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Effects of specialization on genetic differentiation in sister species of bark beetles

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We investigated the relative importance of resource use and geography on genetic differentiation in the sister-species pair of generalist and specialist bark beetles: *Dendroctonus ponderosae* and *D. jeffreyi* (Coleoptera: Scolytidae). In two regions, where the distributions of these species overlap, we collected specimens of the generalist from multiple host species and specimens of the specialist from its single host species. Using allozyme techniques, we uncovered genetic differentiation between generalist populations on different host species in the same region (one locus in each region). However, a much stronger pattern of differentiation was found between specialist populations in the two distantly separated regions (three loci). With mtDNA, we found no significant differentiation between regions in the specialist, or among host species in the generalist, although there was some differentiation between regions in the generalist (AMOVA, $P < 0.05$). Overall, the generalist populations maintained approximately 10 times the genetic variation in mtDNA as the specialist populations, which suggests that the specialist either has generally smaller population sizes than the generalist, or has experienced a historical population bottleneck.

Keywords: *Dendroctonus*, gene flow, genetic differentiation, phytophagous insects, speciation, specialization.

Introduction

Phytophagous insects comprise approximately one-quarter of the recognized species on earth, and the majority of these specialize to some degree in their diets (Bernays & Chapman, 1994). Although the diversity of herbivorous insects has long been supposed to be a direct consequence of specialized plant-feeding, the mechanisms by which specialization might influence diversity are still the subject of much debate (Bush, 1975; Futuyma & Mayer, 1980; Jaenike, 1990). Proponents of sympatric speciation suggest adaptation of insects to particular host-plant species serves to isolate insect populations, a process which may eventually lead to speciation (Bush, 1975). In this scenario, genetic differentiation should be observed between populations of an insect species utilizing different host species. Genetic differentiation among host-plant species may also be observed as a result of adaptive deme formation (i.e. local adaptation of phytophagous insects to specific hosts), a hypothesis which has been supported by recent meta-analysis (Van Zandt & Mopper, 1998).

Alternatively, under an allopatric model, particular host-plant species might influence diversification if their patchy distributions effectively fragment populations of herbivores, and reduce the possibility of gene flow between populations (Barton & Charlesworth, 1984). Genetic drift, or a combination of drift and local selection pressures, then creates genetic differentiation between populations. In this scenario, the expectation would be that herbivorous insect species with a more restricted diet breadth (i.e. specialists) would be more prone to genetic differentiation than insect species with a greater diet breadth (Peterson & Denno, 1998). Genetic differentiation resulting from habitat fragmentation, and the subsequent geographical isolation of populations, is well documented (Hall *et al.*, 1995; Lacy & Lindenmayer, 1995).

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However, a recent review of genetic differentiation patterns in herbivorous insects found surprisingly little support for the common assumption that specialists should show more pronounced genetic structuring than generalists (Peterson & Denno, 1998). The authors demonstrated, via meta-analysis, that dispersal ability had a much stronger influence on genetic differentiation than did differences in resource use between generalists and specialists. It is possible, however, that the traditional classification of species as generalists or specialists is insufficiently accurate to permit the detection of consistent differences via meta-analysis. For example, a species restricted to *Pinus* or *Quercus*, but able to use a large number of North American species, might face less fragmentation in the local distribution of resources than a specialist on *Liquidambar* or *Larix*, of which only single species are native to North America. Moreover, the traditional definition of generalists does not distinguish between those in which a variety of hosts is used by a single population in a single place, from those in which local populations may strongly prefer fewer and different hosts from place to place (Fox & Morrow, 1981; Thompson, 1994). Indeed, poor dispersal abilities might foster selection for broadened local host use in some species, while enabling local differentiation in host use in others.

To examine the possible relationship between host specialization and genetic differentiation in phytophagous insects, we compared levels of genetic differentiation between two sister species of bark beetles (Coleoptera: Scolytidae) that differ strongly in diet breadth. Using the phylogeny estimate of the bark beetle genus *Dendroctonus*, we identified a pair of sister species whose distributions overlap: *D. ponderosae*, whose 'generalist' habits are ancestral, and *D. jeffreyi* whose 'specialist' habits are derived (Kelley & Farrell, 1998). *Dendroctonus jeffreyi* is associated with *Pinus jeffreyi* whereas its sister species, *D. ponderosae*, attacks a majority of the *Pinus* species in its broad range (11 species), as do most other *Dendroctonus* species (Kelley & Farrell, 1998). Moreover, *D. ponderosae* has been reported utilizing multiple host species in the same area, with high estimates of gene flow between populations, so this is not simply a case of undiscovered cryptic species (Stock & Amman, 1980; Sturgeon & Mitton, 1986).

Thus, a novel aspect of our study is the use of a phylogeny estimate to establish directionality in the evolution of specialization, in order to evaluate its correlates and consequences. Furthermore, comparing sister species allows for the greatest possible control for the potentially confounding effects of age (for sister species are the same age by definition (Mitter *et al*., 1988)) and phylogeny on levels of genetic differentiation. In addition, by collecting these two species in the same areas, we attempted to control for potential effects of environment and geographical separation on genetic subdivision. (For the remainder of this paper, we will be referring to *D. jeffreyi* as the 'specialist' and *D. ponderosae* as the 'generalist'.)

Using data from allozyme and mitochondrial DNA polymorphism, and standard techniques of population genetic analysis, we asked which of the host-plant driven diversification mechanisms mentioned above best explained the patterns of genetic variation observed in *D. ponderosae* and *D. jeffreyi*. We asked the following questions about the population structure of the two species. (i) Is the generalist more differentiated on its various host species than the specialist on its single host species? (ii) What are the relative effects of geographical distance on genetic differentiation in the specialist and generalist? (iii) Are there any differences in the overall amount of genetic variation between the specialist and the generalist?

Materials and methods

Beetle collections

We collected samples of both species in two different regions of California where earlier infestations had been identified by colleagues: around Mammoth Lakes, CA and in Lassen National Forest (Fig. 1). In both regions, the generalist was collected on multiple host species, whereas the specialist was collected at multiple sites in both areas (Fig. 1). In 1995 and 1996, we collected *D. ponderosae* in Lassen from *Pinus ponderosa* (ponderosa pine), *P. contorta* (lodgepole pine) and *P. lambertiana* (sugar pine), while also collecting *D. jeffreyi* at multiple sites within Lassen (Table 1; Fig. 1). In those same two years, we also collected *D. ponderosae* from *P. contorta* and *P. flexilis* (limber pine) in the Mammoth area, and *D. jeffreyi* at several different sites around Mammoth (Table 1; Fig. 1).

Because the beetles were relatively scarce at the time of the study (i.e. there was not a serious outbreak of either species), our choice of sample trees was limited. The numbers of trees and sites listed in Table 1 represent all of the infested trees at all of the sites we were able to find in the two years of the study. However, because a single infested tree may contain thousands of beetles, even a single tree provides a good basis for sampling the population, particularly when infestation rates are low. Beetles were selected from galleries in all accessible parts of each tree. Because beetles in the same gallery are related (except for the mating pair), we sampled only 2–3 beetles from each gallery system. Adults and larvae were removed from underneath the bark of dying host trees using a hatchet or a small axe to

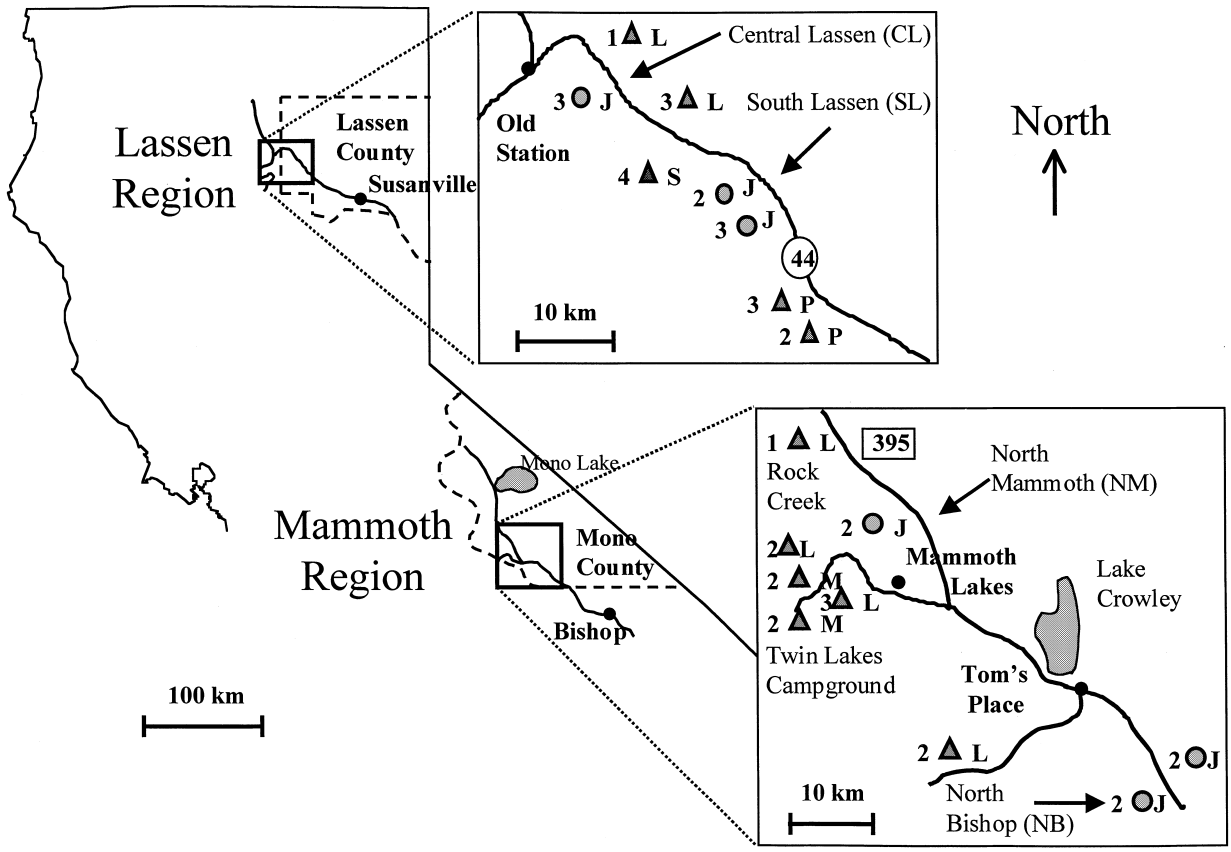

Fig. 1 Collection localities of *Dendroctonus ponderosae* (△) and *D. jeffreyi* (o) in California. Letters next to the symbols indicate the host species that individuals were collected from: P, ponderosa pine (*Pinus ponderosa*); S, sugar pine (*P. lambertiana*); L, lodgepole pine (*P. contorta*); M, limber pine (*P. flexilis*); and J, Jeffrey pine (*P. jeffreyi*). The numbers next to the symbols indicate the number of host trees sampled at that particular locale (see Table 1). There were four specific collection sites of *D. jeffreyi* that are indicated on the figure: Central Lassen, South Lassen, North Mammoth, North Bishop.

peel away the bark. Individuals were kept on ice in plastic 50 mL centrifuge tubes with a small amount of phloem and bark inside the vials. The live beetles were transported back to the University of Colorado where they were frozen in a −70°C freezer.

Allozyme electrophoresis

Genotypes for individuals of both species were determined with horizontal starch gel electrophoresis. Out of nine loci surveyed, we found three polymorphic enzymes common to both species for comparison of their genetic population structure: esterase (EST), peptidase (PEP) and phosphoglucose isomerase (PGI). Other studies have reported finding AAT and AcP polymorphic in *D. ponderosae* (Stock & Amman, 1980; Sturgeon & Mitton, 1986). However, we were unable to resolve AAT after trying numerous buffer systems, and AcP was monomorphic in the *D. jeffreyi* samples we examined. Five other enzymes (IDH, MDH, ME, PGM and PMI) were monomorphic. All three enzymes we used resolved well on a discontinuous Tris-borate buffer system. Sample preparation and electrophoresis were performed in the same manner as Sturgeon & Mitton (1986).

DNA preparation and PCR

For genomic DNA preparations, we used 150 μL of sonicated beetle tissue taken directly out of the allozyme sample after preparation. Genomic DNA was isolated and purified using procedures and materials from the QIAamp Tissue Kit (QIAGEN, Chatsworth, CA). DNA was eluted from the QIAamp spin columns with 150 μL of 10 mM Tris-HCl pH 8.0. Using 1 μL of the DNA extraction, we then amplified a 2120 bp fragment of the mtDNA genes cytochrome oxidase I and II (*COI* and *COII*) using the polymerase chain reaction (PCR). For all the samples, we used two universal insect primers, S1541 (known as 'Zeus', designed by the R. Harrison lab: 5′-TGA (G/T)C(C/T) GGA ATA

Table 1 Sample sizes of *Dendroctonus ponderosae* and *D. jeffreyi* analysed with allozymes and RFLP techniques in the Lassen National Forest and Mammoth Lakes regions of California in 1995 and 1996. Sites within regions correspond to areas indicated in Fig. 1

				Individuals analysed	
Region	Year	Host species	No. trees	Allozymes	RFLP
Dendroctonus ponderosae					
Lassen Region					
Off Rt. 44 S. Lassen	1995	*Pinus ponderosa*	3	35	15
Off Rt. 44 S. Lassen	1996	*P. ponderosa*	2	35	15
North Lassen	1995	*P. contorta*	1	30	15
North Lassen	1996	*P. contorta*	3	40	15
Central Lassen	1995	*P. lambertiana*	4	45	30
Mammoth Region					
Twin Lakes	1995	*P. contorta*	3	20	10
Rock Creek	1995	*P. contorta*	2	25	15
Twin Lakes	1996	*P. contorta*	2	25	10
Central Lassen	1996	*P. jeffreyi*	3	20	10
Twin Lakes	1995	*P. flexilis*	2	30	15
Twin Lakes	1996	*P. flexilis*	2	38	15
Dendroctonus jeffreyi					
Lassen Region					
South Lassen	1995	*P. jeffreyi*	3	50	18
South Lassen	1996	*P. jeffreyi*	2	40	18
Central Lassen	1996	*P. jeffreyi*	3	66	18
Mammoth Region					
North Mammoth	1995	*P. jeffreyi*	2	90	18
North Bishop	1995	*P. jeffreyi*	2	32	18
North Bishop	1996	*P. jeffreyi*	2	28	18

(C/G)TA GGA (C/G)CA TC-3′) and C2-N-3661 (Simon *et al*., 1994). Using 1 μL of extracted DNA, we amplified double-stranded DNA product under the following conditions: 10 pmole of each primer, 200 μM of each dNTP, 2.0 mM $MgCl_2$, 1× buffer provided by Promega (Madison, WI) and 1 unit of Taq DNA polymerase (Promega) in 100 μL total volume. Typical thermal cycling conditions were a 95°C denaturing step for 1 min followed by a 47°C annealing for 1 min and a 72°C extension for 2 min. This series of steps was repeated 35 times and ended in an indefinite 4°C refrigeration period until the reaction tubes were removed from the PCR machine.

Restriction fragment length polymorphism (RFLP) analysis

In order to survey for mtDNA variation, we used the SEQUENCHER 3.0 DNA alignment program (Gene Codes Corporation, Ann Arbor, MI) to map cut sites for 12 different restriction enzymes based on the *COI* sequence from *D. ponderosae* and *D. jeffreyi*: *Aci*I, *Ase*I, *Bam*HI, *Ban*I, *Dpn*II, *Hae*III, *Hha*I, *Hin*P1I, *Msp*I, *Nla*III, *Pst*I and *Spe*I. Using the PCR-amplified mtDNA gene fragments, we first surveyed populations of *D. ponderosae* and *D. jeffreyi* (24 individuals for each species from all hosts and sites) for restriction fragment length polymorphisms (RFLPs). Those enzymes that uncovered polymorphism were then used to digest the rest of the individuals for the RFLP study: 150 in *D. ponderosae* and 100 in *D. jeffreyi*. Samples for RFLP analysis were chosen at random to represent all collection sites and all trees and host species within each site. Restriction digest reactions were performed in microtitre plates to save time and reduce plastic waste (the plates could be washed out and re-used). Nine μL of DNA (straight from the PCR reactions) was placed in the microtitre plate wells, along with 11 μL of master mix that included: 2 μL of the appropriate 10× (buffer supplied with the enzyme), 0.2 μL BSA (if required), and 0.1 μL restriction enzyme all up to 11 μL in water. The microtitre plates were then covered with cellulose tape and placed in an incubator set at the appropriate temperature for 6 h. Finally, the digested DNA bands were separated on a 2% agarose gel with 0.5 μL 20 mg/mL ethidium bromide added per 10 mL gel.

Data analysis

Calculation of allozyme allele frequencies, and analyses of allele frequencies were all performed using the population genetics software POPGEN Version 1.2 (Yeh *et al.*, 1997). Contingency table (χ^2) analyses of allele frequencies were performed in a hierarchical manner: within each region (LNF and ML) and then between regions (following Stock & Amman (1980) and Sturgeon & Mitton (1986)). Within regions, we compared allele frequencies among generalists on the various host species, and among specialists among sites within the region. For the specialist, samples were considered within a site if they were separated by a distance of less than 10 km, beyond the typical dispersal distance of *Dendroctonus* beetles (Berryman, 1982). In both the specialist and generalist, these groups included individuals from multiple trees at that site or from the same host species (Table 1). For the comparison between regions, we pooled results from all individuals of both species within the two regions.

We used GDA to perform bootstrapping of F_{ST}s averaged over loci (Lewis & Zaykin, 1997). With this procedure, we asked whether average F_{ST} values were significantly different from zero (i.e. was there significant differentiation between populations). MtDNA RFLP data were analysed using AMOVA (Schneider *et al.*, 1997), which makes estimates of variance components for *F*-statistics analogues. These *F*-statistics analogues, ϕ-statistics, are comparable to F_{ST}, and incorporate sequence divergence between haplotypes (Excoffier *et al.*, 1992). Levels of significance for the ϕ-statistics were generated from 10 000 random permutations. ARLEQUIN was used to calculate standard haplotype F_{ST}s for the mtDNA data. To estimate the number of polymorphic nucleotide sites in the *COI–COII* region of mtDNA, we used the formula: $P_{sites} = C/(4(j) + 6(k))$, where C is the total number of polymorphic restriction sites found, and j and k are the total number of (monomorphic and polymorphic) 4-cutter and 6-cutter sites, respectively. This estimate makes the assumption that each polymorphic restriction site is polymorphic at only one nucleotide position.

Results

Genetic population structure among host plants

Allozymes Protein electrophoresis revealed some genetic differentiation among host species in the generalist, *D. ponderosae*. In Mammoth, contingency (χ^2) tests comparing allele frequencies between samples of *D. ponderosae* taken from lodgepole pine and samples taken from limber pine were significantly heterogeneous at the *PGI* locus, but not at *EST* and *PEP* (Table 2a). In Lassen, contingency tests revealed significant differentiation between *D. ponderosae* samples taken from lodgepole, ponderosa and sugar pine at the *EST* locus, but not at *PGI* or *PEP* (Table 2b). In comparison, *D. jeffreyi* showed no significant differentiation at any locus among sample sites in either Lassen or Mammoth (Table 2a,b).

Table 2 Comparison of specialist and generalist sister species of *Dendroctonus* in levels of differentiation in three allozyme loci and mtDNA, in (a) Mammoth and (b) Lassen (see Fig. 1). We treated beetles collected from different local sites (in the specialist) and different host species (in the generalist) as separate populations

	D. jeffreyi (specialist)		*D. ponderosae* (generalist)	
Locus	χ^2	d.f.	χ^2	d.f.
(a) Mammoth Lakes, CA				
EST	0.83 NS	3	8.99 NS	6
PGI	7.49 NS	4	8.61 *	2
PEP	0.80 NS	1	0.00 NS	0
	F	d.f.	*F*	d.f.
mtDNA	1.42 NS	1, 48	1.62 NS	1, 75
(b) Lassen NF, CA				
EST	7.81 NS	3	23.8 *	14
PGI	10.9 NS	5	3.51 NS	6
PEP	3.54 NS	1	9.71 NS	8
	F	d.f.	*F*	d.f.
mtDNA	0.16 NS	1, 52	1.36 NS	2, 75

*$P < 0.05$.

mtDNA In contrast to the allozyme data, mtDNA did not reveal any population structure associated with host species. AMOVA analyses of mtDNA haplotypes in *D. ponderosae* did not detect any subdivision associated with hosts in Mammoth or Lassen (Table 2a,b).

Genetic population structure between Mammoth and Lassen

Allozymes Comparison of *D. ponderosae* populations between Mammoth and Lassen also revealed some differentiation. Contingency tests found significant differentiation between populations of *D. ponderosae* at the *PEP* locus, but not at *EST* or *PGI* (Table 3). Comparisons of differentiation between populations of *D. jeffreyi* in Mammoth and Lassen were more striking: contingency tests showed significant differentiation at all of the allozyme loci (Table 3).

mtDNA AMOVA analysis also showed significant subdivision between *D. ponderosae* populations in the two regions (Table 3). Although contingency tests of allozyme allele frequencies showed significant differentiation between *D. jeffreyi* populations in Lassen and Mammoth, AMOVA analyses did not reveal any significant subdivision between the two regions (Table 3). This may have been because we found only three total mtDNA haplotypes in *D. jeffreyi*, and the AMOVA analysis uses haplotype networks to calculate variances (Excoffier *et al*., 1992). That is, the test of differentiation between areas may have been less sensitive for *D. jeffreyi* than for *D. ponderosae*, because of the low level of variability of mtDNA in *D. jeffreyi*.

Table 3 Comparison of specialist and generalist sister species of *Dendroctonus* in levels of differentiation in three allozyme loci and mtDNA, between the two regions in California, Lassen and Mammoth. The populations being compared were composed of samples pooled from individual beetles collected from all the various sites (for the specialist) and host species (for the generalist)

	Lassen NF vs. Mammoth Lakes			
	D. jeffreyi (specialist)		*D. ponderosae* (generalist)	
Locus	χ^2	d.f.	χ^2	d.f.
EST	17.05 **	6	9.55 NS	7
PGI	11.91 *	5	3.62 NS	3
PEP	23.95 ***	3	12.38 *	4
	F	d.f.	*F*	d.f.
mtDNA	2.68 NS	1, 99	7.97 *	1, 149

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Comparisons of overall differentiation

Mean allozyme F_{ST}s and their confidence limits were used to determine whether differentiation between regions was greater than differentiation between host species (in *D. ponderosae*) or sample sites within areas (in *D. jeffreyi*). This also allowed a contrast of differentiation between the specialist and generalist. We detected significant overall differentiation (mean $F_{ST} > 0$) between Lassen and Mammoth only in the specialist, *D. jeffreyi*. Mean F_{ST} values were not significantly different among sites or hosts in the same region for either *Dendroctonus* species, or between regions in *D. ponderosae* (Table 4).

Allozyme allelic diversity

Allelic frequencies for all loci and populations in both species are presented in Table 5. *Dendroctonus*

Table 4 Results from bootstrap analyses of differentiation across three allozyme loci. Mean F_{ST} values and 95% confidence intervals (CI) are given for all the various comparisons of populations in both *Dendroctonus ponderosae* and *D. jeffreyi*. Mean F_{ST} values with negative confidence intervals are not significantly different from zero

Population comparison	UL		$\hat{F}_{ST}$		LL
D. jeffreyi (specialist)					
(1) Between regions	0.074	≥	0.022	≥	+0.009 **
(2) Within Mammoth	0.009	≥	0.007	≥	−0.013 NS
(3) Within Lassen	0.041	≥	0.013	≥	−0.007 NS
D. ponderosae (generalist)					
(1) Between regions	0.022	≥	0.012	≥	−0.002 NS
(2) Within Mammoth	0.024	≥	0.013	≥	−0.007 NS
(3) Within Lassen	0.010	≥	0.007	≥	−0.005 NS

UL, upper limit; LL, lower limit.
**$F_{ST} > 0$.

Table 5 Allele frequencies for three polymorphic loci common to both *Dendroctonus ponderosae* and *D. jeffreyi*. Frequencies are presented for populations on various hosts and pooled within each of the regions

	Dendroctonus ponderosae							*Dendroctonus jeffreyi*					
	Between regions		Within Mammoth		Within Lassen			Between regions		Within Mammoth		Within Lassen	
Locus	ML	LNF	L	M	P	S	L	ML	LNF	NM	NB	SL	CL
EST													
N	136	170	80	56	65	45	60	150	156	90	60	66	90
1	0.017	0.027	0.009	0.036	0.029	0.012	0.033	0.000	0.013	0.000	0.000	0.015	0.011
2	0.299	0.209	0.296	0.304	0.164	0.209	0.258	0.000	0.051	0.000	0.000	0.000	0.089
3	0.229	0.215	0.280	0.125	0.231	0.326	0.117	0.053	0.122	0.056	0.050	0.121	0.122
4	0.161	0.232	0.169	0.143	0.216	0.209	0.267	0.287	0.282	0.311	0.250	0.334	0.244
5	0.161	0.205	0.144	0.196	0.224	0.163	0.217	0.633	0.500	0.611	0.667	0.500	0.500
6	0.109	0.091	0.085	0.161	0.097	0.069	0.100	0.027	0.026	0.022	0.033	0.030	0.022
7	0.000	0.018	0.017	0.036	0.037	0.012	0.000	0.000	0.005	0.000	0.000	0.000	0.011
PGI													
N	140	178	82	58	70	45	63	150	156	90	60	66	90
1	0.017	0.019	0.000	0.052	0.014	0.022	0.024	0.347	0.354	0.300	0.417	0.272	0.413
2	0.977	0.949	1.000	0.931	0.957	0.934	0.952	0.453	0.538	0.500	0.383	0.652	0.457
3	0.006	0.028	0.000	0.017	0.029	0.033	0.024	0.180	0.069	0.200	0.150	0.030	0.098
4	0.000	0.003	0.000	0.000	0.000	0.011	0.000	0.007	0.019	0.000	0.017	0.015	0.022
5								0.016	0.020	0.000	0.034	0.030	0.010
PEP													
N	132	168	78	54	65	42	61	150	143	85	58	62	88
1	0.006	0.000	0.000	0.000	0.008	0.012	0.000	0.000	0.066	0.000	0.000	0.032	0.089
2	1.000	0.926	1.000	1.000	0.900	0.941	0.943	0.571	0.730	0.540	0.620	0.726	0.734
3	0.000	0.042	0.000	0.000	0.062	0.048	0.016	0.429	0.197	0.460	0.380	0.242	0.167
4	0.000	0.024	0.000	0.000	0.023	0.000	0.041	0.000	0.007	0.000	0.000	0.000	0.011
5	0.000	0.003	0.000	0.000	0.008	0.000	0.000						

LNF, Lassen National Forest; ML, Mammoth Lakes; L, lodgepole pine; M, limber pine; P, ponderosa pine; S, sugar pine; NM, North Mammoth; NB, North Bishop; CL, Central Lassen; SL, South Lassen; *N*, sample size. (See Table 1 and Fig. 1 for details on collection locales and hosts.)

ponderosae showed more variation at *EST* and *PEP* than *D. jeffreyi*, but less at *PGI*. Overall there were no significant differences in allelic diversity between the two species ($\chi^2_2 = 3.0$, NS). Although no pairs of populations were fixed for different alleles, some alleles were not detected in certain populations. For instance, individual *D. ponderosae* collected from the Mammoth Lakes region were homozygous for *PEP* allele *2*. Similarly, we did not find some *EST* alleles (*1*, *2* and *7*) or *PEP* alleles (*1* and *4*) in the Mammoth populations of *D. jeffreyi*. In all cases, the ''missing'' alleles made up less than 7% of the total, and more extensive sampling might have uncovered them in the populations (Table 5).

Mitochondrial DNA haplotype diversity

Unlike the overall allelic diversity in allozymes, there were marked differences between *D. ponderosae* and *D. jeffreyi* in mtDNA diversity. Out of the 12 restriction enzymes used to survey for polymorphism in the mtDNA genes *COI* and *COII*, eight of the 12 uncovered polymorphism in *D. ponderosae*, whereas only two of the 12 revealed polymorphism in *D. jeffreyi*. We estimated that around 8.2% of nucleotide positions in the *COI* and *COII* gene regions were polymorphic in *D. ponderosae* compared with only 0.8% in *D. jeffreyi*. The results were also dramatic in terms of the total haplotype diversity uncovered in the two species. Data from the eight restriction enzymes showing polymorphism in *D. ponderosae* uncovered 31 distinct haplotypes, whereas the same set of enzymes revealed only three different haplotypes in *D. jeffreyi*.

Discussion

Our comparison of population structure within sister species of generalist and specialist herbivores had two important results: (i) the generalist showed somewhat greater genetic differentiation between host species than the specialist did on the same host species over similar geographical distances (Table 2), and (ii) the specialist showed the strongest levels of differentiation between the two geographical regions, Lassen and Mammoth (Tables 3 and 4). Similar to several other population genetic studies, we found significant subdivision at two loci in *D. ponderosae* samples living on different host species (Stock & Amman, 1980; Sturgeon & Mitton, 1986). In Mammoth, host-associated populations of *D. ponderosae* showed significant differentiation at *PGI* (Table 2a), whereas host species populations in Lassen were differentiated at the *EST* locus (Table 2b). In contrast, the monophagous sister species *D. jeffreyi* showed no evidence of differentiation at these loci over similar spatial scales (Table 2a,b; Fig. 1).

However, the evidence we found for differentiation among host species in the generalist was limited to one locus out of four in each of the regions, and a different locus in each case (Table 2). Moreover, the level of differentiation between populations on different host-plant species calculated over all allozyme loci was not significant (Table 4). Thus, although this and previous studies have provided some evidence of host-associated genetic differentiation in these beetles, the inconsistency of the differentiation from place to place suggests that it may be short-lived and is later swamped by gene-flow with other populations. Studies of other insects have shown that host-plant preferences differ among populations of the same species, which promotes isolation and differentiation (Thompson, 1993). However, such differentiation may most often only be temporary.

On the other hand, we found a strong pattern of differentiation between geographically separated populations, particularly in the specialist. In both the generalist and specialist, we found significant genetic differentiation at multiple loci between the two regions, Lassen and Mammoth, separated by 400 km (Table 3; Fig. 1). The generalist showed significant differentiation at the *PEP* locus and in mtDNA, whereas the specialist showed significant differentiation at all allozyme loci, though not in mtDNA (Table 3). When we compared levels of differentiation across all allozyme loci, *D. jeffreyi* showed significant overall levels of differentiation whereas *D. ponderosae* did not (Table 4). The fact that *D. ponderosae* shows significant mtDNA differentiation between regions, but *D. jeffreyi* does not (Table 3), may be caused by the paucity of haplotype diversity in *D. jeffreyi*.

These results suggest that geographical separation may play a stronger role in the isolation of populations than associations with different *Pinus* species in these beetles. Why should a specialist experience the effects of physical distance on population structure more acutely than a generalist? Perhaps because the distribution of the specialist's single host is patchier and less dense than the combined distribution of all the generalist's hosts, we might infer that populations of the specialist should also be less continuous as a result. In the study area, *D. ponderosae* uses eight *Pinus* whereas *D. jeffreyi* is monophagous on *P. jeffreyi* (Critchfield & Little, 1966; Wood, 1982). Reduced gene flow as a result of habitat fragmentation has often been reported in the conservation biology literature (Hall *et al*., 1995; Lacy & Lindenmayer, 1995).

Our finding of greater differentiation in a specialist compared to a generalist follows expectation, but seems to run counter to the study of Peterson & Denno (1998). We suggest that this simply indicates a difference in scale

and in precision of the comparisons made in each study. The specialist in the present study uses only a single host species, while the generalist uses most of the pine species in its broad range, and up to eight species in the region considered here. In fact, this same generalist, *D. ponderosae*, is considered a specialist in the literature (Peterson & Denno, 1998).

In order to determine whether the patterns found in this study are common and consistent, more studies are needed comparing genetic differentiation between generalist and specialist sister species. Also, because of the restrictions of the collection scheme (i.e. collecting two closely related species in the same geographical areas on multiple hosts), we were only able to sample in two geographical areas. It would be preferable if more areas could be sampled, though this may be easier said than done. These caveats aside, we feel that this study provides a 'blueprint' for how future studies on this topic might be undertaken.

Genetic variation: generalist vs. specialist

Overall levels of allozyme variation between *D. ponderosae* and *D. jeffreyi* were similar. In the three shared polymorphic loci we examined, each species had the same total number of alleles, though allele frequencies varied between populations (Table 4). In sharp contrast, however, we found substantial (10-fold) differences in the amounts of genetic diversity in mtDNA between the two species. Eight restriction enzymes uncovered 31 different haplotypes in *D. ponderosae* compared with only three in *D. jeffreyi*.

A comparable study of *COI* haplotype diversity in a pair of chrysomelid beetle generalist and specialist sister species also reported restricted mtDNA variability in the specialist (Dobler & Farrell, in press). The *Chrysochus* milkweed beetle species, *C. cobaltinus* and *C. auratus*, a generalist and specialist, respectively, use species of *Asclepias* and *Apocynum*. Samples of beetles screened with 10 restriction enzymes digests (for the same gene fragment as in the present study) revealed five haplotypes in the generalist that differed in frequencies among Californian populations (this species also showed differences in local host preferences). The much more widespread specialist (from the Rockies to the east coast) bears a single haplotype across its range, so haplotype diversity is not just a function of range size alone. Because contrasts of sister species offer phylogenetic control for other life-history variation that might influence population structure, consistent differences between resource (or habitat) specialists and generalists may yet be discovered as such studies become more common.

Thus, at least in mtDNA, these generalists appear to maintain a great deal more genetic diversity than their sister specialists. Why might there be such a disparity between these classes of genetic marker (allozymes vs. mtDNA)? First, restriction digest surveys usually reveal more variation than allozymes, which only detect amino acid substitutions affecting allozyme electrophoretic mobility (Mitton, 1997). Secondly, mtDNA has 1/4 the effective population size of diploid nuclear DNA and should coalesce much more quickly than nuclear genes, which makes mtDNA much more sensitive to factors that restrict effective population size and shorten coalescence times (Moore, 1995). These factors include patterns of dispersal, mating systems, sex ratios, historical bottlenecks, and smaller overall population sizes. Because dispersal patterns and mating behaviours are known to be extremely similar between *D. ponderosae* and *D. jeffreyi* (Wood, 1982), the disparity in mtDNA genetic diversity probably results from either an historical bottleneck (perhaps during speciation) or from smaller long-term population sizes in the specialist.

Distinguishing between these two alternatives, historical bottlenecks or differences in population sizes, is difficult. Circumstantial evidence for an historical bottleneck comes from the modern-day distribution of the two species: the range of *D. jeffreyi* is peripatric on the edge of *D. ponderosae*'s range (Wood, 1982). Small ancestral populations may have become isolated on *P. jeffreyi* and experienced a population bottleneck. Alternatively, the specialist might generally have smaller effective population sizes, which may have played a role in reducing the levels of mitochondrial variation by increasing the importance of genetic drift in the specialist compared with the generalist (Whitlock & Barton, 1997). Both *D. jeffreyi* and *D. ponderosae* are known to experience episodes of flushes and crashes in population density (Berryman, 1982), and theory predicts that populations which undergo frequent bottlenecks with small population sizes should have reduced amounts of genetic variation (Kimura & Ohta, 1971; Chesser, 1983). If *D. jeffreyi* is more prone to the effects of genetic drift, this may help explain the disparity in levels of genetic differentiation in mtDNA observed between the two species.

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