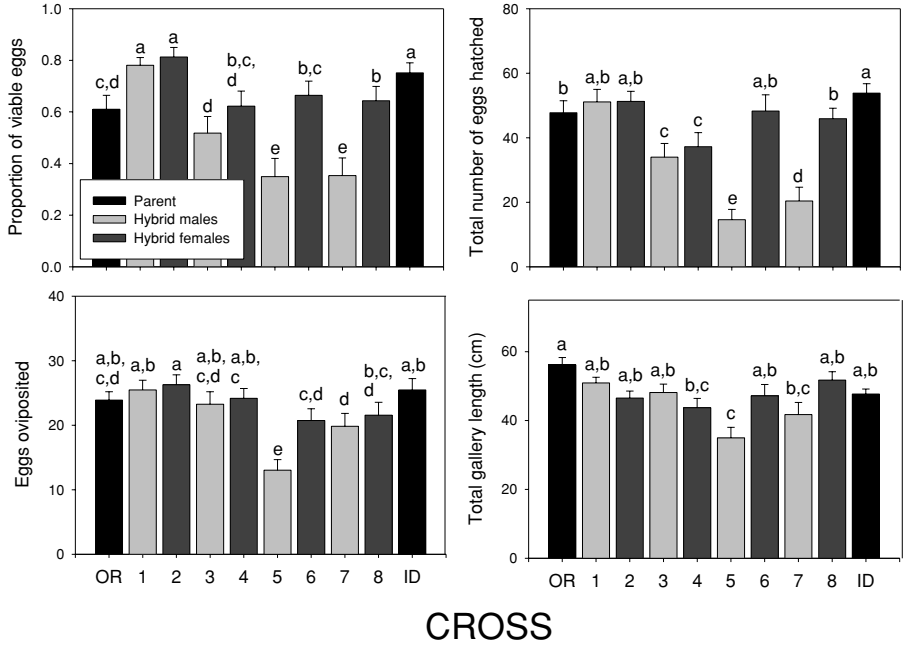

Figure 4. Mean reproductive fitness (+1 SEM) of OR and ID populations and their hybrids as assessed in backcrosses. Crosses: (1) = (OR♀ × ID♂)♂ × ID♀, (2) = (OR♀ × ID♂)♀ × ID♂, (3) = (OR♀ × ID♂)♂ × OR♀, (4) = (OR♀ × ID♂)♀ × OR♂, (5) = (ID♀ × OR♂)♂ × ID♀, (6) = (ID♀ × OR♂)♀ × ID♂, (7) = (ID♀ × OR♂)♂ × OR♀, (8) = (ID♀ × OR♂)♀ × OR♂. Bars with the same letter are not significantly different from one another.

Table 7. Proportion of F_1 offspring that were fertile (≥1 egg hatched) in backcrosses using populations on either side of the proposed reproductive threshold (OR×ID).

	Backcross			
	(OR♂×ID♀) hybrid backcrossed to ID	(OR♂×ID♀) hybrid backcrossed to OR	(OR♀×ID♂) hybrid backcrossed to OR	(OR♀×ID♂) hybrid backcrossed to ID
Male hybrid	0.68 ($n = 28$)	0.84 (25)	0.87 (24)	1.00 (23)
Female hybrid	0.96 (23)	1.00 (27)	1.00 (23)	1.00 (29)

hybrid males were capable of producing offspring. Intraspecific variation in the degree of male sterility is not uncommon, and has been observed in multiple species of *Drosophila* (Reed and Markow 2004; Kopp and Frank 2005), and is thought to appear primarily during the early stages of species formation. We found no evidence of the more advanced stage of postzygotic isolation, hybrid inviability, within the *D. ponderosae* populations analyzed, which is consistent with multiple crossing studies utilizing various populations from throughout this species range (Hay 1956; Lanier and Wood 1968; B. J. Bentz et al. unpubl. data). Not only was there no difficulty producing hybrid male and female offspring, but all hybrid cohorts had sex ratios similar to intrapopulation crosses and published estimates (Amman and Cole 1983; Cerezke 1995).

The onset of hybrid male sterility appeared to occur abruptly in the CA × ID cross, rather than incrementally along a clinal gradient, among increasingly divergent population crosses found surrounding the Great Basin Desert. We did not find significant evidence of sex-specific decrease in the proportion of viable eggs or total egg hatch in backcrosses using hybrids derived from less geographically distant crosses. Subsequent crosses between populations bounding the transition from compatibility to hybrid male sterility (OR and ID) showed evidence of less-severe incompatibilities that appear to be primarily unidirectional. We describe the incompatibilities as primarily unidirectional because only one direction of the cross (ID♀ × OR♂) produced hybrid males with substantial decreases in both the proportion of viable eggs and total egg hatch. The unidirectional loss of reproductive fitness in these hybrid males is in contrast to the clear bidirectional hybrid male sterility seen between more geographically distant crosses (CA × ID). This intriguing result suggests that a reproductive barrier exists between the OR and ID populations, and with increased geographic distance from ID, we see more postzygotic isolation.

Postzygotic isolation is thought to evolve as a byproduct of adaptation to divergent environments and is typically observed between lineages showing detectable morphological, ecological, and/or genetic divergence. The apparent reproductive barrier that exists between OR and ID is perplexing given the relative proximity of these populations (262 km), their use of a common host tree species (*P. contorta*), overall morphological and ecological similarity, and shallow genetic distance (Mock et al. 2007). Numerous studies have been conducted on *D. ponderosae* because of its ecological and economic impacts and there is currently no indication of substantial divergent ecological adaptation between OR and ID populations. Critical life-history traits such as body size and development time do appear to differ between northern and southern populations (Bentz et al. 2001), yet these two traits do not appear to differ significantly between OR and ID populations (R. R. Bracewell et al. unpubl. data). Phylogeographic analyses of the beetle did not find evidence of historical allopatry and subsequent secondary contact near OR and ID, suggesting a hybrid zone (Mock et al. 2007). However, analyses of three host tree species have described Pleistocene glacial refugia harboring disjunct host tree populations and zones of secondary contact (Latta and Mitton 1999; Richardson et al. 2002; Godbout et al. 2008) roughly geographically concordant with our observed OR/ID reproductive boundary. *Dendroctonus ponderosae* is considered a *Pinus* generalist and using historical tree distributions as a proxy for historical beetle distributions would at a minimum require historical data on all host tree species. Currently, these data are not available, although linking multiple phylogeographic analyses from a variety of tree species has provided valuable insight into glacial refugia during the Pleistocene (Jaramillo-Correa et al. 2009) which could help highlight potential modes of speciation in tree-colonizing insect species (Kelley et al. 1999).

Because *D. ponderosae* mating takes place under the bark, a direct observation of mating behavior and copulation is difficult (Reid 1958). Mating was not directly observed in our crosses, although it is suspected to have occurred in sterile backcrosses for several reasons. Sterile hybrid galleries were often quite long and full of unhatched eggs, whereas unmated females typically produced short galleries devoid of eggs. We also found no significant increase with respect to hybrid failure to oviposit or create galleries, which, if we had, might suggest courtship and mating difficulties leading to the premature abandonment of the gallery. Furthermore, studies investigating species boundaries between *D. ponderosae* and its sibling species *D. jeffreyi*, have demonstrated that even these two separate species mate under laboratory conditions and show sperm transfer, gallery construction, and oviposition, yet exhibit the more advanced stage of postzygotic isolation and produced inviable hybrids (Lanier and Wood

1968). We did find that backcrosses using reproductively compromised hybrid males resulted in fewer average eggs oviposited and slightly decreased total gallery length, suggesting that the male/female coordinated behavior in brood production may be slightly altered when using reproductively compromised hybrid males.

A somewhat surprising result was the many significant differences detected in several fitness measures among F_1 intrapopulation crosses (Table 3). We did not find a clear tendency for geographically proximal populations to be similar in all fitness values, or reproductively isolated groups to consistently significantly differ (Table 3). In general, our results are consistent with previous studies on *D. ponderosae* that have detected differences in life-history traits among populations (Bentz and Mullins 1999; Bentz et al. 2001) and further establish *D. ponderosae* as a complex of populations that exhibit significant differences in some life-history parameters.

GENETIC MECHANISMS CAUSING POSTZYGOTIC ISOLATION

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). Endosymbiont-induced infections via *Wolbachia* infections are well known to cause rapid postzygotic isolation in insects (Hoffmann et al. 1986; Turelli and Hoffmann 1991). However, incompatibilities typically manifest as hybrid inviability, not hybrid male sterility (Werren 1997; Stouthamer et al. 1999), and our data are not consistent with patterns of *Wolbachia*-induced cytoplasmic incompatibilities. Additionally, screening of *D. ponderosae* using a PCR-based method from Jeyaprakash and Hoy (2000) failed to detect *Wolbachia* in populations that showed incompatibilities (i.e., CA and ID) (R. R. Bracewell unpubl. data). Although an endosymbiotic contribution cannot be completely ruled out, our described inheritance pattern of incompatibilities in *D. ponderosae*, and the lack of detection during screening (R. R. Bracewell unpubl. data), makes a *Wolbachia* influence unlikely.

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, 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. Limited chromosomal research has been undertaken on *D. ponderosae*, although early work describes the karyotype as $n = 11 +$ neo-XY (Lanier and Wood 1968). The current configuration is thought to have been derived from an ancestral state of 12 XY_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). Sex chromosomes appear to play a large role in Haldane's rule (Laurie 1997; Presgraves 2008), although, little is known about the neo XY condition in *D. ponderosae*, and whether intraspecific chromosomal variation exists that might contribute to reproductive incompatibilities.

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 thought to explain Haldane's rule, suggesting that X-linked recessives are expressed in hemizygous individuals through negative epistatic interactions between the X chromosome and autosomes. Dominance theory is considered the most universal explanation for Haldane's rule (Coyne and Orr 2004), although individual cases of hybrid male sterility have been associated with different mechanisms (reviewed in Laurie 1997). Comparisons between *Drosophila* species have shown that species with larger X chromosomes tend to express hybrid male sterility at lower genetic distances, consistent with dominance theory and the increased likelihood of negative epistatic interactions due to the increase in hemizygosity (Turelli and Begun 1997). The largest chromosomes in *D. ponderosae* are the sex chromosomes, and if we assume that incompatibilities involving X-linked loci are responsible for hybrid male sterility, our results suggest that sex-linked incompatibilities have independently accumulated in both the CA and ID/UT populations because reciprocal crosses between these populations produced sterile males (i.e., hybrid males had X chromosomes derived from both CA and ID/UT populations). Detection of bidirectional incompatibilities is not uncommon in cases of hybrid male sterility (Coyne and Orr 1989, 1997), although one might expect unidirectional incompatibilities in less-divergent populations crosses that would precede bidirectional incompatibilities in more divergent population crosses. In fact, we do see some evidence of the progressive development of postzygotic incompatibilities. We found a weaker, primarily unidirectional incompatibility in the form of reduced reproductive output from hybrid males in the proximate OR $\times$ ID cross and strong bidirectional incompatibilities in the distant CA $\times$ ID cross. The underlying genetic mechanism contributing to incompatibilities is currently unknown, and further inquiry into potential chromosomal and genic mechanisms is needed.

HYBRID MALE STERILITY AND A LACK OF NEUTRAL MOLECULAR GENETIC SIGNAL

Although the exact genetic mechanism causing hybrid male sterility in *D. ponderosae* is unknown, 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 large quantities of sterile male hybrids (CA × ID, CA × UT), or produce hybrid males with reduced fecundity (OR × ID) (Mock et al. 2007). Molecular genetics is commonly used to infer species boundaries, population subdivision, and patterns of gene flow, yet our results suggest that some postzygotic isolation may go undetected with an analysis based solely on neutral molecular markers. The apparent mismatch between the observation of postzygotic isolation and the lack of clear discontinuities at molecular markers may be reconciled in multiple ways: (1) incomplete hybrid male sterility is an ineffective isolating mechanism because gene flow can still occur via the few fertile males and fully fertile females, (2) the onset of hybrid male sterility is recent and molecular differentiation at neutral loci has yet to occur.

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 mitochondrial haplotypes (Mock et al. 2007). Additionally, the amount of genetic differentiation (both nuclear and mtDNA) between populations that produce reproductively compromised hybrid males 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 (Marshall et al. 2002). Assuming that genetic divergence has occurred at roughly similar rates, this observation is consistent with a very recent post-Pleistocene onset of postzygotic isolation within *D. ponderosae*. Such rapid onset of partial postzygotic isolation has also been suggested in the yellow-rumped warbler (Brelsford and Irwin 2009). Particularly interesting is our detection of strong bidirectional incompatibilities, because theory suggests that unidirectional incompatibilities likely involve few loci, whereas bidirectional incompatibilities often involve many (Turelli and Moyle 2007, and references therein). Our results therefore suggest neutral molecular markers failed to accurately describe what must be substantial genetic differentiation, or that contrary to predictions from Turelli and Moyle (2007) very few loci are involved in the incompatibility. Interpretation of *D. ponderosae* molecular genetic data could be influenced by population size, because analyses based on neutral markers rely on drift to create allele frequency differences. *Dendroctonus ponderosae* population sizes are known to be substantial, particularly during outbreaks. A recent outbreak has affected over 14 million ha in B.C., Canada (Safranyik et al. 2010) 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. Additionally, gene flow may very well still be occurring between partially reproductively isolated *D. ponderosae* populations, even if hybridization does lead to males with reduced fitness. Cyclical eruptive outbreaks might facilitate mating if outbreaks exceed typical ecological boundaries and overwhelm weak postzygotic reproductive barriers between geographically adjacent populations.

CONCLUSIONS

Our understanding of the evolution of postzygotic isolation is often based on organisms that have diverged hundreds of thousands of years ago, making the timing and relative importance of postzygotic isolation during speciation difficult to determine. Understanding these aspects of reproductive isolation requires systems in the early stages of divergence. *Dendroctonus ponderosae* gives insight into the role of geographic/genetic divergence on postzygotic isolation by capturing a spectrum of postzygotic isolation conforming to Haldane's rule. We suspect the onset of these incompatibilities is quite recent. The amplification from weak unidirectional incompatibilities between proximate populations to strong bidirectional incompatibilities between distant populations suggests a progression of postzygotic reproductive isolation and speciation in its earliest stages. Future research in this system should be directed at uncovering the potential genetic mechanism(s) contributing to hybrid male sterility and describing sterile hybrid male physiology.

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