

Genetic Relationships Between Two Sibling Species of Bark Beetle (Coleoptera: Scolytidae), Jeffrey Pine Beetle and Mountain Pine Beetle, in Northern California¹

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ABSTRACT

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The genetic relationship between two sibling bark beetle species from northern California, the Jeffrey pine beetle, *Dendroctonus jeffreyi* Hopkins, and the mountain pine beetle, *D. ponderosae* Hopkins, was examined by electrophoresis. The genetic differences found between the Jeffrey pine beetle and the mountain pine beetle support their current designation as separate species. Two gene loci were fixed for different alleles in the two groups and provide strong evidence that gene flow does not occur between them. At several other loci, less striking but nevertheless significant differences were observed. The level of overall genetic similarity between the Jeffrey pine beetle and the mountain pine beetle was much lower than that found between conspecific populations of either group.

In northern California, the ranges of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, and the Jeffrey pine beetle, *D. jeffreyi* Hopkins, overlap (Fig. 1), and infestations of both beetles can be found in mixed or adjacent stands of Jeffrey pine, *Pinus jeffreyi* Greville and Balfour, ponderosa pine, *P. ponderosa* Lawson, and

other pine species. The two beetles are considered sibling species because they are difficult to distinguish morphologically, and the galleries they excavate in their host trees are identical. Differences in their anatomies are subtle; the Jeffrey pine beetle is generally somewhat larger than the mountain pine beetle, and its pronotal punctures are smaller and more lightly impressed (Hopkins 1909, Lanier and Wood 1968). In practice, the character most commonly used to distinguish the two beetle species is host preference. The mountain pine beetle is found in ponderosa pine and other pine species except Jeffrey pine; the Jeffrey pine beetle is found only in Jeffrey pine. However, identifying of beetle species by their host, then using host specificity as evidence of species integrity, is circular; corroborating evidence is required to validate the species and the reality of their host associations. Generally, structural divergence parallels genetic divergence and speciation, so that morphological differences provide reliable criteria for species identification. In sibling species, where genetic divergence and reproductive isolation have not been accompanied by conspicuous structural differentiation, there is a need to augment traditional morphological approaches in taxonomy with other approaches.

Until 1963, Hopkins' (1909) separation of mountain pine beetle and Jeffrey pine beetle, based primarily on host preferences, was the accepted classification. Since then, relationships between the two groups have been repeatedly reevaluated as new approaches and additional taxonomic criteria became available. Wood (1963) synonymized the Jeffrey pine beetle and the mountain pine beetle because of their anatomical similarity. Thomas (1965) concurred with this synonymy, based on his studies of larval and pupal morphology. However, observations of attack patterns (Eaton 1956) and the generally larger size of beetles from Jeffrey pine in California (Hopkins 1909) raised doubts about the *ponderosae-jeffreyi* synonymy. In addition, the two groups reacted differently to resin vapors from the different host trees; i.e., nonhost resin vapors were more toxic than host resin vapors (Smith 1963, 1965). A comprehensive study of this complex by Lanier and Wood (1968) showed a complete failure of *D. jeffreyi* to produce fertile off-

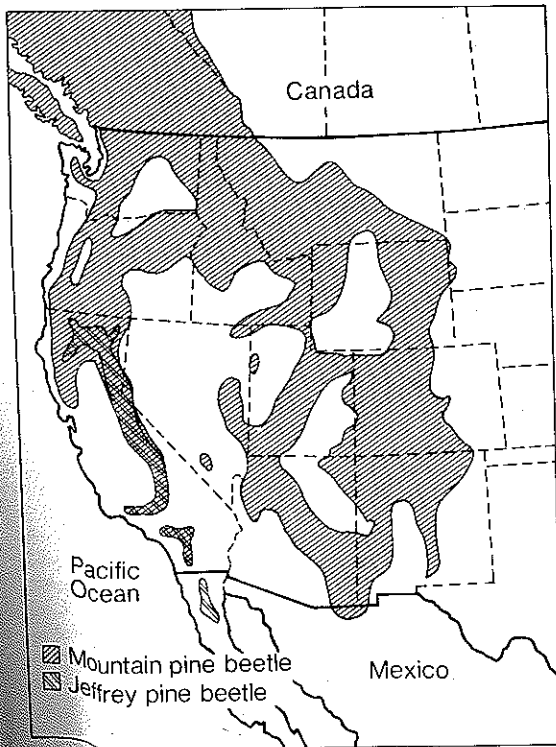


FIG. 1.—Approximate geographic distribution of Jeffrey pine beetle and mountain pine beetle.

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spring when crossed with *D. ponderosae*, and differences in survival of laboratory broods, egg incubation time, karyotypes, and width and sculpturing of the pronotum. Their work reestablished *D. jeffreyi* as a separate species. Later work by Renwick and Pitman (1979) indicated that the pheromones produced by the two species are also different.

In cases such as this, where morphologically similar species occur sympatrically, electrophoretic analysis can be helpful and sometimes definitive, providing estimates of the extent of gene flow between the groups. By using electrophoresis, variant allelic forms at numerous enzyme-producing gene loci can be identified. Differences within and between groups and levels of genetic similarity between groups can be estimated. In addition, non-interbreeding groups (species or geographically separated populations) may be characterized by fixation or near fixation for different alleles at one or more loci (Ayala and Powell 1972, Huettel and Bush 1973, Berlocher 1979). Such diagnostic loci may provide convincing evidence for reproductive isolation and validate species separation in cases where sympatric groups occur (e.g., May et al. 1977a). Biochemical keys, based on electrophoretically diagnostic loci, have been developed to distinguish species where other criteria are lacking or are unreliable (Mahon et al. 1976, Miles 1979, Berlocher 1980).

The objective of this work was to examine and compare, using electrophoretic techniques, the genetic makeup of mountain pine beetle and Jeffrey pine beetle populations in California, to determine if biochemical genetic evidence would support their status as separate species.

Materials and Methods

In summer 1980, samples of Jeffrey pine beetle and mountain pine beetle were collected from 10 sites near Lake Tahoe in northern California and western Nevada and from 1 site in southern Oregon (Table 1, Fig. 2.) We were unable to find both beetle species in a single mixed stand; samples were taken from adjacent stands of Jeffrey pine, ponderosa pine, and lodgepole pine, *Pinus contorta* Douglas. Although our initial aim was to collect from 10 infested trees at each site, 10 such trees did not occur at many of the sites, so most samples were taken from 1 to 3 trees.

We separated bark sections (20 by 25 cm) from the infested trees, then removed beetles from the brood gallery in the pieces of free bark with forceps. Beetles were placed in petri dishes with several layers of damp paper towel and transported to the laboratory in an ice chest. Nearly 1,300 beetles were obtained from the 11 sites: 447 Jeffrey pine beetles from Jeffrey pine, 549 mountain pine beetles from ponderosa pine, and 298 mountain pine beetles from lodgepole pine.

All larvae were laboratory-reared to the adult stage using methods described by Bedard (1966). Most beetles were reared on phloem tissue from the same host species in which they were collected. To determine if the observed electrophoretic differences might be environmentally induced (i.e., due to host differences), 100 Jeffrey pine beetle larvae were reared on ponderosa pine phloem

and 100 mountain pine beetle larvae were reared on Jeffrey pine phloem. Adults resulting from these rearings on alternative host phloem were then compared electrophoretically.

Eight enzyme systems used by Stock and Amman (1980) and three additional enzymes were assayed. Buffer and stain recipes are shown in Table 2. Gels were made from a 13% (wt/vol) solution of hydrolyzed potato starch (Electrostarch) and the appropriate buffer. Electrophoresis continued at a maximum of 75 mA until a red food-dye marker had migrated 7 cm toward the anode (ca. 3.5 h). After staining, variant allozymes at each locus were identified by their mobilities (relative to the dye marker), and genotype frequencies for each sample were recorded.

A preliminary examination of allozyme patterns of 12 offspring from a single mountain pine beetle cross was made to determine whether banding patterns observed on the gels reflected simple Mendelian inheritance patterns.

Genotype frequencies at each locus were compared with those expected for a random-mating population. To compare samples at individual loci, contingency chi-square tests were used. Nei's (1975) heterozygosity and average heterozygosity were calculated to estimate and compare levels of genetic diversity at single loci and over all loci. Levels of similarity of conspecific samples and species were compared over all loci by Nei's (1972) indices of genetic identity and genetic distance. Finally, a dendrogram was constructed from the genetic distance values, using a single-linkage, unweighted approach (Sneath and Sokal 1973).

Results

Data on 17 gene loci were obtained from assays of the 11 enzyme types (Table 3, Fig. 3). Of the 17 loci, 6 (AAT1, AcP, EST1, LAP2, PEP, and PGI) were polymorphic in most or all samples. The remaining 11 loci (AGP1, AGP2, AGP3, CK, EST2, EST3, IDH, LAP1, MDH1, MDH2, and TO or SOD) were monomorphic in all samples examined. At CK, one mountain pine beetle from Cottonwood Creek was heterozygous for a rare allelic form. Percent polymorphism varied little among samples, ranging from 35% (6/17) in eight samples to 30% (5/17) in the remaining four samples. The esterase system was most variable of all loci examined; products of two monomorphic loci appeared on the cathodal gel slice and two polymorphic loci appeared on the anodal slice. However, interpretable bandings patterns were resolved only for the slower of the two polymorphic loci (EST1) and the two monomorphic loci. An extra band ($rf = 0.78$) was observed in 4% of Jeffrey pine beetles examined. These extra bands appeared sporadically and apparently independently of variation observed among allozymes of EST1 in mountain pine beetle and PGI in Jeffrey pine beetles. They could represent products of an infrequently resolved or expressed additional gene locus or evidence of foreign protein, such as that of a parasite (May et al. 1977b, Castrovillo and Stock 1981). Dissection of beetles in this study revealed a high incidence of nematode parasites.

Table 1.—Collection information for mountain pine beetle and Jeffrey pine beetle samples obtained May to July 1980

Site	Abbreviation	Tree species present ^a	Host species	No. of trees sampled	Life stage(s)	No. of beetles collected	Date
Mountain pine beetle							
Incline Village	IV	JP, PP	pp	1	Larvae	117	30-V-80
Winema National Forest	WNF	LPP, PP	PP	2	Larvae	96	25-VI-80
Lake Almanor	LA	JP, PP	PP	2	Larvae	184	26-VI-80
Truckee	TR	PP, LPP, JP	PP	2	Larvae, pupae	157	28-VI-80
Sattley	SAT	PP, JP	PP	2	Larvae, pupae	189	29-VI-80
Cottonwood Creek	CWC	White fir, PP, JP	PP	2	Pupae, adults	194	30-VI-80
Kyburz Flat	KF	JP, PP	PP	2	Pupae	100	30-VI-80
Meyers	ME	LPP	LPP	10	Larvae	970	29-V-80
Jeffrey pine beetle							
Camp Richardson	CR	White fir, incense cedar, LPP, JP	JP	10	Larvae	808	30-V-80
Yuba Pass	YP	JP, sugar pine, white fir	JP	2	Pupae, adults	203	30-VI-80
Taylor Creek	TC	JP, white fir	JP	1	Pupae, adults	123	1-VII-80
Fallen Leaf Lake	FL	JP, LPP, white fir, incense cedar	JP	3	Adults	270	1-VII-80

^aPP, ponderosa; LPP, lodgepole pine; JP, Jeffrey pine.

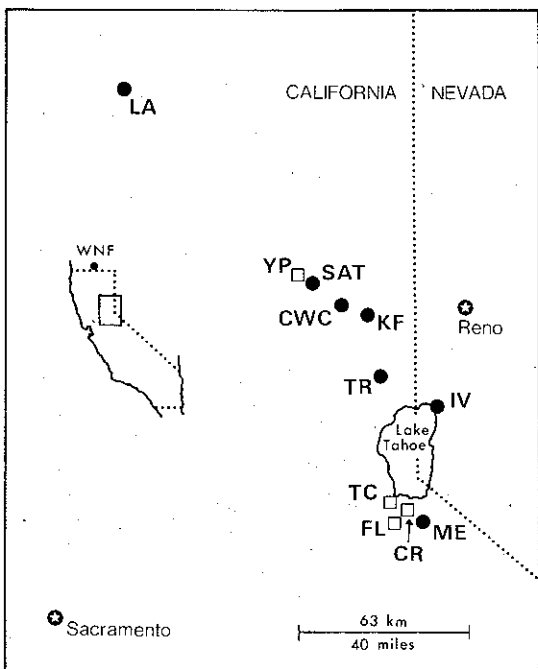


FIG. 2.—Collection sites of Jeffrey pine beetle (□) and mountain pine beetle (●).

Distributions of AAT1, AcP, LAP2, PEP, and PGI genotypes in the sibling group tested were close to expected Mendelian ratios. In addition, comparison of mountain pine beetles and Jeffrey pine beetles reared on host and nonhost phloem revealed no differences attributable to host effects.

Within-Species Variation

Genotype proportions observed at certain loci in some samples were not in agreement with random-mating (Hardy-Weinberg) expectations. At AAT1, both Camp Richardson Jeffrey pine beetles and Sattley mountain pine beetles had a deficiency of heterozygous .51/.39 genotypes and a concomitant excess of homozygous .39/.39 genotypes. At EST1, genotype proportions in three mountain pine beetle samples (from Cottonwood Creek, Truckee, and Sattley) were significantly different from Hardy-Weinberg expectations; all three had an excess of homozygous .64/.64 genotypes. At LAP2, an excess of heterozygous .43/.35 genotypes was observed in two Jeffrey pine beetle samples (from Cottonwood Creek and Truckee). These genotype disproportions could not be interpreted as consistent with any of the common explanations for such phenomena (e.g., sampling of closely related groups, the presence of a silent allele, the Wahlund effect) except, possibly, selection of some type.

Some significant differences in allele frequencies occurred among samples within species (Table 3). Camp Richardson Jeffrey pine beetles had a lower frequency of AAT1(.51) and a higher frequency of AAT1(.39) than other Jeffrey pine beetle samples. Of the mountain pine beetles, only the Sattley sample contained AAT1 (.51). Fallen Leaf Lake Jeffrey pine beetles had a significantly lower frequency of AcP (0.35) than other samples of this species. Kyburz Flat and Incline Village mountain pine beetles had a lower frequency of AcP (.35) than other mountain pine beetle samples. At EST1 and LAP2, Jeffrey pine beetle samples showed considerable variation among localities. Sattley mountain pine beetles were significantly different, at EST1, from other samples of this species. At PGI, Sattley mountain pine

Table 2.—Assay information for Jeffrey pine beetle and mountain pine beetle enzyme systems

Enzyme	Abbreviation	Buffer system ^a	Cofactor (5 mg) ^b	Stain recipe:		Missing solution (100 ml) ^d
				Other components ^c		
Aspartate aminotransferase Acid phosphatase	AAAT	RW	—	70 mg of Fast Garnet (GBC) salt	AAAT buffer	
	ACP	AC	—	30 mg of α -naphthyl acid phosphate	0.1 M monobasic phosphate buffer	
α -Glycerophosphate dehydrogenase	AGP	AC	NAD	100 mg of DL- α -glycerophosphate	RW gel buffer	
				2 mg of PMS 7 mg of NBT 7 mg of MTT		
Creatine kinase	CK	AC	NADP	20 mg of phosphocreatine	RW gel buffer	
				30 mg of ADP 100 mg of glucose 100 units of hexokinase 60 units of G-6-PDH 2 mg of PMS 7 mg of NBT 7 mg of MTT		
Esterase	EST	RW	—	1.5 ml of α -naphthylacetate	Esterase buffer	
				1.5 ml of β -naphthylacetate 5 ml of propanol 1 ml of Fast Garnet solution		
Isocitrate dehydrogenase	IDH	AC	NADP	30 mg of isocitric acid	RW gel buffer	
				2 mg of PMS 7 mg of NBT 7 mg of MTT		
Leucyl aminopeptidase Malate deh	LAP	RW	—	10 mg of L-leucyl- β -naphthylamide	0.1 monobasic phosphate buffer	
	MDH	AC	NAD	20 mg of Fast Garnet salt	RW gel buffer	
Malate dehydrogenase				20 ml of 0.5 N DL-NA-malate		
				2 mg of PMS 7 mg of NBT 7 mg of MTT		
Peptidase	PEP	RW	—	10 mg of 0-diansidine dissolved in 5 mg of acetone	RW gel buffer	
				5 mg of amino acid oxidase 5 mg of peroxidase 20 mg of glycyl-leucine		
Phosphoglucose isomerase	PGI	RW	NADP (2 mg)	10 mg of fructose	RW gel buffer	
				4 units of G-6-PDH 1 mg of PMS 3 mg of NBT 3 mg of MTT		
Tetraazolum oxidase	TO	AC	NADP	50 mg of glucose-1-phosphate	RW gel buffer	
				2 mg of glucose-1, 6-diphosphate 80 units of G-6-PDH 2 mg of PMS 7 mg of NBT 7 mg of MTT		

^aRW = Ridgway buffer system (Ridgway et al. 1970), Ridgway stock concentrate = 3000 ml of distilled water + 108.9 g of Tris + 31.53 g of citric acid, Ridgway tank buffer (pH 8.1) = 3,000 ml of distilled water + 7.56 g of lithium hydroxide + 55.65 g of boric acid, Ridgway gel buffer (pH 8.5) = 30 ml RW tank buffer + 297 ml of RW stock concentrate + 2673 ml of distilled water, AC, Aminopropyl morpholine/citric acid buffer (Clayton and Trettak 1972); stock (tank) buffer (pH 6.1) = 25.22 g of citric acid + ca. 36 ml of N-(3-aminopropyl)-morpholine + 3,000 ml of distilled water; gel buffer = 150 ml stock buffer + 2,850 ml of distilled water.

^bPMS, Nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate.
^cPMS, Phenazine methosulfate; NBT, nitroblue tetrazolum; MTT, MTT, tetrazolum; ADP, adenosine 5'-diphosphate; G-6-PDH, glucose-6-phosphate dehydrogenase, α -Naphthylacetate = 0.2 g of α -naphthylacetate + 10 ml of distilled water + 10 ml of acetone, β -naphthylacetate = 0.2 g of β -naphthylacetate + 20 ml of acetone, Fast Garnet solution = 0.8 g of Fast Garnet (GBC) salt + 20 ml of distilled water.

^dAAAT buffer = 600 ml of RW stock concentrate + 900 ml of distilled water + 2 g of α -ketoglutaric acid + 5 g of aspartic acid, Esterase buffer = 50 ml of "A" buffer (27.6 g of monobasic NaPO₄ + 1,000 ml of distilled water) + 10 ml of "B" buffer (28.2 g of anhydrous dibasic Na₂HPO₄ + 1,000 ml of distilled water) + 40 ml of distilled water.

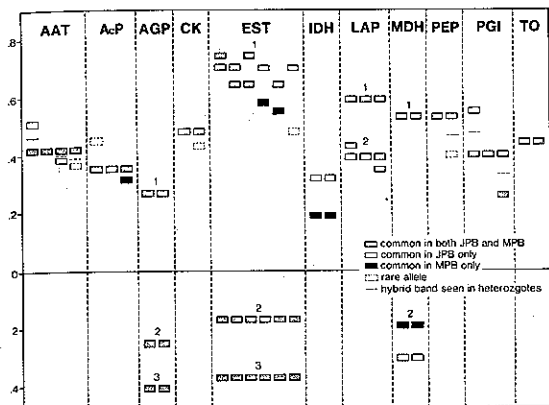


FIG. 3.—Representative genotypes at 17 loci assayed in Jeffrey pine beetle and mountain pine beetle.

beetles had a significantly lower frequency of the common allele, PGI(.40), than other mountain pine beetle samples. There was no indication that mountain pine beetles from lodgepole pine (at the Meyers site) were genetically different from mountain pine beetles in ponderosa pine at the other sites.

When overall diversity was compared among samples within species, we found that Yuba Pass Jeffrey pine beetles were markedly less diverse (average heterozygosity = 11%) than other samples of this species (14 to 15%). Sattley mountain pine beetles were considerably more diverse (17%) than other mountain pine beetle samples (13 to 14%).

Genetic identity values, calculated over all loci, ranged from 0.987 to 0.997 among Jeffrey pine beetle samples and from 0.984 to 0.999 among mountain pine beetle samples.

Between-Species Differences

Major differences occurred between Jeffrey pine beetles and mountain pine beetles at several loci (Table 3). IDH and MDH2 were fixed for different alleles in the two species. At AAT1 and PGI, Jeffrey pine beetles were more diverse; at AcP and EST1, the converse was true. At AAT1, Jeffrey pine beetles had an allele that was not present in most mountain pine beetle samples. All but a very few Jeffrey pine beetles lacked EST1 (.55) and EST1(.48), which were relatively common in the mountain pine beetle samples. PEP was the only polymorphic locus for which differences between samples of the species were not significant.

Estimates of genetic identity between species samples ranged from 0.798 (Yuba Pass Jeffrey pine beetles vs. Kyburz Flat mountain pine beetles) to 0.845 (Camp Richardson Jeffrey pine beetles vs. Truckee mountain pine beetles). This range was considerably lower than that for conspecific samples (0.987–0.999). The overall genetic identity level between species was 0.83. These relationships are reflected in the dendrogram shown in Fig. 4.

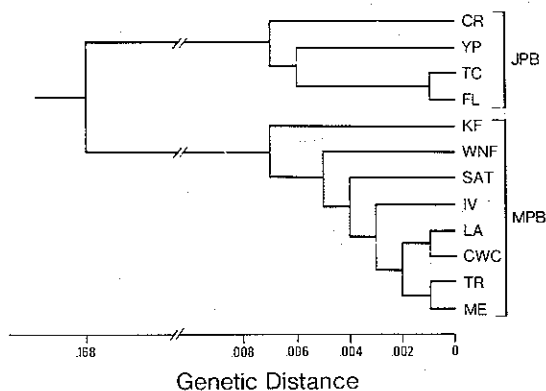


FIG. 4.—Species and population relationships, based on samples of Jeffrey pine beetle and mountain pine beetle.

Discussion

The genetic differences found between Jeffrey pine beetle and mountain pine beetle support their current designation as separate species. Two loci, IDH and MDH2, are fixed for different alleles in the two beetle groups. This is strong evidence that gene flow does not occur between them. If interbreeding was occurring, at least some individuals heterozygous at IDH and MDH2 should have been found. Thus, IDH and MDH2 may be considered diagnostic characteristics which can be added to those identified by earlier workers. At several other loci, less striking, but still significant, differences were observed. Mountain pine beetles were more heterozygous at AcP and EST1 than were Jeffrey pine beetles. Conversely, Jeffrey pine beetles were more heterozygous at AAT1 and PGI. Nevertheless, a large percentage of the loci were monomorphic and identical in all groups. This pattern suggests relatively recent separation of the two species.

The overall level of genetic similarity (0.83) between Jeffrey pine beetle and mountain pine beetle was considerably lower than has been found among any conspecific mountain pine beetle populations. For example, genetic identity values calculated over the same 17 loci, for mountain pine beetles collected from several sites from near the Canadian border to southern Utah, ranged only from 0.963 to 0.999 (Stock and Amman, unpublished data).

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