Genetic Structure of *Dendroctonus mexicanus* (Coleoptera: Curculionidae: Scolytinae) in the Trans-Mexican Volcanic Belt

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ABSTRACT It is assumed that geographic isolation of *Dendroctonus* Erichson species populations or their plant hosts determines genetic structure. This structure can be analyzed with respect to the biogeographic pattern that describes the species in a region. The Trans-Mexican Volcanic Belt (TMVB) is located between the Neartic and Neotropical regions and is a center of diversification and endemism of trees in the genus *Pinus* L. *Dendroctonus mexicanus* Hopkins is polyphagous within *Pinus* species and has a continuous geographic distribution across the TMVB. We explored whether the population genetic structure of *D. mexicanus* is reflective of the distribution pattern of the *Dendroctonus* species that occur in the TMVB. Twelve gene loci were analyzed by isozyme electrophoresis in 17 populations found on pines from the *Leiophyllae* subsection. Allele frequencies, average heterozygosity, heterozygosity by locus, deviations from Hardy–Weinberg equilibrium (HWE), F-statistics among populations, and average genetic flow were calculated. Genetic structure was determined using the relationship between F_{ST} versus geographic distances among populations. Genetic relations among populations were established by neighbor-joining and principal components analysis by using Nei's genetic distances. Dendrogram reliability was assessed by bootstrap analysis and cophenetic correlation coefficient by using the Mantel test. Results show that heterozygosity of *D. mexicanus* is similar to other scolytids. A high proportion of loci were out of HWE by homozygous excess, which may be explained by multiple factors. The scarce number of fixed alleles, the allele variation pattern, pairwise genetic distances, and F-statistics suggest a model of isolation by distance for *D. mexicanus* in the TMVB resulting from recent dispersal events.

KEY WORDS *Dendroctonus*, bark beetle, genetic structure, isozyme, Mexico

The diversification of bark beetles is associated with the origin of the gymnosperms and angiosperms (Sequeira et al. 2000) and with the way in which the species share resources in space and time (Sturgeon and Mitton 1982). The study of genetic structure in both geographic and phylogenetic contexts is fundamental to understanding the differentiation processes affecting populations of these insects. It is assumed that geographic isolation of *Dendroctonus* Erichson per se and the use of different hosts have been the determining factors in the differentiation of their populations (Anderson et al. 1979, 1983; Namkoong et al. 1979; Stock and Guenther 1979; Stock et al. 1979, 1984; Stock and Amman 1980; Higby and Stock 1982; Sturgeon and Mitton 1986; Roberds et al. 1987; Langor and Spence 1991). Although these studies have documented significant genetic differences among populations, it has been difficult to discern whether these differences are a result of geographic isolation or processes of local adaptation to hosts.

Genetic differentiation of *Dendroctonus* populations apparently is a complex process, where local adaptations to particular hosts, the geographic distribution of the insects and their hosts, as well as the distinct geomorphologic histories of the areas where insects are distributed determine different levels of allopatry or sympatry of populations that affect the genetic structure in space and time. Examples of this complexity are found in studies of genetic differentiation in *Dendroctonus ponderosae* Hopkins conducted in small areas of its geographic range, which extends from southern British Columbia in Canada to Baja California in Mexico (Stock and Guenther 1979; Stock and Amman 1980, 1985; Higby and Stock 1982; Stock et al. 1984; Sturgeon and Mitton 1986; Langor and Spence 1991). The geographic distribution of this species is embedded in different geomorphological areas whose ecological and historical conditions are distinct, which makes it difficult to compare the genetic com-

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position of populations from different locations. In addition, this insect colonizes 11 species in the genus *Pinus* L. that overlap in geographic distribution (Farjon and Styles 1997, Kelley and Farrell 1998). In some regions where multiple host species coexist, *D. ponderosae* is capable of using several of them. In this situation, it is difficult to associate observed genetic differences with specific factors that might contribute to these differences.

Specialization in the use of hosts may be an important factor influencing the differentiation of phytophagous insects (Thompson 1994). The importance of specialization has been investigated in *Dendroctonus* by assessing the correspondence of the generalist or specialist nature of the species and their molecular phylogeny (Kelley and Farrell 1998). Geographically distinct populations of *Dendroctonus brevicomis* LeConte from the eastern and western portions of their distribution have been reproductively isolated by use of geographically separated varieties of *Pinus ponderosa* Douglas ex Lawson (Kelley et al. 1999). Levels of genetic differentiation were compared between *Dendroctonus jeffreyi* Hopkins and *D. ponderosae*, which are sister species that differ in diet breadth (*D. jeffreyi* only colonizes *Pinus jeffreyi* Balfour, whereas *D. ponderosae* colonizes numerous species of *Pinus* but not *P. jeffreyi*). Slight genetic differences were found among populations of these species colonizing different hosts in the same locality, but marked differences were found among geographically separated populations, particularly among populations of the specialist species (Kelley et al. 2000). Although these results suggest the degree of specialization of these species and the pattern of geographic distribution of their hosts influence isolation of the populations, they also indicate that the balance among dispersion, gene flow, and geographic isolation is a fundamental component affecting their differentiation.

A different way of analyzing the effect of geographic isolation of populations on genetic structure, which is not in conflict with the generalist or specialist condition of the taxa, is recognizing a priori the individual distribution and overlapping patterns that describe the distributions of species within a genus across a predetermined geographic region. These patterns of distribution are a reflection of how species share geographic space and are the result of the combined actions of various factors such as competition and type of habitat as well as ecological and historical factors of the geographic region (Brown and Lomolino 1988). Thus, once the pattern of geographic distribution of the species in a region is determined, and the biotic and morphotectonic history of the region is known, it is possible to analyze the effect of geographic isolation on the spatial genetic structure at a mesoscale level (Guisan and Hofer 2003).

The geographic distribution pattern of the genus *Dendroctonus* in Mexico (Fig. la) suggests that the Trans-Mexican Volcanic Belt (TMVB) has been a corridor for its species (Salinas-Moreno et al. 2004), despite that for their host genus *Pinus*, it has been a center of diversification and endemism (Farjon and Styles 1997). In the TMVB, *Dendroctonus mexicanus* Hopkins presents a nearly continuous geographic distribution over a wide elevation range (1,600–2,800 m) (Fig. 1b); coexists with *Dendroctonus adjunctus* Blandford, *Dendroctonus approximatus* Dietz, *Dendroctonus frontalis* Zimmermann, *Dendroctonus parallelocollis* Chapuis, and *Dendroctonus valens* LeConte; and is a generalist species that colonizes nearly all known *Pinus* spp. in the region (Salinas-Moreno et al. 2004).

In this study, we examine the hypothesis that the population genetic structure of *D. mexicanus* reflects the distribution pattern of the *Dendroctonus* species that occur in the TMVB. In particular, we use isozyme analysis to determine whether the genetic structure of *D. mexicanus* exhibits a model of isolation by distance in a east–west direction as is predicted from the analysis of the overlapping patterns of distribution of *Dendroctonus* species in the TMVB (Salinas-Moreno et al. 2004).

Materials and Methods

Study Area. The TMVB is the most recently formed mountain chain of the Mexican territory; it is situated between the 19° and 21° N latitude, dividing Mexico in half (Fig. 1c). The TMVB is considered a transitional area between Neartic and Neotropical regions. The western portion of this region dates to the Mid-Cenozoic, whereas the eastern region dates to the Late Cenozoic. Its southern part is separated from the Sierra Madre del Sur by the depression of the Balsas and its northeastern portion extends toward the Sierra Madre Oriental across a series of small mountain ranges (Ferrusquía-Villafranca 1998). Internally, the TMVB is formed by a series of plains extending from the Pacific Coast to the Atlantic, interrupted by mountains and volcanoes. It includes the highest mountains of Mexico, which are of Plio-Pleistocene origin, and which reach elevations between 4,090 and 5,650 m. The predominant elevation of the TMVB ranges between 1,500 and 2,500 m.

Study Species. *D. mexicanus* occurs in all mountain systems from Mexico, colonizes 24 of the 42 *Pinus* species reported in the country (Farjon and Styles 1997), and has three to six generations per year, depending on temperature and elevation (Cibrián-Tovar et al. 1995). In the TMVB, *D. mexicanus* has four overlapping generations per year and predominantly colonizes *Pinus* spp. from the *Leiophyllae*, *Oocarpae*, and *Pseudostrobi* subsections, which have discontinuous distributions in this region (Salinas-Moreno et al. 2004).

Collection and Electrophoresis. Seventeen geographically distinct populations of *D. mexicanus* from the TMVB were studied (Fig. 1d). The distances between populations varied from 6.9 (San Rafael-Amecameca) to 361 km (Chimalhuacan-Milpillas), and the elevation of collection sites ranged from 2,200 to 2,980 m. All samples were taken from pines in the *Leiophyllae* subsection, particularly *Pinus leiophylla* variety *leiophylla* Schiede ex Schlechtendal & Chamisso.

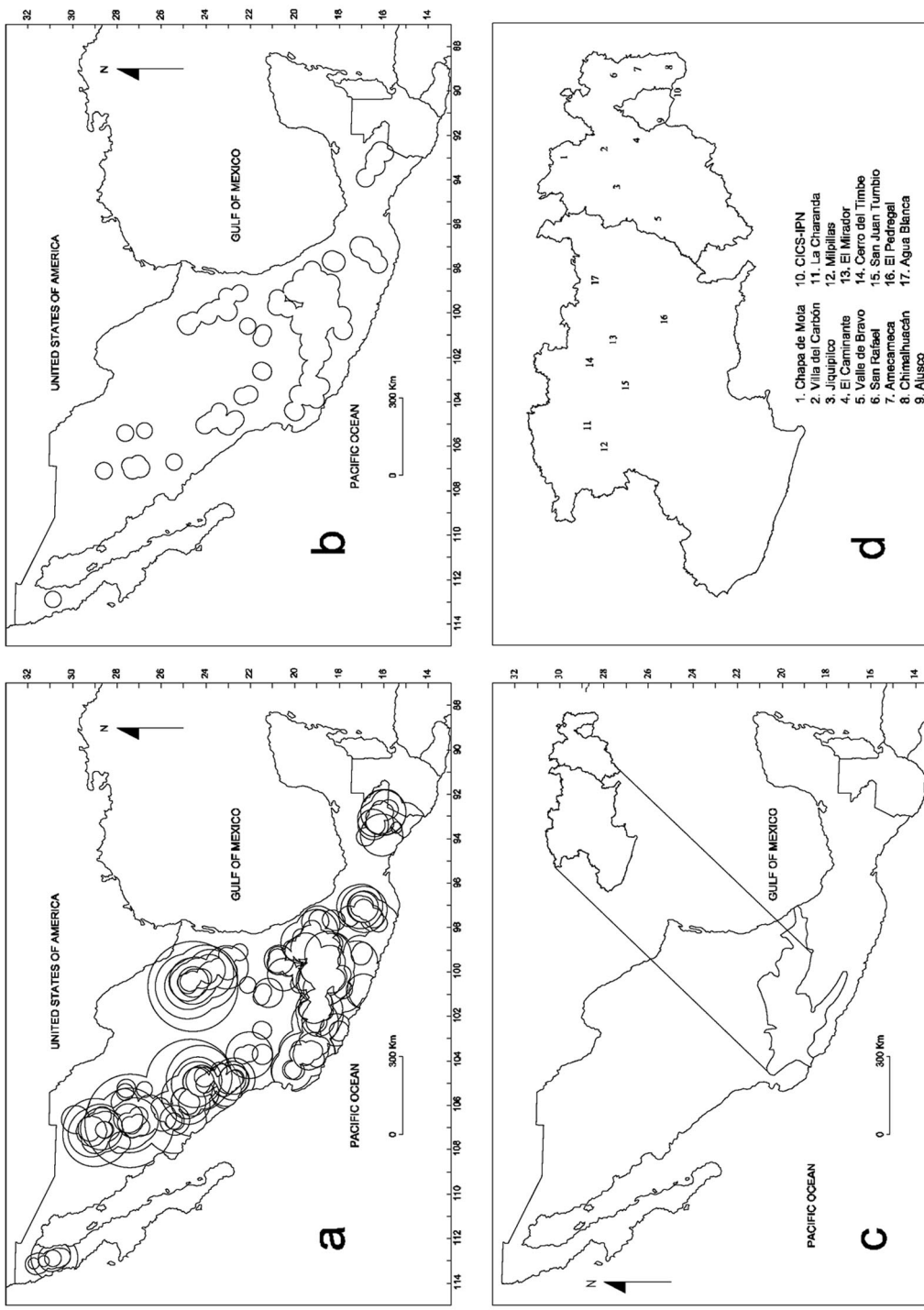

Fig. 1. (a) Composite pattern of 12 species from the genus *Dendroctonus* in Mexico (Salinas-Moreno et al. 2004). (b) Geographic distribution of *D. mexicanus* (Salinas-Moreno et al. 2004). (c) Location of TMVB in Mexico. (d) Populations studied of *D. mexicanus* from TMVB.

Adults were removed directly from pines, transported to the laboratory in liquid nitrogen, and later transferred to −70°C. Species identification was based on external morphological characters, chromosome number, and morphology of the seminal rod of the male genital capsule (Perusquía 1978, Wood 1982, Salinas-Moreno et al. 1994). One thousand seven hundred beetles were analyzed using enzyme electrophoresis on horizontal starch gels (12.5%) by using the methods of Richardson et al. (1986). In total, 16 enzymes were assayed, but only esterase (EST.3.1.1.1), acid phosphatase (ACP.3.1.3.2), glutamate-oxaloacetate-transaminase (GOT 2.6.1.1), isocitrate dehydrogenase (IDH 1.1.1.42), leucyl-amino-peptidase (LAP 3.4.11), malate dehydrogenase (MDH 1.1.1.37), and malic enzyme (ME 1.1.1.40) were used for analysis because they displayed clearly resolved band patterns.

Analysis. Allele frequencies were calculated by sex for each population and 95% confidence intervals were obtained with the approximation of a normal distribution by means of the following relationship:

$$p \pm Z_{0.05} \sqrt{p(1-p)/2n}.$$

Heterozygosities by locus were transformed to $\log_{10}$ for analysis of variance (ANOVA) to test for differences among populations (Barker et al. 1986).

Because the number of individuals analyzed differed and was relatively low in some populations, the genotypic frequencies of each locus were compared with those expected by Hardy–Weinberg Equilibrium (HWE) by using a test of exact probabilities instead of chi-squared distribution (Elston and Forthofer 1977). The nonparametric sequential test of Bonferroni was used to avoid a type I error in interpretation of the exact probabilities (Rice 1989). The magnitude and the direction of deviations from HWE were quantified by the index of fixation (F_{IS}).

Geographic variation of allele frequencies of the polymorphic loci among populations was analyzed statistically with jackknifing of F_{ST}-specific locus estimations across all populations (Weir and Cockerham 1984). A one-tailed *t*-test was used to test the hypothesis that $F_{ST} \neq 0$.

To assess genetic structure, F-statistics of Weir and Cockerham (1984) were estimated. To test the significance of the F-statistics, standard errors of the estimated averages were obtained with the jackknife method across loci, and 95% confidence intervals were determined using bootstrap analysis (Felsenstein 1985).

Indirect estimates of gene flow (*Nm*), the mean number of migrants exchanged among populations per generation was calculated using the expression $F_{ST} = 1/(1 + 4\,Nm(n/n-1)^2)$ (Chakraborty and Leimar 1987). To examine whether the genetic structure describes an isolation by distance pattern, linear regression of least squares between $F_{ST}/1 - F_{ST}$ (using θ_{ST}) and geographic distances among the populations was determined (Rousset 1997). Ordinary linear regressions were carried out with untransformed and log-transformed geographic distance values.

Because the significance of this relationship cannot be evaluated with ordinary regression methods, the nonparametric Mantel test was used to determine whether the correlation between the matrix of geographic distance (Gd, transformed and untransformed) and the matrix of F_{ST} values [sensu $F_{ST}/(1 - F_{ST})$] was significantly greater than the correlation between the matrix of geographic distance and the randomized matrix of the F_{ST} values (Manly 1997). Elements of the observed F_{ST} matrix were standardized to obtain a mean of zero and a variance of one. The randomized matrices were generated by row and column permutation. The randomization was performed 5,000 times to establish a 95% confidence interval (Manly 1997).

Deviation from a slope of zero was determined by regression on the reduced major axis (RMA) (Kermack and Haldane 1950). The standard error for the RMA slope deviation was estimated by the relationship $SE = b_{RMA}\sqrt{(1 - r^2/n)}$, where r^2 is the coefficient of determination, and *n* is the sample size. Asymmetric 95% confidence intervals were established by the relationship $LC = b_{RMA}((B + 1)^{1/2} \pm (B)^{1/2})$, where $B = t^2((1 - r^2)/n - 2)$, and *t* is the Student's *t* for $n - 2$ df (Clark 1980, McArdle 1988).

To determine whether particular loci contribute more than others to the microgeographic model of spatial variation, a jackknife method was conducted on gene loci. The corresponding RMA functional regression was calculated, excluding each locus in each of the runs. In total, 12 slopes was obtained. The equivalence of these slopes with respect to the total RMA slope was established by the relationship $T_{12} = |\log b_1 + \log b_2| / ((1 - r^2_1)/n1 + (1 - r^2_2)/n^2)^{1/2}$, which is an approximation of the Student's *t*, with 2 + 2/(variety $(T_{12}) - 1$) df (McArdle 1988).

Genetic differences among population pairs were calculated using Nei's distance (Nei 1972). The neighbor-joining method was used to construct the corresponding dendrogram. The reliability of the groupings was evaluated with a bootstrap analysis.

Finally, a principal components analysis by using pairwise genetic distances was conducted to detect more complex models of genetic structure among the populations (Legendre and Legendre 1998). This method facilitates the detection of nonhierarchical aspects of variation at a microgeographic level and allows observation of the relationship among populations in a multidimensional space. A minimum spanning tree was superimposed on the spatial scenario described by the populations as a way of testing whether the proximities among the populations were adequately preserved by the multidimensional representation (Legendre and Legendre 1998).

Analyses were performed using GDA, version 1.0 (Lewis and Zaykin 2001); NTSYS-PC, version 2.02j (Rohlf 1998); and PHYLIP, version 3.5c (Felsenstein 1997).

Results

There were no significant differences in allelic frequencies between males and females; therefore, these data were combined in subsequent analyses. More-

Table 1. Allele frequencies at 12 polymorphic loci in 17 populations of *D. mexicanus* in the TMVB

	Chapa de Mota	Villa del Carbón	Jiquipilco	El Caminante	Valle de Bravo	San Rafael	Amecameca	Chimalhuacán	Ajusco	CICS-IPN	La Charanda	Milpillas	El Mirador	Cerro del Timbe	San Juan Tumbio	El Pedregal	Agua Blanca
EST-1 (A)	0.868 ± 0.780	0.685 ± 0.089	0.722 ± 0.086	0.790 ± 0.073	0.725 ± 0.058	0.773 ± 0.064	0.750 ± 0.058	0.700 ± 0.097	0.781 ± 0.059	0.786 ± 0.059	1.000	0.673 ± 0.070	0.468 ± 0.072	0.312 ± 0.065	0.318 ± 0.063	0.359 ± 0.081	0.592 ± 0.072
EST-1 (B)	0.132 ± 0.078	0.315 ± 0.089	0.278 ± 0.086	0.210 ± 0.073	0.275 ± 0.058	0.227 ± 0.064	0.250 ± 0.058	0.300 ± 0.097	0.219 ± 0.059	0.217 ± 0.059	0.409 ± 0.074	0.327 ± 0.070	0.532 ± 0.072	0.688 ± 0.065	0.682 ± 0.063	0.641 ± 0.081	0.408 ± 0.072
n	38	54	54	62	120	88	45	45	98	103	88	98	95	101	110	71	92
EST-2 (A)	0.294 ± 0.110	0.295 ± 0.079	0.341 ± 0.083	0.375 ± 0.083	0.344 ± 0.063	0.229 ± 0.058	0.279 ± 0.068	0.223 ± 0.086	0.297 ± 0.064	0.478 ± 0.054	0.360 ± 0.068	0.319 ± 0.060	0.317 ± 0.068	0.297 ± 0.057	0.297 ± 0.057	0.478 ± 0.086	0.342 ± 0.077
EST-2 (B)	0.397 ± 0.110	0.371 ± 0.079	0.326 ± 0.083	0.279 ± 0.083	0.330 ± 0.063	0.438 ± 0.058	0.541 ± 0.068	0.479 ± 0.086	0.381 ± 0.064	0.382 ± 0.054	0.380 ± 0.068	0.425 ± 0.060	0.366 ± 0.068	0.410 ± 0.057	0.410 ± 0.057	0.366 ± 0.086	0.322 ± 0.077
EST-2 (C)	0.309 ± 0.110	0.333 ± 0.079	0.333 ± 0.083	0.346 ± 0.083	0.326 ± 0.063	0.333 ± 0.063	0.180 ± 0.068	0.298 ± 0.086	0.322 ± 0.064	0.140 ± 0.054	0.260 ± 0.068	0.257 ± 0.060	0.317 ± 0.068	0.293 ± 0.057	0.293 ± 0.057	0.157 ± 0.086	0.336 ± 0.077
n	34	66	66	68	112	105	86	47	101	68	100	113	93	128	128	67	76
EST-3 (A)	0.306 ± 0.093	0.179 ± 0.084	0.138 ± 0.077	0.254 ± 0.075	0.284 ± 0.074	0.200 ± 0.057	0.231 ± 0.057	0.262 ± 0.096	0.444 ± 0.065	0.402 ± 0.060	0.597 ± 0.079	0.722 ± 0.070	0.750 ± 0.075	0.631 ± 0.074	0.659 ± 0.066	0.514 ± 0.083	0.378 ± 0.076
EST-3 (B)	0.694 ± 0.093	0.821 ± 0.084	0.893 ± 0.077	0.746 ± 0.075	0.746 ± 0.074	0.800 ± 0.057	0.769 ± 0.057	0.738 ± 0.096	0.556 ± 0.065	0.598 ± 0.060	0.403 ± 0.079	0.278 ± 0.070	0.250 ± 0.075	0.369 ± 0.074	0.341 ± 0.066	0.486 ± 0.083	0.622 ± 0.076
n	49	42	40	67	74	100	108	42	117	92	77	72	65	84	104	72	82
ACP-1 (A)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
n	50	45	40	80	70	70	70	45	70	55	60	63	57	100	85	50	71
ACP-2 (A)	0.698 ± 0.095	0.708 ± 0.083	0.833 ± 0.068	0.621 ± 0.068	0.633 ± 0.065	0.240 ± 0.059	0.265 ± 0.062	0.377 ± 0.094	0.356 ± 0.061	0.437 ± 0.060	0.481 ± 0.058	0.485 ± 0.070	0.482 ± 0.077	0.504 ± 0.067	0.472 ± 0.068	0.493 ± 0.084	0.608 ± 0.060
ACP-2 (B)	0.302 ± 0.095	0.292 ± 0.083	0.167 ± 0.068	0.379 ± 0.068	0.367 ± 0.042	0.760 ± 0.059	0.735 ± 0.062	0.623 ± 0.094	0.644 ± 0.061	0.563 ± 0.060	0.519 ± 0.058	0.515 ± 0.070	0.518 ± 0.077	0.496 ± 0.067	0.528 ± 0.068	0.507 ± 0.084	0.392 ± 0.060
n	58	60	60	103	109	104	102	53	125	119	107	101	85	113	108	71	134
GOT (A)	0.160 ± 0.060	0.115 ± 0.056	0.127 ± 0.058	0.102 ± 0.040	0.114 ± 0.042	0.105 ± 0.030	0.099 ± 0.028	0.106 ± 0.052	0.188 ± 0.053	0.333 ± 0.050	0.106 ± 0.046	0.160 ± 0.050	0.161 ± 0.066	0.106 ± 0.039	0.165 ± 0.049	0.191 ± 0.056	0.077 ± 0.034
GOT (B)	0.660 ± 0.060	0.569 ± 0.056	0.545 ± 0.058	0.558 ± 0.040	0.557 ± 0.042	0.567 ± 0.030	0.566 ± 0.028	0.570 ± 0.052	0.569 ± 0.053	0.521 ± 0.050	0.683 ± 0.046	0.634 ± 0.050	0.702 ± 0.066	0.626 ± 0.039	0.657 ± 0.049	0.515 ± 0.056	0.720 ± 0.034
GOT (C)	0.180 ± 0.060	0.315 ± 0.056	0.328 ± 0.058	0.341 ± 0.040	0.329 ± 0.042	0.328 ± 0.030	0.336 ± 0.028	0.324 ± 0.052	0.243 ± 0.053	0.146 ± 0.050	0.211 ± 0.046	0.206 ± 0.050	0.317 ± 0.066	0.268 ± 0.039	0.178 ± 0.049	0.294 ± 0.056	0.203 ± 0.034
n	75	65	67	113	114	215	228	71	109	48	90	97	62	123	115	97	123
IDH-1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
n	40	40	40	45	50	40	70	50	50	55	55	45	45	42	55	55	55
IDH-2 (A)	0.361 ± 0.087	0.346 ± 0.083	0.333 ± 0.086	0.639 ± 0.069	0.649 ± 0.070	0.292 ± 0.044	0.118 ± 0.033	0.274 ± 0.074	0.470 ± 0.077	0.0358 ± 0.077	0.310 ± 0.065	0.330 ± 0.060	0.217 ± 0.064	0.324 ± 0.071	0.372 ± 0.064	0.454 ± 0.071	0.129 ± 0.043
IDH-2 (B)	0.639 ± 0.087	0.654 ± 0.083	0.667 ± 0.086	0.361 ± 0.069	0.351 ± 0.070	0.708 ± 0.044	0.882 ± 0.033	0.726 ± 0.074	0.530 ± 0.077	0.642 ± 0.077	0.690 ± 0.065	0.670 ± 0.060	0.783 ± 0.064	0.676 ± 0.071	0.628 ± 0.064	0.546 ± 0.071	0.871 ± 0.043
n	61	65	60	97	94	209	190	73	84	144	100	106	83	88	113	97	124
LAP (A)	0.449 ± 0.092	0.390 ± 0.097	0.440 ± 0.092	0.389 ± 0.073	0.452 ± 0.058	0.415 ± 0.051	0.372 ± 0.049	0.444 ± 0.096	0.450 ± 0.099	0.565 ± 0.079	0.468 ± 0.057	0.435 ± 0.070	0.482 ± 0.077	0.463 ± 0.064	0.492 ± 0.065	0.507 ± 0.083	0.441 ± 0.064
LAP (B)	0.551 ± 0.092	0.620 ± 0.097	0.560 ± 0.092	0.611 ± 0.073	0.548 ± 0.058	0.585 ± 0.051	0.628 ± 0.049	0.556 ± 0.096	0.550 ± 0.099	0.435 ± 0.079	0.532 ± 0.057	0.565 ± 0.070	0.518 ± 0.077	0.537 ± 0.064	0.508 ± 0.065	0.493 ± 0.083	0.559 ± 0.064
n	59	50	58	90	146	188	196	54	50	92	110	93	85	122	119	72	119
MDH-1 (A)	0.344 ± 0.097	0.322 ± 0.071	0.287 ± 0.069	0.325 ± 0.087	0.256 ± 0.056	0.771 ± 0.052	0.294 ± 0.046	0.167 ± 0.067	0.450 ± 0.070	0.500 ± 0.070	0.535 ± 0.071	0.658 ± 0.060	0.500 ± 0.081	0.485 ± 0.071	0.500 ± 0.069	0.548 ± 0.154	0.483 ± 0.076
MDH-1 (B)	0.666 ± 0.097	0.678 ± 0.071	0.713 ± 0.069	0.675 ± 0.057	0.744 ± 0.056	0.229 ± 0.052	0.706 ± 0.046	0.833 ± 0.067	0.550 ± 0.070	0.500 ± 0.070	0.465 ± 0.071	0.342 ± 0.060	0.500 ± 0.081	0.515 ± 0.071	0.500 ± 0.069	0.452 ± 0.154	0.517 ± 0.076
n	48	87	87	134	121	121	199	63	100	60	100	114	77	98	105	21	86
MDH-2 (A)	0.329 ± 0.104	0.329 ± 0.066	0.294 ± 0.070	0.724 ± 0.052	0.772 ± 0.059	0.790 ± 0.053	0.716 ± 0.049	0.713 ± 0.082	0.681 ± 0.063	0.860 ± 0.082	0.433 ± 0.077	0.435 ± 0.060	0.108 ± 0.057	0.138 ± 0.055	0.173 ± 0.052	0.186 ± 0.051	0.671 ± 0.073
MDH-2 (B)	0.671 ± 0.104	0.671 ± 0.066	0.706 ± 0.070	0.276 ± 0.052	0.228 ± 0.059	0.210 ± 0.053	0.284 ± 0.049	0.287 ± 0.082	0.319 ± 0.063	0.140 ± 0.082	0.567 ± 0.077	0.565 ± 0.060	0.892 ± 0.057	0.863 ± 0.055	0.827 ± 0.052	0.814 ± 0.051	0.329 ± 0.073
n	41	102	85	145	101	119	169	61	108	57	82	124	60	80	107	118	82
ME (A)	0.385 ± 0.085	0.216 ± 0.058	0.233 ± 0.069	0.270 ± 0.057	0.259 ± 0.068	0.708 ± 0.045	0.776 ± 0.053	0.573 ± 0.101	0.538 ± 0.080	0.365 ± 0.070	0.750 ± 0.058	0.578 ± 0.060	0.734 ± 0.070	0.697 ± 0.060	0.619 ± 0.066	0.742 ± 0.057	0.097 ± 0.038
ME (B)	0.615 ± 0.095	0.784 ± 0.058	0.767 ± 0.096	0.730 ± 0.057	0.741 ± 0.068	0.292 ± 0.045	0.224 ± 0.053	0.427 ± 0.101	0.462 ± 0.080	0.635 ± 0.070	0.250 ± 0.058	0.422 ± 0.060	0.266 ± 0.070	0.303 ± 0.060	0.381 ± 0.066	0.258 ± 0.057	0.903 ± 0.038
n	65	102	75	122	83	207	122	48	78	115	110	116	79	119	109	118	119

Table 2. Summary statistics (means and SE in parentheses) describing genetic variation in 17 populations of *D. mexicanus* from the TMVB

Pop	Mean sample size across loci	Mean no. of alleles per locus	% polymorphic loci	Mean heterozygosity	
				Direct count	HW expected
Chapa de Mota	51.5 (3.6)	2.0 (0.2	83.3	0.296 (0.063)	0.380 (0.058)
Villa del Carbón	63.4 (5.6)	2.0 (0.2)	83.3	0.268 (0.051)	0.375 (0.057)
Jiquipilco	61.0 (4.7)	2.0 (0.2)	83.3	0.270 (0.062)	0.357 (0.059)
El Caminante	93.8 (8.9)	2.0 (0.2)	83.3	0.301 (0.052)	0.381 (0.057)
Valle de Bravo	99.5 (7.7)	2.0 (0.2)	83.3	0.279 (0.046)	0.381 (0.057)
San Rafael	131.3 (17.1)	2.0 (0.2)	83.3	0.291 (0.057)	0.378 (0.057)
Chimalhuacán	54.3 (3.0)	2.0 (0.2)	83.3	0.291 (0.056)	0.378 (0.057)
Amecameca	154.2 (22.1)	2.0 (0.2)	83.3	0.289 (0.040)	0.343 (0.055)
Ajusco	90.7 (7.1)	2.0 (0.2)	83.3	0.287 (0.045)	0.414 (0.060)
CICS-IPN	84 (9.1)	2.0 (0.2)	83.3	0.291 (0.053)	0.389 (0.060)
La Charanda	89.9 (5.3)	2.0 (0.2)	83.3	0.302 (0.054)	0.407 (0.058)
Milpillas	94.8 (6.8)	2.0 (0.2)	83.3	0.300 (0.051)	0.409 (0.058)
El Mirador	73.8 (4.5)	2.0 (0.2)	83.3	0.296 (0.059)	0.369 (0.060)
Cerro del Timbe	99.5 (6.9)	2.0 (0.2)	83.3	0.298 (0.058)	0.390 (0.060)
San Juan Tumbio	104.8 (5.4)	2.0 (0.2)	83.3	0.303 (0.050)	0.401 (0.059)
El Pedregal	77.3 (6.7)	2.0 (0.2)	83.3	0.295 (0.060)	0.408 (0.060)
Agua Blanca	96.9 (7.4)	2.0 (0.2)	83.3	0.287 (0.077)	0.364 (0.061)

A locus was considered polymorphic if the frequency of the most common allele was 0.95 or less.

over, examination of external morphological characters, chromosome number, and the shape of the seminal rods confirmed our species determination. The number of insects analyzed varied both by enzyme and by population (Table 1). For the seven enzymatic systems studied, 12 presumed gene loci were found, of which 10 were polymorphic (EST-1, EST-2, EST-3, ACP-2, GOT, IDH-2, LAP, MDH-1, MDH-2, and ME) by using the criterion that the frequency of the most common allele was <95%. The loci ACP-1 and IDH-1 were monomorphic, and both were fixed for the same allele in all populations.

Genetic Variability. Allelic frequencies for the loci analyzed from 17 populations are shown in Table 1. The average sample size across loci per population ranged from 51.5 individuals in Chapa de Mota to 154.2 in Amecameca. The average number of alleles per locus was similar for all populations (2.0), as was the percentage of polymorphic loci (83%) (Table 2). The average heterozygosity of each locus was variable and statistically significant among the 17 populations ($F_{obs} = 27.420 > F_{critical\ (2),\ 16,\ 153} = 1.938$; $P < 0.05$). The average heterozygosity, both by direct count and by HWE, showed heterogeneous behavior, although the range of variation for both measures was small among the samples. The Villa del Carbón population exhibited the lowest average observed heterozygosity ($H_o = 0.268$), whereas the San Juan Tumbio population exhibited the highest ($H_o = 0.303$). In the case of expected heterozygosity, Amecameca exhibited the lowest value ($H_e = 0.343$) and Ajusco the highest value ($H_e = 0.414$).

The relationship between the expected heterozygosity and the longitudinal position of the populations in the TMVB showed a statistically significant correlation ($r_{obs} = 0.5610 > r_{critical(2)15} = 0.482$; $P > 0.05$); the highest expected heterozygosity was found in the western populations of the TMVB. The same pattern was seen in the observed heterozygosity; however, the statistical significance was slightly below the critical value ($r = 0.469 < r_{15} = 0.482$; $P > 0.05$).

The average heterozygosity by direct count ($0.268 < H_{CD} < 0.303$) and the expected heterozygosity by HWE ($0.343 < H_{HW} < 0.414$) for *D. mexicanus* lie within the range of values found in other scolytid species in the genera *Dendroctonus*, *Ips* DeGeer, and *Conophthorus* Hopkins (Table 3).

Hardy–Weinberg Equilibrium. We found by exact test 121 significant deviations in gene frequencies ($P < 0.05$) from HWE of 170 comparisons made over all loci and all populations (Table 4). The sequential Bonferroni adjustment of *P* values showed that these HWE deviations could not be the result of chance alone. The proportion not in HWE by population varied from 60% in the populations of Villa del Carbón, CICS-IPN, La Charanda, and Chimalhuacán to 90% at Ajusco and San Juan Tumbio. The loci EST-3, ACP-2, and MDH-2 were those most often found outside of HWE, given that they were found in HWE in only two of the 17 populations. However, MDH-1, GOT, and EST-2 were shown to be in equilibrium in 10 (58%), eight (47.05%), and seven (41.17%) populations, respectively.

The majority of the deviations from HWE in the populations were due to a deficiency of heterozygotes (102 tests); only a small fraction showed excess of individual heterozygotes (19 tests). Excess of heterozygotes was only observed in eight populations for the LAP enzyme, four populations for GOT, three populations for EST-2, two populations for MDH-2, and one population for IDH-2 and MDH-1.

Geographic Variation. Geographic analysis of allele frequencies shows that the F_{ST} value varied widely among alleles of the same locus, and among loci (Table 5); however, of all the comparisons, including that of the mean, only the F_{ST} variations for EST-1, EST-3, MDH-2, and ME were statistically significant at the 1% level.

The allele frequencies show distinct geographic tendencies. For example, the frequencies of the fast allele (B) (rapidly migrating allele) of the EST-2, EST-3, and MDH-1 loci diminish in an east–west di-

Table 3. Levels of allozyme variation reported for populations of *Dendroctonus*, *Ips* and *Conophtorus*

Species	H_e	P	No. Loci	No. Populations	Reference
Dendroctonus					
D. brevicomis	0.363 ± 0.135	100	5	1	Namkoong et al. (1979)
D. frontalis	0.354 ± 0.019	100	5	6	"
D. frontalis	0.166 ± 0.006	45	6	5	Anderson et al. (1979)
D. psuedotsugae	0.288 ± 0.014	50	1	2	Stock et al. (1979)
D. ponderosae	0.084 ± 0.004	13	15	6	Stock and Guenther (1979)
D. ponderosae	0.124 ± 0.003	25	15	4	Stock and Amman (1980)
D. frontalis	0.351 ± 0.005	86	5	3	Florence and Kulhavy (1981)
D. jeffreyi	0.136 ± 0.008	34	17	4	Higby and Stock (1982)
D. ponderosae	0.146 ± 0.003	33	17	8	"
D. terebrans	0.123 ± 0.003	73	8	4	Anderson et al. (1983)
D. ponderosae	0.119 ± 0.002	29	18	15	Stock et al. (1984)
D. ponderosae	0.334 ± 0.006	100	5	5	Sturgeon and Mitton (1986)
D. adjunctus	0.225 ± 0.057	72	18	1	Bentz and Stock (1986)
D. approximatus	0.218 ± 0.059	61	18	1	"
D. brevicomis	0.233 ± 0.056	67	18	4	"
D. frontalis	0.217 ± 0.064	61	18	1	"
D. ponderosae	0.156 ± 0.059	50	18	2	"
D. pseudotsugae	0.228 ± 0.061	72	18	3	"
D. rufipennis	0.236 ± 0.063	67	18	4	"
D. simplex	0.182 ± 0.055	61	18	3	"
D. terebrans	0.249 ± 0.065	61	18	1	"
D. valens	0.189 ± 0.052	67	18	2	"
D. micans	0.053 ± 0.034	27	15	1	Stock et al. (1987)
D. frontalis	0.324 ± 0.005	85	8	4	Roberds et al. (1987)
D. ponderosae	0.108 ± 0.002	30	14	8	Langor and Spence (1991)
D. adjunctus	0.320 ± 0.005	69	13	4	Zúniga et al. (unpublished data)
D. micans	0.026 ± 0.050	6.25	16	1	Kegley et al. (1997)
D. punctatus	0.075 ± 0.050	37.5	16	1	"
D. jeffreyi	0.004 ± 0.003	28	21	10	Six et al. (1999)
Ips					
I. calligraphus	0.114 ± 0.010	71	7	4	Anderson et al. (1983)
I. confusus	0.073 ± 0.027	55	20	3	Cane et al. (1990)
I. grandicollis	0.085 ± 0.025	60	20	1	"
I. hoppingi	0.095 ± 0.039	50	20	2	"
I. latidens	0.099 ± 0.025	65	20	1	"
I lecontei	0.173 ± 0.040	65	20	2	"
I paraconfusus	0.095 ± 0.023	65	20	2	"
I pini	0.159 ± 0.032	80	20	2	"
Conophtorus					
C. banksianae	0.047 ± 0.006	50	10	9	de Groot et al. (1992)
C. coniperda	0.084 ± 0.007	57	10	11	"
C. resinosae	0.040 ± 0.005	51	10	9	"

H_e (±SE) is the mean expected heterozygosity. P represents the percent of polymorphisms.

Table 4. Probabilities that the observed gene frequencies in 17 populations of *D. mexicanus* in the TMVB conform to those expected under Hardy-Weinberg equilibrium, using the exact test

	EST-1	EST-2	EST-3	ACP-2	GOT	IDH-2	LAP	MDH-1	MDH-2	ME
Chapa de Mota	0.099	0.285	**0.000**	**0.004**	0.606	**0.027**	**0.000**	**0.009**	**0.014**	**0.001**
Villa del Carbón	**0.004**	**0.039**	**0.001**	**0.004**	0.136	0.272	0.077	0.052	**0.000**	**0.000**
Jiquipilco	**0.000**	**0.000**	**0.014**	**0.006**	0.327	0.078	**0.000**	0.187	**0.000**	**0.000**
El Caminante	**0.017**	**0.009**	0.103	0.093	**0.013**	**0.002**	**0.000**	**0.001**	0.099	**0.000**
Valle de Bravo	**0.000**	**0.006**	1.000	**0.038**	**0.001**	**0.001**	**0.045**	0.153	**0.000**	**0.000**
San Rafael	**0.002**	**0.000**	**0.001**	**0.034**	**0.000**	**0.000**	**0.000**	0.136	**0.012**	0.868
Chimalhuacán	0.126	**0.004**	**0.029**	**0.020**	**0.000**	0.150	**0.000**	0.039	**0.001**	0.076
Amecameca	0.167	0.078	**0.016**	**0.010**	0.057	**0.015**	**0.000**	0.054	**0.003**	**0.017**
Ajusco	**0.000**	**0.003**	**0.001**	**0.003**	**0.000**	**0.009**	0.164	**0.026**	**0.000**	0.043
CICS-IPN	**0.003**	0.231	**0.000**	**0.000**	1.000	**0.000**	0.290	1.000	**0.007**	**0.000**
La Charanda	**0.000**	0.139	**0.000**	**0.000**	0.086	**0.010**	0.848	0.075	**0.043**	**0.000**
Millpillas	**0.000**	0.565	**0.000**	**0.001**	**0.001**	0.660	**0.038**	0.680	**0.018**	**0.000**
El Mirador	0.153	**0.024**	**0.000**	**0.009**	0.222	0.194	**0.009**	**0.039**	**0.014**	**0.000**
Cerro del Timbe	**0.000**	0.143	**0.000**	**0.002**	**0.007**	**0.000**	**0.000**	**0.027**	0.343	**0.000**
San Juan Tumbio	**0.000**	**0.008**	**0.015**	**0.001**	0.312	**0.000**	**0.000**	**0.019**	**0.038**	**0.000**
El Pedregal	**0.001**	0.332	**0.019**	**0.000**	**0.008**	**0.000**	0.102	0.257	**0.000**	**0.000**
Agua Blanca	**0.000**	**0.000**	**0.004**	0.370	**0.024**	**0.030**	0.576	**0.000**	**0.000**	0.289

Significant values are given in bold.

Table 5. Locus-specific and overall F_{ST} values across 17 populations of *D. mexicanus* in the TMVB

Locus	F_{ST}	SE	*P*
EST-1	0.127	0.036	s***
EST-2	0.011	0.005	ns**
EST-3	0.145	0.033	s***
ACP-2	0.079	0.027	ns**
GOT	0.013	0.004	ns**
IDH-2	0.096	0.004	ns**
LAP	0.005	0.004	ns**
MDH-1	0.097	0.039	ns**
MDH-2	0.258	0.044	s***
ME	0.218	0.051	s***
Overall	0.104	0.007	ns**

Significance of variation among populations was determined by one-tailed *t*-test of independence, $**P < 0.05$, $***P < 0.01$.

Table 6. Estimates of F-statistics calculated separately for each locus for 17 populations of *D. mexicanus* in the TMVB

Locus	F_{IS}	F_{IT}	F_{ST} (θ)
EST-1	0.390	0.466	0.125
EST-2	0.208	0.217	0.011
EST-3	0.347	0.443	0.146
ACP-2	0.281	0.339	0.080
GOT	0.202	0.213	0.013
IDH-2	0.314	0.380	0.095
LAP	−0.053	−0.047	0.005
MDH-1	0.120	0.204	0.095
MDH-2	0.315	0.493	0.259
ME	0.323	0.469	0.215
Mean (SD)	0.236 ± 0.042	0.314 ± 0.053	0.100 ± 0.027
95% CI	0.152–0.309	0.210–0.407	0.0–0.157

Means and SE were obtained by jackknifing across loci. Confidence interval was obtained by bootstrapping across loci.

rection, whereas the fast allele (B) for EST-1 and MDH-2 loci increase. The ACP-2 and IDH-2 loci show another tendency; the frequencies of the fast alleles (B) diminish in the populations located in the central part of the TMVB and increase in frequency considerably toward the eastern part of this area. However, the fast allele (B) of the ME locus reaches a high frequency in the populations in the central part of the TMVB and a low frequency in the populations of the east and west regions. Finally, the fast allele (B) of the LAP and GOT loci are homogeneous throughout the TMVB.

Genetic Structure. Table 6 shows the F-statistics of the polymorphic loci, their standard errors, 95% confidence intervals, and significant deviations from zero. The data show important deviations from random pairing in the populations of *D. mexicanus.* The F_{IS} (*f*) value was variable by more than a factor of three across the loci. Positive values were observed in nine of 10 loci analyzed, of which EST-1, EST-3, IDH-2, MDH-1, MDH-2, and ME were statistically different from zero ($P < 0.05$). The LAP enzyme showed a negative value, which was also significantly different from zero ($P < 0.05$).

The F_{IT} (F)-statistic was less variable across loci, with its values differing by a factor of two. Positive values were found in nine out of 10 loci analyzed confirming an excess of homozygotes within populations. Finally, F_{ST} (θ) showed values for MDH-2 and ME that were statistically different from zero between the loci and across populations even though the mean was not different from zero.

A comparison of the magnitude of statistical values showed that the F_{IS} values were greater than the F_{ST} values. On average, the former was two times greater than the latter, which indicates that the largest part of the F_{IT} values is explained by the variation observed within populations (23%) and not by differences among them (9.9%).

Based on the mean value of F_{ST} (θ), the average number of migrants per generation (*Nm*) among the populations was 1.992 (Table 7). The regression between $F_{ST(\theta)}/(1 - F_{ST(\theta)})$ and geographic distance shows that the populations of *D. mexicanus* in the TMVB describe a model of isolation by distance (Fig. 2a,b). The line of ordinary regression and RMA between these variable explains ≈20% of the total variation, if it is assumed that the populations are located in linear habitats, and 30%, if they are located in bidimensional habitats.

Slopes of the RMA regression lines were greater than those of the ordinary linear regressions, and they were included within the 95% confidence limits (CL) (Table 7). The slopes of both the ordinary regression and of the RMA indicate the estimated number of individuals that have migrated per generation as described by a linear or bidimensional model of habitats, is far greater than the estimated average for all the populations, by using the relationship $Nm = 1/4\ (n/n-1)^2\ [(1/F_{ST} - 1)]$ (Table 7). Likewise, estimation of individual migrants using ordinary linear regressions is greater than those that result from the slopes of the RMA (Table 7).

Genetic Differentiation. Genetic distances between pairs of populations are presented in Table 8. Valle de Bravo and El Caminante were genetically most similar ($D_{Nei} = 0.003$), whereas Valle de Bravo and El Mirador were genetically most dissimilar ($D_{Nei} = 0.182$). The mean genetic distance was 0.079 ± 0.001.

Table 7. Equations of ordinary linear regressions slopes (b_1) and SE, reduced major axis slopes (b_{RMA}), and confidence intervals (CL), regression coefficients (r^2), and number of migrants per generation (*Nm*) based on slopes of 17 populations of *D. mexicanus* in the TMVB

Regression	Linear $y = b_1x + b_0$	SE	RMA $y = b_{RMA}x + b_0$	CL	r^2		*Nm*	
					Linear	RMA	Linear	RMA
F_{ST} (θ) versus Gd	$y = 0.003x + 0.0670$	0.0045	$y = 0.006x + 0.1638$	$0.0053 < b_{RMA} < 0.0067$	0.223	0.230	333	166
F_{ST} (θ) versus log Gd	$y = 0.040x - 0.0793$	0.00501	$y = 0.071x - 0.2253$	$0.0063 < b_{RMA} < 0.0078$	0.318	0.324	25	14
F_{ST} (θ) (avg)								1.992

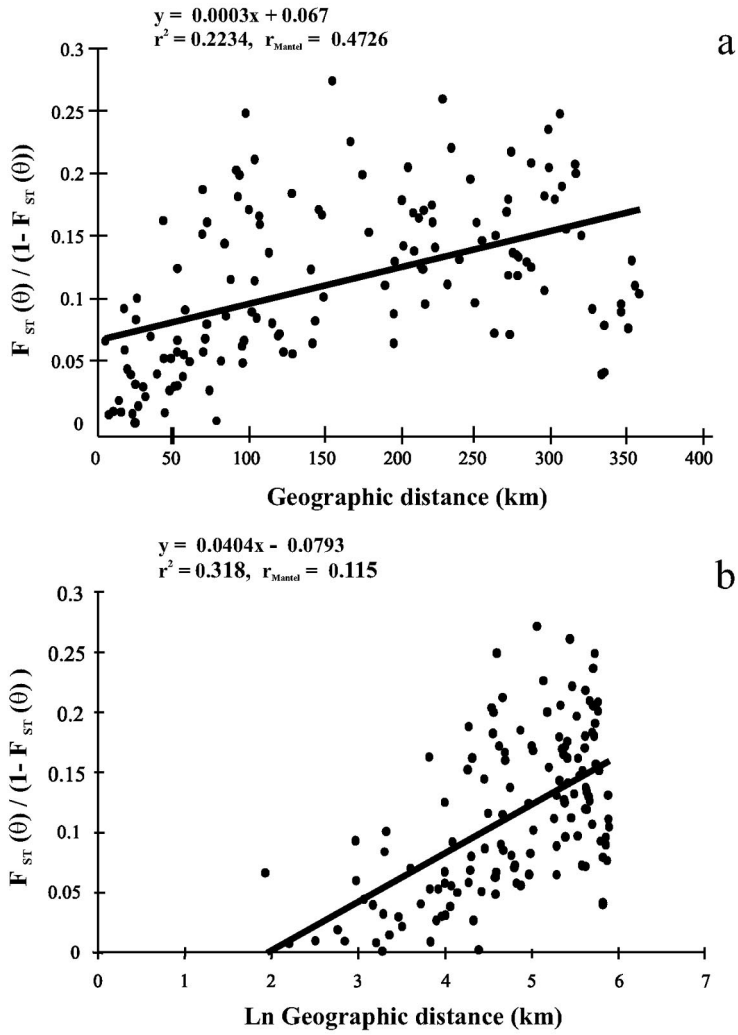

Fig. 2. Scatterplots showing the relationship between $F_{ST\ (\theta)}$ from Weir and Cockerham (1984) and geographic distance untransformed (a) or ln transformed (b) between populations of *D. mexicanus* in the TMVB. Regression statistics are shown in each graphic.

The majority of bootstrap values located at the nodes were >50% (Fig. 3b). Two large groups were clearly defined, one formed by the populations located in the western region of the TMVB (La Charanda, Milpillas, Cerro del Timbe, San Juan Tumbio, El Mirador, and El Pedregal), and the other composed of populations located in the central and eastern part of this region.

The populations of this second group describe three subgroups, which are defined by their geographic closeness. The correlation between genetic distance and geographic distance was significant (r_{Mantel} = 0.4435, P = 0.004), which confirms that the populations in this mountain system describe a model of isolation by distance (Fig. 3a). Likewise, the relationships of the populations in the multidimensional space show that the model of isolation by west–east distance prevails among them. Populations located in the center and east of this region apparently show a pattern of north-south differentiation (Fig. 3c). The first three principal components of the analysis explained 100% of the observed variation, specifically the first component explained 78.89% of variation, the second component 16.05%, and the third component 5.06%.

Discussion

Genetic Variability. Comparisons of heterozygosity and average polymorphism within *Dendroctonus* with that found in other taxonomic groups show that the values of these estimators are, with the exception of *Dendroctonus micans* (Kugelann), among the highest reported within Coleoptera, other insects, and invertebrates in general (Powell 1975, Nevo 1978, Nevo et al. 1984, Ward et al. 1992). Quantitative comparisons between the average heterozygosity of *D. mexicanus*

Table 8. Nei's genetic distance (Nei 1972) above the diagonal and geographic distance (km) below the diagonal among 17 populations of *D. mexicanus* in the TMVB

	ChM	VC	Jiq	EC	VB	SR	Ame	Chi	Aju	CIC	Cha	Mil	EM	CT	SJT	EP	AB
ChM		0.013	0.016	0.041	0.048	0.104	0.052	0.083	0.046	0.064	0.056	0.055	0.087	0.088	0.082	0.083	0.051
VC	12.3		0.004	0.038	0.044	0.124	0.060	0.102	0.068	0.081	0.086	0.085	0.116	0.098	0.094	0.095	0.039
Jiq	28.7	23.8		0.047	0.053	0.150	0.075	0.122	0.088	0.097	0.102	0.104	0.131	0.114	0.111	0.107	0.052
EC	59	45.8	36.9		0.003	0.095	0.049	0.097	0.034	0.042	0.097	0.094	0.177	0.155	0.140	0.129	0.055
VB	98.8	97.5	74	80.6		0.107	0.046	0.100	0.037	0.042	0.102	0.100	0.182	0.157	0.141	0.132	0.056
SR	109	95.9	99.8	71.2	148.5		0.038	0.057	0.036	0.054	0.063	0.072	0.145	0.139	0.136	0.117	0.100
Ame	121.5	107	106	73.1	144.5	19.5		0.015	0.043	0.063	0.057	0.083	0.122	0.119	0.127	0.107	0.098
Chi	115.5	102	106	74.5	150.5	6.9	15.9		0.026	0.044	0.058	0.077	0.120	0.109	0.108	0.100	0.064
Aju	71.9	59.9	54.7	21.5	97.9	50.6	52.7	54.8		0.020	0.035	0.033	0.095	0.093	0.080	0.075	0.061
CIC	104	89.9	85.9	54.5	125	273	19.7	27.9	33.4		0.068	0.059	0.146	0.146	0.130	0.117	0.045
Cha	265	274	256.5	279.5	204.5	349	353.5	349	338	330		0.010	0.022	0.025	0.025	0.024	0.083
Mil	275	280.5	265.5	286.5	212	358	361	356	336	338	9.08		0.029	0.038	0.032	0.041	0.070
EM	216	223.5	207.5	230.5	157.5	300.5	305	301	281	276	49.7	58.2		0.009	0.010	0.026	0.127
CT	217.5	225.5	211	236.5	169.5	308.5	312.5	309.5	289	289	55	63	24.8		0.004	0.011	0.121
SJT	233.5	241.5	224	249	177.5	318.5	322.5	319	298	298	32	41.3	17.3	26.6		0.014	0.107
EP	192.5	198.5	181.5	203.5	130.5	274	277	273	252	253	76	83.5	26.9	46.3	45.5		0.128
AB	122.5	131	117.7	146	86.9	215	219	218	198	198	143.5	151.5	93.9	95	109.5	71.6	

Chapa de Mota (ChM), Villa del Carbón (VC), Jiquipilco (Jiq), El Caminante (EC), Valle de Bravo (VB), San Rafael (SR), Amecameca (Ame), Chimalhuacán (Chi), Ajusco (Aju), CICS-IPN (CIC), Charanda (Cha), Milpillas (Mil), El Mirador (EM), Cerro del Timbe (CT), San Juan Tumbio (SJT), El Pedregal (EP), Agua Blanca (AB).

and that of other *Dendroctonus* as reported previously, by using the T method of Tukey and the adjustment of Tukey–Kramer for unequal sample sizes (Zar 1996), indicated that the observed differences in average heterozygosity among species are not statistically significant ($q = 0.041 < q_{0.005,12,4} = 4.19$).

High *Dendroctonus* genetic variability may be explained by greater heterozygosity in epidemic populations than endemic populations (Roberds et al. 1987), by development on trees with thick bark (Stock and Amman 1985, Sturgeon and Mitton 1986, Langor and Spence 1991, Amman and Stock 1995), or by development in the mid-bole of the tree (Florence and Kulhavy 1981). However, some studies have shown that hosts are heterogeneous resources with respect to chemical, physical, and biological characteristics, and their traits are difficult to associate with the variability of the insects (Powell 1967, Raffa and Berryman 1982, Safrayink 1983, Langor 1989, Langor et al. 1990). Thus, it is necessary to develop precise experimental designs that elucidate how much of the variation observed in *Dendroctonus* is intrinsic to the species and how much is a response to pressures of differential selection associated with microhabitat.

Hardy–Weinberg Equilibrium. HWE deviation found in *D. mexicanus* by heterozygote deficiency agrees with that observed in *D. frontalis* (Florence and Kulhavy 1981), *D. ponderosae* (Stock and Gunther 1979, Stock and Amman 1980, Sturgeon and Mitton 1986, Langor and Spence 1991), and *D. jeffreyi* (Six et al. 1999). Additionally, the magnitude of the F_{IT} and F_{IS} statistics, although not statistically different from zero, suggests that deviations from HWE are due to the presence of a certain degree of endogamy in the populations of *D. mexicanus.* Similar results have been found in *D. frontalis*, *D. ponderosae*, and *D. jeffreyi* (Stock and Gunther 1979, Stock and Amman 1980, Florence and Kulhavy 1981, Sturgeon and Mitton 1986, Langor and Spence 1991, Six et al. 1999).

There are several potential explanations for these deviations, none of which are mutually exclusive. First, heterozygotes could be emerging before homozygotes. Second, there could be a differential genetic contribution of males and females in the overall population. Third, there could be null alleles present that do not produce enzymatic activity. Fourth, there may be selection against heterozygotes; however, this explanation seems unlikely given that if there were selection against heterozygotes, we would have observed a preponderance of one homozygous class over another, which was not the case. Finally, there may be nonrandom mating or a Wahlund effect (the reduction in the observed frequency of heterozygotes from that expected because of lumping of subpopulations; Sturgeon and Mitton 1986). The latter effect could be the consequence of two phenomena, that is, an effect of genetic sampling due to short dispersal of the insects, the possibility of overlapping generations. Again, neither of these explanations is mutually exclusive.

Dispersal studies have shown that many insects find their hosts within the neighborhood of the emergence site (short dispersal of <1 km), whereas others fly long distances at random to find a suitable host (long dispersal of >1.5 km) (Byers 1995). This evidence suggests that beetle subpopulations inside of the same forested area could be responding to different selective pressure or genetic drift. Additionally, when populations are at endemic levels and susceptible hosts are limited, the possibility of overlapping generations may increase, particularly in species such as *D. mexicanus* that have multiple generations and in which multiple broods per generation are produced. This increases the potential for inbreeding, as genetic relatives are more likely to mate when resources are scarce and beetles are colonizing the same (reinfesting) or nearby trees (Ramírez-Delgado 1994).

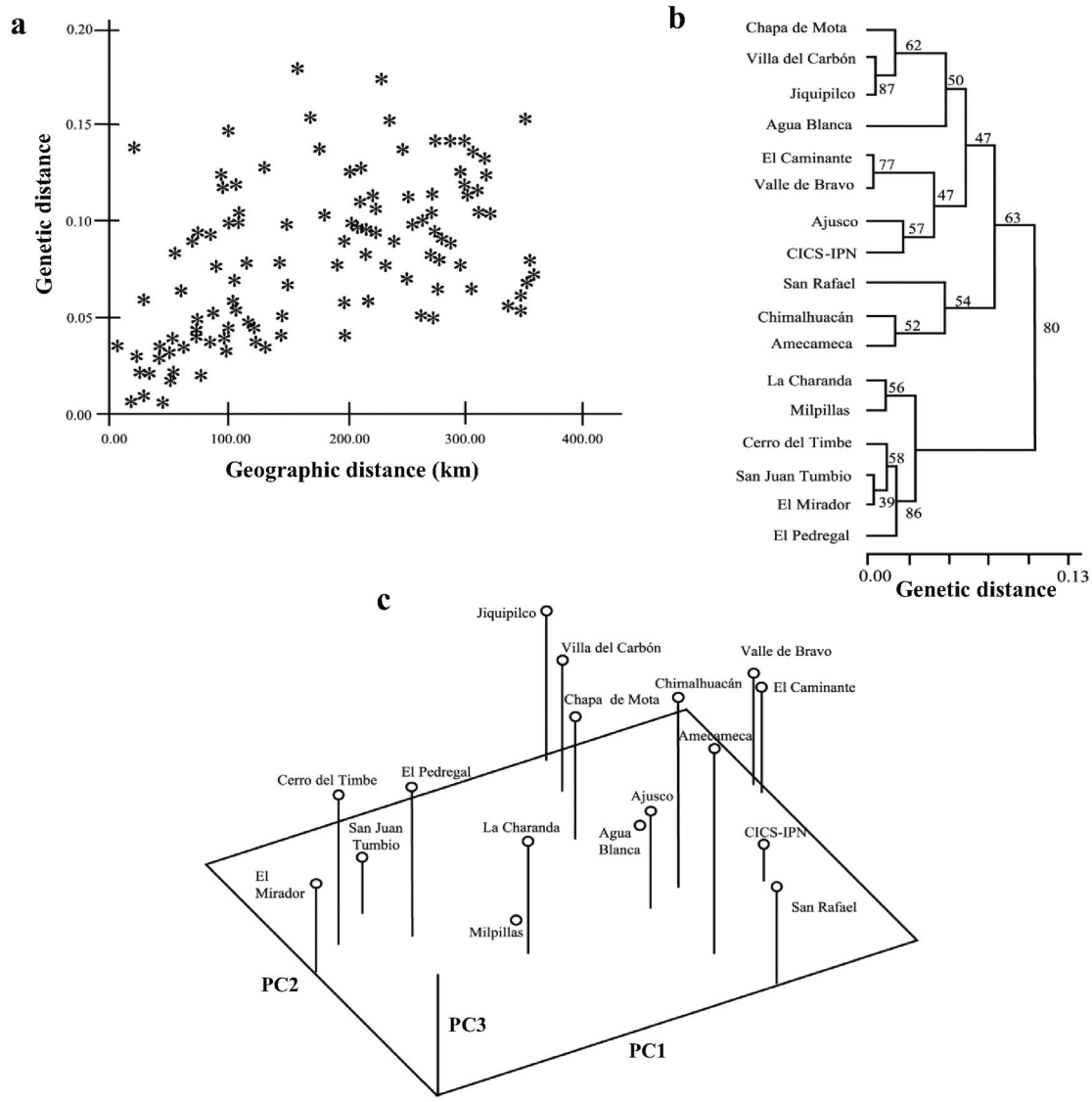

Fig. 3. (a) Scatterplots showing the relationship between genetic distance (Nei 1972) and geographical distance (kilometers) between populations of *D. mexicanus* in the TMVB; $r = 0.443$, $P = 0.004$. (b) Neighbor-joining tree based on isozymes using the Nei (1972) distances of 17 populations of *D. mexicanus* in the TMVB; $r = 0.91$, $P = 0.004$. (c) Principal component analysis of 17 populations of *D. mexicanus* in a multidimensional space.

The geographically distinct populations sampled in this study are single samples of insects that inhabit a wider forested area. Additionally, they were at endemic levels after recent outbreaks, which increases the probability of overlapping generations, inbreeding, and alteration of the distribution of their genotypic composition. These situations can explain, independent of high gene flow, the excess of homozygous and the exclusive genetic variation observed within populations; both phenomena are consequences of a Wahlund effect.

Genetic Structure. The general tendency of the lines of ordinary regression and RMA shows that populations whose geographic distances are small exhibit high levels of genetic differentiation and pairs of populations with large geographic distances exhibit low levels of differentiation. Although these results indicate that the model of isolation by distance cannot be completely explained by the relationship between F_{ST} and geographic distance, the Mantel test indicates that the relationship is significant, and not a result of chance.

Few isozyme studies have been conducted on scolytids that explicitly relate geographic organization of genetic variation with historical events (founder effects, bottlenecks, and so on). However, recent phylogeographic studies using isozymes and rapid amplification of DNA ends or mitochondrial markers in *D.*

brevicomis (Kelley et al. 1999), *D. jeffreyi* (Six et al. 1999), *D. ponderosae* (Kelley et al. 2000), *Ips typographus* (L.) (Stauffer et al. 1999), *Pityogenes chalcographus* (L.) (Ritzengruber 1990), and *Tomicus piniperda* (L.) (Carter et al. 1996) suggest that the genetic structure of these species results from historical and biogeographic events. For example, phylogeographic analysis of *I. typographus* clearly shows that the actual genetic structure of this species has been determined by glacial and postglacial events, which occurred during the Plio-Pleistocene in Europe, and is associated with the fragmentation of its habitat (hosts) and bottlenecks its populations have experienced (Stauffer et al. 1999).

Paleoclimatic evidence indicates that the glacial events of the Plio-Pleistocene in the TMVB did not have an important effect on the contraction of the areas of distribution of the coniferous forests in this region (Bryant and Holloway 1985, Millar 1998). Thus, the scarce number of fixed alleles, the geographic pattern of allele frequencies, and the *F* values suggest that the genetic structure of isolation by distance of *D. mexicanus* in the TMVB results from dispersal events. Indeed, the estimations of the number of individuals that have migrated per generation among populations from TMVB indicate that gene flow is higher between them. The robustness of this tendency is consistent, given that all the RMA regression lines, by systematically excluding one locus, show the same behavior and none of them alone determine the relationship between gene flow and geographic distances (data not shown).

The study of distribution range of *Dendroctonus* in Mexico supports the idea that isolation by distance is the result of dispersal events. The distribution pattern of the genus in the TMVB suggests that its species, including *D. mexicanus*, uses this mountain system as a broad corridor with few barriers for its dispersal (Salinas-Moreno et al. 2004). In addition, our results suggest that geographic distribution of hosts has had little effect on genetic structure of *D. mexicanus*, because the beetles used in this study were collected from distinct pines belonging to the *Leiophyllae* subsection, which exhibit a discontinuous distribution in the TMVB (Farjon and Styles 1997). Likewise, theoretical studies using meta-analysis found for phytophagous insects that the balance between dispersal and geographic isolation has a greater effect over genetic differentiation of populations than geographic distribution of hosts (Peterson and Denno 1998, but see Van Zandt and Mopper 1998).

Genetic Differentiation. The genetic differentiation observed among populations of *D. mexicanus* in the TMVB, the absence of exclusive alleles, and the absence of new electromorphs support the model of isolation-by-distance observed. The separation of the eastern and western populations of *D. mexicanus* in the dendrogram, and the small differences in genetic distance found among alleles suggest that independent of the geographic history of the TMVB, we are dealing with genetically homogenous populations of recent origin. Additionally, the analysis of principal components and the tree of minimum distance indicate that observed differences among populations are small and cumulative in a west–east direction, whereas geographically close populations describe a coherent pattern of association determined by their geographic location.

The magnitude of Nei's genetic distance among most divergent populations suggests the existence of cryptic species. However, we suspect that genetic and morphological differences observed among these populations are not sufficient to consider them as two genetically distinct groups. Other studies conducted with enzymatic markers on geographic populations of *D. brevicomis*, *D. frontalis*, *D. jeffreyi*, *D. ponderosae*, *Dendroctonus pseudotsugae* Hopkins, and *Dendroctonus terebrans* (Olivier) have described similar geographic patterns as that of *D. mexicanus* (Anderson et al. 1979, 1983; Namkoong et al. 1979, Stock and Guenther 1979, Stock et al. 1979, 1984; Higby and Stock 1982; Roberds et al. 1987; Six et al. 1999). For example, in some of these studies significant genetic difference among some populations of these species were observed, but the values of genetic distance found suggest that these differences have resulted from relationships among the dispersion potential of the insect and geographic isolation. Additionally, interpopulation crosses carried out with some *Dendroctonus* species from allopatric and sympatric populations, and from distinct hosts in the same locality demonstrate that the observed genetic differences are labile (Langor 1989, Langor and Spence 1991), a situation contrary to that which occurs with genetic differences observed among geographically isolated populations or cryptic species.

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