

HISTORICAL DEMOGRAPHY AND PRESENT DAY POPULATION STRUCTURE OF THE GREENFINCH, *CARDUELIS CHLORIS*—AN ANALYSIS OF mtDNA CONTROL-REGION SEQUENCES

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Abstract.—Genetic variability within and among 10 geographically distinct populations of Greenfinches (*Carduelis chloris*) was assayed by directly sequencing a 637 BP part of the mtDNA control region from 194 individuals. Thirteen variable positions defined 18 haplotypes with a maximum sequence divergence of 0.8%. Haplotype ($h = 0.28\text{--}0.77$) and nucleotide ($\pi = 0.058\text{--}0.17\%$) diversities within populations were low, and decreased with increasing latitude ($h:r_s = -0.81$; $\pi:r_s = -0.89$). The distribution of pairwise nucleotide differences fit better with expectations of a “sudden expansion” than of an “equilibrium” model, and the estimates of long term effective population sizes were considerably lower than current census estimates, especially in northern European samples. Selection is an unlikely cause of observed patterns because the distribution of variability conformed to expectations of neutral infinite alleles model and haplotype diversity across populations was positively correlated with heterozygosity (H_E) in nuclear genes ($r_s = 0.74$, $P < 0.05$). Hence, a recent bottleneck, followed by serial bottlenecking during the process of post-Pleistocene recolonization of northern Europe, together with recent population expansion provide a plausible explanation for the low genetic diversity in the north. Genetic distances among populations showed a clear pattern of isolation-by-distance, and 14% of the haplotypic variation was among populations, the rest being distributed among individuals within populations. In accordance with allozyme and morphological data, a hierarchical analysis of nucleotide diversity recognized southern European populations as distinct from northern European ones. However, the magnitude of divergence in mtDNA, allozymes and morphology were highly dissimilar (morphology > mtDNA > allozymes).

Key words.—Bottleneck, *Carduelis chloris*, control-region, effective population size, genetic diversity, geographic variation, mitochondrial DNA.

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A pattern common to many organisms in widely different taxa is that populations inhabiting recently glaciated areas are genetically less variable than populations of the same species occurring in the areas where populations have been established for longer periods of time. Latitudinal trends in levels of genetic variability have been found in plants (Cwynar and Macdonald 1987; Lagercrantz and Ryman 1990), insects (Cooper et al. 1995), mammals (Sage and Wolff 1986) including humans (Cann et al. 1987; Vigilant et al. 1991; Takahata 1995) and birds (Merilä et al. 1996), indicating that all these taxa are responding to some common latent factor. Two plausible explanations for this pattern include shared historical influences on demography (e.g., Hewitt 1996) and selection favoring heterozygous (allozymes) individuals in the south or/and a common haplotype in the north (e.g., Excoffier 1990; Ballard and Kreitmann 1995). If the low genetic variability in northern temperate populations reflects loss of variation due to bottlenecks, this means that in addition to the surveyed markers, many functionally important loci (e.g., quantitative trait loci, MHC) have been affected too (Lande 1980; Hartl and Clark 1989). Consequently, evolutionary potential in the face of similar selection pressures might differ considerably between southern and northern populations. Thus, analyses of several unlinked genetic systems are required to ascertain that the observed differences are not brought about by selection on the particular markers.

We discovered recently that the average heterozygosity in

different populations of greenfinches (Aves: Carduelinae: *Carduelis chloris*), as measured by allelic variation in nuclear genes, is extremely low and decreases with increasing latitude across continental Europe (Merilä et al. 1996). The low overall variability, about one-third that of other passerines, and the northward decline were tentatively explained in terms of a Pleistocene bottleneck and gradual loss of variation associated with serial bottlenecking during the recolonization of northern Europe. Here we investigate further the genetic variability and continent-wide population structure of the greenfinch by examining nucleotide sequence variation in two segments of the mitochondrial DNA control-region. We chose to use the control-region as a genetic marker because of its high variability (e.g., Vigilant et al. 1991; Baker et al. 1993; Wenink et al. 1993) and sensitivity to demographic changes in populations (e.g., Wilson et al. 1985; Avise et al. 1987). In particular, we asked the following questions: (1) does the amount of genetic variation present within different greenfinch populations decline with increasing latitude as in the case of nuclear genes; (2) do the mtDNA data support the hypothesis of recent severe bottleneck(s) or could the pattern be explained by selection acting on mtDNA variants; (3) how do the magnitude and pattern of population structure in mtDNA compare to that observed in allozymes and quantitative traits; and (4) what can be said about the genetic structure of maternal lineages?

MATERIALS AND METHODS

Populations Sampled

Tissue samples were collected in 1992–1994 from 11 different greenfinch populations (Fig. 1). The sampling localities

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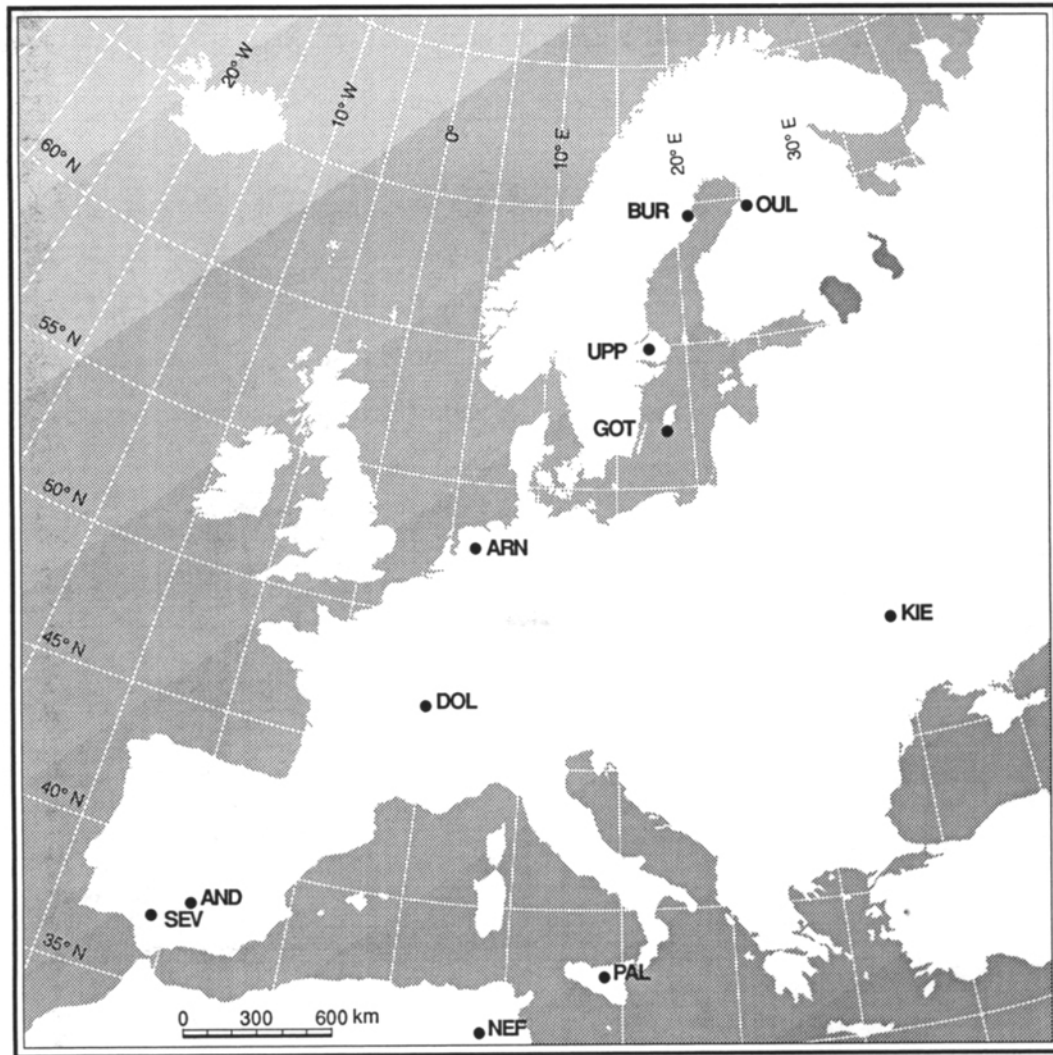


FIG. 1. Map of the locations for the 11 samples of greenfinches analyzed in this study. For locality abbreviations, see Materials and Methods.

were (abbreviation, coordinates, sample size): Oulu (OUL, 65°01'N/25°30'E, $n = 14$), Bureå (BUR, 64°37'N/21°10'E, $n = 20$), Uppsala (UPP, 59°50'N/17°50'E, $n = 19$), Gotland (GOT, 57°04'N/18°13'E, $n = 20$), Arnhem (ARN, 51°55'N/05°05'E, $n = 19$), Dole (DOL, 47°05'N/05°03'E, $n = 20$), Kiev (KIE, 50°26'N/30°29'E, $n = 20$), Andujar (AND, 38°03'N/04°03'W, $n = 17$), Seville (SEV, 37°23'N/06°00'W, $n = 25$), Palermo (PAL, 38°07'N/13°07'E, $n = 15$) and Nefza (NEF, 32°56'N/06°56'E, $n = 4$). These localities are the same as in the earlier survey of allozyme variation in this species (Merilä et al. 1996), except that a few populations representing extensively sampled regions were omitted. The single north African locality (Nefza), represented by four individuals only, was included to unravel possible unique haplotypes; but it was excluded from all comparisons of diversity and population structure. To include only breeding birds, collecting was restricted to the species breeding season (March–July). However, samples from Kiev were obtained in September but still most likely represent locally born juveniles.

Sequenced Regions—An Overview

Using the procedures described below, we first obtained nearly the whole control-region sequence for 17 individuals from five different populations to identify variable regions for further sequencing efforts. This was done by using several pairs of overlapping primers described in Baker and Marshall (1997). This initial screening revealed no variable positions at the 5'-region close to tRNA^{glu}, nor in the central domain ("conserved sequence block," Baker and Marshall 1997). Therefore, we proceeded to amplify and sequence 260 and 377 bp portions of two regions around the central domain, henceforth called "Region I" and "Region II" according to Wenink et al. (1993), respectively (see below).

DNA Isolation and Amplification

Total DNA was extracted from muscle using a phenol-chloroform extraction protocol. In short, an approximately 0.2 g piece of tissue was incubated 8 h at 56°C in a mixture

of 350 µL STE buffer, 40 µL SDS (10%) and 3 µL proteinase-K. Samples were further mixed with 500 µL phenol (0.1 M Tris-HCl pH 8 saturated) and centrifuged to separate phenol from template. The phenol extraction was then repeated and the supernatant was further mixed with 500 µL chloroform (1/25 volume of isoamylalcohol) and centrifuged to remove phenol and chloroform residues from the DNA. Most samples were further ethanol precipitated while some were directly amplified by diluting 5 µL of template in 95 µL dH₂O.

Amplification took place in a 25 µL volume containing 10 mM Tris pH 8.3, 2.5 mM MgCl₂, 0.16 mg/mL BSA, 50 mM KCL, 0.01% gelatin, 0.05 mM each dNTP, 0.4 µM each primer (5 pmol/µL), and 0.2 µL Taq polymerase (AmpliTaQ[®] or Boeringer Mannheim) in a Perkin Elmer-Cetus thermal cycler using the following profile: 93°C for 45 sec, 46°C for 45 sec, 72°C for 90 sec for 38 cycles. The primers used to amplify Region I were the forward primer CRtPRO (Baker and Marshall 1997) located in the tRNA^{pro} upstream from the control region, and one of the following internal reverse primers: FRCI3', F389 or MATS (all described in Baker and Marshall 1997). The Region II was amplified using an internal forward primer F304 (Baker and Marshall 1997) and an universal reverse primer 1261 (Baker and Marshall 1997) situated in tRNA^{phe}. Amplified DNA fragments were electrophoresed in 2% agarose gels with 1 × TA buffer (Sambrook et al. 1989) and visualized in UV light in the presence of ethidium-bromide. Bands in agarose gels were excised and the DNA was cleaned using the GeneClean kit (Bio 101 Inc.) following the manufacturer's instructions.

Sequencing

Double-stranded DNA was sequenced directly with the dideoxynucleotide chain-termination method (Sanger et al. 1977) using Perkin Elmer AmpliCycle[®] sequencing kit following the guidelines provided with the kit. Some individuals were processed with Sequenase kit (United States Biochemical, ver. 2.0) according to manufacturer's directions with some modifications for double-stranded sequencing. The primers used in sequencing of Region I were MATS and FRCI. Region II was sequenced with F304. The sequencing reaction products were separated electrophoretically in 5% Long Ranger gels for 2.5–4.0 h at 60 W in a BRL sequencing apparatus. All sequencing was performed in one direction only.

Descriptive Statistics

Estimates of genotypic (= haplotypic) diversity (h), analogous to heterozygosity in nuclear genes was calculated as: $h = (n/[n - 1])(1 - f_i^2)$, where f_i is the frequency of i th haplotype in sample of n individuals (Nei 1987). Nucleotide diversity (π) was calculated as:

$$\pi = (n/n - 1) \left(\sum_{ij} f_i f_j p_{ij} \right), \quad (1)$$

where p_{ij} is the estimated sequence divergence between the i th and j th haplotype (= number of base substitutions per base pair, and f_i and f_j are their frequencies (Nei 1987). No

correction was made for differences in substitution rates for different pairs of nucleotides as this will have little effect on estimates when p is low as in this study (Nei 1987; Kumar et al. 1993). Confidence limits (95%) for h and π were obtained with a bootstrap procedure (1000 permutations). The mean sequence divergence within each population (p) was calculated as the mean of all possible comparisons (k) of n individuals where $k = n * (n - 1)/2$. Estimates of inter-population sequence divergence (p_A) were corrected for polymorphisms within each population (i.e., $p_A = p_{xy} - (p_x + p_y)/2$, where p_{xy} is mean sequence divergence between all individuals in populations x and y , and p_x and p_y are the mean sequence divergences in populations x and y , respectively (Nei 1987, pp. 276–277). Evolutionary relationships between different haplotypes were estimated using maximum parsimony analysis performed with PAUP (Swofford 1993). Attempts to gain additional resolution by applying coalescent theory (Excoffier and Langaney 1989; Crandall and Templeton 1993) were unsuccessful.

Neutrality Test

Evidence is accumulating to suggest that mtDNA polymorphism may sometimes be influenced by direct or indirect forces of selection (Ballard and Kreitmann 1995; Kreitman and Akashi 1995). If so, this means that inference based on mtDNA variation may violate the equilibrium assumption of most population genetic models. As a test of neutrality of mtDNA variants, we tested whether sequence variation in different populations conforms to a neutral infinite alleles distribution (Tajima 1989a). Tajima (1989a) suggested that the magnitude of difference, as measured by his D statistics, in the estimate of $\Theta = 4N_e\mu$ (N_e = effective populations size, μ = substitution rate per genome per generation) derived from number of variable sites (S) and the average number of nucleotide differences (k) could be used to detect departures from neutrality. However, this test assumes that populations have not been bottlenecked and recently expanded as this would lower the D values and thus increase the probability of rejecting neutrality on false grounds (Tajima 1989b; Ballard and Kreitmann 1995). However, if the null hypothesis of neutrality of mtDNA is rejected, a comparison with variation in nuclear loci may provide a way of distinguishing between the competitive hypothesis. Significance of D was determined from tables given by Tajima (1989a).

Analyses of Historical Demography

To investigate whether reductions in population size have occurred in the past, two different approaches were adopted. First, we estimated the long-term effective female population size from the equation (Wilson et al. 1985; Avise et al. 1988):

$$N_e = [10^6 p * s]/g, \quad (2)$$

where p is the mean pairwise sequence divergence (in %) within the given population, s is the evolutionary rate (= % substitutions/genome/Myr), g is the generation time, and the multiplier 10^6 is required to bring the estimate to Myr (Wilson et al. 1985). We adopted the estimate of $s = 14.8\%$ (Wenink et al. 1996) and $g = 1$. Confidence intervals (CI, 95%) for this quantity were estimated by bootstrapping p . However,

note that CIs indicate uncertainty due to sampling error alone, and do not address uncertainties in generation time and substitution rate.

Another way of gaining insight into the historical demography of the populations is based on the examination of the distribution of pairwise sequence differences within populations (e.g. Slatkin and Hudson 1991; Rogers and Harpending 1992; Harpending et al. 1993; Rogers 1995). We used this information to test whether current haplotypic variation in the greenfinch might fit better with expectations of the "equilibrium" model (constant long term N_e) or with the "sudden expansion" model which postulates a recent expansion in population size (Rogers 1995). Furthermore, the parameters derived from mismatch distributions can be used to get rough estimates of three demographic parameters of interest: effective female population size before (N_0) and after (N_1 = current size) a hypothesized expansion, and the time (t) to start of the possible expansion in generations (Rogers and Harpending 1992). The expected distribution of pairwise differences ($i = 0 \dots$) under equilibrium conditions is

$$F_i = \theta^i / (\theta + 1)^{i+1} \quad (3)$$

where $\theta = 2Nu$ is the expected number of pairwise differences (Watterson 1975). The actual values were obtained by setting θ = observed number of pairwise differences. The expected distribution of pairwise differences under the expansion model was given by Rogers (1995) as

$$G_i(\tau) = \theta_0^i / (1 + \theta_0)^{i+1} \sum_{j=0}^i (1 + \theta_0/\theta_1)^j * [(\tau^j * e^{-\tau})/j!] \quad (4)$$

θ_0 was estimated as $\sqrt{v - m}$, where m and v are the observed mean and variance of pairwise sequence differences and τ was estimated as $m - \theta_0$ (Rogers 1995). Following Rogers (1995), θ_0 was set to 0.0 if $v < m$. The initial population size (N_0) can be estimated by equating $\theta_0 = 2N_0u$ (see above) where $u = 2\mu k$, where μ is the mutation rate (14.8%/Myr; Quinn 1992; Wenink et al. 1993) and k is the length of the sequence (637 bp). An estimate for current population size (N_1) can be derived in a similar way using the fact the vertical intercept (F_0) of the observed distribution of pairwise differences equals $1/(1 + \theta_1)$ (Rogers and Harpending 1992). To obtain estimates of time since expansion we note that $\tau = 2ut$ where t is the time elapsed between N_0 and N_1 (Rogers and Harpending 1992).

Analysis of Population Structure

Three different statistics were calculated to test for differentiation among populations. First, we performed a χ^2 test following Hudson et al. (1992), and second, a hierarchical analysis of population differentiation (Excoffier et al. 1992) using the program WINAMOVA (vers. 1.04; Excoffier 1992). This method allows estimation of the fraction of genetic variance attributable to different hierarchical levels based on geographic distribution of haplotypes and the matrix of squared-distances between all pairs of haplotypes. Two alternative distance metrics were used to assess robustness of the results: (1) minimum number of mutational steps separating a pair of haplotypes; and (2) simple equidistant metrics ("all hap-

lotypes different") which reduces the analyses to the equivalent of F -statistics (Excoffier et al. 1992). The significance of different variance components was tested with randomization procedure (2000 permutations) available in WINAMOVA. The third method was the hierarchical analysis of Holsinger and Mason-Gamer (1996) based on pairwise g_{ST} estimates calculated between all pairs of populations in the data. These statistics were used to produce a dendrogram of relationships between populations in which the mean time to coalescence of haplotypes drawn from the same node is smaller than the mean time to coalescence of haplotypes drawn from populations belonging to different nodes. The significance of g_{ST} values was tested with randomization.

Quantitative Traits, Allozymes and mtDNA

To provide a directly comparable and quantitative comparison of divergence in different suites of traits, we calculated F_{ST} estimates also for allozyme and quantitative traits. Only the nine populations (OUL, BUR, UPP, GOT, ARN, KIE, DOL, AND and SEV) for which data on all traits were available were included in these comparisons. The data for allozymes and quantitative traits are described in Merilä et al. (1996) and Merilä (1997), respectively. F_{ST} for allozymes was estimated using the theta estimator obtained with FSTAT (Goudet 1995). As the quantitative trait data are purely phenotypic, the among population component of variance was estimated simply as the proportion of variation among populations using a one-way ANOVA. As the different quantitative traits, representing 16 linear skeletal dimensions, are inter-correlated to different degrees, we choose to use mean of the 16 traits to represent population structure in quantitative traits. Geographic distances between pairs of populations were measured along the circle track using the locality coordinates, and significance of matrix correlations (r_M) was determined by using the permutation approach described in Cheverud et al. (1989).

RESULTS

Characterization of Control Region Sequences

Thirteen variable positions (all transitions), defining 18 distinct haplotypes were found among the 194 typed individuals (Table 1). Six of the variable sites were situated in Region I (260 bp) and seven in Region II (367 bp), and consequently, both of the sequenced regions seem to have comparable amounts of variation. Hence, data on both regions were pooled in the following analyses. The mean pairwise nucleotide divergence (p) among these haplotypes was 0.39% (SE = 0.0001 %, $n = 153$) and 0.12% among all individuals in the entire sample ($n = 18\,721$ comparisons). Assuming a rate of 14.8% divergence per Myr, then $p = 0.78\%$ between the two most divergent haplotypes indicates that the common ancestor of all current greenfinch haplotypes existed about 53 000 years ago. Furthermore, over 50% of pairwise comparison among 18 haplotypes show divergence $\leq 0.3\%$, indicating that majority of haplotypes are not older than approximately 20 000 years. This is also reflected in the results of the maximum parsimony analysis of the 18 haplotypes: seven equally long trees were found (tree length = 17 steps)

TABLE 1. Haplotypes found in 11 different greenfinch populations.

#	Haplotype [†]	Population ^{††}											Σ
		OUL	BUR	UPP	GOT	ARN	KIE	DOL	AND	SEV	PAL	NEF	
1	TTATGG ATTCAGG	12	16	15	17	13	15	9	5	10	5	1	118
2	TTATGG ACTCAGG	0	1	0	0	5	1	7	8	5	0	0	27
3	TTACGG ATTCAGG	1	1	1	0	1	1	2	0	1	7	0	15
4	CTATGG ATTCAGG	0	0	0	0	0	0	0	4	6	0	3	13
5	TTACGG ATTCAGG	1	0	2	0	0	1	1	0	0	0	0	5
6	TTATGG ACTCGGG	0	0	0	0	0	0	0	0	0	2	0	2
7	TTATGG ATTCAGG	0	2	0	0	0	0	0	0	0	0	0	2
8	TTATGG ATTTAGG	0	0	1	1	0	0	0	0	0	0	0	2
9	CTATAG ATTCAGG	0	0	0	0	0	0	0	0	1	0	0	1
10	TCATGG ACTCAGG	0	0	0	0	0	0	1	0	0	0	0	1
11	TTACAG ATTCAGG	0	0	0	0	0	1	0	0	0	0	0	1
12	TTACGA ATTCAGG	0	0	0	1	0	0	0	0	0	0	0	1
13	TTACGG ATTTAGG	0	0	0	1	0	0	0	0	0	0	0	1
14	TTATGG ATCCAGG	0	0	0	0	0	0	0	0	1	0	0	1
15	TTATGG ATTCAGA	0	0	0	0	0	0	0	0	1	0	0	1
16	TTATGG ATTCGGG	0	0	0	0	0	0	0	0	0	1	0	1
17	TTATGG GTTCAGG	0	0	1	0	0	0	0	0	0	0	0	1
18	TTGTGG ATTCAGG	0	0	0	0	0	1	0	0	0	0	0	1
Σ		14	20	20	20	19	20	20	17	25	15	4	194

[†] Variable nucleotide sites listed in sequential order.

^{††} See methods for population abbreviations.

but the strict consensus tree resolved only two distinct nodes—the one consisting of haplotypes 4 and 9, and the other consisting of 5 and 11, respectively (Fig. 2). Hence, most haplotypes are separated by one or few mutations and their evolutionary relationships are not well resolved.

Variation within Populations

The most common haplotype was found in all populations, while the majority of the haplotypes (10/18) were singletons (Table 1). Haplotype and nucleotide diversity estimates within each of the sampled populations were low, indicating that the probability of randomly choosing two individuals with the same haplotype is quite high (Table 2). Diversity esti-

mates did not differ significantly among different populations due to large *SEs* (Table 2), but there was a strong negative correlation between latitude and haplotype diversity ($r_s = -0.87$, $z = -2.62$, $P = 0.009$), as well as between latitude and nucleotide diversity ($r_s = -0.89$, $z = -2.67$, $P = 0.0075$; Fig. 3). Furthermore, a significant among-region difference in both haplotype and nucleotide diversity estimates is evident if pooled southern (Spain and Italy) and northern European samples are compared (Table 2). Given the low haplotype and nucleotide diversity in northern European populations, the question becomes whether this can be explained by selection or historical demography of populations.

Neutral Expectation

As a test of neutrality we compared the distribution of haplotypic variability within populations to that expected under the neutral infinite allele model (Tajima 1989a). The null hypothesis of neutrality was rejected only in the case of Bureå sample, though two other northern European populations were also close to the critical level (Table 2). However, combining either southern or northern European samples or both, we cannot reject the null hypothesis of neutrality of studied variation. Furthermore, a strong positive correlation between average expected heterozygosity, as enumerated from variation in nuclear genes (Merilä et al. 1996), and nucleotide diversity ($r_s = 0.85$, $z = 2.44$, $P = 0.014$), as well as between haplotype diversity further indicates that selection is an unlikely cause of clinal variation in mtDNA variability (Fig. 4). Therefore, we proceed to examine evidence for possible historical explanations.

Distribution of Pairwise Sequence Differences and Long-Term Effective Population Size

Estimated long-term female population sizes for different populations (Table 2) should be taken as rough approxima-

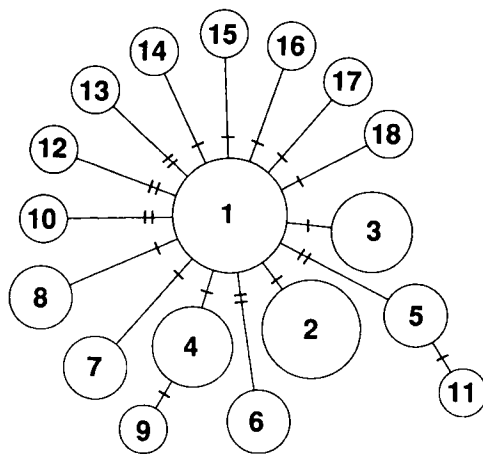


FIG. 2. Maximum parsimony network of 18 haplotypes found in 11 greenfinch populations. The network is a strict consensus solution of seven equally parsimonious alternatives. Each cross-bar between two haplotypes represents one mutational event. The size of the circles are indicative of the relative frequency of different haplotypes in the total sample.

TABLE 2. Haplotype (h) and nucleotide (π) diversities, Tajima's (1989) D statistic and estimated long-term effective female population sizes (N_e) for 10 samples of greenfinches. n = number individuals, n_H = number of distinct haplotypes. C.I._{95%} = 95% confidence interval obtained with bootstrap.

Locality	n	n_H	h	C.I. _{95%}	$\pi^{\dagger}$	C.I. _{95%}	D	N_e	C.I. _{95%}
Oulu	14	3	0.275	0.00–0.56	0.064	0.000–0.126	–1.67	4300	0–8500
Bureå	20	4	0.362	0.10–0.61	0.058	0.016–0.096	–2.38**	4100	1100–7500
Uppsala	20	5	0.461	0.11–0.68	0.108	0.032–0.174	–1.57	7000	2000–12,000
Gotland	20	4	0.284	0.00–0.52	0.076	0.000–0.144	–1.64*	5100	0–10,000
Arnhem	19	3	0.447	0.19–0.66	0.120	0.032–0.200	–1.76*	5000	2000–7500
Kiev	20	6	0.485	0.21–0.61	0.080	0.032–0.114	–1.10	8400	2200–14,000
Dole	20	5	0.694	0.49–0.78	0.152	0.090–0.204	–0.78	11,000	6000–15,500
Palermo	15	4	0.695	0.45–0.75	0.162	0.074–0.224	–0.28	12,000	6000–15,500
Andujar	17	7	0.667	0.43–0.71	0.146	0.092–0.162	–1.31	9600	6000–16,000
Sevilla	25	3	0.766	0.60–0.82	0.166	0.112–0.162	0.08	11,000	8000–14,000
North ^{††}	133	12	0.458	0.36–0.56	0.100	0.068–0.130	–1.72*	5000	2500–7100
South ^{†††}	57	9	0.788	0.71–0.82	0.184	0.152–0.210	–0.79	11,000	7000–14,000
Pooled	190	18	0.612	0.53–0.68	0.134	0.110–0.154	–1.75*	7000	5000–9000

[†] $10^2 \pi$, ^{††} Oulu-Dole, ^{†††} Palermo-Seville, * $0.05 < P < 0.10$, ** $P < 0.01$

tions, but they are strikingly low (≈ 4000 – $12,000$) as compared to current census sizes (assuming: $N_{e,\text{female}} = 1/2N_{\text{pairs}}$) of greenfinches only from France ($> 500,000$), Sweden ($\approx 100,000$) and Finland ($\approx 75,000$; Cramp and Perrins 1994), and those estimated for various other species of birds (e.g., Ball and Avise 1992). That this discrepancy is not sensitive for possible overestimation of the substitution rate of 14.8% (Wenink et al. 1993) is illustrated by a simple calculation: assuming a conservative current N_e of one million females, then the substitution rate needed to bring the present mtDNA data to this value from equation (2) is about

120 times smaller than the one we used. Furthermore, using the method of Rogers and Harpending (1992), the estimates for current effective female population size (N_1 ; Table 3) are strikingly similar to those obtained in Table 2. In the southern populations the current effective female population size was estimated to be around 18,000 individuals, whereas in the northern populations, it was estimated at only around 4000 individuals (Table 3). Hence, although there is a large uncertainty on true values of estimated parameters, at least a qualitative difference seems to exist between northern and southern European populations. The discrepancy between current census estimates and estimates based on mtDNA data is not difficult to understand: the estimated time to most recent common ancestor for the whole dataset is roughly 3600 to 5300 years (Table 3), indicating that most present day greenfinches coalesce to a common ancestor that lived after the last glaciations. Hence, a recent population bottleneck followed by population expansion could account for the discrepancy. Indeed, a comparison of the “sudden expansion” and “equilibrium” models revealed that for most comparisons, and especially so for the larger datasets (= combined southern and northern populations, total data), the fit was

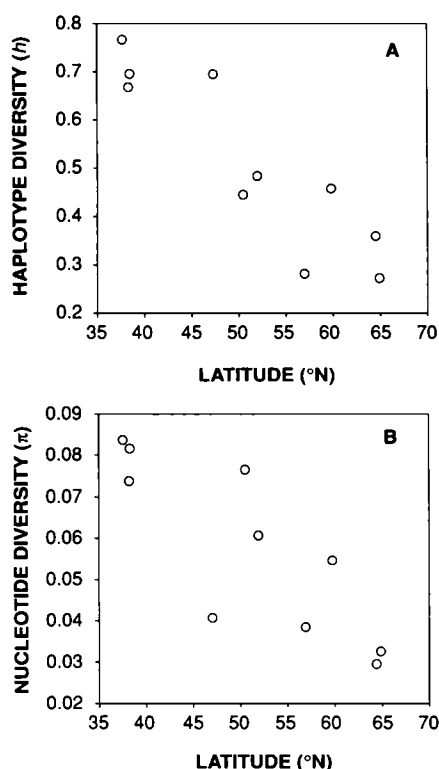


FIG. 3. Haplotype and nucleotide diversities as a function of latitude. (A) Haplotype diversity: $r_s = -0.87$, $z = -2.58$, $P = 0.009$; (B) Nucleotide diversity $r_s = -0.89$, $z = -2.67$, $P = 0.008$.

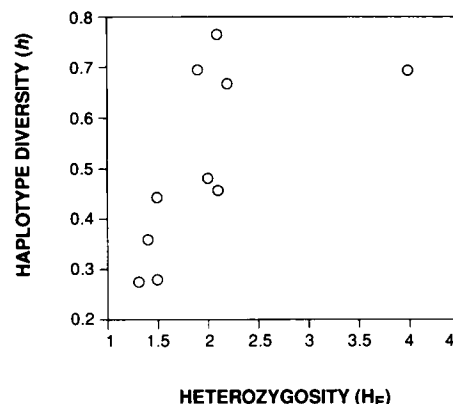


FIG. 4. Correlation between haplotype diversity and average expected heterozygosity (H_E) as enumerated by allelic variation in nuclear genes ($r_s = 0.739$, $z = 2.34$, $P = 0.019$). Data on H_E from Merilä et al. (1996).

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TABLE 3. Results of pairwise sequence difference analyses showing estimates of mean sequence divergence (MSD), approximate time to the beginning of the expansion (t), effective female population size at the beginning (N_0) and at the end (=today) of the expansion (N_1), as well as the mean square errors measuring the fit of equilibrium (MSE_1) and expansion (MSE_2) models.

Population	MSD	t	C.I. _{95%} [†]	N_0	C.I. _{95%} [†]	N_1	C.I. _{95%} [†]	MSE_1	MSE_2	MSE_1/MSE_2 [‡]
OUL	0.41	800	0–4000	0	0–2700	1900	0– 6800	0.003	0.005	0.7
BUR	0.39	2000	500–3700	0	0–0	2900	600– 8100	0.004	0.002	2.0
UPP	0.66	2200	0–5600	400	0–2600	4000	600–10,200	0.001	0.002	0.4
GOT	0.48	400	0–4300	1900	0–2700	2100	0– 5400	0.007	0.009	0.7
KIE	0.51	1500	0–5500	2400	0–4400	4100	600–11,200	0.000	0.001	0.3
ARN	0.76	2600	1100–3800	0	0–0	5000	1300– 8600	0.000	0.001	0.3
DOL	1.05	5100	2800–7800	0	0–1200	10,400	5100–18,700	0.011	0.002	7.1
PAL	1.12	5400	1800–7600	0	0–2800	10,600	4500–18,900	0.012	0.147	0.08
AND	0.98	4800	3200–5400	0	0–0	9700	5000–12,700	0.021	0.007	3.1
SEV	0.93	5400	3600–6900	0	0–0	15,100	8000–24,200	0.016	0.002	6.8
North	0.64	2500	900–4100	900	0–2100	4400	2800– 6500	0.000	0.000	8.1
South	1.18	6100	5100–7200	0	0–0	18,500	13,300–24,300	0.009	0.002	4.8
Total	0.84	4400	3600–5300	0	0–0	8100	5800–11,100	0.003	0.000	8.5

[†] All confidence intervals obtained by bootstrapping MSD (500 replicates).
[‡] A ratio (MSE_1/MSE_2) > 1 indicates better fit to expansion than to equilibrium model.

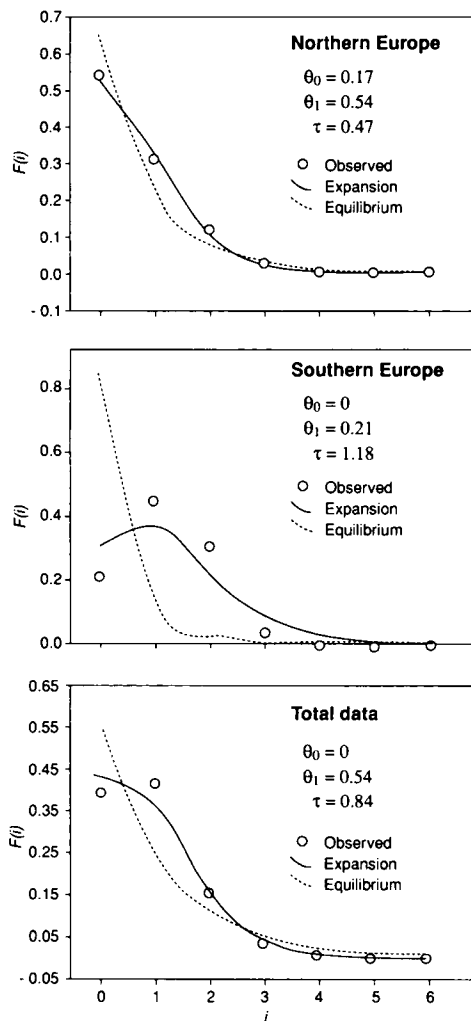


FIG. 5. Observed and expected distributions of pairwise sequence differences under the “Equilibrium” model and the “Sudden expansion” model (Rogers 1995) in northern European, southern European, and combined samples of greenfinches, respectively. For symbols, see Materials and Methods.

about 5–8 times better to the sudden expansion than the equilibrium model (Fig. 5; Table 3).

Population Structure and Phylogeography

A comparison of pairwise sequence divergence estimates and geographic distances separating pairs of populations by means of matrix correlation revealed that differentiation in mtDNA is geographically ordered ($r_M = 0.51$, $P < 0.001$; Fig. 6). χ^2 tests (Hudson et al. 1992) revealed significant population structuring across all populations ($\chi^2_{170} = 289.7$, $P < 0.001$), among southern European localities ($\chi^2_{24} = 49.47$, $P < 0.005$) but not between northern European localities ($\chi^2_{60} = 82.51$, $P = 0.082$). Further analysis of population subdivision with the AMOVA approach (Excoffier et al. 1992) revealed that 13.5% of haplotypic variation was among populations, the rest being distributed among individuals within populations. Furthermore, adding “region” into the model to distinguish southern and northern European populations revealed that 12% of the haplotypic variability

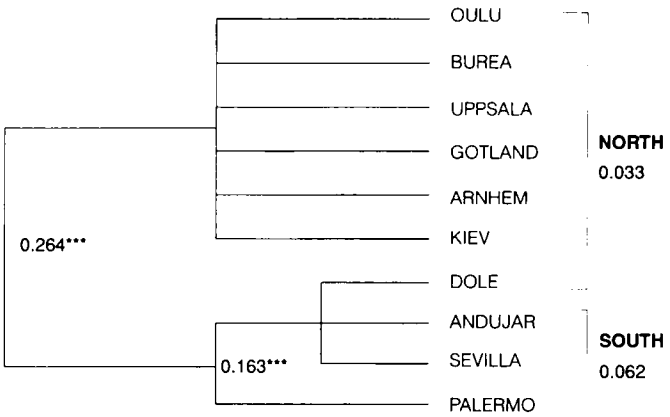


FIG. 6. Hierarchical analysis of haplotype diversity in greenfinch. The number at each node is the distance (g_{ST}) between its daughter nodes at the next hierarchical level. Numbers at the right are estimates among populations within the two main lineages. Non-significant distances are collapsed to polytomies (***) $P < 0.001$.

TABLE 4. Hierarchical analysis of molecular variance among different greenfinch populations with two different matrices of haplotype divergence. P = The probability of getting a larger value than that observed by change.

Variance component	Variance	%	Φ -statistics	P
<i>Equidistance matrix</i>				
Among regions	0.042	13.0	$\Phi_{CT} = 0.130$	0.001
Among populations/ regions	0.021	6.5	$\Phi_{SC} = 0.074$	<0.001
Within populations	0.260	80.5	$\Phi_{ST} = 0.194$	0.999
<i>Mutational step matrix</i>				
Among regions	0.021	4.9	$\Phi_{CT} = 0.049$	0.021
Among populations/ regions	0.036	8.5	$\Phi_{SC} = 0.090$	<0.001
Within populations	0.368	86.6	$\Phi_{ST} = 0.135$	0.999

was between regions, 7% being among populations within regions, and most being among individuals within populations (Table 4). This pattern is changed somewhat if a mutational step matrix is used: the difference among regions accounts then for only 5% of all variation (Table 4). The hierarchical approach of Holsinger and Mason-Gamer (1996) produced a dendrogram which clearly separates the southern populations from the northern ones (Fig. 6), though the Dole population is indicated to be more closely related to the southern populations than to the northern populations. The topography of the dendrogram in (Fig. 6) is very similar to that produced by the Neighbor-Joining analysis of the sequence divergence estimates, except that in the latter analysis the Dole population clusters with the northern European populations. Hence, despite the low degree of absolute divergence and the short time to common ancestry (Table 3), some population subdivision is evident.

Comparison of Population Structure in Different Datasets

Because of its higher mutation rate and smaller effective population size, we expect more population structuring in mtDNA than in allozymes. A comparison of the mtDNA estimates presented here with those obtained from allozymes (Merilä et al. 1996) seems to confirm this expectation (Table 5). However, differentiation both in allozymes and nuclear genes is much less than in quantitative traits (Table 5). Despite the grossly different magnitudes of differentiation in different sets of traits, the pairwise genetic and morphometric distances among populations are significantly correlated (mtDNA-morphology: $r_M = 0.59$, $P < 0.001$; allozymes-morphology: $r_M = 0.56$, $P < 0.001$), as well as between geographic distances separating pairs of populations (mtDNA-geography: $r_M = 0.61$, $P < 0.001$; allozymes-geography: $r_M = 0.46$, $P = 0.001$). Furthermore, divergence in allozymes, as measured by Rogers' (1972) genetic distances and with average sequence divergence in mtDNA are significantly, albeit weakly, correlated ($r_M = 0.26$, $P = 0.046$).

DISCUSSION

The Pattern

The two most striking aspects of our results were the low haplotype (and nucleotide) diversity in northern European as

TABLE 5. Comparison of population structure in mtDNA, nuclear genes and quantitative traits of greenfinches.

Type of trait	Variance component (%)	
	Among population	Within population
mtDNA (F_{ST}) [†]	11.3	88.7
Allozymes (F_{ST})	2.2	97.2
Quantitative traits (s^2_A) [‡]	28.0	72.0

[†] Variance components for mtDNA estimated using equidistance matrix.

[‡] Added variance component from a one-way ANOVA.

contrasted to southern European greenfinch populations, and the starlike phylogeny of different haplotypes. The strong negative correlation between haplotype (or nucleotide) diversity and latitude is noteworthy for two reasons: (1) it is consistent with the outcome of our previous analyses of variation in nuclear genes (Merilä et al. 1996), indicating that a common latent factor might be involved in the determination of levels of intrapopulation variation both in nuclear and mitochondrial genomes in the greenfinch; and (2) the clinal reduction in genetic diversity from south to north (Fig. 3) is oriented in a direction of presumed recolonization path from a glacial refuge. As this finding parallels the pattern detected in the human control region sequences (Cann et al. 1987; Vigilant et al. 1991; see also: Takahata 1995), and low within-population genetic diversity occurs commonly in other northern temperate zone animal and plant populations as well (e.g., Sage and Wolff 1986; Lagercrantz and Ryman 1990), this suggest a common cause that determines the distribution of genetic diversity within species is shared among many unrelated taxa.

Selection

One possible explanation for lower haplotype diversity in the north is positive selection favoring the common haplotype. We regard this explanation as highly unlikely for three reasons. First, the results of the neutrality tests (Tajima 1989a) gave no reason to reject the null hypothesis of neutrality. Although some of the individual tests indicated significant deviations from a neutral expectation, this could be expected also if the equilibrium assumption is violated: unless the populations under study have been of constant size for considerable periods of time, this approach cannot strictly separate selective and demographic explanations (Tajima 1989b; Ballard and Kreitmann 1995). Second, the different measures of mtDNA diversity in different populations were strongly correlated with heterozygosity in nuclear loci in the same populations. The strong correlation in levels of genetic diversity between the two sets of (presumably) unlinked markers indicates that selection is an unlikely explanation. Furthermore, the nature of avian allozyme polymorphism fits well to neutral expectations (Barrowclough et al. 1985; see also: Nei and Graur 1988), and we have also confirmed that allozyme variation in this species does not fit well to the pattern expected under selective models such as over dominance (Merilä et al. 1996). In addition, the allozyme variability in northern European populations is very low (Merilä et al. 1996), and such low variabilities in both nuclear and mitochondrial genomes are difficult to explain except in terms

of population bottleneck (Wilson et al. 1985 their fig. 9). Finally a similar reduction in levels of genetic diversity toward the north has been observed in several other independent studies (e.g., Sage and Wolff 1986; Lagercrantz and Ryman 1990; Takahata 1995), and it is unlikely that many unrelated organisms and different marker systems would be influenced by similar selection regimes. Therefore, we conclude that the low haplotype and nucleotide diversity in northern European greenfinch populations is unlikely to be explained by natural selection.

Demographic Explanations

The analysis of pairwise sequence differences revealed highly truncated distributions in all but three populations, giving generally better fit to "expansion" than to "equilibrium" models (Rogers and Harpending 1992; Rogers 1995). All within-population distributions of pairwise nucleotide differences (reproducible from data in Table 1) were strongly L-shaped (left truncated) which are rather untypical when compared with distributions published so far (e.g., Harpending et al. 1993; Marjoram and Donnelly 1994; Baker and Marshall 1997). By contrast, these distributions resemble those obtained in simulations of strongly bottlenecked populations (Marjoram and Donnelly 1994; their fig. 6). Because southern European populations tended to a more unimodal distribution (Fig. 6), they might have had a longer time to recover from a population bottleneck, whereas northern European populations might have not reached a new equilibrium (Rogers and Harpending 1992).

Bottlenecking hypotheses predict low long-term population sizes because they are determined by the harmonic (rather than the arithmetic) mean which gives heavy weight to occasional low numbers (Hartl and Clark 1989; p. 83). Consistent with this, we found the estimated long-term female population sizes (Table 2) to be much smaller than current census estimates, and those estimated for other birds (e.g. Ball and Avise 1992). This holds even if we used the low 2% substitution rate for the whole mtDNA genome, and even more so if the generation time we have used ($g = 1$) was an under-estimate. Taken together, the data are consistent with a historical bottleneck followed by population expansion subsequent to the last glaciations.

Population Structure and Process of Colonization

We found only slight shifts in haplotype frequencies among different populations, and the deepest split corresponds to that between southern and northern European populations. Beyond this, there was little evidence for any distinct lineages which could be indicative of multiple origins of present-day populations from different glacial refuges. Although this does not strictly rule out the possibility of the greenfinch having survived in several different refuges, it is consistent with the hypothesis that the clinal patterns of genetic and quantitative trait variation across Europe do not represent secondary intergradation between previously isolated populations (Merilä 1997). Instead, northern European greenfinches may have been derived from a single ancestral Iberian stock which has successively spread over the continent. This expansion is probably recent, as judged from the fact that the colonization

of northern Fenno-Scandia has occurred in this century (Cramp and Perrins 1994), and from the result that most present day greenfinches were indicated to coalesce to a common ancestor that lived after Pleistocene glaciations. The spread of the greenfinch to the north may have followed closely that of agriculture as the occurrence of greenfinches in northern Europe (and in New Zealand) is strongly clustered around human settlements. Indeed, a stepping-stone like dispersal pattern is obvious at the edge of the dispersal front in northern Finland (Hyytiä et al. 1983). If this mode of dispersal was prevalent during the recolonization of north, it could account for the progressive northward decline of nuclear and mtDNA variability. In other words, the leading edge of the colonization front could have been composed of small isolated demes which have provided "stepping-stones" for future dispersal, thereby successively reducing the genetic diversity due to chance effects and genetic drift (e.g., Sage and Wolff 1986; Hewitt 1996).

An alternative explanation which could account for higher genetic diversity in southern populations is that these populations consist of a mixture of several previously isolated populations (cf. Excoffier 1990). Such a mixing would increase their diversity and explain the apparently reduced variation in the north (the "normal" condition). However, this will not change the conclusions about severe bottleneck: the low diversity and starlike phylogeny of haplotypes remain unchanged, though the interpretation about serial bottlenecking would be challenged.

With few exceptions (Avise and Nelson 1989; Wenink et al. 1993; Zink 1994), studies of mtDNA variation among bird populations have revealed little geographic structuring in mtDNA lineages (e.g., Avise et al. 1988; Zink and Dittmann 1993a,b; Seutin et al. 1995). This could be either due relatively high levels of present-day gene flow or due to shared ancestral polymorphism. However, a common factor shared by this and many other studies is that they have been conducted with northern-temperate zone passerines currently inhabiting recently deglaciated areas. Hence, the lack of population structuring in the present-day populations does not necessarily imply extensive on going flow, but may instead indicate the retention of shared ancestral polymorphisms spread over large geographical ranges by recent population expansions. Furthermore, if our interpretation of population bottleneck followed by expansion about 5000–8000 years ago is correct, this means that much of the historical information carried in the greenfinch genome was lost at that period.

Concordance between Morphology, Allozymes and mtDNA

Although population subdivision is expected to have the same effect on geographic distribution of variation in single locus and polygenic traits, so far they are both neutral (Rogers and Harpending 1983). This expectation does not apply to comparisons between nuclear and mitochondrial genes, because the effective population size for mtDNA genes is only one-fourth as large as for nuclear genes, allowing faster divergence among demes. Hence, unless migration is strongly female-biased, or selection is acting on nuclear or mtDNA markers (or both), we should expect more population structuring for mtDNA than for nuclear genes. In accordance with

the results of previous empirical studies (Avise et al. 1979; Desalle et al., 1987; Hale and Singh 1987; Crease et al. 1990), the results of this study lend support to this prediction. Furthermore, the ability of mtDNA to recover more geographic structure than allozymes is also evident from matrix correlations: the correlation between geographic distances and sequence divergence ($r_M = 0.51$) was significantly ($P < 0.001$) higher than that between geographic and Rogers genetic distances of allozymes ($r_M = 0.46$). Notably, the divergence in quantitative traits exceeded greatly that in nuclear markers and mtDNA, a pattern to be expected if the divergence of quantitative traits has been promoted by natural selection favoring different genotypes in different parts of the distribution range. However, differentiation in quantitative traits could also reflect nongenetic environmental influences, although evidence exists to favor natural selection as cause of population differentiation in the greenfinch (Merilä 1997). Nevertheless, as demonstrated earlier for the Canada goose (*Branta canadensis*; van Wagner and Baker 1990) our data clearly shows that genetic and morphometric divergence between different greenfinch populations has proceeded in a correlated fashion.

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