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Corresponding Editor: G. Wagner

Evolution, 50(6), 1996, pp. 2548–2557

GENETIC POPULATION STRUCTURE AND GRADUAL NORTHWARD DECLINE OF GENETIC VARIABILITY IN THE GREENFINCH (*CARDUELIS CHLORIS*)

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Key words.—Bottleneck, genetic variation, heterozygosity, latitudinal variation.

Received September 27, 1995. Accepted April 22, 1996.

Distribution of genetic variation among and within different populations and their historic relationships are central for

understanding the mechanisms of microevolution, and ultimately, speciation. Although the relative importance of different modes of speciation is still disputed (Lynch 1989; Chesser and Zink 1994), vicariance events caused by glaciations probably had a major impact on the speciation process and many present-day patterns of phenotypic and genetic

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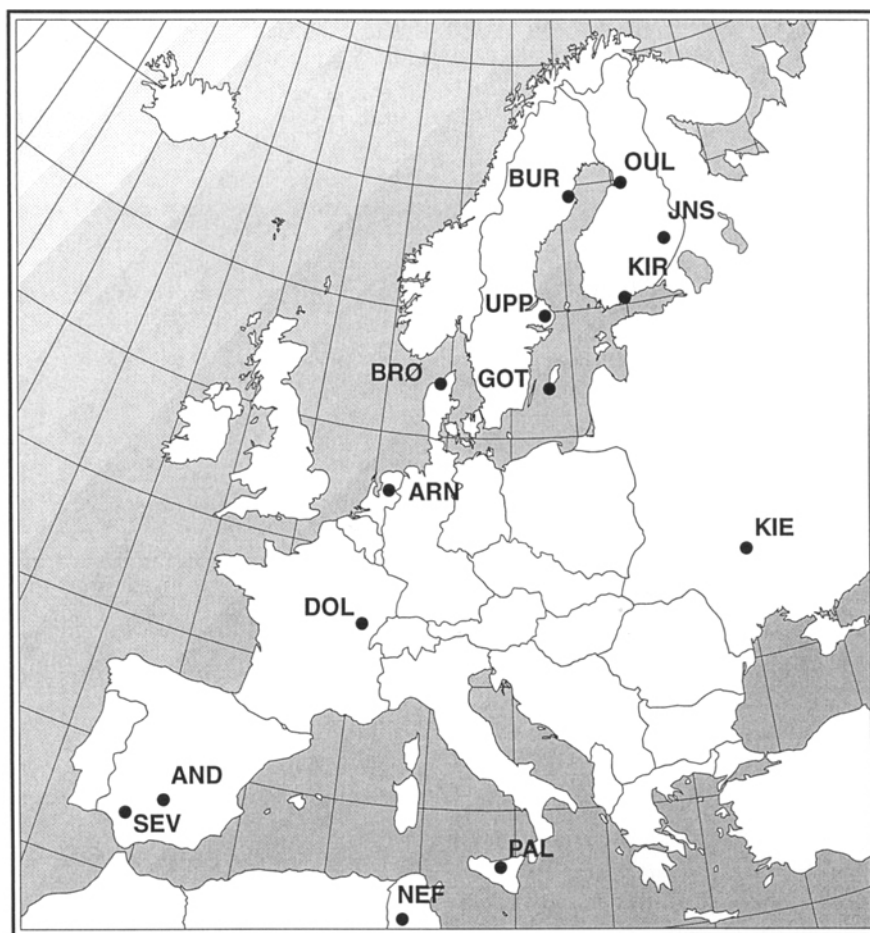


FIG. 1. Map of collecting localities. For locality abbreviations, see Appendix 1.

variation have their origins in Pleistocene events (e.g. Sage and Wolff 1986; Hewitt 1988; Avise 1992; Cooper et al. 1995; but see: Zink and Slowinski 1995). In Europe, Pleistocene glaciations are believed to have created several refuges from which northern Europe was subsequently reinvaded after the retreat of ice sheets (e.g., Harrison 1982; Cooper et al. 1995). Several species were split into different refuges resulting in extensive subspeciation and even in formation of new species (e.g., Harrison 1982; Hewitt 1988). However, as a contrast to many well-worked examples of population differentiation and speciation on the North American continent (e.g., Avise 1992), relatively little is known about large-scale patterns of genetic variability in the European fauna (but see Cooper et al. 1995).

We investigated genetic variability and population structure of the greenfinch (*Carduelis chloris*), a widespread Palearctic passerine showing clinal body size and plumage variation across Europe (Voous 1952). In particular, the three main goals were to: (1) identify possible distinct genetic lineages that would be indicative of a historic split between different populations; (2) compare levels of genetic variation within populations among different geographical regions; and (3) infer the possible processes that could be responsible for the observed patterns.

MATERIALS AND METHODS

The Species

The greenfinch (*C. chloris*, Fringillidae) is a seed-eating, medium-sized passerine having a wide Palearctic distribution. It is a partial migrant and is known to move regularly up to several hundred km (Cramp and Perrins 1994), but little is known about its natal or breeding dispersal. However, Greenwood and Harvey (1977) reported some cases of likely effective dispersal over distances of 150 km. Voous (1952) ascribed the greenfinch to seven different races using plumage and morphological criteria, indicating that gene flow between populations might be, or might have been, restricted enough to lead to local differentiation. Two of these subspecies (*C. c. chloris* and *C. c. aurantiiventris*) are included to the present study (Appendix 1).

Sampling

Fourteen samples of greenfinches comprising 472 specimens were collected during the 1992–1994 breeding seasons from localities shown in Figure 1. Sample sizes, collecting localities, and dates are listed in Appendix 1. Birds were captured with mist nets, anesthetized, and dissected im-

mediately in the field. Tissue samples, consisting of heart, liver, muscle, and kidney tissue (approx. 2.0 g) were preserved in liquid nitrogen, transferred to the laboratory and maintained in a freezer below -70°C until used in electrophoresis.

Electrophoresis

For each individual, a small piece (about 0.02 g) of each of four tissue types was ground together in approximately 1.2 mL ice-cold Tris buffer (1 M, pH 7.0) and used as such in electrophoresis. To resolve peptidases, tissues were ground in a di-thio-threitol (pH 6.0). Horizontal starch (11%) gel electrophoresis was performed at 4°C by using standard procedures as described in Murphy et al. (1990). The 40 scored loci and their Enzyme Commission number (International Union of Biochemistry 1984) were: ACON 1 and 2 (4.2.1.3); ACP (3.1.3.2); AK 1 and 2 (2.7.4.3); α -GPDH 1 and 2 (1.1.1.8); CK 1, 2 and 3 (2.7.3.2); EAP 1 and 2 (3.4.23.24); ES 1, 2 and 3 (3.1.1.1); FL-ES (3.1.1.1); G6PDH (1.1.1.49); GLUD (1.4.1.2); GOT 1 and 2 (2.6.1.1); GP 1, 2, and 3 (non-specific protein, "general protein"); GPI (5.3.1.9); ICD 1 and 2 (1.1.1.42); LDH 1 and 2 (1.1.1.27); MDH 1 and 2 (1.1.1.37); MPI (5.3.1.8); NP (2.4.2.1); PEP "A" and "B" (3.4.11); 6PGD (1.1.1.43); PGM 1 and 2 (2.7.5.1); SOD 1 and 2 (1.15.11); and SDH (1.1.1.14). Details of electrophoretic conditions are (with minor modifications) the same as in St. Louis and Barlow (1988) and Baker et al. (1990).

Electrophoretic variation was presumed to reflect allelic differences at the loci assayed. For the interpretation of zymogram patterns we used the quaternary structure of the enzymes known from other species (Evans 1987; Baker et al. 1990). Multiple isozymes of a particular protein were numbered sequentially with integers beginning with one for the most anodal form, and different alleles were given an alphabetical code so that the most common allele at a locus was designed with A. Alleles in different populations were calibrated to each other by running individuals from different populations on the same gels. Staining protocols follow those of Barrowclough and Corbin (1978), Cole and Parkin (1981), and Harris and Hopkinson (1976) with minor modifications (available from the authors on request).

Calculations

Allele frequencies and their variances were calculated from genotype frequencies observed in zymograms using the BIOSYS-1 package (Swofford and Selander 1989). Genotype frequencies were tested for Hardy-Weinberg (H-W) equilibrium using exact tests based on the Markov chain method available in the GENEPOP package (Raymond and Rousset 1995). Significant departures from H-W equilibrium were detected in only five cases, namely in PGI (OUL, UPP), EST-2 (ARN), and PEP-B (BUR, AND). All were slight heterozygote deficiencies and could be expected by chance alone (sequential Bonferroni adjustment for 54 tests, $P > 0.05$ in all tests; Rice 1989; Lessios 1992). All loci were also tested for linkage disequilibrium using exact tests (Raymond and Rousset 1995), but significant deviations were detected only in three of the 247 possible tests ($P > 0.05$; sequential Bonferroni correction). For each population, we computed average

observed (direct count) and average expected heterozygosity (unbiased estimate of Nei 1978) using BIOSYS-1. However, to reduce the variances of heterozygosity estimates, we also calculated observed heterozygosity as the ratio of the number of heterozygous loci in an individual to the total number of loci assayed in that individual, and averaged these values over individuals (Corbin 1981; Evans 1987). Differences among populations in average heterozygosity and mean number of alleles per locus were assessed with Kruskal-Wallis analysis of variance (Sokal and Rohlf 1981; Archie 1985). Data for comparison of genetic variability measures with other species were obtained from Evans (1987). If the same species or subspecies occurred several times, only the first reported estimate was included.

As population bottlenecks are predicted to produce an excess of alleles segregating in low frequencies (Chakraborty et al. 1980; see also Barrowclough et al. 1985), we compared observed and expected numbers of rare ($q \leq 0.05$) and common alleles ($p \geq 0.95$) in each of the study populations. The expected number of alleles in these frequency classes were calculated using the infinite-allele model with varying mutation rate (model IV, Chakraborty et al. 1980). The discrepancy (R^2) between expected (E) and observed (O) number of alleles in a given frequency class was calculated as: $R^2 = (O - E)^2 / E$, and its significance was tested with a randomization procedure using 9999 permutations.

F-statistics were used to investigate the extent of population structuring and genetic differentiation. F_{ST} , defined as the standardized genetic variance among (sub)populations in the total sample (Wright 1965), was estimated using two different algorithms. First, we calculated G_{ST} using the BIOSYS-1 (Swofford and Selander 1989) program to obtain Nei's (1986) estimate of F_{ST} . This was done to allow comparison with other studies as G_{ST} has been by far the most common method to estimate F_{ST} . However, as G_{ST} does not include correction terms for sample size and the number of populations examined, Weir and Cockerham (1984; see also Porter 1990; Cockerham and Weir 1993) have argued that their theta (θ) estimator is a more accurate descriptor of F_{ST} . Hence, we also calculated θ estimates using the FSTAT program (Goudet 1995). Pairwise G_{ST} and θ estimates were calculated using Slatkin's (1993) isolation-by-distance program. To assess differentiation in absolute scale, we also calculated Rogers' (1972) and Nei's standard genetic distance between pairs of populations. A matrix of Rogers' distances was subjected to cluster analysis using the neighbor-joining (N-J) algorithm of Saitou and Nei (1987). As the input order of OTUs is known to affect the outcome of this analysis (Felsenstein 1993), 15 matrices differing in the input order of the populations were used to evaluate the robustness of the N-J tree.

Two approaches were used to estimate gene flow. First, because N is the effective population size and m is the fraction of population replaced by immigrants in each generation, $Nm = (1/F_{ST} - 1)/4$ describes the average number of individuals per generation migrating between populations (Wright 1943; Slatkin and Barton 1989). F_{ST} in this equation was estimated using both G_{ST} and θ estimators as described above. The second approach was Slatkin's (1985) private alleles method which is based on the finding that Nm can be estimated from knowledge of the average frequency of private alleles, $p(i)$

TABLE 1. Mean ($\pm$ SE) genetic variability measures (A = number of alleles per locus, P% = percent polymorphic loci, H_O and H_E = average observed and expected heterozygosity), expected and observed numbers of rare alleles at 40 loci in 14 greenfinch populations. The expected number is calculated according to model IV of Chakraborty et al. (1980); n = average sample size per locus.

Population (n)	A	P%	H_O^a	H_E^{a*}	Exp	Obs	R^2	P
Oulu (14.0)	1.13 $\pm$ 0.06	10.0	1.3	2.0 $\pm$ 1.4	3.9	3	0.20	0.69
Joensuu (4.0)	1.08 $\pm$ 0.04	7.5	2.5	2.3 $\pm$ 1.4	3.3	0	3.31	< 0.01
Kirkkonummi (47.0)	1.20 $\pm$ 0.08	15.0	1.3	1.5 $\pm$ 0.7	3.6	7	3.11	0.54
Bureå (50.0)	1.17 $\pm$ 0.07	15.0	1.4	1.8 $\pm$ 0.9	4.4	5	0.07	0.85
Uppsala (49.7)	1.23 $\pm$ 0.08	20.0	2.1	2.3 $\pm$ 1.2	5.7	6	0.01	0.91
Gotland (49.9)	1.17 $\pm$ 0.07	15.0	1.5	1.5 $\pm$ 0.8	3.7	5	0.48	0.66
Brønderslev (51.0)	1.30 $\pm$ 0.10	12.5	2.7	2.7 $\pm$ 1.4	6.8	9	0.72	0.72
Arnhem (23.0)	1.25 $\pm$ 0.08	20.0	1.9	2.3 $\pm$ 0.9	5.0	7	0.82	0.74
Kiev (50.9)	1.30 $\pm$ 0.11	12.5	1.5	1.6 $\pm$ 0.8	3.9	11	12.64	0.03
Dole (30.9)	1.15 $\pm$ 0.06	15.0	1.9	2.1 $\pm$ 1.0	4.8	4	0.13	0.82
Andujar (55.0)	1.25 $\pm$ 0.09	15.0	2.2	2.4 $\pm$ 1.3	5.3	9	2.60	0.67
Seville (26.0)	1.17 $\pm$ 0.08	12.5	2.1	2.6 $\pm$ 1.3	5.8	5	0.11	0.90
Palermo (15.0)	1.13 $\pm$ 0.05	12.5	4.0	3.6 $\pm$ 1.7	7.3	0	7.33	< 0.01
Nefza (4.0)	1.17 $\pm$ 0.07	15.0	5.6	5.9 $\pm$ 2.6	9.1	0	9.11	> 0.01
Mean (33.6)	1.19 $\pm$ 0.02	14.1	2.3	2.5 $\pm$ 0.1				

^a Multiplied by 100; * Nei's (1978) unbiased estimate.

as: $Nm = \exp\{\ln[p(i)] + 2.44\} - 0.505$. Because sample sizes differed among populations, Nm values from the private allele method were corrected by dividing Nm with the ratio of the average sample size to 25 (Slatkin 1985). These calculations were carried out with option 4 available in the GENEPOP package. Both these methods assume that migration, random genetic drift, and mutation have reached equilibrium and that populations have been demographically stable (e.g., Chakraborty and Leimar 1987). They also assume an n -island model of migration, an assumption which is relatively insensitive to the geometry of populations (Crow and Aoki 1984; Slatkin 1985; Slatkin and Barton 1989).

To examine the relationship between geographic distance, gene flow, and different measures of genetic differentiation, the matrix of geographic distances between sites were calculated using the geographic coordinates for each site (Appendix 1). This was done by measuring the shortest distance between two points as given by the great circle track connecting two points (e.g., Korsström 1927). All distances were rounded to the nearest 10 km. Statistical testing of matrix correlations (r_M) was performed using Mantel's statistics (e.g., Cheverud et al. 1989). One thousand permutations were used in each test and P -values give proportion of permutations with P -value higher than observed.

RESULTS

Variation within Populations

Of the 40 loci we assayed for variation, 18 (45%) were monomorphic (ACON-2, GOT-2, PGM-1, MDH-2, GLUD, SOD-2, ICD-1, CK-2, CK-3, AK-1, AK-2, FI-EST, EAP-1, EAP-2, α -GPDH-2, GP-1, GP-2, GP-3) and fixed for the same allele in all 14 populations. The remaining 22 (55%) loci were polymorphic in at least one of the populations (Appendix 2). Both average percentage of polymorphic loci ($P = 14.1\%$ SE = 0.89%; $\leq 99\%$ criteria) and the average number of alleles per locus ($A = 1.19$ SE = 0.02) were very low, and did not differ significantly among sampled populations ($P: \chi^2_{13} < 6.92, P > 0.90$; A: Kruskal-Wallis, $H_{13,40} = 6.83, P = 0.91$, Table 1). Likewise, the levels of average hetero-

zygosity were low ($H_E = 0.025 \pm 0.013, n = 14$; Table 1), and all measures of intrapopulation variation were well below levels observed in the closely related chaffinch (*Fringilla coelebs*; $P = 24.5 \pm 1.5, H_E = 0.048 \pm 0.002, n = 12$ populations; Baker 1992) and other passerines ($P = 24.2 \pm 1.3, H_E = 0.063 \pm 0.005, n = 61$ species/subspecies; Evans 1987).

Although there were no significant differences in mean heterozygosity among populations when tested using different loci as sampling units (Kruskal-Wallis, $H_E: H_{13,560} = 5.94, P = 0.95$; $H_O: H_{13,560} = 5.60, P = 0.93$), heterozygosities differed significantly when observed individual heterozygosities were used (Kruskal-Wallis, $H_{13,472} = 39.49, P = 0.0002$). This was mostly due to fact that average heterozygosity decreased with increasing latitude across the populations ($r_s = -0.58, z = 2.09, P = 0.036$). No correlation was detected between latitude and proportion of polymorphic loci ($r_s = -0.13, z = 0.46, n = 14, P = 0.64$) or between latitude and mean number of alleles per locus ($r_s = -0.13, z = 0.61, n = 14, P = 0.61$).

Number of Rare and Common Alleles

As we found indications that greenfinch populations might have been bottlenecked in the recent past (see above), we further examined the prediction (Chakraborty et al. 1980; Barrowclough et al. 1985) that an excess of rare alleles would be found from most severely affected populations. A comparison of expected and observed numbers of rare alleles in different populations revealed three significant deficiencies of rare alleles, and one significant excess (Table 1). However, while 70% (7/10) of the northern and central European populations had an excess of rare alleles, this was true only in 25% (1/4) of the southern populations (Fig. 2). Although this difference was not significant, it is worth noting that all 14 private alleles (found in one population only) occurred in northern populations ($\chi^2_1 > 10, P < 0.0001$). Furthermore, the average heterozygosity was strongly negatively correlated with excess/deficiency of rare alleles (Fig. 3), as could be expected if low heterozygosities reflect recent bottlenecking.

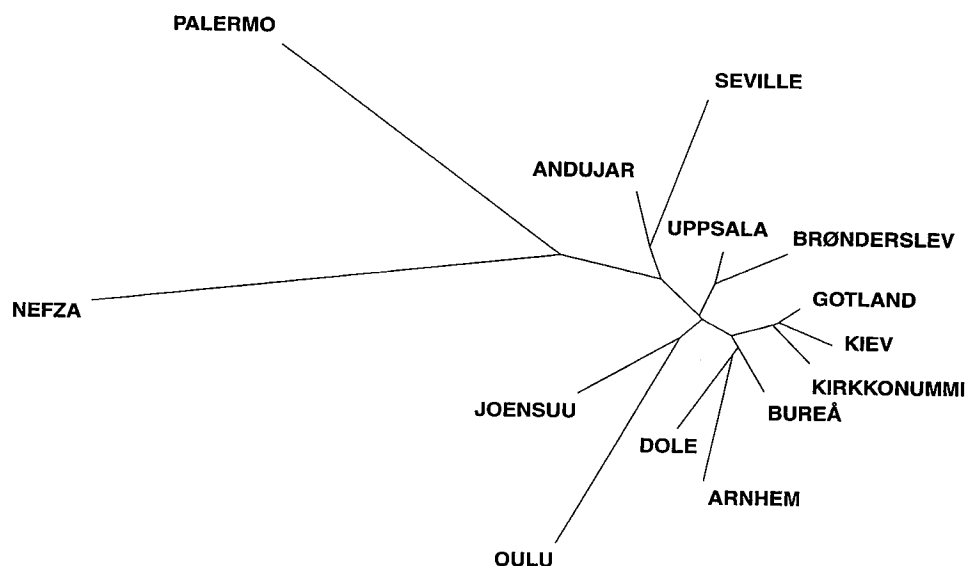


FIG. 2. An unrooted neighbor-joining tree depicting genetic similarity of different greenfinch populations based on Rogers' (1972) genetic distances.

Observed and expected numbers of common alleles were in good agreement in all populations ($R^2 < 0.12$, $P = 1.0$ in all comparisons).

Differentiation among Populations

There were no fixed allele differences between populations, and exact tests for population differentiation revealed significant allele differences only in six of the 22 polymorphic loci (6GPDH, PEP-A, PEP-B, PGI, EST-2, G6PDH; $P < 0.05$). F_{ST} estimated by θ revealed that only 4.1% of genetic

variation detectable with allozymes can be attributed to differences between populations, whereas 95.9% of it was within populations ($\theta = 0.041$). However, although θ and G_{ST} based estimates were highly correlated ($r_s = 0.96$, $z = 4.34$, $n = 22$ loci, $P < 0.001$), F_{ST} estimates based on G_{ST} were consistently higher ($G_{ST} = 0.092$) than θ based estimates (Wilcoxon signed rank, $z = 4.13$, $P < 0.001$). The low degree of genetic differentiation between populations was also evident from small values of Rogers' (D_R [mean $\pm$ S.E.] = 0.013 ± 0.0008) and Nei's genetic (D_N [mean $\pm$ S.E.] = 0.005 ± 0.0005) distances.

Although greenfinch populations are only weakly structured, N-J tree of Rogers' genetic distances revealed a geographic component to population differentiation (Fig. 2). The two southernmost populations (Palermo, Nefza) are clearly divergent from all other populations and form a monophyletic cluster together with the two Iberian populations (Andujar, Seville). Likewise, the two northernmost Finnish populations, Oulu and Joensuu, seem to have diverged from the rest of European populations, whereas remaining populations are only weakly differentiated (Fig. 2). Geographic structuring is further supported by a significant positive matrix correlation between Rogers' genetic distances and geographic distance separating pairs of populations ($r_M = 0.44$, $n = 91$, $P < 0.001$).

Estimates of Gene Flow

Although different methods used to estimate gene flow gave somewhat different results (private alleles: $Nm = 12.6$; θ : $Nm = 5.84$; G_{ST} : $Nm = 2.47$), all these global estimates indicate that exchange of alleles between different greenfinch populations is extensive. Despite the apparently high gene flow, $\log Nm$ was strongly negatively correlated with $\log$ distance among localities (G_{ST} : $b = -0.70$, $P < 0.001$; θ : $b = -0.88$, $P = 0.002$), indicating that the gene flow might be more restricted than the results above indicate. In fact,

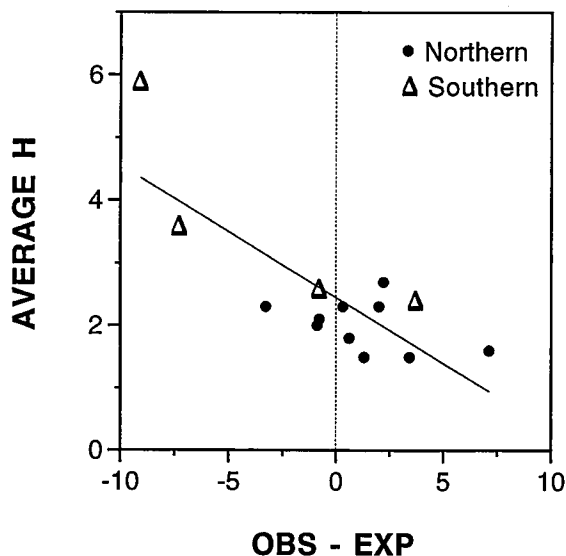


FIG. 3. Average heterozygosity as a function of difference in expected and observed numbers of rare alleles in 14 populations of the greenfinch, *Carduelis chloris*. Expected number of rare ($q \leq 0.05$) alleles was calculated with the infinite alleles model with varying mutation rate (Chakraborty et al. 1980). The regression line is shown for purely illustrative purposes ($r_s = 0.47$, $z = 1.69$, $n = 14$, $P < 0.05$, one-tailed).

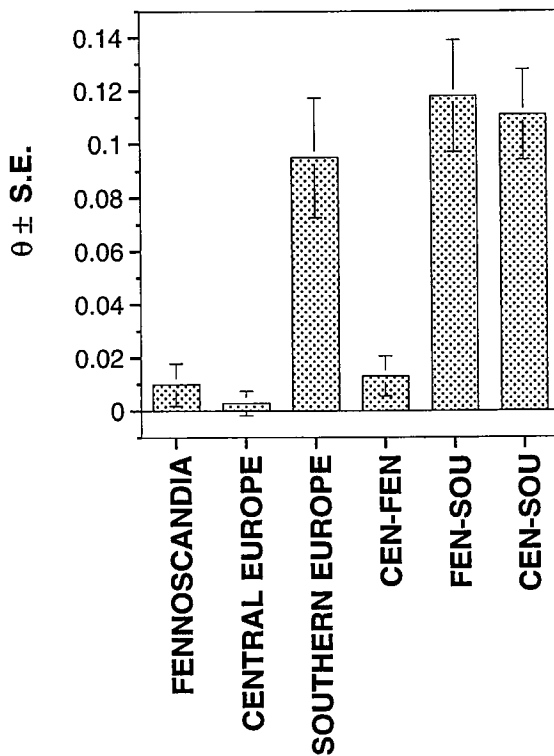


FIG. 4. Comparison of F_{ST} ($\pm$ SE) values as estimated by θ within and between three different geographic regions. Fenno-Scandia = OUL, JNS, KIR, BUR, UPP, GOT, BRØ; central Europe = ARN, DOL, KIE; southern Europe = AND, SEV, PAL, NEF.

when the F_{ST} estimates are compared between different regions, gene flow rates are high and homogeneous within and between Northern and Central European populations, but are much lower between southern and northern European localities (G_{ST} , Kruskal Wallis: $H_{5,91} = 39.56$, $P < 0.001$; θ , $H_{5,91} = 42.76$, $P < 0.001$; Fig. 4). Levels of gene flow between southern European populations seem to be of the same magnitude as that between northern Europe and southern Europe (Fig. 4). Furthermore, seven of the nine pairwise estimates in which Nm was less than one involved north-south comparisons (two comparisons between central and south had $Nm < 1$), whereas all within-region comparisons had $Nm > 1$. The more restricted gene flow among southern European populations as compared to northern European populations is not due to longer geographic distances between southern as compared northern European populations: if we control for effects of geographic distance using residuals from the regression $\log(Nm)$ on $\log(\text{geographic distance})$, gene flow is more restricted among southern than among central or northern European populations (ANOVA: $F_{2,29} = 5.37$, $P = 0.01$).

DISCUSSION

Variation within Populations

Low genetic variability in island (e.g., Soulé and Yang 1973; Gorman et al. 1975; Schmitt 1978; Gyllensten et al. 1985) and introduced populations (e.g., Baker and Moeed 1987; St. Louis and Barlow 1988) as compared to mainland

and source populations is well documented and can be predicted from population genetic theory (Nei et al. 1975; Chakraborty and Nei 1977; see also Verspoor 1988; McCommas and Bryant 1990; Leberg 1992). Average levels of genetic variation within greenfinch populations are extremely low as compared to most other passerine species (Barrowclough 1983; Corbin 1983). This low level of genetic variation cannot be ascribed to any methodological bias (cf. Simon and Archie 1985) as the scored loci and methods used to detect allelic variation were basically the same as in a study of the closely related chaffinch (*F. coelebs*), which possesses about double the heterozygosity of the greenfinch (Baker et al. 1990; Baker 1992). Hence, we suggest that greenfinches have lost genetic variation due to founder events, population bottlenecks, or both. This interpretation is supported by the finding that populations sampled in southern Europe are more variable than northern European populations—a finding consistent with the general tendency for populations in areas glaciated during the Pleistocene to exhibit reduced genetic variability (e.g., Highton and Webster 1976; Bellemin et al. 1978; Väisänen and Lehtälä 1984; Singh and Rhomberg 1987). Latitudinal differences in variability could be expected as the colonizing populations are prone to founder events and serial bottlenecks stemming from the greater demographic instability of these marginal populations. For example, Cwynar and MacDonald (1987) explained the progressive decline in levels of allelic diversity toward north in lodgepole pine as a consequence of small size of the founding populations, which in turn have given rise to new founding populations. Likewise, Sage and Wolff (1986) suggested that the low genetic variability of Dall sheep has arisen by a similar stepping-stone pattern of range establishment that has led to serial loss of alleles. Such a stepping-stone process could also have been involved with colonization of northern Europe by greenfinch.

Although we found no general tendency for an excess of rare alleles in different greenfinch populations, as could be expected in populations that have gone through a bottleneck recently, a negative correlation between average heterozygosity and the difference in expected and observed numbers of rare alleles in different populations further support the bottlenecks hypothesis. These tests also indicate that variation at the assayed loci fits expectations of neutral models (Chakraborty et al. 1980; Barrowclough 1983). For example, selective models such as overdominance are expected to produce excess numbers of alleles at intermediate frequencies, something that clearly was not found in this study.

Gene Flow and Historical Relationships among Populations

Most temperate zone passerines are characterized by relatively large, panmictic populations with F_{ST} values usually less than 0.06 (Barrowclough 1983). The greenfinch is no exception to this pattern, and population structuring in northern Europe was as weak as in other European passerines studied so far (Evans 1987; Parkin 1987; Gelter et al. 1989). The observed disjunction between southern and northern European greenfinch populations, albeit slight, indicates reduced exchange of migrants between these regions. This find-

ing is consistent with current taxonomy in which Iberian, north African, and Sicilian populations of the greenfinch belong to the subspecies *aurantiventris*, whereas northern European birds belong to the nominate race *chloris* (Voous 1952; Cramp and Perrins 1994). Similar discontinuity between southern and northern European populations has been observed in other species as well: Iberian populations of chaffinch (*F. coelebs*) have differentiated genetically from their northern European congeners to a similar degree as in greenfinches (Baker 1992), and morphometric and behavioral studies of the chiffchaff (*Phylloscopus collybita*, Salomon 1987), and other species as well (e.g., Harrison 1982; Cooper et al. 1995), have revealed the same disjunction, suggesting that Pyrenees form, or have formed, a dispersal barrier between populations of various organisms. However, because northern European greenfinch populations inhabit areas that were glaciated during the Pleistocene, and populations near the northern limit of the species range (Oulu, Bureå) are known to have been established during this century (Cramp and Perrins 1994), it is not possible to distinguish between current gene flow and shared ancestral polymorphism as the cause of low interpopulational divergence in northern Europe (Chakraborty and Leimar 1987). Therefore, little can be said about current levels of gene flow among northern European populations, but the N_m values among several southern European populations clearly indicate restricted dispersal. Furthermore, the allozyme data indicate that gene flow between southern and northern European populations is so low (often $N_m < 1$) that the effects of any selection would not be swamped by gene flow. Hence, adaptations for local selection pressures are possible, and the observed differences in body size and plumage characteristics might be examples of this (Voous 1952; Merilä, in press). However, because such differences have been reported among different northern European populations as well (Voous 1952; Merilä, in press), the possibility of environmentally induced differences without any genetical basis remains to be investigated.

ACKNOWLEDGMENTS

We thank S. Ulfstrand and two anonymous referees for helpful comments on earlier drafts of this manuscript. For their help and hospitality during our visits to different localities we thank: L. Andersson, H. van Balen, B. Faivre and family, E. Feldsted-Jensen, B. Frochot, D. Hermant, J. Markkola, L. Mäenpää, A. Møller, T. Tallgren, J. Palomino-Lopez and family, P. Palviainen, T. Redondo, J. Soler, M. Soler, J. Sorjonen, and A. Tokar. Special thanks are due to M. Peck for his help and expertise in the laboratory, J. Bergsten for fine cooking during the field trips, and A. Lindholm for always finding the way to the nearest McDonalds. Authors acknowledge the financial support from the Swedish Natural Science Research Council (MB), the Royal Swedish Academy of Sciences (JM), Stiftelsen Zoologisk Forskning (JM), Uppsala University (JM), and the Natural Sciences and Engineering Research Council of Canada (to AJB).

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APPENDIX 1

Collecting localities, their acronyms, coordinates, collecting dates, sample sizes (n), and subspecies designations (according to Voous 1952).

Oulu, OUL, 65°01'N/25°30'E, 92 May, $n = 14$, <i>chloris</i>
Joensuu, JNS, 62°36'N/29°48'E, 92 May, $n = 4$, <i>chloris</i>
Kirkkonummi, KIR, 60°05'N/24°25'E, 92 May, $n = 47$, <i>chloris</i>
Bureå, BUR, 64°37'N/21°10'E, 92 July & August, $n = 50$, <i>chloris</i>
Uppsala, UPP, 59°50'N/17°50'E, 92 April & 93 May, $n = 50$, <i>chloris</i>
Gotland, GOT, 57°04'N/18°13'E, 92 July, $n = 51$, <i>chloris</i>
Brønderslev, BRØ, 57°15'N/10°00'E, 93 April, $n = 51$, <i>chloris</i>
Arnhem, ARN, 51°55'N/05°05'E, 92 April, $n = 23$, <i>chloris</i>
Dole, DOL, 47°05'N/05°03'E, 93 April, $n = 31$, <i>chloris</i>
Kiev, KIE, 50°26'N/30°29'E, 92 October, $n = 51$, <i>chloris</i>
Andujar, AND, 38°03'N/04°03'W, 93 March, $n = 55$, <i>aurantiiventris</i>
Seville, SEV, 37°23'N/06°00'W, 93 March, $n = 26$, <i>aurantiiventris</i>
Palermo, PAL, 38°07'N/13°07'E, 94 June, $n = 15$, <i>aurantiiventris</i>
Nefza, NEF, 32°56'N/06°56'E, 94 June, $n = 4$, <i>aurantiiventris</i>

APPENDIX 2

Allele frequencies in 22 polymorphic loci in 14 greenfinch populations. Locus-specific sample sizes given in *italic* after the name of the locus.

Locus	Locality													
	OUL	JNS	KIR	BUR	UPP	GOT	BRØ	ARN	KIE	DOL	AND	SEV	PAL	NEZ
6GPDH	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>50</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	.964	1.000	.947	.910	.890	.950	.873	.891	.930	.839	.964	.981	.900	.750
B	.036		.021	.070	.110	.00	.118	.065	.050	.161	.036	.019	.100	.250
C			.032	.020			.010		.010					
D						.00		.043	.010					
PEPA	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	.980	.990	1.000	.990	.978	1.000	1.000	.955	.981	.833	.625
B				.020	.010			.02			.045	.019	.167	.375
C							.010							
PEPB	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	.571	.750	.862	.840	.750	.830	.755	.891	.843	.855	.782	.750	.800	.750
B	.393	.250	.128	.160	.210	.304	.216	.109	.127	.145	.200	.154	.20	.250
C	.036		.011		.040		.029		.029		.018	.096		
PGI	<i>14</i>	<i>4</i>	<i>46</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	.929	1.000	1.000	1.000	.980	1.000	.971	.978	1.000	1.000	.982	1.000	1.000	.750
B							.029	.02			.009			.125
C	.071				.020						.009			.125
MPI	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	.875	.989	1.000	.970	.980	.980	.935	.990	.984	.982	.981	1.000	1.000
B		.125	.011		.030	.020	.00	.043	.010	.016	.018	.000		
C								.022			.019			
ACP	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>50</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	.990	1.000	1.000	1.000	.980	1.000	1.000	1.000	1.000	1.000
B					.010				.020					
GOT1	<i>14</i>	<i>4</i>	<i>47</i>	<i>49</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>50</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	.968	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B			.032											
EST1	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>49</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.980	1.000	1.000	1.000	1.000	1.000
B									.020					
EST2	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	.964	.875	.926	.890	.910	.940	.824	.891	.951	.887	.755	.615	.400	.875
B	.036	.125	.074	.110	.090	.060	.137	.109	.039	.113	.236	.346	.600	.125
C											.009	.038		
D							.19							
PGM2	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	.989	1.000	1.000	.990	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000
B						.010			.010					
C			.011											
NP	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>38</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.978	1.000	1.000	1.000	1.000	1.000	1.000
B								.022						
ACON1	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>49</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B						.010								
MDH1	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.984	1.000	1.000	1.000	1.000
B										.016				
LDH1	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B				.010										
LDH2	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.984	1.000	1.000	1.000	1.000
B										.016				
SDH	<i>14</i>	<i>4</i>	<i>47</i>	<i>50</i>	<i>50</i>	<i>50</i>	<i>51</i>	<i>23</i>	<i>51</i>	<i>31</i>	<i>55</i>	<i>26</i>	<i>15</i>	<i>4</i>
A	1.000	1.000	1.000	1.000	.990	1.000	.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B					.010		.010							

APPENDIX 2. Continued.

Locus	Locality													
	OUL	JNS	KIR	BUR	UPP	GOT	BRØ	ARN	KIE	DOL	AND	SEV	PAL	NEZ
SOD1	14	4	47	50	50	50	51	23	51	31	55	26	15	4
A	1.000	1.000	1.000	1.000	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B							.010							
ICD2	14	4	47	50	50	50	51	23	51	31	55	26	15	4
A	1.000	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B				.010										
CK1	14	4	47	50	50	50	51	23	51	31	55	26	15	4
A	1.000	1.000	1.000	1.000	1.000	1.000	.990	1.000	1.000	1.000	.991	1.000	1.000	1.000
B							.010				.009			
EST3	14	4	47	50	50	50	51	23	51	31	55	26	15	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.978	1.000	1.000	1.000	1.000	1.000	1.000
B								.022						
α-GPH1	14	4	47	50	50	50	51	23	51	31	55	26	15	4
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.990	1.000	1.000	1.000	1.000	1.000
B									.010					
G6PDH	14	4	47	50	50	50	51	23	51	31	55	26	15	4
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	.933	.875
B													.067	.125