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# LANDSCAPE STRUCTURE AND HIERARCHICAL GENETIC DIVERSITY IN THE BROOK CHARR, *SALVELINUS FONTINALIS*

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*Abstract.*—Explaining the extent, causes, and consequences of biotic distributions in space is fundamental to our understanding of how species evolve and cope with particular environments. Yet, identifying extrinsic barriers to migration imposed by landscape structure and predicting their impacts on intraspecific genetic diversity remains a major challenge in population biology. In this study, 30 populations (771 individuals) of brook charr (*Salvelinus fontinalis,* Salmonidae) representing six major river drainages from Maine, USA, were characterized at six microsatellite loci to quantify the role of landscape features, such as habitat size, altitude, contemporary and historical connectivity, in shaping genetic diversity at three spatial scales: within lakes, within river drainages, and among river drainages. Within-population expected heterozygosity was negatively correlated with altitude, whereas no significant correlation was observed with lake size. Conversely, the extent of heterozygote deficiency within lakes was negatively associated with habitat size. The hierarchical analysis of genetic variance revealed that the extent of among-drainage differentiation was unexpectedly low relative to the pronounced population structuring within drainage. Geographically proximate St. John and Penobscot River drainages were characterized by opposite effects of altitude and geographic distance in shaping the pattern of population differentiation within drainages. The geographic pattern of differentiation among drainages could not be accounted for either by an isolation by distance or by a stepwise range expansion model. Overall, this study provided evidence for the role of contemporary landscape features in shaping the observed pattern of genetic diversity at smaller geographic scales (within and among populations within river drainage). On a broader geographic scale, contemporary landscape structure appeared to be only a minor factor determining the observed pattern of genetic structuring among drainages. These results add to the increasing evidence for nonequilibrium conditions between drift and migration in a wide array of animal taxa. The development of more realistic theoretical descriptions of nonequilibrium population structure thus appears to be important to better understand the relative influence of historical and ecological factors in shaping genetic variation in young habitats, such as recently deglaciated areas.

*Key words.*—Fish, isolation by distance, landscape, microsatellite, migration model, nonequilibrium.

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In species composed of large numbers of mobile individuals, extrinsic barriers to migration imposed by landscape features are particularly important in determining population genetic structure. Yet, the identification of such barriers and the prediction of their impacts in shaping intraspecific genetic diversity remain a major challenge in population biology (Slatkin 1985; Sork et al. 1999; Wiegand et al. 1999). Studies dealing with the effect of landscape in shaping population structure have largely focused on the consequences of habitat fragmentation caused by recent anthropogenic disturbance (e.g., Aldrich et al. 1998; Gibbs 1998; Van Dongen et al. 1998). Although obviously relevant, such studies generally lack a historical perspective on the influence of landscape structure on temporal dynamics of genetic connectivity among natural populations (but see Fahrig and Merriam 1994; McCauley 1995; McCauley et al. 1995). Consequently, there is a need for detailed empirical studies that simultaneously quantify the effects of individual landscape features at various geographic scales (Shaw et al. 1994; Kudoh and Whigham 1997; Keyghobadi et al. 1999; Sork et al. 1999).

In this study, we use microsatellite loci to test specific hypotheses about the role of historical and contemporary landscape features in shaping the hierarchical pattern of genetic diversity in the brook charr, *Salvelinus fontinalis* Mitchill. This salmonid is endemic to eastern North America (McCrimmon and Campbell 1969) and inhabits lakes connected by streams, which are themselves potential habitats for the species (Power 1980). Thus, opportunities for migration are highly constrained by patterns of hydrographic networks. The extensive life history (Hutchings 1990) and genetic (Angers and Bernatchez 1998) differences found at all geographic scales in brook charr suggest that genetic exchanges are extremely reduced among populations from different lakes, which therefore act as discrete and independent demographic units. Assuming that habitat and effective population size are correlated, we first tested the hypothesis that lake area should be positively correlated with the levels of intrapopulation genetic diversity (Crow and Kimura 1970; Frankham 1996; Hanfling and Brandl 1998).

Although rarely taken into account (but see Hernandez-Martich and Smith 1990; Shaw et al. 1991, 1994; Angers et al. 1999), altitude may also influence the levels of diversity in freshwater habitats. High-altitude populations are expected to be more physically isolated, either because of increased probability of physical barriers to gene flow (e.g., impassable waterfalls) and/or due to more pronounced founder effects, assuming that the number of colonists decreased with altitude. Therefore, a second hypothesis was that the extent of genetic divergence among populations should correlate with the altitudinal difference in their locations. Because movements of brook charr have been shown to be geographically restricted in riparian habitats (Gowan and Fausch 1996), we also tested the hypothesis that isolation by distance should contribute to population differentiation within river drainages. Migration among river drainages can only be achieved by anadromous individuals. Direct (Smith and Saunders

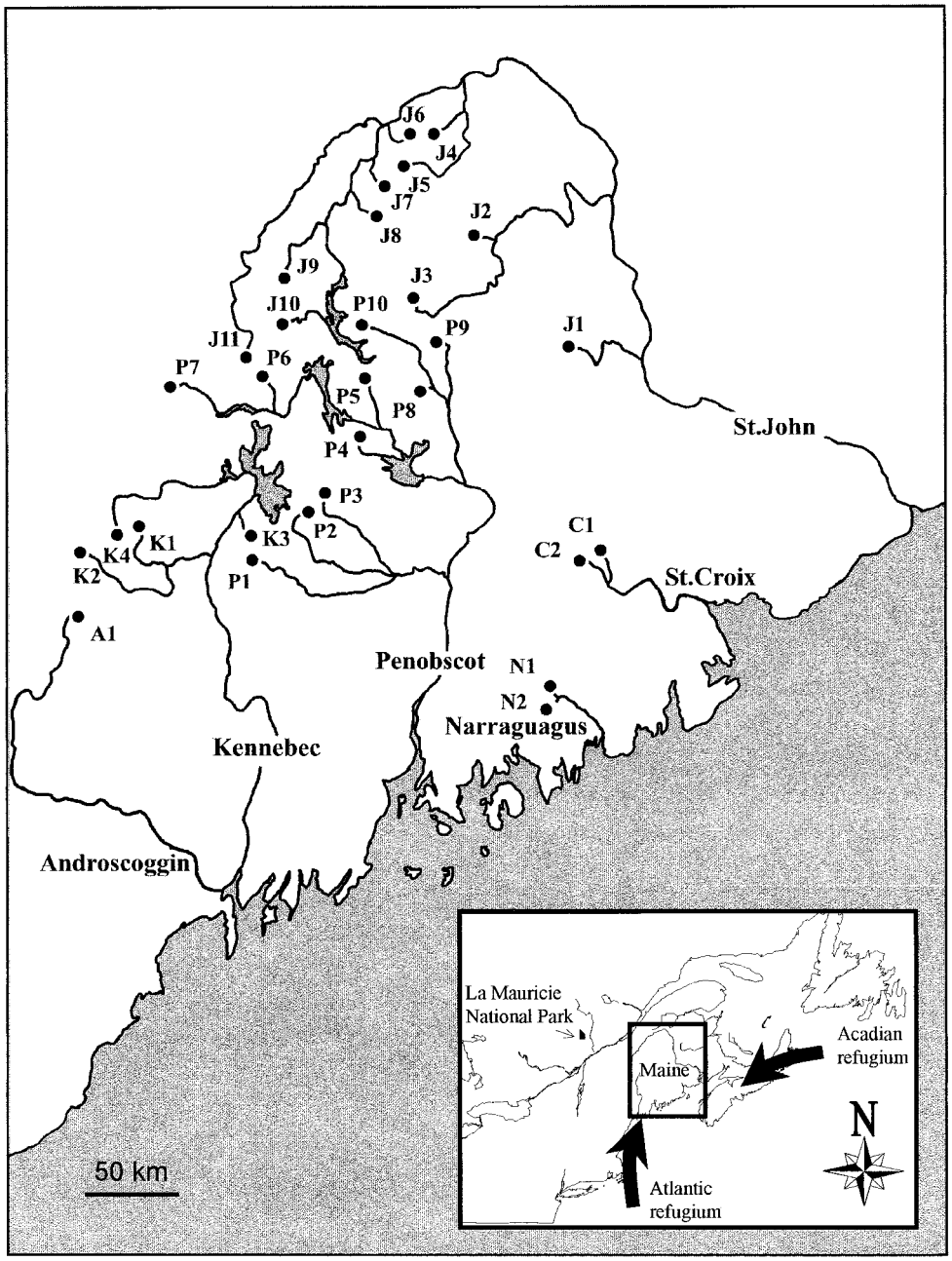


FIG. 1. Sampling sites in Maine. Population codes correspond to locations in Table 1. Approximate locations for glacial refugia are from Schmidt (1986).

1958) as well as indirect estimates (V. Castric, unpubl. data) suggest that dispersal of anadromous individuals is limited to coastal waters and geographically restricted. Therefore, isolation by distance is also predicted to occur among drainages.

North American biota have been extensively influenced by the last glacial age (Pielou 1991). No freshwater habitats were available in Maine until the region was deglaciated, approximately 12,700–13,000 years ago (Borns et al. 1985; Dyke and Prest 1987). Following glacier retreat, a marine invasion (saltwater intrusions) in the coastal plain prevented establishment of freshwater fish communities until at least 12,100–11,000 years ago (Stuiver and Borns 1975). Recolonization from the closest glacial refugia (so-called Acadian and Atlantic; Danzmann et al. 1998; Fig. 1) was therefore impossible before this time. Eurhyaline fishes, such as brook charr, most likely took advantage of their physiological tolerance to saltwater to reinvade previously ice-covered areas via coastal dispersal (Black et al. 1986). Thus, given the relatively short evolutionary time since the postglacial settlement of brook charr populations, we tested the hypothesis that the patterns of genetic differentiation among brook charr from different drainages still reflect the sequence of successive founding events from one river drainage to another.

TABLE 1. Location and physical features of the sampling sites in Maine. Habitat size was not quantified for streams, rivers, and brooks.

| Population | Code | Drainage | *N* | Latitude | Longitude | Altitude (m) | Size (ha) |
|---|---|---|---|---|---|---|---|
| Kennebago Lake | A1 | Androscoggin | 25 | 70°46′21″ | 45°08′08″ | 359 | 688 |
| Upper Flood Lake | C1 | Ste. Croix | 22 | 67°53′22″ | 45°22′36″ | 183 | 100 |
| Trout Lake | C2 | | 30 | 67°49′05″ | 45°22′05″ | 241 | 20 |
| B-Stream | J1 | St. John | 32 | 67°54′41″ | 46°11′54″ | 183 | — |
| Little Machias River | J2 | | 30 | 68°33′10″ | 46°43′18″ | 207 | — |
| Brown Brook Pond | J3 | | 30 | 68°54′24″ | 46°25′02″ | 414 | 24 |
| Third Wallagrass Lake | J4 | | 18 | 68°49′16″ | 47°06′44″ | 301 | 182 |
| Deboullie Lake | J5 | | 23 | 68°51′21″ | 46°57′54″ | 344 | 1060 |
| Third Pelletier Brook Lake | J6 | | 31 | 68°53′25″ | 47°02′00″ | 379 | 336 |
| McQueen Brook | J7 | | 34 | 69°07′15″ | 46°54′12″ | 293 | — |
| Robbins Brook Pond | J8 | | 28 | 69°10′01″ | 46°43′27″ | 405 | 109 |
| Ross Lake | J9 | | 26 | 69°37′21″ | 46°29′36″ | 366 | 1171 |
| Johnson Pond | J10 | | 22 | 69°34′54″ | 46°19′10″ | 319 | 797 |
| Lost Pond | J11 | | 17 | 69°47′32″ | 46°06′44″ | 599 | 182 |
| Rock Pond | K1 | Kennebec | 22 | 70°23′29″ | 45°27′42″ | 499 | 502 |
| Massachussets Bog | K2 | | 16 | 70°47′27″ | 45°20′37″ | 509 | 121 |
| Round Pond | K3 | | 30 | 69°45′38″ | 45°24′54″ | 449 | 243 |
| Prick Pond | K4 | | 23 | 70°31′02″ | 45°26′43″ | 753 | 2 |
| Clark Meadow Brook | N1 | Narraguagus | 15 | 68°08′44″ | 44°40′49″ | 84 | — |
| Narraguagus Lake | N2 | | 8 | 68°08′41″ | 44°39′29″ | 68 | 1724 |
| Little Moxie Pond | P1 | Penobscot | 31 | 69°43′28″ | 45°18′35″ | 397 | 295 |
| Horseshoe Pond | P2 | | 23 | 69°24′10″ | 45°30′30″ | 448 | 648 |
| Baker Pond | P3 | | 28 | 69°24′45″ | 45°32′12″ | 503 | 4 |
| Beaver Pond | P4 | | 22 | 69°09′27″ | 45°48′12″ | 320 | 12 |
| Sourdnahunk Lake | P5 | | 31 | 69°05′47″ | 46°02′30″ | 421 | 565 |
| Bean Pot Pond | P6 | | 20 | 69°42′42″ | 46°03′20″ | 407 | 210 |
| Clish Pond | P7 | | 29 | 70°15′33″ | 46°01′10″ | 550 | 85 |
| Hathorn Pond | P8 | | 29 | 68°46′01″ | 46°00′22″ | 274 | 61 |
| Hay Pond | P9 | | 29 | 68°45′09″ | 46°10′03″ | 195 | 542 |
| Fourth Lake | P10 | | 19 | 69°10′06″ | 46°16′13″ | 298 | 26 |

## MATERIALS AND METHODS

### *Sampling Design*

A total of 771 individuals were collected from Maine at 30 locations (22 lakes and eight brooks) representing six major river drainages during summers 1997 and 1998 (Table 1, Fig. 1). There is no record that these populations have ever been stocked, so we considered them to be native. Adipose fins were removed nondestructively and preserved in 95% ethanol.

### *Detection of Microsatellite Polymorphism*

Total DNA was extracted following a quick lysis protocol (Olsen et al. 1996). Quantification of genetic variation was performed using six microsatellite loci specifically developed for brook charr (SFO-8, SFO-12, SFO-18, SFO-23; Angers et al. 1995), brown trout (*Salmo trutta,* MST-85; Presa and Guyomard 1996), or Atlantic salmon (*Salmo salar,* SSA-197; O'Reilly et al. 1996). Two simultaneous triplex polymerase chain reactions (PCRs; SFO-8, SFO-12, MST-85 and SFO-18, SFO-23, SSA-197) were conducted using 1 μl of the DNA extract (Hébert et al. 2000). A volume of 0.8 μl of each PCR product was mixed with 2 μl of blue formamide containing 10% of GS350 internal size standard (TAMRA 350 bp) and loaded on a 5% polyacrylamid gel for a 2.25-h electrophoresis at 3000 V using an ABI 377 automated DNA sequencer (Perkin-Elmer, Foster City, CA). The fragment sizes were determined in reference to a size standard run in each lane using the softwares GENESCAN version 2.1 and GENOTYPER version 2.0 (Perkin-Elmer). A reference brook charr sample with known allele size (Angers and Bernatchez 1997) was run on each gel to ensure homogeneity of results across gels and to avoid scoring bias when comparing our results to previously published datasets (Angers and Bernatchez 1998; Hébert et al. 2000).

### *Genetic Data Analysis*

For each hierarchical level (within population, among populations within drainage, among drainages), we first estimated the parameters of genetic polymorphism and then quantified the correlation between these estimates and landscape physical features.

#### *Intrapopulation genetic diversity*

Within-population genetic diversity was quantified as the number of alleles per locus (*A*), observed heterozygosity ($H_O$), and the unbiased estimate of heterozygosity corrected for the sampling bias ($H_E$; Nei 1987). Deviations from Hardy-Weinberg equilibrium (HWE) in each site were investigated using an approximation of an exact test based on a Markov chain iteration as implemented in GENEPOP version 3.1 (Raymond and Rousset 1995). Multilocus values of significance for HWE tests were obtained following Fisher's method to combine probabilities of exact tests. Critical significance levels for multiple testing were corrected following the sequential Bonferroni procedure (Rice 1989).

The extent of deviation from HWE proportions was quan-

tified by Weir and Cockerham's (1984) estimator of $F_{IS}(f)$ at each locus and at each site using GENETIX version 4.0 (Belkhir et al. 1998). We tested whether the same loci consistently exhibited stronger $f$ using Kendall's concordance method (Sokal and Rohlf 1981).

We then considered the possible impact of habitat size and altitude on population parameters estimates $H_E$ and $f$ (whenever $f$ was positive). Because it was impossible to reliably quantify habitat size for brooks, this parameter was only quantified for lakes. Because $H_E$ and $f$ may not be distributed normally, all correlations were nonparametrically tested using Spearman $R$ available in STATISTICA version 4.5 (Statsoft 1993).

*Hierarchical population structure*

The null hypothesis of homogeneity in allele frequencies was tested using the exact test for population differentiation in GENEPOP. The extent of population differentiation was then quantified by Weir and Cockerham's (1984) estimator of $F_{ST}(\theta)$, based on the variance in allele frequencies and Michalakis and Excoffier's (1996) estimator of $\rho_{ST}(\phi_{ST})$, incorporating variance in allelic size. These two parameters behave differently with regard to drift and mutation and their comparison can provide insights into the historical time frame involved in population differentiation (Rousset 1996; Goodman 1998). We then performed a hierarchical analysis of genetic variation (AMOVA) implemented in ARLEQUIN version 1.1 (Schneider et al. 1997) to quantify the amount of genetic variance imputable to drainage subdivision.

Cavalli-Sforza and Edwards's (1967) chord distance ($D_{CE}$) was used to construct a population phenogram using Saitou and Nei's (1987) neighbor-joining algorithm. $D_{CE}$ was chosen because it leads to a higher probability of depicting the correct tree topology (Takezaki and Nei 1996) and because this measure involves no assumption regarding constant population size or equal mutation rates among loci. Previously published data for brook charr from La Mauricie National Park, Canada (Angers and Bernatchez 1998) were also included in this analysis to compare the congruence in overall pattern of population structuring between geographically remote areas. SEQBOOT, GENEDIST, NEIGHBOR, and CONSENSE computer programs implemented in PHYLIP version 3.5 (Felsenstein 1993) were used to build the tree from allele frequencies data obtained from GENETIX. Confidence in tree topology was assessed by bootstrapping over loci (2000 iterations).

*Patterns of differentiation within drainages*

As shown in Rousset (1997), a linear relationship is expected between waterway distance and $F_{ST}/(1 - F_{ST})$ when dispersal is geographically limited in a linear array of populations. Mantel tests (Mantel 1967) were thus used to test the significance of this correlation in St. John and Penobscot, the two drainages in which a sufficient number of sites were sampled. Probability values for no correlation were estimated using the permutation procedure ($n = 2000$) in GENETIX. To assess whether populations were at migration-drift equilibrium under isolation by distance in one dimension, we computed the slope of $\log[F_{ST}/(1 - F_{ST})]$ against log(distance). Equation (7) in Rousset (1997) predicts a positive slope of approximately one under equilibrium conditions.

Physical barriers to migration, such as impassable waterfalls, are expected to have a strong impact on genetic exchanges among populations. We assumed that such barriers were more frequent among sites separated by high altitudinal differences, and consequently defined the sum of altitude differences as the sum of altitude variation along the shortest waterway between each population pair. Thus, if populations 1 and 2 have elevation $h_1$ and $h_2$, respectively, and their first common node on the hydrographic network has elevation $h_N$, the sum of altitude differences between populations 1 and 2 is $(h_1 - h_N) + (h_2 - h_N) = h_1 + h_2 - 2h_N$. This distance would be greatest between two populations located on mountain tops, moderate between one population of high altitude and one of low altitude, and lowest between two low-altitude populations. It is unclear, however, what relationship should be expected between the sum of altitude differences and genetic differentiation, because there is no specific theoretical background to support the use of $F_{ST}/(1 - F_{ST})$ in such circumstances. Consequently, we used $D_{CE}$ genetic distance, for which basic assumptions are less restrictive than for $F_{ST}$. Mantel tests were used to test the correlation between $D_{CE}$ and the sum of altitudinal differences (2000 permutations).

*Differentiation patterns among drainages*

We also tested whether isolation by distance occurred among drainages. Genetic differentiation among drainages (subscript D) was estimated by $F_{DT}$, the hierarchical $F$-statistic associated with this component of genetic variance in the AMOVA framework based on allele frequency data. Coastal distances were measured on 1:50,000 topographic maps by closely following the contemporary coastline from one river mouth to the other. Mantel tests were used to test the significance of the regression of pairwise $F_{DT}/(1 - F_{DT})$ on coastal distances (2000 permutations).

Brook charr populations in Maine most likely originated from either an Atlantic or an Acadian refugium (Danzmann et al. 1998) and probably recolonized previously ice-covered areas via coastal dispersal (Black et al. 1986). Therefore, we tested whether the observed patterns of genetic differentiation among drainages could be explained by a postglacial recolonization from these putative refugia. Slatkin (1993) provides one of the only available frameworks to predict the expected patterns of genetic differentiation under nonequilibrium conditions. A directional and gradual stepwise range expansion, referred to as Good's model in Slatkin (1993), is expected to result in greater divergence among earlier founded populations than among more recently founded ones, independently of the geographic distance among them. For example, assuming that recolonization of Maine watersheds by brook charr occurred from southwest to northeast, Androscoggin drainage (see Fig. 1) would have been colonized first and one would predict southwestern populations to be most highly differentiated because they underwent genetic drift for a longer time. Good's model was thus used to assess whether the observed pattern of genetic differentiation fitted the alternative expectations of either southwest-northeast recolo-

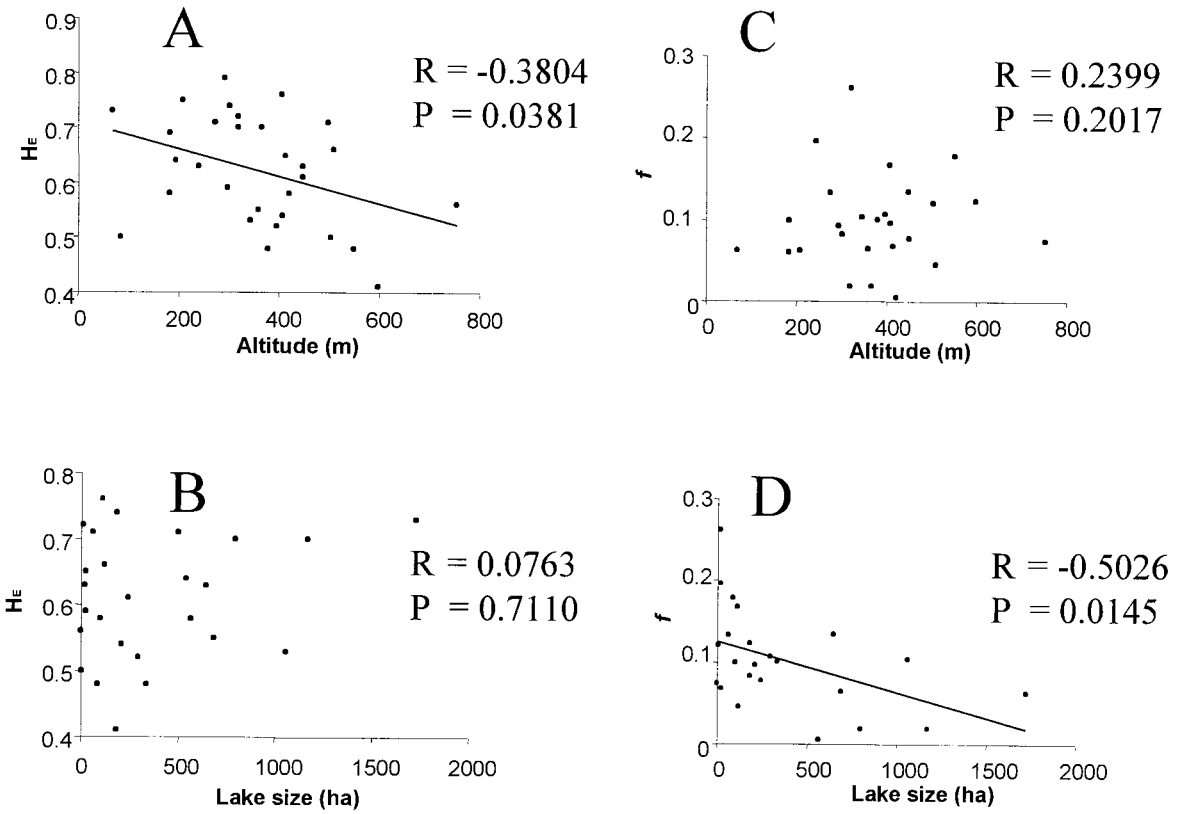


FIG. 2. Relationships between physical features of lakes (lake size and altitude), expected heterozygosity (A, B) and *f*, $F_{IS}$ estimate (C, D). Correlations were tested using Spearman's *R*.

nization from an Atlantic refugium or a northeast-southwest recolonization from an Acadian refugium. Under the hypothesis of an Atlantic origin of Maine populations, we plotted for each pair of drainages $(1/F_{DT} - 1)/4$ against *i*, the coastal distance between the oldest drainage of a given pair and the Androscoggin River (the drainage that would have settled first under this scenario). Mantel's test was used to test the significance of the correlation (2000 iterations). Conversely, under the hypothesis of an Acadian origin, we considered the regression of $(1/F_{DT} - 1)/4$ against *j*, the coastal distance between the oldest drainage of a given pair and the St. John River.

## RESULTS

### *Intrapopulation Genetic Diversity*

All six microsatellite loci were highly polymorphic, with the number of different alleles observed across all populations (*A*) ranging from 10 (SSA-197) to 57 (SFO-8) and mean within population unbiased heterozygosity $H_E$ ranging from 0.40 (SFO-12) to 0.81 (SFO-8; Appendix). The exact test of HWE showed a significant global trend toward heterozygote deficiency ($f = 0.0807$, $P < 0.001$). Seven populations exhibited significant departures from Hardy-Weinberg proportions following sequential Bonferroni correction ($P = 0.0017$, $\alpha = 0.05$, $k = 30$). Details on *f*-values are presented in Appendix. This global deficiency was not caused by one particular locus, as four out of the six screened loci deviated significantly from Hardy-Weinberg expectations following Bonferroni correction ($P = 0.008$, $\alpha = 0.05$, $k = 6$; Appendix). Furthermore, Kendall's test of concordance showed that the ranks of all loci according to *f*-values were not consistent across populations ($P = 0.4653$).

### *Intrapopulation Diversity Versus Landscape Features*

We observed a significant negative correlation ($P = 0.0381$) between altitude and mean expected heterozygosity ($H_E$), whereas no significant association ($P = 0.7110$) between lake size and $H_E$ was apparent (Fig. 2A, B, respectively). Conversely, the extent of heterozygote deficiency (*f*) was not associated with altitude ($P = 0.2017$) but was negatively correlated ($P = 0.0145$) with lake size (Fig. 2C, D, respectively). No correlation was found between lake size and altitude ($P = 0.312$), which ensured the independence of altitude and lake size effects. Noticeably, three out of the four populations exhibiting the highest allelic diversity (J1, J2, J7) were from riparian habitats (stream, river, and brook, Appendix).

### *Hierarchical Population Structure*

Highly significant heterogeneity in allele frequencies was found in all pairwise comparisons for at least one locus ($P < 0.001$; data not shown), which confirmed that brook charr found in different locations compose genetically distinct populations. Overall $F_{ST}$ and $\rho_{ST}$ estimates were very similar but pairwise comparisons were highly variable (mean $F_{ST}$ =

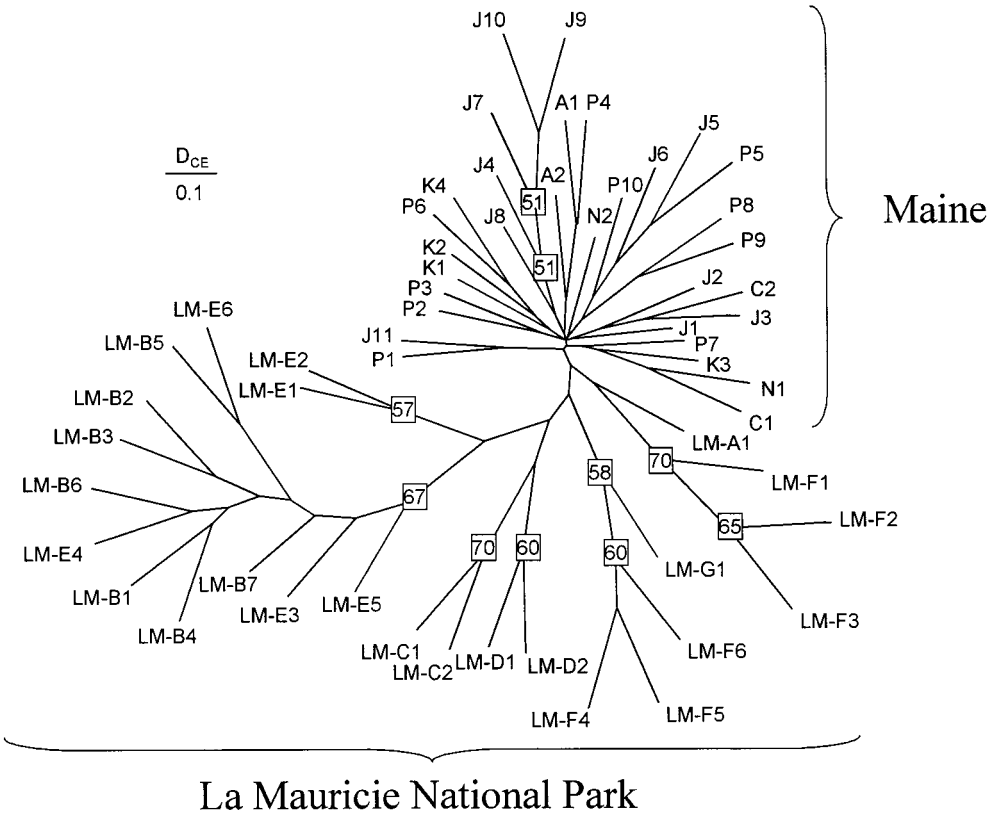


FIG. 3. Neighbour-joining tree relating the 30 populations sampled in Maine and also incorporating La Maurice National Park (Quebec, Canada) populations. Codes for La Mauricie (prefix LM) are as indicated in Angers and Bernatchez (1998). The tree was constructed using the chord distance of Cavalli-Sforza and Edwards (1967). Bootstrap values are based on 2000 replicates.

0.216, range = 0.016–0.465; mean $\rho_{ST}$ = 0.208, range = 0.011–0.718).

The hierarchical analysis of genetic variance revealed that a significant ($F_{DT}$ = 0.037, $P$ = 0.002) component of allelic variance was explained by drainage structure. However, the extent of among-drainage differentiation was very low relative to the pronounced interpopulation structuring within drainage ($F_{SD}$ = 0.203). When assuming a stepwise mutation model, the estimate of differentiation among drainages was lower than $F_{DT}$ ($\phi_{DT}$ = 0.024) and not significantly different from zero ($P$ = 0.2082).

The population phenogram inferred from $D_{CE}$ distance further illustrated the overall lack of population grouping by drainage or any other type of hierarchical clustering among Maine populations. The tree was starlike, with all branches of approximately equal length, and generally poorly supported (Fig. 3). When compared with La Mauricie National Park, Maine populations tightly clustered together and were genetically more similar as illustrated by shorter branch lengths, despite the fact that they were sampled over a much broader geographic scale (Maine: 86,156 km$^2$ vs. La Mauricie: 544 km$^2$).

### *Contrasting Patterns of Differentiation within Drainages*

The Penobscot and St. John River drainages were characterized by differential effects of landscape features on the genetic structure. In the St. John drainage, a strong positive correlation was observed between $D_{CE}$ and the sum of altitudinal differences (Fig. 4A; Mantel test, $Z$ = 3423, $P$ = 0.0055). However, no pattern of isolation by distance was apparent, as the extent of population differentiation did not depart significantly from a randomized association between $F_{ST}/(1 - F_{ST})$ and waterway distance (Fig. 4B; Mantel test, $P$ = 0.533). The Penobscot drainage populations showed the reverse pattern. Genetic divergence was not correlated with the sum of altitudinal differences (Fig. 4C; Mantel test, $Z$ = 5306, $P$ = 0.576), whereas waterway distance was marginally correlated with $F_{ST}/(1 - F_{ST})$ (Fig. 4D; Mantel test, $Z$ = 5458, $P$ = 0.0556). The slope (0.33) of $\log[F_{ST}/(1 - F_{ST})]$ on log(distance) in this drainage was far from the value of one expected at migration-drift equilibrium.

### *Patterns of Differentiation among Drainages*

The geographic pattern of differentiation among drainages could not be accounted for either by isolation by distance or by a stepwise range expansion model. We observed, however, a statistical trend (Mantel test, $Z$ = 2.3032, $P$ = 0.0755) for a reversed isolation by distance pattern, that is, greater genetic similarity with increased coastal distance among drainages. Good's model yielded nonsignificant outcomes for the two alternative hypotheses of gradual recolonization from an Atlantic or an Acadian refugium (Mantel tests $Z$ = 734, $P$ = 0.424 and $Z$ = 910, $P$ = 0.286, respectively).

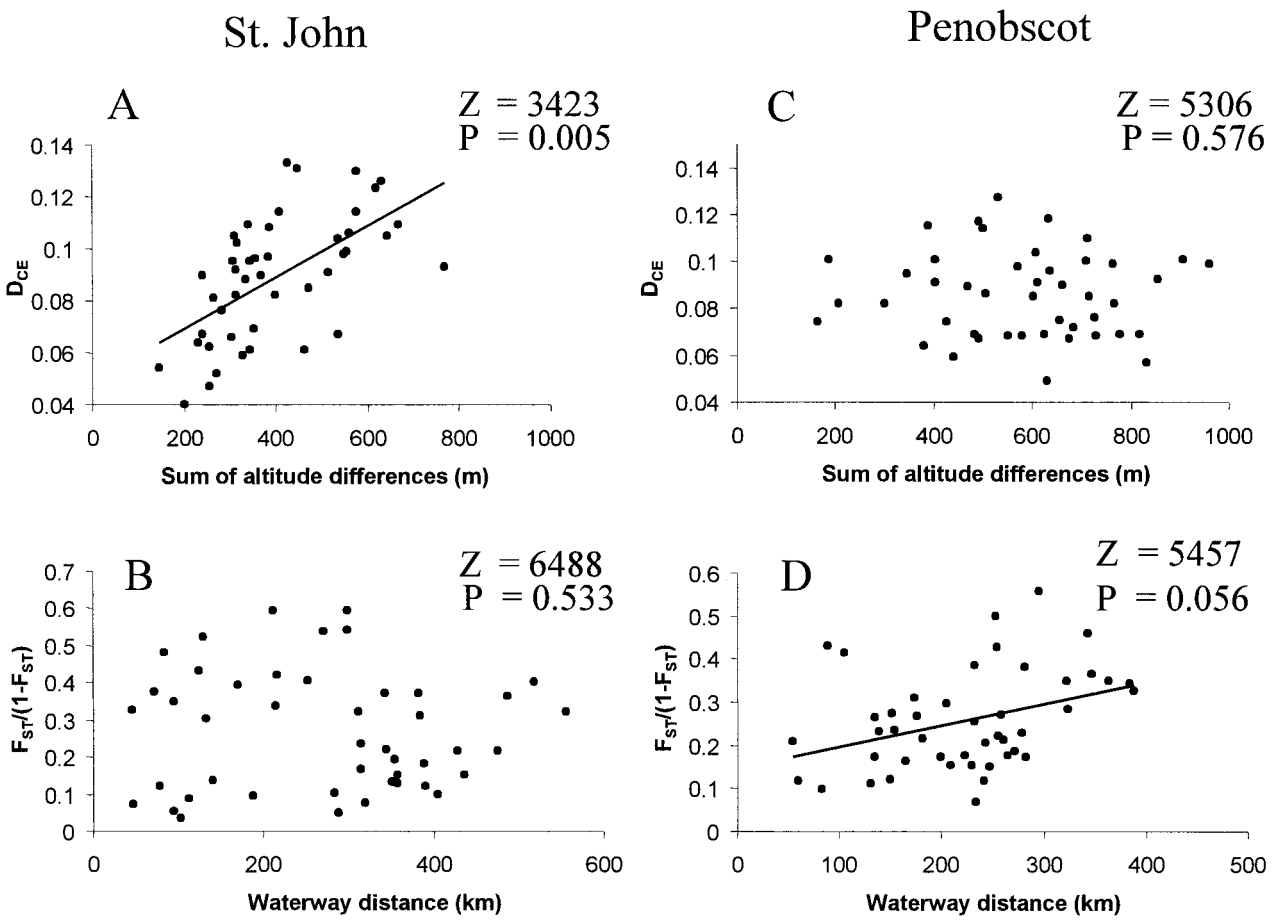


FIG. 4. Genetic divergence among populations as a function of sum of altitudinal differences and waterway distance within St. John (A, B, respectively) and Penobscot River drainages (C, D, respectively).

## DISCUSSION

The main objective of this study was to test specific hypotheses regarding the role of historical and contemporary landscape features in shaping the pattern of genetic diversity in the brook charr, *S. fontinalis* Mitchill. Overall, this study provided evidence for the role of contemporary landscape in shaping the observed pattern of genetic diversity at smaller geographic scales (within and among populations within river drainage). The detected consequence of habitat features, however, generally differed from a priori predictions. On a broader geographic scale, the role of contemporary landscape features appeared to be only a minor factor determining the observed pattern of genetic structuring among drainages.

The finding of lower heterozygosity in populations of higher elevation was congruent with the results of recent empirical studies in other fishes, such as the guppy *Poecilia reticulata* (Shaw et al. 1994); mosquitofish, *Gambusia holbrooki* (Hernandez-Martich and Smith 1990); and brown trout, *Salmo trutta* (Hamilton et al. 1989). At higher altitude, populations are more likely to be isolated by waterfalls, whereby unidirectional downstream gene flow is more likely to occur. Habitats at higher altitudes may also have become isolated earlier. As for all regions covered by glaciers, an isostatic rebound (crustal upheaval) occurred in Maine following removal of the weight of the 2.2-km thick ice sheet (Stuiver and Borns 1975). This in turn may have caused a lag in the timing of population settlement in habitats of different altitudes. Although habitat size is commonly used to infer population census size and thus diversity, such a trend was not detected in this study. This could first reflect the difficulty of reliably quantifying habitat size. Namely, surface lake area alone may not accurately reflect the complex ecological interactions that determine the carrying capacity of lacustrine habitats for brook charr. For instance, the availability of spawning grounds (Blanchfield and Ridgway 1997) and the relative abundance of other species (Magnan 1988) could also influence charr abundance in different lakes. Alternatively, the absence of correlation between habitat size and genetic diversity may indicate the persistent effect of founder events. Freshwater communities in Maine are approximately 11,000 years old (Stuiver and Borns 1975) and brook charr populations may be even younger because they could not become established prior to their invertebrate preys. Consequently, it is likely that mutation-drift equilibrium may not have been reached yet. As such, the levels of intrapopulation diversity may be more reflective of the allelic diversity in founding populations of differential abundance, rather than contemporary censuses.

Highest allelic diversity was observed in stream populations despite much smaller habitat size, as was recently ob-

served for brook charr populations (Angers et al. 1999; Hébert et al. 2000). This suggests that lacustrine and stream populations are characterized by contrasting demographic dynamics. For instance, restricted gene flow among genetically differentiated subpopulations within streams would lead to increased genetic diversity relative to a single panmictic population of the same census size (Whitlock and Barton 1997). A more detailed comparison of fine-scale genetic and demographic structure between lacustrine and riverine populations is required to confirm this hypothesis.

Our data revealed a significant heterozygote deficiency, which was mainly attributed to a significant trend toward higher heterozygote deficiencies in smaller lakes. Technical artifacts were unlikely to cause this pattern, as the observed deficit was not locus-specific. Moreover, previous studies using the same loci (Angers and Bernatchez 1998; Hébert et al. 2000) found no evidence of departures from Hardy-Weinberg equilibrium. At least two alternative explanations could account for this observation, the most likely being admixture of differentiated gene pools. Dynes et al. (1999) recently provided evidence for a slight genetic differentiation between benthic and limnetic lacustrine forms of brook charr in another area of the species range. The possible existence of such sympatric forms in Maine is reinforced by the fact that this area is a known zone of secondary contact between genetically and morphologically differentiated glacial races of other fishes, such as whitefish, *Coregonus clupeaformis* (Bernatchez and Dodson 1990), and rainbow smelt, *Osmerus mordax* (Taylor and Bentzen 1993). Also of note, sympatric pairs of lake whitefish are known to occur mainly in smaller lakes (G. Lu, pers. comm.). Increased probability of relatedness among sampled individuals could also conceivably explain the more pronounced heterozygote deficiencies in smaller lakes. Limited number of spawners could generate a Wahlund effect due to genetic differences among fish using different spawning grounds at a given reproduction event in smaller lakes. Increased availability of spawning areas in larger lakes may potentially buffer this effect. We are currently investigating this possibility.

The overall level of differentiation observed in brook charr populations from Maine was relatively modest when compared to that reported in previous studies. For example, the overall $F_{ST}$-value was 40% lower than that reported by Angers and Bernatchez (1998) in La Mauricie National Park at a much smaller spatial scale ($F_{ST} = 0.37$ with the greatest linear geographic distance of 42 km compared to a maximum of 375 km in this study). The observed level of population differentiation more resembled that observed recently on a much smaller geographic scale ($F_{ST} = 0.28$, maximum linear distance = 13 km) by Hébert et al. (2000). Furthermore, starlike tree topology, low bootstrap support, and the hierarchical analysis of molecular variance all indicated that hierarchical population structuring by drainage was very weak. These results are also in sharp contrast with most studies of genetic variation in freshwater fishes, which generally reported that drainage structure represent a major constraint to gene flow (Gyllenstein 1985; Currens et al. 1990; Carvalho et al. 1991; Ward et al. 1994; Estoup et al. 1998; but see Avise and Felley 1979; Hernandez-Martich and Smith 1990). Lack of structuring by drainage could result from extensive gene flow via coastal dispersal among drainages. However, highly significant population differentiation has been recently observed among neighboring (less than 10 km) anadromous populations in other areas (V. Castric, unpubl. data), suggesting that this factor alone would be insufficient to homogenize allele frequencies among drainages in Maine. Artificial stocking with populations from one drainage into another cannot be entirely ruled out. However, such practices have not been reported in the region (F. Bonney, unpubl. data) and thus appear unlikely to have heavily weakened a signal of drainage differentiation.

Paleodrainages have been deeply modified by the isostatic rebound since the glacial retreat. Consequently, temporal instability in drainage isolation could also reduce genetic differentiation among populations from separate river systems. For instance, although their river mouths are separated by approximately 200 km, the headwaters of the St. John and Penobscot drainages are geographically proximate in central Maine. Thus, hydrographic changes such as drainage capture or disrupted stream connections could have intermittently allowed genetic exchanges among populations from different drainages. In such a case, however, formerly connected populations should cluster together, which was clearly not the case here. Finally, it is also possible that the apparent lack of structuring by drainage partly reflect departure from migration-drift equilibrium, implying that time since population founding (approximately 2000 generations) has been insufficient for genetic differences to accumulate at this spatial scale.

Another salient feature of this study was the observation that geographically proximate drainages (Penobscot and St. John River) were characterized by differential effects of landscape features on genetic structure. Isolation by distance was responsible for shaping population differentiation in the Penobscot River drainage, whereas a correlation between genetic divergence and altitudinal differences was observed within the St. John drainage. The sum of altitude differences was used as a surrogate for the number of impassable falls and thus for the intensity of physical isolation. A significant effect of this parameter on population structuring in the St. John River therefore indicates that restricted gene flow caused by these barriers may be mainly responsible for population divergence within the St. John relative to the Penobscot River drainage. Because the variance around $F_{ST}$ estimates is not known under pure isolation by distance models, the lack of correlation between $F_{ST}/(1 - F_{ST})$ and distance does not entirely rule out the possibility that dispersal is not geographically restricted within the St. John drainage. However, the sharp contrast between both drainages strongly suggested that the landscape features shaping genetic structure are not concordant.

At a larger scale of landscape structure, Good's model failed to detect a signature of progressive recolonization, either from a northern or from a southern refugium. Given the rarity of empirical tests of this model (Jackman and Wake 1994; Hutchison and Templeton 1999), and the relatively small number of comparisons we used to apply it, we cannot rule out the possibility that limited statistical power was responsible for this outcome. Alternatively, rapid range expansion (Slatkin 1993), rather than stepwise recolonization,

could possibly account for the unexpected trend for increased genetic similarity among more geographically distant drainages. Under this scenario, isolation by distance is expected among nearby but not among distant populations until the system reaches migration-drift equilibrium. Sampling over a broader geographical scale may be needed to clarify this issue.

In summary, this study revealed substantial variation in the influence of landscape features on contemporary patterns of genetic structure in brook charr, both spatially and temporally. Other empirical studies in freshwater fishes (Ryman 1983; McClenaghan et al. 1985; Crozier and Ferguson 1986; Moran et al. 1995; Baer 1998; Hansen and Mensberg 1998) also reported limited correlations between indirect estimates of gene flow and potential for dispersal among populations within structured habitats. Similarly, nonequilibrium conditions between drift and migration are increasingly reported for a wide array of animal taxa, particularly when considering population structure over a broad geographic scale (e.g., Larson et al. 1984; Boileau et al. 1992; Hellberg 1995; Baer 1998). The development of more realistic theoretical descriptions of nonequilibrium population structure thus appears to be important for a better understanding of the relative influence of environmental and ecological factors in shaping genetic variation in young habitats, such as recently deglaciated areas (Slatkin 1993; Templeton 1998; Beerli and Felsenstein 1999; Hutchison and Templeton 1999).

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## Appendix

Microsatellite polymorphism and departures from Hardy-Weinberg proportions. *A*, number of alleles observed in the sample; $H_E$ and $H_O$, expected and observed heterozygosities; *f*, Weir and Cockerham's (1984) $F_{IS}$ estimate; *P*(HW) is the combined *P*-value of exact test of HWE using Fisher's method. Significant values following appropriate sequential Bonferroni correction are in bold (k = 30 for multilocus values, k = 6 for single-locus values and single-locus global *f*).

| | | Populations | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A1 | C1 | C2 | J1 | J2 | J3 | J4 | J5 | J6 | J7 | J8 |
| SFO-12 | *A* | 3 | 3 | 4 | 6 | 4 | 3 | 4 | 2 | 1 | 5 | 6 |
| | $H_E$ | 0.15 | 0.50 | 0.57 | 0.57 | 0.64 | 0.50 | 0.67 | 0.05 | 0 | 0.64 | 0.71 |
| | $H_O$ | 0.16 | 0.36 | 0.39 | 0.47 | 0.60 | 0.53 | 0.50 | 0.05 | 0 | 0.74 | 0.57 |
| | *f* | −0.049 | 0.282 | **0.314** | 0.185 | 0.064 | −0.058 | 0.261 | 0 | — | −0.146 | 0.197 |
| SFO-18 | *A* | 5 | 5 | 3 | 9 | 6 | 2 | 5 | 3 | 4 | 7 | 7 |
| | $H_E$ | 0.48 | 0.67 | 0.65 | 0.80 | 0.75 | 0.50 | 0.64 | 0.27 | 0.31 | 0.71 | 0.71 |
| | $H_O$ | 0.32 | 0.62 | 0.57 | 0.84 | 0.77 | 0.30 | 0.75 | 0.20 | 0.32 | 0.53 | 0.57 |
| | *f* | 0.34 | 0.078 | 0.124 | −0.052 | −0.025 | 0.408 | −0.176 | 0.273 | −0.053 | 0.255 | 0.201 |
| SFO-23 | *A* | 15 | 4 | 9 | 15 | 13 | 9 | 11 | 11 | 4 | 21 | 9 |
| | $H_E$ | 0.87 | 0.59 | 0.79 | 0.90 | 0.89 | 0.79 | 0.86 | 0.87 | 0.69 | 0.95 | 0.87 |
| | $H_O$ | 0.80 | 0.59 | 0.83 | 0.83 | 0.78 | 0.83 | 0.75 | 0.95 | 0.63 | 0.91 | 0.78 |
| | *f* | 0.083 | −0.007 | −0.044 | 0.075 | 0.124 | −0.057 | 0.137 | −0.105 | 0.082 | 0.043 | 0.111 |
| SFO-8 | *A* | 14 | 7 | 8 | 13 | 15 | 8 | 10 | 12 | 4 | 21 | 12 |
| | $H_E$ | 0.87 | 0.79 | 0.68 | 0.88 | 0.93 | 0.73 | 0.89 | 0.84 | 0.57 | 0.95 | 0.78 |
| | $H_O$ | 0.88 | 0.77 | 0.38 | 0.70 | 0.87 | 0.57 | 0.80 | 0.74 | 0.27 | 0.81 | 0.58 |
| | *f* | −0.015 | 0.023 | **0.438** | 0.206 | 0.071 | 0.224 | 0.106 | **0.118** | **0.54** | **0.153** | **0.254** |
| SSA-197 | *A* | 2 | 2 | 3 | 3 | 3 | 3 | 4 | 5 | 4 | 5 | 6 |
| | $H_E$ | 0.04 | 0.50 | 0.54 | 0.23 | 0.42 | 0.63 | 0.60 | 0.55 | 0.59 | 0.66 | 0.71 |
| | $H_O$ | 0.04 | 0.59 | 0.43 | 0.19 | 0.38 | 0.69 | 0.61 | 0.52 | 0.64 | 0.58 | 0.58 |
| | *f* | 0 | −0.182 | 0.198 | 0.181 | 0.099 | −0.099 | −0.019 | 0.041 | −0.098 | 0.127 | 0.193 |
| MST-85 | *A* | 12 | 5 | 6 | 9 | 11 | 5 | 6 | 5 | 6 | 11 | 7 |
| | $H_E$ | 0.88 | 0.42 | 0.56 | 0.77 | 0.87 | 0.76 | 0.77 | 0.61 | 0.70 | 0.82 | 0.76 |
| | $H_O$ | 0.88 | 0.20 | 0.45 | 0.88 | 0.83 | 0.73 | 0.67 | 0.39 | 0.71 | 0.74 | 0.71 |
| | *f* | −0.004 | **0.532** | **0.202** | −0.136 | 0.046 | 0.041 | 0.132 | **0.365** | −0.013 | 0.104 | 0.058 |
| Global | *A* | 8.5 | 4.33 | 5.5 | 9.16 | 8.66 | 5 | 6.66 | 6.33 | 3.83 | 11.67 | 7.83 |
| | $H_E$ | 0.55 | 0.58 | 0.63 | 0.69 | 0.75 | 0.65 | 0.74 | 0.53 | 0.48 | 0.79 | 0.76 |
| | $H_O$ | 0.51 | 0.52 | 0.51 | 0.65 | 0.70 | 0.61 | 0.68 | 0.48 | 0.43 | 0.72 | 0.63 |
| | *f* | 0.0648 | 0.0993 | **0.1962** | **0.0605** | 0.0629 | 0.0678 | 0.0826 | 0.1036 | **0.1002** | 0.0937 | **0.1671** |
| | *P*(HW) | 0.1606 | 0.0046 | <0.0001 | 0.0004 | 0.0705 | 0.1812 | 0.1812 | 0.0004 | 0.0005 | 0.0049 | 0.0054 |

| | | Populations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | J9 | J10 | J11 | K1 | K2 | K3 | K4 | N1 | N2 | P1 |
| SFO-12 | *A* | 3 | 3 | 1 | 4 | 3 | 2 | 3 | 3 | 2 | 3 |
| | $H_E$ | 0.33 | 0.57 | 0 | 0.69 | 0.51 | 0.34 | 0.56 | 0.58 | 0.50 | 0.16 |
| | $H_O$ | 0.35 | 0.60 | 0 | 0.73 | 0.50 | 0.21 | 0.22 | 0.60 | 0.50 | 0.14 |
| | *f* | −0.034 | −0.056 | — | −0.062 | 0.012 | 0.379 | **0.619** | −0.041 | 0 | 0.155 |
| SFO-18 | *A* | 7 | 6 | 3 | 3 | 3 | 6 | 3 | 2 | 4 | 3 |
| | $H_E$ | 0.77 | 0.69 | 0.47 | 0.46 | 0.55 | 0.72 | 0.09 | 0.13 | 0.68 | 0.38 |
| | $H_O$ | 0.73 | 0.79 | 0.24 | 0.55 | 0.56 | 0.73 | 0.09 | 0.13 | 0.25 | 0.37 |
| | *f* | 0.052 | −0.156 | 0.506 | −0.18 | −0.015 | −0.018 | −0.011 | −0.037 | 0.646 | 0.028 |
| SFO-23 | *A* | 11 | 14 | 5 | 15 | 12 | 11 | 5 | 8 | 7 | 8 |
| | $H_E$ | 0.87 | 0.86 | 0.70 | 0.89 | 0.74 | 0.82 | 0.74 | 0.79 | 0.87 | 0.75 |
| | $H_O$ | 0.88 | 0.89 | 0.71 | 0.86 | 0.81 | 0.73 | 0.70 | 0.71 | 1.00 | 0.69 |
| | *f* | −0.13 | −0.041 | −0.003 | 0.037 | −0.096 | 0.103 | 0.062 | 0.103 | −0.167 | 0.084 |
| SFO-8 | *A* | 13 | 13 | 4 | 14 | 14 | 15 | 5 | 8 | 7 | 9 |
| | $H_E$ | 0.87 | 0.92 | 0.51 | 0.85 | 0.93 | 0.92 | 0.70 | 0.75 | 0.85 | 0.80 |
| | $H_O$ | 0.77 | 0.88 | 0.59 | 0.86 | 0.87 | 0.88 | 0.84 | 0.60 | 0.75 | 0.63 |
| | *f* | 0.117 | 0.048 | −0.151 | −0.006 | 0.071 | 0.034 | −0.208 | 0.208 | 0.125 | 0.218 |

APPENDIX
Continued.

| | | Populations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | J9 | J10 | J11 | K1 | K2 | K3 | K4 | N1 | N2 | P1 |
| SSA-197 | $A$ | 5 | 4 | 2 | 4 | 2 | 3 | 4 | 2 | 3 | 3 |
| | $H_E$ | 0.57 | 0.34 | 0.30 | 0.66 | 0.44 | 0.24 | 0.60 | 0.50 | 0.64 | 0.18 |
| | $H_O$ | 0.54 | 0.29 | 0.24 | 0.59 | 0.38 | 0.27 | 0.64 | 0.80 | 0.63 | 0.13 |
| | $f$ | 0.049 | 0.152 | 0.22 | 0.1 | 0.159 | −0.105 | −0.063 | −0.647 | 0.028 | 0.294 |
| MST-85 | $A$ | 10 | 6 | 2 | 6 | 6 | 6 | 4 | 3 | 7 | 9 |
| | $H_E$ | 0.78 | 0.81 | 0.49 | 0.69 | 0.80 | 0.61 | 0.67 | 0.25 | 0.85 | 0.83 |
| | $H_O$ | 0.84 | 0.65 | 0.41 | 0.73 | 0.69 | 0.53 | 0.64 | 0.27 | 1.00 | 0.82 |
| | $f$ | −0.083 | 0.197 | 0.158 | −0.062 | 0.145 | 0.122 | 0.05 | −0.077 | −0.191 | 0.005 |
| Global | $A$ | 8.17 | 7.67 | 2.83 | 7.67 | 6.67 | 7.17 | 4 | 4.33 | 5 | 5.83 |
| | $H_E$ | 0.70 | 0.70 | 0.41 | 0.71 | 0.66 | 0.61 | 0.56 | 0.50 | 0.73 | 0.52 |
| | $H_O$ | 0.68 | 0.68 | 0.36 | 0.72 | 0.63 | 0.56 | 0.52 | 0.52 | 0.69 | 0.46 |
| | $f$ | 0.0196 | 0.0193 | 0.123 | −0.0173 | 0.0455 | 00776 | 0.0741 | −0.0409 | 0.0629 | 0.1069 |
| | $P$(HW) | 0.1076 | 0.2440 | 0.2180 | 0.9949 | 0.9656 | 0.3971 | 0.0123 | 0.0304 | 0.4617 | 0.0223 |

| | | Populations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | Global |
| SFO-12 | $A$ | 5 | 2 | 2 | 4 | 2 | 2 | 2 | 3 | 3 | 14 |
| | $H_E$ | 0.50 | 0.04 | 0.51 | 0.20 | 0.50 | 0.19 | 0.22 | 0.24 | 0.42 | 0.29 |
| | $H_O$ | 0.41 | 0.04 | 0.10 | 0.21 | 0.35 | 0.21 | 0.10 | 0.17 | 0.37 | 0.26 |
| | $f$ | 0.171 | 0 | **0.817** | −0.06 | 0.307 | −0.102 | 0.525 | 0.279 | 0.128 | **0.1508** |
| SFO-18 | $A$ | 7 | 5 | 6 | 6 | 3 | 4 | 6 | 6 | 4 | 14 |
| | $H_E$ | 0.76 | 0.59 | 0.71 | 0.46 | 0.19 | 0.43 | 0.76 | 0.82 | 0.70 | 0.59 |
| | $H_O$ | 0.48 | 0.36 | 0.59 | 0.38 | 0.20 | 0.45 | 0.70 | 0.78 | 0.53 | 0.42 |
| | $f$ | **0.371** | **0.398** | 0.171 | 0.158 | −0.063 | −0.049 | 0.072 | 0.048 | 0.254 | **0.1176** |
| SFO-23 | $A$ | 10 | 13 | 17 | 13 | 7 | 11 | 11 | 6 | 7 | 45 |
| | $H_E$ | 0.78 | 0.90 | 0.93 | 0.89 | 0.81 | 0.82 | 0.89 | 0.59 | 0.84 | 0.85 |
| | $H_O$ | 0.66 | 0.93 | 0.91 | 0.90 | 0.75 | 0.66 | 0.79 | 0.64 | 0.95 | 0.87 |
| | $f$ | 0.161 | −0.038 | 0.019 | −0.011 | 0.07 | 0.207 | **0.106** | −0.085 | −0.135 | 0.0339 |
| SFO-8 | $A$ | 9 | 9 | 14 | 15 | 7 | 10 | 19 | 12 | 7 | 57 |
| | $H_E$ | 0.77 | 0.76 | 0.87 | 0.90 | 0.71 | 0.64 | 0.94 | 0.86 | 0.70 | 0.78 |
| | $H_O$ | 0.78 | 0.68 | 0.79 | 0.90 | 0.65 | 0.38 | 1.00 | 0.95 | 0.84 | 0.86 |
| | $f$ | −0.009 | 0.104 | 0.095 | 0.001 | 0.092 | **0.423** | −0.069 | −0.114 | −0.205 | **0.0952** |
| SSA-197 | $A$ | 2 | 2 | 2 | 4 | 4 | 2 | 6 | 4 | 2 | 10 |
| | $H_E$ | 0.22 | 0.04 | 0.47 | 0.51 | 0.43 | 0.17 | 0.67 | 0.54 | 0.10 | 0.42 |
| | $H_O$ | 0.24 | 0.04 | 0 | 0.52 | 0.50 | 0.18 | 0.62 | 0.67 | 0.11 | 0.41 |
| | $f$ | −0.116 | 0 | **1** | −0.007 | −0.159 | −0.08 | 0.069 | −0.242 | −0.029 | 0.0473 |
| MST-85 | $A$ | 8 | 7 | 9 | 7 | 4 | 6 | 9 | 8 | 6 | 24 |
| | $H_E$ | 0.77 | 0.67 | 0.82 | 0.52 | 0.58 | 0.62 | 0.80 | 0.77 | 0.77 | 0.82 |
| | $H_O$ | 0.72 | 0.59 | 0.82 | 0.55 | 0.47 | 0.50 | 0.48 | 0.91 | 0.89 | 0.89 |
| | $f$ | 0.064 | 0.119 | 0.004 | −0.059 | 0.194 | 0.194 | **0.402** | −0.186 | −0.163 | **0.0684** |
| Global | $A$ | 6.83 | 6.33 | 8.33 | 8.17 | 4.5 | 5.83 | 8.83 | 6.5 | 4.83 | 6.61 |
| | $H_E$ | 0.63 | 0.50 | 0.72 | 0.58 | 0.54 | 0.48 | 0.71 | 0.64 | 0.59 | 0.61 |
| | $H_O$ | 0.55 | 0.44 | 0.53 | 0.58 | 0.49 | 0.40 | 0.62 | 0.69 | 0.61 | 0.65 |
| | $f$ | **0.1347** | 0.1207 | **0.262** | 0.0049 | 0.0963 | 0.1778 | **0.1334** | −0.0823 | −0.0425 | **0.0807** |
| | $P$(HW) | <0.0001 | 0.0069 | <0.0001 | 0.4094 | 0.3083 | 0.0059 | <0.0001 | 0.2830 | 0.1467 | <0.0001 |