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SPATIAL PATTERN OF ALLOZYME VARIATION IN A CONTACT ZONE OF *PINUS PONDEROSA* AND *P. ARIZONICA* (PINACEAE)[1]

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The spatial distribution of genotypes for nine polymorphic allozyme loci was examined in a contact zone between *Pinus ponderosa* var. *scopulorum* and another tree regarded as either a separate species, *Pinus arizonica*, or variety, *Pinus ponderosa* var. *arizonica*, in southern Arizona. Previous work had identified a steep elevational cline for a key taxonomic trait, number of leaf-needles per fascicle, on the south slope of Mt. Lemmon. The present results indicate that the taxa are not fully interbreeding in this contact zone, because allozyme genotypes are considerably more spatially structured than expected for the dispersal characteristics of pines. The amount of spatial differentiation is also much less than that observed for needle number. It appears that this is due to the lack of differentiation for allozyme gene frequencies for the two types of trees, which is further evidenced by analysis of samples from two other populations away from the contact zone. It is likely that if the two taxa were isolated in the past, it was not for long enough nor complete enough to allow mutation-drift to create substantial differentiation between them. Another possible explanation is that introgression after recontact is so advanced that any differences have been erased throughout the Santa Catalina mountain range.

Key words: allozymes; contact zone; genetic differentiation; geography; mutation drift; *Pinus arizonica*; *Pinus ponderosa*; spatial genetic structure.

Ponderosa pine (*Pinus ponderosa* Law.) has a broad range throughout much of the western United States, and it extends south along the Rocky Mountains into the southwestern United States, where it reaches a transition zone. Whereas in the broad plateau country in northern Arizona and New Mexico, ponderosa pine is generally considered to occur as a single taxon, *Pinus ponderosa* var. *scopulorum* Engelm., in southern Arizona it co-occurs with and possibly interbreeds with another tree, variously regarded as a separate species, *Pinus arizonica* Engelm. (Perry, 1991; Farjon and Styles, 1997), or variety, *Pinus ponderosa* var. *arizonica* (Engelm) Shaw (Kearney and Peebles, 1964). Taxa in this region differ by a number of highly heritable seed cone and leaf-needle traits, including numbers of needles per fascicle (Rehfeldt et al., 1996; Rehfeldt, 1999). *Pinus ponderosa* has three needles whereas *arizonica* is a five-needle pine (e.g., Niebling and Conkle, 1990). *Pinus arizonica*'s distribution extends much farther south, well into the central mountains of Mexico. The five-needle form may be better adapted to warmer conditions. In certain regions of Arizona, New Mexico, and northwestern Mexico the ranges of the two taxa overlap (Perry, 1991; Flora of North America Editorial Committee, 1993; Farjon and Styles, 1997; Martin et al., 1998). In southern Arizona, *Ponderosae* populations are relatively small and scattered, confined to high mountains. In this area, three-needle trees tend to occur on mountain tops and the five-needle trees occur on slopes and canyons at somewhat lower elevations (F. W. Telewski, unpublished data).

A detailed study of one such transitional population, on Mt. Lemmon in the Santa Catalina Mountains, near Tucson, Arizona, revealed a striking spatial distribution of needle-number types (Epperson et al., 2001). Nearly all trees are pure three-needle types near the summit, and pure five-needle types ~1000 m downslope, while toward the middle of the transect many trees have characteristics of hybrids, containing a mix of three, four, and five needles. The spatial differentiation of needle numbers indicated that there is very strong selection acting, at either the intra- or interspecific level. The data also suggested the occurrence of low levels of hybridization. In the Mt. Lemmon population the amount of spatial autocorrelation for needle numbers is extremely high (Epperson et al., 2001), indeed possibly the highest reported for any genetic trait within a plant population (Epperson, 2003). It is completely inconsistent with the much weaker amounts of autocorrelation expected for neutral genes in a freely interbreeding population having the seed and pollen dispersal characteristics of *Pinus* species (Epperson, Huang, and Li, 1999).

In this study we investigated the spatial distribution of nuclear allozyme variants in the Mt. Lemmon contact zone, in order to determine the amount of spatial differentiation for nuclear genomic variation for genes that mutate at normal rates. This can be compared to the spatial differentiation of the ecotypic trait, needle number. If there is spatial structuring similar to that found for needle number, it would strongly indicate that the two types had been reproductively isolated (and probably geographically separated) for a very long time and that since recontact introgression has been insubstantial. No or

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little spatial structuring, beyond that expected for a freely interbreeding population, together with little differentiation, could be consistent with either low or high rates of hybridization, because the two taxa may not have been separated long enough for mutation drift to have created much differentiation since separation. Little spatial structure in the transect population, but high differentiation (in populations away from the transect), would indicate that the taxa had been reproductively isolated for a long period and that hybridization has been common since recontact.

MATERIALS AND METHODS

Sample—We assayed allozyme variation for the 81 trees originally identified in 1996, 71 of which were used in the earlier transect study on needle traits (Epperson et al., 2001), and we added a number of trees at the transect site as well as in two other populations in the region. All needle materials for allozyme analysis were collected in 1999. We also assayed or reassayed needle numbers for all trees, and based on this expanded sample, we reanalyze the spatial distribution of needle number.

In total, 105 sampled *Ponderosae* trees are located along an elevational gradient, beginning at ~2758 m (9080 feet [ft]) on the south-facing slope of Mt. Lemmon, Santa Catalina Mountains, Pima County, Arizona, and extending down to an elevation of ~2402 m (7880 ft). This gradient zone is dominated by *Ponderosae* but contains a transition from a primarily three-needle-leaved population of ponderosa pine (*Pinus ponderosa* var. *scopularum*) at the summit of Mt. Lemmon to a primarily five-needle-leaved population (*Pinus ponderosa* var. *arizonica* or *Pinus arizonica*) at the bottom of the transect. The belt transect has approximate dimensions, measured in horizontal distances, of 1.25 km by 100 m and an area of ~12.5 ha. The average slope is ~30%.

In 1996, the criteria for selecting the 81 trees included the availabilities of collectable cones with mature seed. Of these, needle numbers for 71 were used in the previous study, and the other 10 were resampled in 1999. Sample trees added in 1999 were intended to achieve three objectives: (1) add samples (six trees) representing the top end of the transect; (2) add samples (ten) extending the bottom end of transect; and (3) fill in (eight) two gap areas where few trees were sampled originally. Attempts were made to spread the added samples equally over each relevant area, but otherwise they were randomly chosen (availability of cones was not a criterion), subject to the constraint that they were at least 20 cm diameter at breast height (dbh) (i.e., at height ~147 cm or 4′10″).

A high-resolution Trimble GeoExplorer Model 17319-32 unit, together with a calibration base station (U.S. Forest Service, Santa Catalina Ranger District, Coronado National Forest, Arizona, USA) was used to obtain Global Positioning Satellite (GPS) coordinates for each sampled tree. Field coordinates were recorded in Universal Trans-Mercatur (UTM) units, calibrated, and plotted using ArcView Geographic Information System (GIS) software.

In addition, in an effort to assay possible outgroups for the five-needle types, samples were collected from 15 trees (>15 cm dbh) in Bear Canyon, located about 10.75 km southeast from the transect, at an elevation of 1829 m (6000 ft). The 15 trees were on the north side of a stream, close to a 1-ha study plot described by Schreve (1917), in a stand consisting mostly of *Ponderosae*. As a possible outgroup for the three-needle pines, 15 three-needle trees (>20 cm dbh) were sampled from just over the summit (and on the opposite side of the Mt. Lemmon Observatory), on the north-facing slope of Mt. Lemmon. Three groups of five trees were sampled at distances of ~0.60, 0.85, and 1.03 km, respectively, north from the top of the transect. Approximate elevations of the three groups were determined as, respectively, 2713 m (8890 ft), 2682 m (8800 ft), and 2658 m (8720 ft). In this zone on the north slope, common tree species included Douglas-fir (*Pseudotsuga menziesii*) and white fir (*Abies concolor*) at higher elevations and southwestern white pine (*Pinus strobiformis*) downslope. Collected needles were kept on ice and then shipped to the laboratory of M. G. Chung and stored at 4°C until protein extraction.

Needle number counts—The numbers of needles per fascicle were counted for several hundred fascicles from three branches from each tree. The number of needles per fascicle was determined, carefully accounting for broken, missing, malformed, or aborted needle leaf formation within each fascicle (Epperson et al., 2001).

Allozyme analysis—Needles were cut into fine pieces and crushed with a mortar and pestle. A potassium phosphate extraction buffer (Mitton et al., 1979) was added to the needles to facilitate crushing and to aid enzyme stabilization. The crushed extract was absorbed onto 4 × 6 mm wicks cut from Whatman 3MM chromatography paper, and the wicks were stored at −70°C until needed for allozyme analysis. Electrophoresis was performed using 11.5% starch gels. Out of 13 enzyme systems that were clearly resolved using three gel/electrode buffer combinations, we focused on nine polymorphic loci that produced very clear banding patterns. Stain recipes were taken from Soltis et al. (1983), except for diaphorase (Cheliak and Pitel, 1984). The genetic basis of allozyme banding patterns was inferred from segregation patterns with reference to typical subunit structure (Weeden and Wendel, 1989; Wendel and Weeden, 1989) and conceptual methods described in Gillet (1998). Putative loci were designated sequentially, with the most anodally migrating allozyme designated *1*, the next *2*, and so on. Similarly, alleles were designated sequentially with the most anodally migrating alleles designated by *a*. A Poulik buffer system, a modification (Haufler, 1985) of Soltis et al.'s (1983) system 6 was used to resolve alcohol dehydrogenase (*Adh-2*), flouresent esterase (*Fe-2*), phosphoglucomutase (*Pgm-1*), and triosephosphate isomerase (*Tpi-2*). A histidine citrate buffer system, a modification (Chung and Kang, 1994) of Soltis et al.'s (1983) system 11 was used to resolve isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh-3*), 6phosphogluconate dehydrogenase (*6pgd-1, 6pgd-2*). Soltis et al.'s (1983) system 7 was used to resolve diaphorase (*Dia*-3).

Statistical analysis—The mean number of needles per fascicle and its standard error (SE) were calculated for each tree using SAS procedure Means (SAS, 1982). For spatial autocorrelation analysis of allozyme genotypes, each allele "A" was characterized separately. If tree *i* was homozygous for allele A, it was assigned the value $X_i = 1.0$. If it were heterozygous for or had no copies of that allele, it was assigned values 0.5 or 0, respectively. Spatial structure was quantified using unweighted Moran's *I* statistics (Sokal and Oden, 1978; Cliff and Ord, 1981). Each pair of trees was assigned to one of a number of mutually exclusive distance classes. Distance classes were formed, generally for 100-m intervals, as in Epperson et al. (2001). Allele *a* of *Dia-3* occurred as only one copy in the entire sample of 105 trees, and it was excluded from spatial analysis as being noninformative. For each allele and distance class, Moran's *I* statistic was calculated using the standard formula

$$I = \frac{n \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} Z_i Z_j}{W \sum_{i=1}^{n} Z^2{}_i} \quad (1)$$

where n is the number of individuals (105); w_{ij} equals 1.0 if ith and jth individuals are in the distance class and zero otherwise; W is twice the total number of pairs for the distance class; $Z_i = X_i - X$, $Z_j = X_j - X$; and X is the grand mean value for all individuals in the sample. Each I value was tested for significant deviation from the expected value, $E(I) = -1/(n - 1)$ under the null hypothesis of a spatially random distribution (Cliff and Ord, 1981). A significant positive value of Moran's *I* indicates that pairs of individuals in the distance class have positively correlated genetic values, whereas a significant negative value indicates that they have negatively correlated values. In addition, each set of *I* statistics for mutually exclusive distance classes, known as an *I* correlogram, was tested for statistical significance, using Bonferroni's criteria. *I* was also calculated for X_i = mean number of needles per fascicle for tree *i*. All spatial autocorrelation results were obtained using the SAAP computer program of Wartenberg (1989). Distance classes were based on the Euclidean horizontal distance between pairs of trees, estimated using

UTM coordinates obtained through the GPS data gathered at the field plot. Because the area is small, the distance values correspond closely to meters.

Several statistical analyses were conducted to determine the degree of allozyme differentiation between the three-needle and five-needle forms. Twelve trees at the bottom of the transect (BH) were chosen with the criteria that they have mean needle numbers closest to 5.0. Similarly, 12 trees at or near the top of the transect (TH) with needle numbers closest to 3.0 were grouped. The pattern of allelic differentiation among these two groups and the two other population samples, Bear Canyon (BC) and north slope of Mt. Lemmon (OH), was assessed using the Genetic Data Analysis program of Lewis and Zaykin (2001) to calculate Weir and Cockerham's (1984) θ estimator of the theoretical measure F_{ST} of population genetic differentiation, and Nei's (1978) measures of genetic identity and distance (which correct for sample sizes), and to conduct the unweighted pair-group method with arithmetic mean (UPGMA).

RESULTS

Allozyme data—Many of the individual values of spatial autocorrelation as well as entire correlograms were statistically significant, indicating overall that the spatial pattern is not random. The values also varied markedly among loci, and this includes the values for the especially important first distance class (Table 1), indicating that the amount of small-scale autocorrelation varies among loci. Large positive values at short distances indicate substantial spatial autocorrelation for those alleles. Importantly, some correlograms have large negative values at long distances, which is generally interpreted as indicating a cline, in addition to smaller scale autocorrelation. Clines are strongly indicated for *Idh,* for alleles *b* and *c* of *Mdh-3,* for alleles *b* and *c* of *6pgd-2,* and for alleles *b* and *c* of *Pgm-1*. In addition, there are noteworthy large negative values at intermediate distances for many alleles.

The overall average correlogram was also calculated (Fig. 1). Values for diallelic loci are included only once because the values for each of the two alleles are completely correlated. Only one value for the triallelic *Adh-2* is included because after excluding the noninformative allele *a,* the remaining two alleles are nearly completely correlated. Approximate standard errors on the average, under the null hypothesis, are shown in Table 1, neglecting the correlations among values of different alleles of the same locus. Such correlations can be substantial, and they are generally either small or, if large, then positive, whereas those among loci should be near zero (Epperson, 2003). Thus the standard errors reported may be considered lower bounds and the resulting statistical test of significance slightly liberal. Nonetheless, because the reported instances of statistical significance have high values for standard normal deviates, they should be considered reliable. The overall correlogram indicates substantial positive autocorrelation at short distances. Further, it smoothly decreases with distance, indicating a weak but real cline along the transect.

Genetic distances and genetic identity measures (Nei, 1978), based on allozyme gene frequencies, among the groups TH, BH, OH, and BC are shown in Table 2. The UPGMA analysis of the genetic distances clustered the three-needle pines at the top of the transect (TH) with the three-needle pines on the north flank of Mt. Lemmon (OH), and the five-needle pines at the bottom of the transect (BH) clustered with the mostly five-needle population located at Bear Canyon (BC). In addition, note that BH is more different from OH than from TH, suggesting some influence of hybridization in the TH in the transect area. Note also that even though BC clusters with BH, the similarity value is considerably less than that between OH and TH. Moreover, BH is more different from OH than is BC. This suggests that BC may be somewhat ''contaminated'' with three-needle genes, more so than BH is. Thus while OH appears to be a good outgroup for three-needle pines, BC appears to be a less pure five-needle population than BH itself.

The degree of differentiation varies considerably among loci. Values of θ calculated by treating combined samples BH + BC (the ''fives'') vs. OH + TH (''threes'') range from −0.02 to 0.25 (Table 3). Similar results were obtained treating the four samples separately (Table 3). In addition, the ''threes'' had six ''private'' alleles (*Adh-2b, Fe-2c, Idhb, 6pgd-1a, 6pgd-2a,* and *Pgm-1c*) not found in the sample of ''fives,'' although it should be noted that five of these (*Adh-2b, Fe-2c, Idhb, 6pgd-2a,* and *Pgm-1c*) occurred only in the OH group, not in TH. In comparison, there were three private alleles for the ''fives'' (*Adh-2c, Mdh-3d,* and *Tpi-2b*), and two of these (*Adh-2c* and *Mdh-3d*) were found only in the BC group. Tables 1 and 3 indicate that large *I* statistics occur only for loci that are differentiated among the needle types. *Tpi-2* is unusual in that it was the most differentiated but did not show autocorrelation in the transect.

Needle number data—Figure 1 also shows the *I* correlogram for the mean number of needles per fascicle. As in the previous analysis (Epperson et al., 2001), a very high level of spatial autocorrelation is indicated by very high positive values for short distances. The cline is evident in the increasingly large negative values at larger distances. Its orientation is with three-needle pines at the top end of the sample area and five-needle pines at the low end.

The mean needle numbers for the 15 trees in the sample from the north slope of Mt. Lemmon was 3.12, with values ranging from 3.00 to 3.70. The median was 3.03, and the one tree with 3.70 accounted for much of the standard deviation, 0.21. Overall, this indicates that the sample from the north slope is indeed nearly pure three-needle pines. The mean value for the 15 trees from Bear Canyon was 4.22. Values ranged from 3.02 to 4.94, with a much larger standard deviation of 0.62, indicating that Bear Canyon is a mixed population, not the pure five we expected. Most of the variation is caused by three trees with numbers near 3.0 (3.02, 3.21, and 3.29), although there were also several trees with values near 4.0, as well as the numerous individuals near 5.0.

DISCUSSION

The degree of autocorrelation of allozyme loci is highly variable but averages to moderate values, for example 0.07 for distance class 1 (0–100 m). The allozyme genotypes are not randomly distributed. Moreover, they show greater average levels of autocorrelation than expected based on neutral isolation by distance models and the dispersal characteristics of *Ponderosae*. Based on estimated density and using a realistic range of distances of dispersal of pollen and seed, we estimated that Wright's (1943) neighborhood size must exceed 384 (Epperson et al., 2001). A neutral locus in a freely interbreeding population at equilibrium or ''quasi-equilibrium'' under isolation by distance should have a value of ~0.01 or less for Moran's *I* statistic for distance class 1 (Epperson, Huang, and Li, 1999).

The average levels of autocorrelation are also far smaller than that observed for the characteristic trait of needle number. The latter values are among the highest ever reported for ge-

TABLE 1. Moran's *I* spatial autocorrelation statistic for each allozyme allele and distance class. Also shown is the allele frequency, *q*, and the upper bound on the distance class (in meters).

	Distance class											
Allele	1 (100 m)	2 (200 m)	3 (300 m)	4 (400 m)	5 (500 m)	6 (600 m)	7 (700 m)	8 (800 m)	9 (900 m)	10 (1300 m)	P^a	*q*
Adh-2a	0.16**	0.11**	−0.08*	−0.14**	−0.18**	−0.09*	−0.06	0.05	0.06	0.06*	0.000	0.943
Adh-2b	0.06*	0.09**	−0.04	−0.09*	−0.09*	−0.09*	−0.07	0.00	0.03	0.07*	0.010	0.031
Adh-2c	0.08**	0.00	−0.06	−0.08*	−0.08*	−0.05	0.04	0.08*	0.03	−0.01	0.045	0.026
Dia-3b	0.08**	−0.02	0.03	−0.03	−0.13**	−0.07	−0.03	−0.02	0.05	0.03	0.024	0.742
Fe-2a	0.03	−0.07	−0.01	0.03	0.00	−0.03	0.06	−0.00	−0.08	−0.08	0.535	0.593
Idha	0.04	0.04	0.00	0.05*	0.04	−0.05	−0.07	−0.10*	−0.11*	−0.11**	0.074	0.974
Mdh-3a	−0.01	0.01	0.03	−0.03	0.01	−0.03	−0.02	−0.04	−0.06	−0.03	1.000	0.021
Mdh-3b	0.05*	0.11**	−0.00	0.06*	−0.02	−0.08	−0.04	−0.13*	−0.16**	−0.14**	0.002	0.057
Mdh-3c	0.04	0.06*	−0.00	0.05	−0.00	−0.03	−0.09	−0.04	−0.17**	−0.11*	0.051	0.892
Mdh-3d	0.01	0.05*	0.04	−0.04	−0.09*	−0.11*	−0.07	−0.03	0.02	0.08*	0.142	0.031
6pgd-1a	0.04*	0.01	−0.00	−0.01	−0.01	−0.02	−0.06	−0.06	−0.08	0.00	0.488	0.015
6pgd-1b	0.23**	0.02	−0.14**	−0.08*	0.02	0.06	−0.21**	−0.23**	−0.09	0.16**	0.000	0.320
6pgd-1c	0.32**	0.07*	−0.16**	−0.16**	−0.14**	−0.03	−0.10*	−0.17**	−0.00	0.16**	0.000	0.490
6pgd-1d	0.06*	0.03	−0.05	−0.09*	−0.14**	−0.06	0.09*	0.06	0.11*	−0.02	0.009	0.175
6pgd-2a	0.09**	0.03	−0.03	−0.08*	−0.05	−0.04	−0.03	−0.03	−0.04	0.01	0.005	0.015
6pgd-2b	0.06*	0.02	−0.03	0.06*	0.04	−0.03	−0.08	−0.09	−0.10	−0.10*	0.106	0.974
6pgd-2c	0.06**	0.01	0.00	−0.00	−0.01	0.01	−0.05	−0.06	−0.05	−0.11**	0.020	0.010
Pgm-1a	−0.06	−0.00	0.01	−0.01	0.02	−0.05	0.01	−0.03	0.01	−0.00	0.808	0.077
Pgm-1b	0.01	0.03	−0.02	−0.01	0.01	−0.05	0.02	−0.01	0.01	−0.11**	0.086	0.856
Pgm-1c	0.21**	−0.00	0.04	0.05*	−0.05	−0.05	−0.04	−0.07	−0.15**	−0.28**	0.000	0.057
Pgm-1d	−0.04	0.04*	−0.03	−0.02	−0.03	−0.02	−0.03	−0.02	0.02	0.02	0.197	0.010
Tpi-2a	−0.03	−0.04	0.04	0.01	−0.06	0.07*	−0.05	0.06	−0.05	−0.05	0.339	0.928
Average	0.07	0.03	−0.02	−0.03	−0.04	−0.04	−0.04	−0.04	−0.04	−0.03		
SE	0.007	0.007	0.007	0.008	0.009	0.009	0.009	0.011	0.012	0.008		
SND[b]	11.4**	5.7**	−1.4	−2.5**	−3.3**	−3.3**	−3.3**	−2.7**	−2.5**	−2.5**		

* Significant at the 5% level, **significant at the 1% level.

[a] Probability value for the test of the null hypothesis that the correlogram is not significant.

[b] Standard normal deviate.

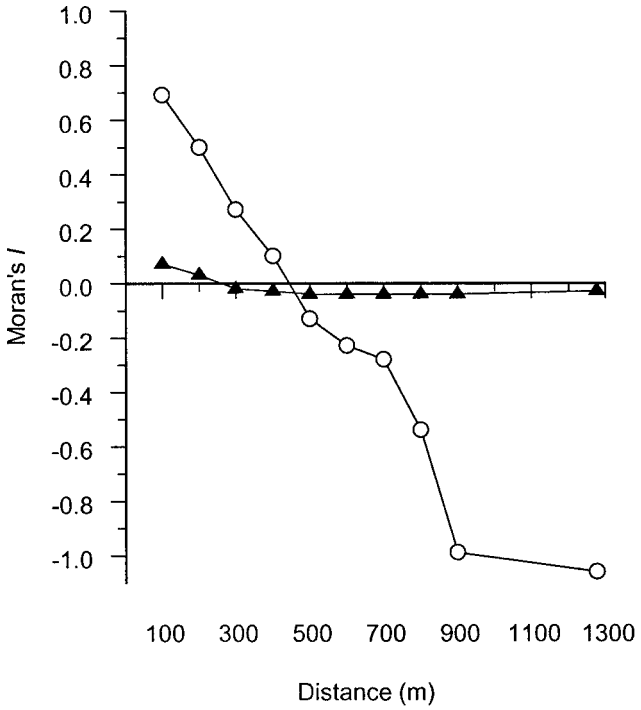

Fig. 1. Correlogram of Moran's autocorrelation statistic, *I*, as a function of distance, for the mean number of needles per fascicle for individual trees (○) and the average for allozyme genotypes (▲).

netic traits in any species (Epperson, 2003), and they are even slightly higher than we observed based on a subset of the present needle data set (Epperson et al., 2001). They show that the population is structured near the theoretical maximum. Moreover, a very strong cline is clearly indicated, as is its orientation.

Loci are generally subject to more or less the same mating system, so why do allozyme genotypes have less spatial structure than does the needle trait? If both were selectively neutral, then they should have similar spatial structures under genetic isolation by distance models for freely interbreeding populations at equilibrium (Epperson, 1995). The strength of the cline for needle number made it clear that selection is operating either on genetic variation for needle number (heritability ~60%: Rehfeldt, 1999) itself, or on other genes of the taxa it represents, with which it may be in strong linkage disequilibrium (Epperson et al., 2001). However, it is unknown whether selection for the taxa is interspecific (i.e., the two types are completely nonhybridizing ''biological species'') or intraspecific (i.e., the two hybridize at substantial levels). In contrast, allozyme variation is generally believed to be selectively neutral. Difference in selection is an important part of the explanation of the difference in spatial structure for allozymes compared to needle number.

We can further examine the role of selection in determining spatial structure in the transect by considering two opposite scenarios about how much hybridization-mediated gene flow has been occurring. First consider the scenario where there has been little or no recent hybridization. It follows that one possibility is that the difference in spatial structure for allozymes and needle number is simply due to there never having been much differentiation for the allozymes between the two taxa. Our analysis using pure three- and pure five-needle pines from the ends of the transect as well as the two other populations clearly shows that the allozymes are much less differentiated. Moreover, those loci that show very low differentiation also show little or no spatial autocorrelation along the transect. For the two loci that were somewhat more differentiated, one, *6pgd-1*, showed much greater autocorrelation than the other seven loci, but the other, *Tpi-2*, did not. However, we should note that the sample sizes used to establish differentiation are small and that the high estimated level of differentiation for *Tpi-2* is due to the five needle-types having a ''private allele'' at moderate frequency. Loci that are not differentiated between the two types should show only a background level of spatial autocorrelation that fits a freely interbreeding population, even if there really are two distinct noninterbreeding populations. Each would have the same type of structure within it, and combining the two over the transect area would result in an overall spatial distribution like that of its two parts. In contrast, if the two were completely differentiated for allozymes, then the spatial distribution of allozyme genotypes should reflect that of the needle types. Difference in rates of mutation between the genes controlling allozyme variation vs. those controlling the needle trait could explain the difference in spatial structures for allozymes vs. needle number. It would have caused differences in the amount of newly generated genetic differentiation that has occurred between the two taxa since they initially became reproductively isolated. However, there

TABLE 2. Nei's (1978) measures[a] of distance (below diagonal) and identity (above diagonal), among four groups of trees,[b] based on all nine allozyme loci.

	Group			
Group	TH	BH	OH	BC
TH		0.980169	0.988986	0.965089
BH	0.020030		0.945892	0.970972
OH	0.011075	0.055627		0.963279
BC	0.035535	0.029458	0.037412	

[a] Measures correct for finite sample size.

[b] TH is a group of 12 three-needle trees near the top (which is near the summit of Mt. Lemmon) of the transect; BH is a group of five-needle trees at the bottom of the transect; OH consists of 15 (mostly three-needle) trees located at high elevations on the opposite side of the summit of Mt. Lemmon; and BC is a group of 15 (mostly five-needle) trees in Bear Canyon, located about 10.75 km southeast from the transect and at a low elevation.

TABLE 3. θ estimates of F_{ST} among all four groups (TH, BH, OH, and BC) of trees described in the text and Table 2, for each locus and combined over loci.

	θ	
Locus	All four groups	''Threes vs. fives''[a]
Adh-2	0.01	0.00
Dia-3	0.07	−0.00
Fe-2	0.05	−0.01
Idh	0.03	0.02
Mdh-3	0.03	−0.02
6pgd-1	0.12	0.16
6pgd-2	−0.01	0.00
Pgm-1	0.06	0.00
Tpi-2	0.16	0.25
Overall	0.08	0.07

[a] Values of θ calculated by treating combined samples BH + BC (the ''fives'') vs. OH + TH (''threes'').

appears to be no reason to expect that the mutation rate for needle number genes is much greater than that for allozyme alleles (e.g., Hedrick, 2000). Selection for needle number during isolation would be the most likely cause for the difference in differentiation. Moreover, apparently the needle genes differentiated in direct response to selection, because it seems somewhat unlikely that hitchhiking would cause such differentiation for needle traits but not allozymes.

In the second scenario where hybridization is common, it could be reducing spatial structure and differentiation for allozymes, while very strong (intraspecific) selection maintains the cline in needle number. As pointed out in our earlier paper (Epperson et al., 2001), the pattern of trees with intermediate numbers of needles in the center of the transect and the preponderance of pure three-needle types at the top and pure five-needle types at the bottom strongly suggest that some hybridization is occurring. Moreover, some reproductive compatibility between the taxa has been demonstrated (Conkle and Critchfield, 1988). But the required amount of gene flow would have to be quite high in order to equalize any substantial differences in allele frequencies for allozymes that might have existed before contact. It is also worth noting that this again would suggest that needle number per se is under direct selection, rather than genetic hitchhiking. It seems unlikely (but not impossible) that genes controlling needle number would maintain strong disequilibrium with other, selected genes while the allozymes would not. It is still possible under the scenario of recent high levels of hybridization that rates of mutation were not high enough to have created much differentiation for allozymes during any period of isolation before the present contact.

Finally, as in our previous paper, we further tested Rehfeldt's (1999) idea that there exists a ''taxon X,'' a primarily three- and four-needle taxon, by conducting an additional spatial autocorrelation analysis of the needle number data, after excluding all individuals having mean needle values ≥ 4.6 (following the Peloquin's [1984] definition of a ''five-needle type''). The following values of Moran's *I* statistics were obtained: 0.04, −0.02, 0.04, −0.03, −0.04, 0.01, −0.04, for the first seven of the distance classes in Fig. 1, and −0.29 for the largest distance class, from 700 to 902 m (exclusion of ''five-needle types'' shortened the transect by ~400 m). The values are very similar to, only somewhat stronger than, those for the analogous statistics calculated in our earlier paper (Epperson et al., 2001). In that paper we argued that although the values for short distances are much smaller than those for the full data set and indicate that the various (non-five-needle) types are indeed highly mixed over short distances, the very large negative value for the longest distance class (700–902 m) indicates strong spatial differentiation over the upper two-thirds of the transect. Inspection of the spatial distribution clearly indicates the orientation of this differentiation: trees with needle numbers nearer to 3.0 occur on the top, whereas (non-five-needle) trees with larger values are concentrated 600–800 m down the mountainside. The fact that the putative hybrid types are concentrated in the transition zone is consistent with their truly being hybrids or advanced introgressed types and suggests that there is no taxon X on Mt. Lemmon.

It would be useful to investigate the transect with molecular markers having very high mutation rates, and we are in the process of identifying polymorphic loci for microsatellites, simple sequence repeats (SSRs). If there is marked differentiation along the transect and for the two other populations, it would suggest that the two taxa have not experienced advanced introgression and that the role of hybridization has been minor. Consequently, this would mean that there has been sufficient time for mutation drift to have differentiated SSRs but not enough for allozyme differentiation, between the two taxa.

In summary, allozyme genotypes show more spatial autocorrelation than expected for a freely interbreeding population, but relatively little differentiation compared to needle number. The contrast suggests that the initial differentiation of needle types was caused by direct selection on the genes controlling needle number. It appears that *P. ponderosa* and *P. arizonica* have not been separated long enough or completely enough for mutation drift to have caused much allozyme differentiation. Thus, those parts of the nuclear genome (apart from the genes controlling ecotypic traits) that have similar or lower mutation rates also should not have differentiated much. The needle data suggests that hybridization does occur on Mt. Lemmon.

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