Evolution, 57(12), 2003, pp. 2852–2864

RECONCILING ACTUAL AND INFERRED POPULATION HISTORIES IN THE HOUSE FINCH (*CARPODACUS MEXICANUS*) BY AFLP ANALYSIS

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Abstract.—The house finch (*Carpodacus mexicanus*) is a native songbird of western North America that was introduced to the eastern United States and Hawaiian Islands in historic times. As such, it provides an unusually good opportunity to test the ability of molecular markers to recover recent details of a known population history. To investigate this prospect, genetic variation in 172 individuals from 16 populations in the western and eastern United States, southeastern Canada, Hawaiian Islands, and Mexico, as well as genetic variation in the closely related purple finch (*Carpodacus purpureus*) and Cassin's finch (*Carpodacus cassinii*) was studied by a semi-automated fluorescence-labeled amplified fragment length polymorphism (AFLP) marker system. A total of 363 markers were generated, of which 258 (71.2%) were polymorphic among species, 166 (61.4%) polymorphic among house finch subspecies, and 157 (60.2%) polymorphic among populations within the *frontalis* subspecies complex. Heterozygosities and interpopulation divergences revealed by the analysis appeared relatively low at all taxonomic levels, but there are few similar studies in avian populations with which to compare results. Whereas the known population history predicts that both eastern and Hawaiian finches should have been derived from within western populations, tree analysis using both populations and individuals as units suggests weak monophyly of eastern populations and indicates that Hawaiian populations are not clearly derived from California populations. However, the genetic distinctiveness of native and recently founded populations was disclosed by analyses of molecular variance as well as by a model-based assignment approach in which 98%, 94%, and 99% individuals from western, Hawaiian, and eastern regions, respectively, were assigned correctly to their populations without using prior information on population of origin, suggesting that these recent introductions have resulted in detectable differentiation without substantial loss of AFLP diversity. Our results indicate that AFLPs are a useful tool for population genetic and evolutionary studies of birds, particularly as a prelude to finding molecular markers linked to traits subjected to recent adaptive evolution.

Key words.—Amplified fragment length polymorphism, analysis of molecular variance, gene diversity, house finch, phylogeography.

Received March 10, 2003. Accepted July 7, 2003.

Studies of population genetic structure provide windows into the roles that selection, mutation, gene flow, and drift play in processes such as local adaptation and speciation (Barton and Clark 1990; Avise 1994; Slatkin 1994; Foster et al. 1998). Because the actual histories of most species are unknown, many population genetic analyses focusing on geographic patterns are necessarily based on the assumption that the analyzed populations are in equilibrium with respect to these forces. In recent years, however, the possibility of nonequilibrium situations in many species has become clearer, and a number of new methods promise to deal better with nonequilibrium situations (Kuhner et al. 1998; Polanski et al. 1998; Templeton 1998; Weiss and von Haesler 1998; Emerson et al. 2001). In the face of complex, nonequilibrium situations, we can expect an enhanced ability to study the process of speciation when we employ molecular genetic tools to species with recent demographies whose details have been recorded historically (Clegg et al. 2002).

The house finch (*Carpodacus mexicanus*) is a cardueline finch with a wide distribution in North America. Before the landscapes were modified by Europeans house finches were found from Oaxaca, Mexico, to central Oregon and east to Colorado and Texas (American Ornithologists' Union 1983; Hill 2002), with several morphologically distinct subspecies in Mexico and on islands in the Pacific and Gulf of California. Moore (1939) recognized 18 subspecies of house finches, but Hill (1996) recently reduced this to 14 subspecies. All of the house finches in the United States and Canada, except for birds in the extreme lower Rio Grande Valley, belong to one supspecies, *C. m. frontalis* (Hill 1996).

House finches probably originating from coastal California and from the subspecies *C. m. frontalis* were introduced to the Hawaiian Islands sometime before 1870 (Grinnell 1911) and to Long Island, New York, in 1940 (Elliot and Arbib 1953). There is no record of the number of house finches that were released in Hawaii, but because the birds were transported slowly in wooden ships, the number has been assumed to be small. Wild house finches were first noticed in eastern North America in New York City in 1940 (Elliot and Arbib 1953), but the number of birds that founded the eastern population is unknown. Hill (2002) speculated that, based on the large number of house finches that were imported from California to East Coast cities in the early 20th century, 50 or more individuals may have founded the eastern population of house finches.

Since they were introduced to the New York City area 60 years ago, the western boundary of the eastern population's range has reached the easternmost extension of some western populations, although populations in the Great Plains are sparse and gene flow across this region is likely low. Despite

the very recent divergence of eastern and western populations of this species, differences in morphological characters, behavior, and plumage colors have been detected in east-west comparisons and at a variety of taxonomic levels both within and between subspecies (Aldrich and Weske 1978; Power 1979; Aldrich 1982; Sprenkle and Blem 1984; Stangel 1985; Wootton 1986). Many of these differences, including hatching order and sex allocation, are now known to be adaptive (Badyaev et al. 2002).

Two unpublished master's theses that examined genetic diversity among native California populations and introduced populations of house finches with mitochondrial DNA restriction fragment length polymorphisms and allozyme analyis showed that house finches in the eastern United States have retained most of the genetic diversity found in house finches sampled in California. Similarly, house finch populations in the Hawaiian Islands have levels of genetic diversity similar to those within California populations, although in some comparisons there was loss of rare alleles (Benner 1991; Vasquez-Phillips 1992). These studies suggest that the founder effect in this species—the shift in allele frequencies accompanying the founding of novel populations—has not been dramatic. The molecular tools used for these studies, namely allozyme electrophoresis and restriction fragment analysis of mitochondrial DNA, are less sensitive than some recently developed methods. In recent years, a variety of DNA-based techniques have been employed to study variation within and among species, for example, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellite, and single nucleotide polymorphism (SNPs; Sunnucks 2000; Brumfield et al. 2003). Advantages of these methods over allozyme include the increased likelihood of neutral variation and their increased saturation and better coverage of the genome (Storfar 1996). Recent methods also detect higher levels of variation and, in the case of mitochondrial DNA, have increased sensitivity to bottlenecks. AFLPs have emerged as a recent molecular method that detects significant levels of DNA polymorphism at a very large number of loci (Vos et al. 1995). AFLP analysis is not technically difficult, yet it generates large numbers of markers spanning the entire genome without requiring any prior sequence knowledge, library construction, or the characterization of DNA probes. In addition, newly developed semi-automated fluorescence-labeled detection of AFLPs and software has improved both fragment scoring and data handling. The dominant nature of the AFLP markers may lead to the underestimation of recessive allele frequencies (Szmidt et al. 1996), and it is still unclear whether AFLPs can or should be analyzed in a genealogical framework within species. These potential problems, however, may be overcome by examining a large number of loci.

Although the AFLP technique is widely used in plant mapping and population genetic studies (Travis et al. 1996), its application to animals, especially for population genetic and evolutionary studies, is still relatively new (Otsen et al. 1996; Ajmone-Marsan et al. 1997; Questian et al. 1999; Busch et al. 2000). Several studies have recently used AFLPs to reconstruct interspecfic relationships (Albertson et al. 1999; De Knijff et al. 2001; Giannasi et al. 2001; Parsons and Shaw 2001), but few studies have applied the technique to questions below the species level.

In choosing the AFLP technique for our study, we were motivated at least as much by its ability to generate large numbers of markers across the genome as by its potential utility in phylogeography. In fact, were our motives purely phylogeographic, we would have chosen single nucleotide polymorphisms over AFLPs as our preferred marker (Brumfield et al. 2003). However, eastern populations of the house finch have recently been invaded by a bacterium, *Mycoplasma gallisepticum*, that escaped from chickens and caused a large decline in house finch numbers (Hochachka et al. 2000). Our long-term goal is to identify genetic loci that have contributed to resistance to this parasite and may have undergone selection as a result of the epizootic. The AFLP method is well suited for generating a large number of markers that, in principle, could ultimately aid in the discovery of loci involved in the evolution of resistance. In addition, to our knowledge, this is the first large-scale geographic survey using the AFLP approach of an avian species below the species level, although some smaller avian studies have been published (Busch et al. 2000).

Materials and Methods

Genetic Resources

We examined 163 individuals sampled from 16 house finch populations from North America and the Hawaiian Islands, as well as samples of two closely related sister species, purple finches (*C. purpureus*; $n = 3$) and Cassin's finches (*C. cassinii*; $n = 6$) in this study (Fig. 1). Detailed information on location, sample sizes per population, and collection dates are presented in Table 1. Importantly, all samples were collected prior to the onset of the *Mycoplasma* epizootic in any given locality, a sampling scheme that ensures that AFLP frequencies are uninfluenced by this disease. Blood samples were stored in Queen's lysis buffer (Seutin et al. 1991) after collection in the field. Tissue samples were immediately frozen in liquid nitrogen after collection, stored long term at −80°C, and transported between laboratories in 100% ethanol at room temperature.

DNA Isolation

DNA extraction was based on the protocol of Kempenaers et al. (1999) with minor modifications. About 0.1 g tissue, or 150 μl blood, were diluted in Queen's lysis buffer (Seutin et al. 1991) and added to 600 μl digestion buffer (100 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, and 1% sodium dodecyl sulfate) with proteinase K (final concentration 50 μg/ml). Tissue digestion took place in a water bath at 55°C for 3–4 h. Before extracting DNA, 5 μl RNase A (10 mg/ml) was added to each sample and incubated at 55°C for another 30 min. DNA extraction was carried out twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and once with an equal volume of chloroform:isoamyl alcohol (24:1). The DNA was precipitated in ethanol by using 1/10 volume of 3 M sodium acetate, spun down, and dried and diluted with TE buffer (10 mM Tris-HCl, pH 8.0,

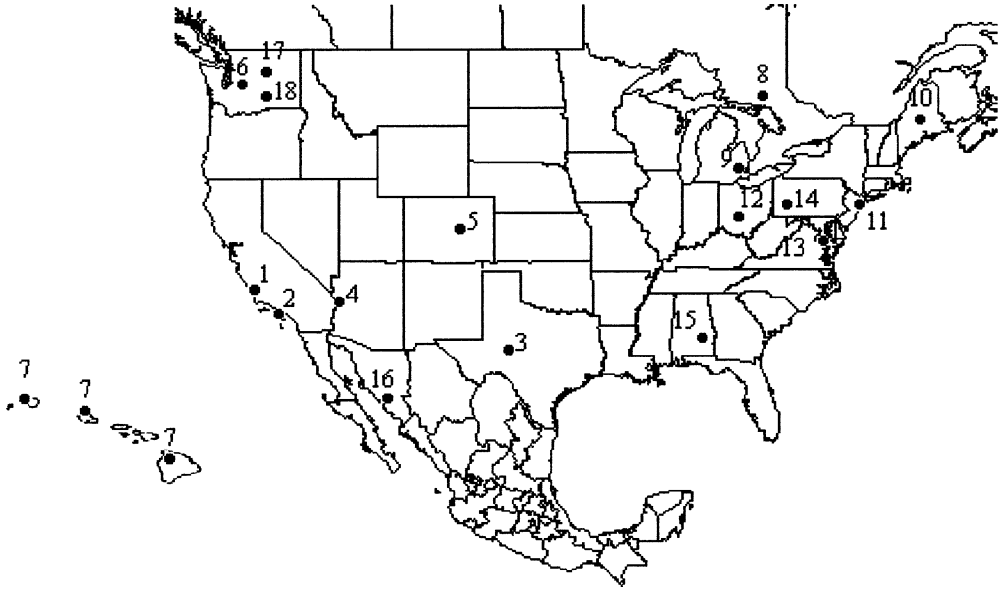

FIG. 1. Geographic locations of house finch populations, as well as outgroups sampled in this study. Circles represent populations. Numbers correspond to populations in Table 1.

1 mM EDTA). The diluted DNA samples were run on electrophorestic gels to determine DNA quality and quantity.

Amplified Fragment Length Polymorphism Procedure and Primer Screening

Our AFLP procedure followed the AFLP plant mapping kit protocol (Perkin Elmer, Foster City, CA) with modifications. Restriction digestion and adaptor ligation were performed simultaneously on 200 ng of genomic DNA using 7.5 units *EcoRI* (New England Biolabs, Beverly, MA), 1.5 units *MseI* (New England Biolabs), and 67 Weiss units of T4 DNA ligase (New England Biolabs). The restriction ligation reaction was performed in 11-μl volume at room temperature overnight. The restriction ligation products were subsequently diluted to 100 μl using $TE_{0.1}$ (20 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Polymerase chain reaction (PCR) amplifications were carried out in two steps as recommended by Vos et al. (1995). Preselective amplifications were performed by using 2 μl of the diluted, restricted, and ligated DNA, 0.5 μl *EcoRI* and *MseI* preselective primers, and 7.5 μl of AFLP core mix supplied in the kit in a final volume of 10 μl. The preselective primers consisted of the adaptor primer sequence with a single nucleotide extension at the 3′ end. This preselective PCR was performed with the following temperature profile: 2 min at 72°C followed by 20 cycles of 20 sec at 94°C, 30 sec at 56°C, and 2 min at 72°C, then a holding step at 60°C for 30 min. A 5-μl aliquot of each preselective amplification product was checked for quality on a 1.5% agarose gel. For selective amplification, 3 μl of the preselective amplified diluted DNA were added to 15 μl of AFLP core mix,

TABLE 1. Population sample information used in the present investigation.

Population	Population code	Species/ subspecies	Country/ State	Locality	Tissue for DNA	Sampling period	Sample size
1	CA-Goleta	*frontalis*	California	Goleta	tissue	March 1984	10
2	CA-Los Alamos	*frontalis*	California	Los Alamos	tissue	April 1989	10
3	TX	*frontalis*	Texas	San Angelo	tissue	September 1991	10
4	AR	*frontalis*	Arizona	Marana, Tucson	tissue	April 1989	10
5	CO	*frontalis*	Colorado	Boulder	tissue	April 1989	10
6	WA	*frontalis*	Washington	Kittitas and King Co.	tissue	1996–2000	6
7	HI	*frontalis*	Hawaii	Oahu, Mauna Kea, Maui	tissue	July 1991	17
8	Canada	*frontalis*	Canada	St. Catharines	tissue	January 1984	10
9	MI	*frontalis*	Michigan	Ann Arbor	blood	February 1991	10
10	ME	*frontalis*	Maine	south of Gorham	tissue	April 1991	10
11	NY	*frontalis*	New York	Mattituck Long Island	tissue	May 1990	10
12	OH	*frontalis*	Ohio	Cleveland	tissue	March 1990	10
13	MD	*frontalis*	Maryland	Laurel	tissue	May 1990	10
14	PA	*frontalis*	Pennsylvania	Quarryville	tissue	May 1990	10
15	AL	*frontalis*	Alabama	Auburn	blood	1995	10
16	Mexico	*griscomi*	Mexico	Guerrero	tissue	1990	10
17	purple finch	*purpureus*	Washington	Clark, Kittitas, Pacific Co.	tissue	1995–1998	3
18	Cassin's finch	*cassinii*	Washington	Asotin, Yakima	tissue	1994–1995	6

1 μl (5 pmol) of selective *MseI* primer, and 1 μl (1 pmol) of the *EcoRI* selective primer labeled with fluorescent dye (FAM, blue; or NED, yellow). Both the *EcoRI* and *MseI* primers used in the selective amplification have three extra nucleotides at the 3′ end to reduce the number of the amplified fragments. The cycle profile for the selective amplification began with a 2-min denaturation at 94°C, then followed by 10 cycles with 20-sec denaturation at 94°C, 30-sec annealing with the temperature decreased each cycle by 1°C from 66°C to 57°C, and elongation 2 min at 72°C. The PCR was continued for 20 cycles with 20 sec at 94°C, 30 sec at 56°C, and 2 min at 72°C, followed by a holding step at 60°C for 30 min.

After checking the PCR products (10 μl) on 1.5% agarose gels, 0.4 μl of blue-labeled and/or 0.75 μl of yellow-labeled amplification products were added to 1.05 μl of loading buffer containing 0.67 μl of deionized formamide, 0.13 μl of blue dextran, and 0.25 μl of GeneScan-500 (Perkin Elmer) ROX (red) labeled size standard. The samples were denatured for 3 min at 90°C, then run on an ABI Prism 377 DNA sequencer (Perkin Elmer) in a 5% Long Ranger (Combrex Bioscience, Rockland, ME) gel for 6 h with a well-to-read distance of 48 cm. The digital gel data was collected by ABI Prism GeneScan analysis software (ver. 3.1.2). Each lane file was analyzed for the presence and absence of AFLP products at approximately 1-bp intervals using Genographer software (Benham et al. 1999). With this labeling system, small fragments typically have a stronger fluorescence signal than larger fragments, and fluorescence signal decreases with increasing fragment size. We thus analyzed different parts of the gel with different intensity indices to visualize the fragments with maximum clarity. Only unambiguously detectable fragments were scored.

To determine the number of fragments generated by different primer pairs, 16 primer combinations (E-AAC, AAG, ACC, ACT/M-CAC, CAG, CTA, CTG, where E is *EcoRI* and M is *MseI*) were used in a preliminary screen of two individuals. Ten individuals from different populations were employed to confirm the preliminary screening results. Based on these results, three primer combinations (E-AAC/M-CTG, E-ACC/M-CAG, E-ACT/M-CTA) were chosen for all individuals. These primer combinations were selected because they produced a manageable number of appropriately sized, polymorphic, and well-separated markers.

Data Analysis

We considered each fragment position as a dominant locus with two states: presence or absence. Amplification products were scored as discrete, binary state (present/absent) for each individual and labeled by primer combination and estimated band size. A data matrix (individual x marker) containing the band scoring information was transformed to allele frequencies under the assumption that each amplified band corresponds to a different AFLP locus.

Because AFLP markers must be analyzed as dominant loci, the Hardy-Weinberg equilibrium assumption was made to estimate population genetic diversity and genetic structure parameters. Nei's (1973) gene diversity was obtained by the POPGENE version 1.31 program (Yeh et al. 1997). Gene diversity was also obtained by a Bayesian method (Holsinger et al. 2002), which does not assume that genotypes within populations are in Hardy-Weinberg proportions. The percentage of polymorphic loci was obtained by Tools for Population Genetic Analysis (TFPGA) software version 1.3 (Miller 1997a). The proportion of shared bands (F) was calculated using the RAPDPLOT program (Black 1996) based on Dice's (1945) similarity coefficient: $S_{xy} = 2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of fragments in common between x and y individuals, and Nx and Ny are the total number of fragments in x and y individuals, respectively (see also Nei and Li 1979). Nucleotide diversity (π) based on F, in principle directly comparable to sequence data, was estimated by the method of Innan et al. (1999). This method of estimating nucleotide diversity is based on the fact that each AFLP product represents a 16-bp sequence assay when using the *EcoRI* and *MseI* restriction enzymes, which have 6-bp and 4-bp recognition sequences, respectively, and three selective nucleotides on each of the two AFLP selective amplification primers. Therefore, each shared AFLP product indicates zero nucleotide differences over 16 bp, whereas polymorphisms reflect at least one nucleotide difference over 16 bp. The actual number of differences that contribute to each polymorphism is a function of F, which can be used to determine the overall number of nucleotide substitutions per site. The standard deviation of π was calculated by the jackknife method (Efron 1982) following Nei and Miller's (1990) approach.

Hierarchical structuring of genetic variation and pairwise Φ_{ST} distances (analogous to F_{ST}-statistics at the molecular level; Excoffier et al. 1992) among populations were measured using analysis of molecular variance (AMOVA) using WINAMOVA version 1.55 (Excoffier et al. 1992; Stewart and Excoffier 1996). Significance levels of the variance components were based on 1000 permutations. A pairwise Euclidean distance matrix and all input files needed for the AMOVA analysis were produced using the AMOVA-PREP program version 1.1 (Miller 1997b), which specifically prepares dominant marker data for Excoffier et al.'s WINAMOVA. The parameter θ^B, which is analogous to F_{ST}, was also calculated by the Bayesian approach (Holsinger et al. 2002), which incorporates uncertainty about the magnitude of within-population inbreeding.

Gene flow between pairs of populations based on the equation $Nm = 0.25(1/F_{ST} - 1)$ was calculated from Φ_{ST}-values under the assumption of an infinite-island model of population structure (Wright 1951). A matrix Mantel correspondence test (Mantel 1967) between genetic distances and geographical distances among populations was carried out using the TFPGA program. The geographical distances among populations were obtained by using Distance Finder (available via http://www.indo.com/distance).

A neighbor-joining tree for individuals were constructed from the mean character distance with PAUP* version 4.0b8 (Swofford 2001). Bootstrap support was evaluated with 1000 replicates. To obtain a tree in which populations are taxonomic units, gene frequency datasets was obtained by POPGENE version 1.31 program (Yeh et al. 1997). Then a bootstrapped neighbor-joining tree was constructed by various modules in the PHYLIP package (Felsenstein 1985) and visualized using TreeView version 1.6.1 (Page 1996).

TABLE 2. Amplified fragment length polymorphism primer pairs used and their amplification results.

Primer pair[1]	Size of fragments scored (bp)	Among *Carpodacus*			Within house finch			Within *frontalis* subspecies		
		Total bands	Polymorphic bands	Polymorphic bands (%)	Total bands	Polymorphic bands	Polymorphic bands (%)	Total bands	Polymorphic bands	Polymorphic bands (%)
E-AAC/M-CTG	80–499	130	84	64.6	92	57	62.0	90	56	62.2
E-ACC/M-CAG	79–489	131	100	76.3	99	64	64.7	94	58	61.7
E-ACT/M-CTA	80–485	102	74	72.6	78	45	57.7	76	43	56.6

[1] E, *EcoRI*; M, *MseI*.

Treating either populations or individuals at taxonomic units with AFLP data obsures details of gene history through pedigrees at several hierarchical levels, because trees at these levels are summaries of many independent gene genealogies. Therefore, a model-based clustering method for using multilocus genotype data to infer population structure and assign individuals to populations was implemented by the STRUCTURE program (Pritchard et al. 2000). This approach provides a coherent Bayesian framework for incorporating the inherent uncertainty of parameter estimates into the inference procedure and for evaluating the strength of evidence for the inferred clustering. We chose to use a burn-in period of 30,000 iterations and collect data for 10^6 iterations under a no-admixture model without using prior population origin information for running the program. For each dataset, we ran three independent simulations of this length. Highly consistent results were produced between independent runs.

RESULTS

Amplified Fragment Length Polymorphism Patterns and Polymorphism

The three primer combinations generate a total of 363 bands that range in size from 79 to 499 bp, of which 258 (71.2%) are polymorphic across all 172 individuals. A total of 166 (61.3%) bands are polymorphic across the 163 house finch individuals, and 157 (60.2%) bands are polymorphic across the 153 individuals representing the 15 populations from the eastern and western United States and Hawaiian Islands (*C. m. frontalis* subspecies; Table 2). Within house finches, the primer pair E-AAC/M-CTG generates the largest number of polymorphic bands and primer pair E-ACT/M-CTA generates the smallest number of polymorphic bands (Table 2). An example of an AFLP pattern reproduced by Genographer software with primer pair E-ACC/M-CAG is shown in Figure 2; visual inspection of this reproduced gel clearly shows the distinct AFLP patterns found between the three species analyzed and between Mexican and U.S. house finch populations. We find 12, 11, and 41 bands specific to (albeit not fixed within) *C. cassini*, *C. purpureus* and *C. mexicanus*, respectively, and three bands specific to but not fixed in the Mexican populations of house finches. However, no population-specific bands are detected among populations within the *C. m. frontalis* subspecies. The complete data matrix is available on the Web at http://depts.washington.edu/scotte/AFLPdata.

Population Genetic Diversity

Population genetic diversity descriptive parameters are summarized in Table 3. Within the *frontalis* subspecies, average heterozygosity ranges from 0.08 to 0.11, with an average of 0.10. Similar but slightly higher values are obtained with the Bayesian approach. The percentage of polymorphic loci ranges from 22.3 to 32.5, with an average of 29.9. Overall, average heterozygosity is relatively low and comparable to the levels for allozyme study (Vasquez-Phillips 1992). Estimated nucleotide diversity ranges from 0.0057 to 0.0085, with an average of 0.0075, implying that a random pair of house finches differs at approximately seven nucleotides per 1000 in the nuclear genome, a level that is relatively high compared to direct estimates in humans and other species from single nucleotide polymorphisms (Brumfield et al. 2003).

There is very little difference in levels of diversity between the western and eastern regions and the Hawaiian Islands. For all the descriptive parameters, the population from Canada shows the highest average heterozygosity and percent polymorphic loci, and the Washington population exhibits the lowest values for all of the descriptive statistics, a result that may be a consequence of the small sample size for this population.

Population Structure

We partitioned the molecular variances into species, subspecies, regions, population, and individual levels. AMOVA results recover deep divisions between the *griscomi* and *frontalis* subspecies within the house finch (Table 4). Of the total molecular variance within the house finch, 21% is attributable to divergence between subspecies and 71% is found among individuals within populations. However, when only the *frontalis* subspecies is considered, the vast majority (87.3%) of the total variance is found among individuals within populations. Similar results are obtained when analyses are carried out within the continental United States or within the eastern or western regions (data not shown).

Phylogenetic Analysis

Neighbor-joining trees are constructed with populations as units (Fig. 3). The phenograms reveal deep divisions between *C. mexicanus* and its congeners, and between *C. griscomi* in Mexico and *C. m. frontalis* subspecies within house finches. One cluster in the tree consisting solely of eastern populations is most closely related to the Goleta, California, population. Contrary to expectation from the known history, Hawaiian populations are not clearly derived from western populations, but instead fall outside all other *frontalis* populations. A similar pattern is found in trees constructed with PAUP programs with individuals as units, although bootstrap support is very low (not shown). Though individuals from each population

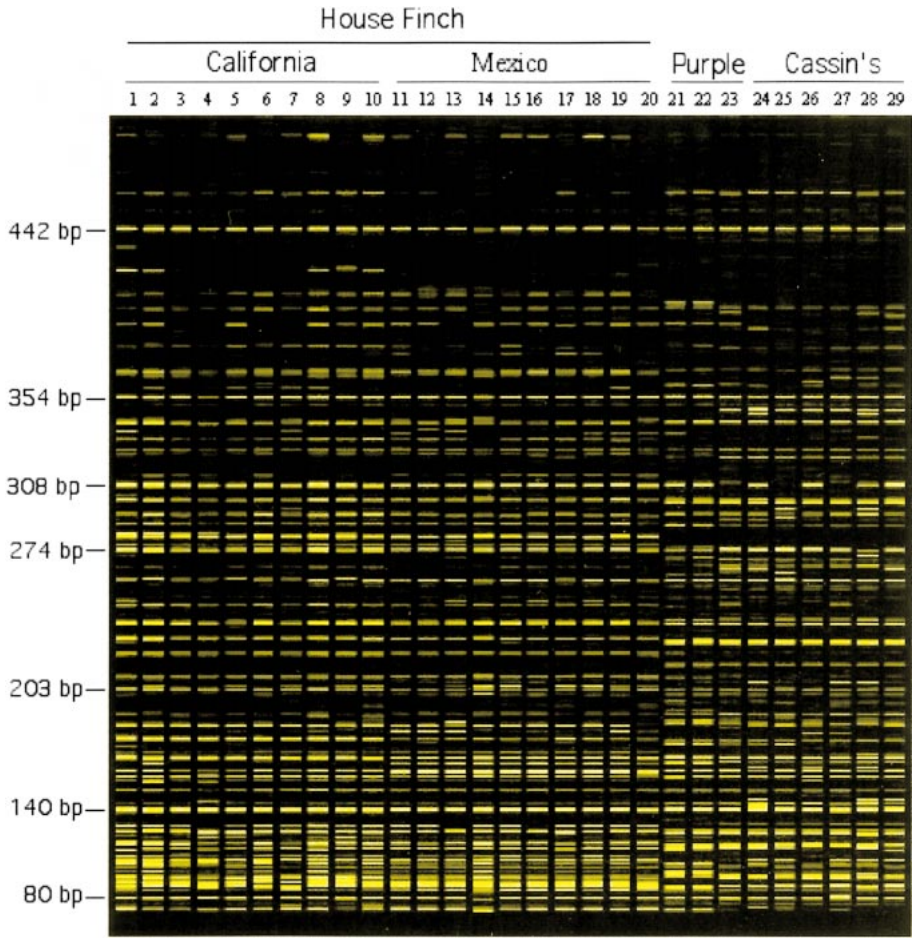

FIG. 2. Example gel reproduced by Genographer software showing amplified fragment length polymorphism products with primer pair E-ACC/M-CAG. Lanes 1–10 are CA-Goleta samples, 11–20 are Mexican population samples, 21–23 are purple finch samples, and 24–29 are Cassin's finch samples.

do not always cluster together, this tendency is evident for many western, eastern, and Hawaiian individuals. We also noticed that one supposed purple finch individual clusters strongly with Cassin's finch samples (100% bootstrap value). This individual was likely misidentified during collection or accessioning. The locality of this individual (Washington state) is consistent with its being a Cassin's finch.

Inferring Population Structure and Individual Assignment from Genotypes

A model-based Bayesian clustering method was used to infer population structure and assign individuals to populations for the house finch *frontalis* subspecies. To infer the number of populations, *k*, a series of populations are assumed for implementing the STRUCTURE program. The posterior probabilities of *k* were presented in Table 5. The natural logarithm of the probability of the data is lowest with $k = 1$ ($\ln = -10964.5$), highest with $k = 3$ ($\ln = -10355.2$), and decreases again for higher *k*. These results indicate that the house finch data contain at most three broadly distinct groups (Fig. 4). In terms of genome proportions, 90% of the genotypes of all individuals were estimated to be 100% from their populations of origin, and 10% of individuals were estimated to possess hybrid or backcross genotypes. One sample from Alabama (AL7) was assigned to the western region, with over 99.7% of its genome belonging to western group. We have checked the origin of this sample and found that it was sampled in the year it was hatched in Auburn, Alabama, and, for this and other reasons, could not be a mislabeled or misidentified sample. It therefore represents a rare case of gene flow from west to east or, equally likely, an inability of the program to correctly determine the genotype of this individual. In addition, two samples from Texas (TX6, TX8) were assigned to the Hawaiian cluster, with genome proportions of 64% and 70%, respectively. The one unassigned Hawaiian sample was still close to its cluster of origin with 60% of its genome belonging to the Hawaiian group. The western and eastern population datasets were also analyzed independently. However, no subpopulation structure was identified within them (data not shown). Like the AMOVA, this result indicates that there is little population structure within the western and eastern regions. When all populations including the Mexican birds were analyzed together, again without using prior population information, as expected, STRUCTURE found that $k = 4$ populations maximized the likelihood, which further confirms other analyses (data not shown).

TABLE 3. Descriptive statistics for amplified fragment length polymorphism variation in *Carpodacus* species.

Population	Average heterozygosity		% polymorphic loci (95% criterion)	Nucleotide diversity ($\times 10^3$)
	Nei[1]	Bayesian[2]		
House finch				
frontalis subspecies				
Western region				
CA-Goleta	0.10 ± 0.17	0.16 ± 0.0048	30.0	7.97 ± 4.15
CA-Los Alamos	0.10 ± 0.17	0.16 ± 0.0049	32.0	8.15 ± 4.04
TX	0.10 ± 0.17	0.15 ± 0.0050	30.0	8.06 ± 3.36
AR	0.10 ± 0.17	0.15 ± 0.0051	32.0	8.41 ± 4.45
CO	0.11 ± 0.17	0.16 ± 0.0048	31.4	5.69 ± 2.34
WA	0.08 ± 0.16	0.16 ± 0.0058	22.3	6.92 ± 3.13
Average	0.10 ± 0.17	0.16 ± 0.0051	29.6	7.54 ± 3.41
Hawaii	0.10 ± 0.16	0.14 ± 0.0038	30.6	6.86 ± 2.42
Eastern region				
Canada	0.11 ± 0.18	0.16 ± 0.0049	32.5	8.47 ± 3.94
MI	0.10 ± 0.18	0.16 ± 0.0047	30.0	7.84 ± 2.15
ME	0.09 ± 0.17	0.15 ± 0.0049	28.7	6.62 ± 1.29
NY	0.09 ± 0.17	0.16 ± 0.0047	29.5	8.09 ± 2.61
OH	0.11 ± 0.17	0.16 ± 0.0049	31.4	7.65 ± 2.47
MD	0.10 ± 0.17	0.16 ± 0.0047	31.1	7.36 ± 1.43
PA	0.10 ± 0.17	0.15 ± 0.0048	27.8	6.66 ± 2.02
AL	0.10 ± 0.17	0.16 ± 0.0048	28.7	7.91 ± 3.63
Average	0.10 ± 0.17	0.16 ± 0.0048	30.0	7.58 ± 2.35
Total average	0.10 ± 0.17	0.15 ± 0.0042	29.9	7.51 ± 0.79
Mexico	0.08 ± 0.15	0.15 ± 0.0051	22.9	8.57 ± 0.97
Purple finch	0.10 ± 0.17	0.27 ± 0.0068	25.6	14.32 ± 5.24
Cassin's finch	0.06 ± 0.14	0.24 ± 0.0065	16.8	8.83 ± 4.85

[1] Obtained by Nei's (1973) method.
[2] Obtained by Bayesian method (Holsinger et al. 2002).

Gene Flow among Populations

A matrix of pairwise F_{ST}-values and the inferred effective number of migrants (*Nm*) estimated for an island model is presented for western populations in Table 6. We focus on western populations in this case because only among these populations can we expect any sort of equilibrium between drift and migration to have been achieved. F_{ST}-values at this level are consistently very low, implying a large number of migrants exchanged between western populations per generation. The θ^B-value (0.067) obtained by the Bayesian method is close to but consistently higher than the F_{ST} results. What little graphical evidence for a relationship between geographic distance and F_{ST} is in the unexpected direction, with low F_{ST} values being associated with larger distances, and the relationship is not significant ($r = -0.297$; upper-tail probability: $P = 0.818$; lower-tail probability: $P = 0.186$; Fig. 5).

DISCUSSION

We employed AFLPs to study population genetics and geographic variation of native and introduced populations of the house finch. Our study is among the largest to date for birds, and, because the recent demographic history of house finches is well documented, it offers an opportunity to compare the known history of a taxon with that inferred by AFLPs. Analysis of nuclear loci allows us to understand more completely the mosaic of genealogical patterns evolving in genomes as a response to historical, demographic, and selective forces. We chose the AFLP technique both to examine the history of nuclear markers in this species and to generate a large number of markers for future studies aimed at determining the genetic basis of resistance to *Mycoplasma* in this species (Roberts et al. 2001). AFLPs suffer from being dominant markers, and it is still unknown how well they perform in comparison to other methods for assaying nuclear gene variation. Nonetheless, they appear to be particularly well suited for studies whose goals are to broadly survey genetic variation and find loci linked to specific phenotypic traits.

Phylogeographic Concordance

We predicted that both the eastern U.S. and Hawaiian populations should cluster phylogenetically with the California population, from which both were derived during the last century. Indeed the AFLP population tree suggested that eastern house finches were derived (albeit weakly) from a population near Goleta, California, a result that is consistent with the known history. However, the Hawaiian population was genealogically distinct not only from California but also from the entire clade of western North American populations. For two reasons, we believe these results stem from the fact that AFLP allele frequencies have shifted significantly in introduced populations. First, the unpublished electrophoretic study also recovered an unweighted pair group method with arithmetic mean (UPGMA) topology in which Hawaiian populations clustered outside not only California populations but all of the eastern and western populations studied (Vasquez-Phillips 1992). Second, the mitochondrial DNA study also found variation consistent with this result (Benner 1991). Here, Hawaiian birds possessed one widespread haplotype that was found in all other eastern and western U.S. popu-

TABLE 4. Analysis of molecular variance (AMOVA) for house finch amplified fragment length polymorphism data.

Source of variation	df	Φ-statistic[1]	% total	*P*-value
Analysis 1				
Among subspecies	1	$\Phi_{CT} = 0.212$	21.2	<0.001
Populations/subspecies	14	$\Phi_{SC} = 0.103$	8.15	<0.001
Individuals/populations	147	$\Phi_{ST} = 0.294$	70.65	<0.001
Analysis 2				
HI vs west vs east	2	$\Phi_{CT} = 0.077$	7.68	<0.001
Populations/regions	12	$\Phi_{SC} = 0.054$	5.03	<0.001
Individuals/populations	138	$\Phi_{ST} = 0.127$	87.29	<0.001
Analysis 3 (only western region)				
Among populations	5	$\Phi_{ST} = 0.045$	4.55	<0.001
Within population	50		95.45	<0.001
Analysis 4 (only eastern region)				
Among populations	7	$\Phi_{ST} = 0.057$	5.72	<0.001
Within population	72		94.28	<0.001

[1] CT, variance among groups of populations; SC, variance among the populations within group; ST, variance among the individuals within a population.

lations, as well as a rarer haplotype that was shared only with a population from Washington state. In addition, one haplotype (no. 3) that was found in very high frequency in the eastern populations but was not found at all in the western or Hawaiian populations. Although this was a relatively low-resolution study by today's standards, employing 16 enzymes to assay about 2.2% of the mitochondrial DNA molecule, the variation uncovered did not appear to link the Hawaiian or eastern populations specifically to the California population. Natural selection on AFLP markers in the eastern population could also be an explanation for the detectable shifts. However, no statistical tests for selection on AFLPs are known at this time.

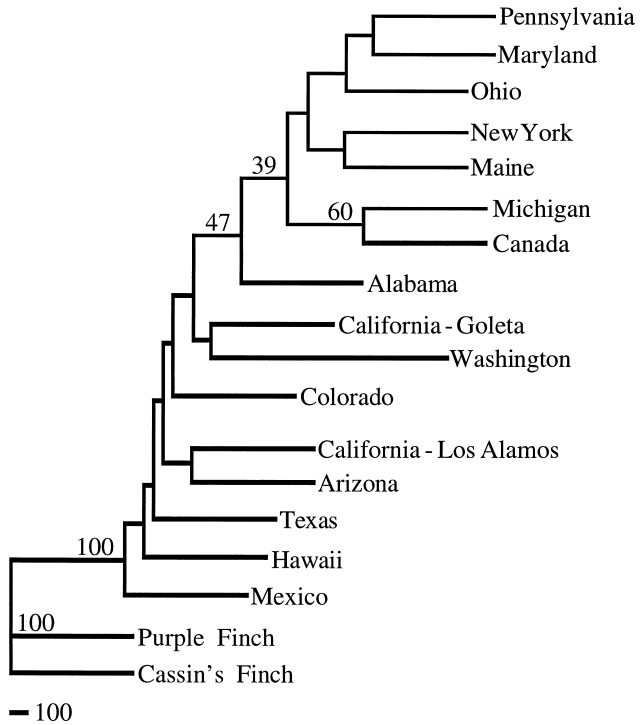

FIG. 3. Neighbor-joining tree based on gene frequency among populations with 363 amplified fragment length polymorphism markers. Bootstrap values (based on 1000 permutations) are indicated for each node when over 39%.

All three genetic markers therefore are consistent with a role for genetic drift in shifting allele frequencies such that the introduced populations are more distinct from their source than historical records would suggest, although this effect is stronger for the Hawaiian population. Still, the AFLP data did not suggest even a mild reduction of diversity indicated by potential bottlenecks in the eastern United States and Hawaiian Islands. As reported by a microsatellite study of the silvereye species complex (*Zosterops*), single founder events did not affect levels of heterozygosity or allelic diversity, nor did they result in immediate genetic differentiation between populations (Clegg et al. 2002). In both Clegg et al.'s and our studies, the number of founding birds was probably well above that required to result in substaintial losses of diversity (Nei et al. 1975). In addition, the duration of the bottleneck in each case was minimized and followed by rapid population expansion, a situation that would not be conducive to loss of AFLP diversity. Both allozymes and mitochondrial DNA have previously been shown to be able to detect founder events (Baker and Moeed 1987; Avise et al. 1988), and it appears that AFLP markers are also capable of detecting such shifts.

The population-assignment tests produced the strongest evidence that both introduced populations were genetically differentiated from their source population. Combined with the known history of eastern and Hawaiian house finches, these results verify the suggestion of genetic differentiation among the western, eastern, and Hawaiian populations in the tree analysis. These results therefore suggest greater confidence

TABLE 5. Inferring the number of clusters, *k*, by STRUCTURE program without using population priority information.

k	ln P ($x \mid k$)	P($k \mid x$)
1	−10964.5	~0
2	−10550.1	~0
3	−10355.2	~1
4	−10445.8	~0
5	−10730.1	~0

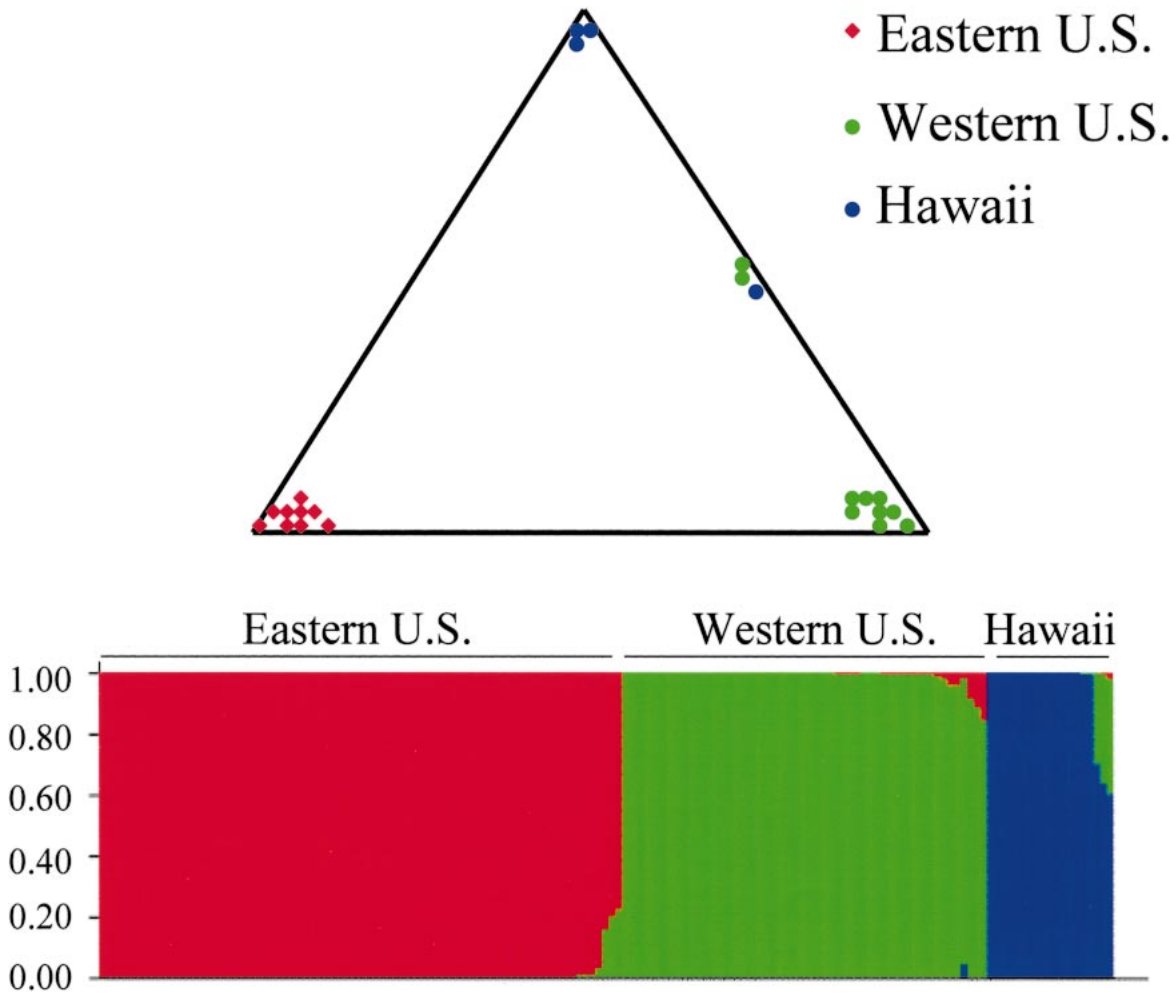

FIG. 4. Tripartite structure of the house finch inferred by STRUCTURE. Upper panel, triangle plot of individuals. Because some of the points overlap each other, the number of individuals is less than the numbers in the text. Individual AL7 from Alabama is assigned a green dot because its genotype is inferred to be 99.7% western. Lower panel, genotypic makeup for each individual in the three clusters. Each individual is represented by a thin vertical line. Genotypic fractions deriving from eastern United States, western United States, and Hawaiian populations are represented by red, green, and blue, respectively.

in the model-based Bayesian clustering method than in distance-based neighbor-joining trees. For example, the status of the Hawaiian population was not clearly resolved in the neighbor-joining tree of individuals (not shown). However, the STRUCTURE program almost exactly assigns all Hawaiian individuals into an independent cluster. Other studies have also reported superior performance of assignment tests versus tree clustering methods (Pritchard et al. 2000; Rosenberg et al. 2001a, b). We have not placed confidence limits on individual assignments, however, so the observed discreteness of the clusters could be less robust than it appears.

Across their range, house finches display substantial variation in body size, bill size and shape, wing and tail length, tarsus length, and plumage coloration (Moore 1939; Aldrich and Weske 1978; Aldrich 1982). Moreover, the size and shape of males and females varies somewhat independently across populations so that there is variation in dimorphism among populations (Badyaev and Hill 2000). The size and shape of males and females in each local population corre-

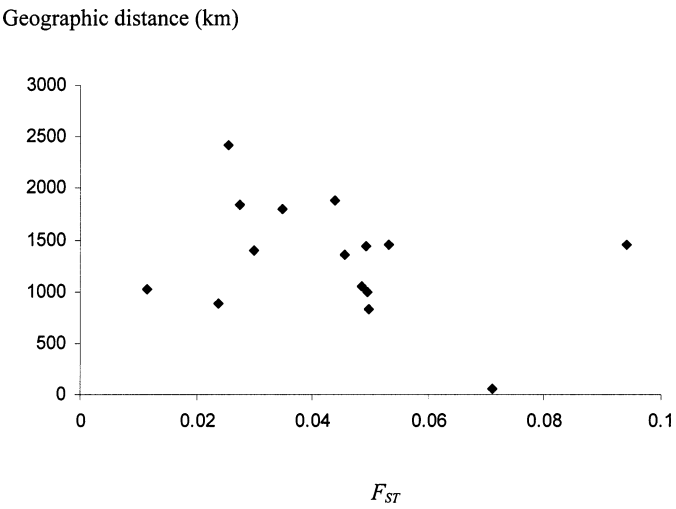

FIG. 5. Relationship between genetic differentiation (F_{ST}) and geographic distance (km) within western populations.

TABLE 6. F_{ST} distance and *Nm* values among populations in the western region. Values below the diagonal indicate pairwise F_{ST}, above the diagonal indicate the effective number of migrants (*Nm*). The upper numbers are values calculated from pairwise Euclidean distances (Excoffier et al. 1992); the bottom values are obtained by the Bayesian approach (Holsinger et al. 2002).

	CA-Goleta	TX	CA-Los Alamos	AR	CO	WA
CA-Goleta		8.875	3.271	4.770	4.831	8.139
		5.058	2.233	3.281	3.106	4.604
TX	0.0274		5.445	21.489	4.894	9.525
	0.0471		3.394	5.789	3.75	4.388
CA-Los Alamos	0.0710	0.0439		10.299	2.407	5.239
	0.1007	0.0686		3.650	2.243	3.266
AR	0.0498	0.0115	0.0237		4.811	6.955
	0.0708	0.0414	0.0641		3.242	3.889
CO	0.0492	0.0486	0.0941	0.0494		4.440
	0.0745	0.0625	0.1003	0.0716		3.432
WA	0.0298	0.0256	0.0456	0.0347	0.0533	
	0.0515	0.0539	0.0711	0.0604	0.0679	

sponds to the most fit phenotype for each sex in that population (Badyaev et al. 2000). Thus, there has been rapid evolution in populations within the *frontalis* subspecies. The variation in AFLP allele frequencies that we observed across populations within *C. m. frontalis* suggests that AFLP allele frequency has evolved rapidly within this subspecies. The Hawaiian population was more distinct from other *frontalis* populations than the known history of the group indicated it should have been, but it has also diverged more in morphology (Vasquez-Phillips 1992; Hill 1994; Badyaev and Hill 2000), and the novel environment of this population may have caused rapid evolution in both morphology and AFLP alleles.

Our population tree, as well as the tree of individual birds clearly indicates that purple finch and Cassin's finch are closely related to each other, whereas they are distinct from all populations of the house finch. Similar conclusions are obtained by Martin and Johnson (1986) based on allozyme loci analysis. They concluded that house finches likely diverged from a common ancestor of Cassin's and purple finches about 8.4 million years ago. Within the house finch clade, our AFLP results suggest that the Mexican population, *C. m. griscomi*, is strongly separated from *C. m. frontalis* populations, implying an old divergence. Based on morphology, Moore (1939) divided house finches into four species with a total of 18 subspecies. He suggested that *griscomi* and *frontalis* subspecies were isolated from each other during the Pleistocene. Hill (1994) also detected a distinct difference between the two subspecies after analyzing six morphological characters. Our results confirm the status of *griscomi* as a subspecies. It is tempting to construct an AFLP clock, perhaps using genetic distance of the proportion of shared fragments as a measure. However, our results suggest that, like allozymes, AFLP allele frequencies can shift rapidly in response to demographic events, making a correlation of such measures with time very weak in the short run.

Levels of Amplified Fragment Length Polymorphism Variability

Depending on the measure of genetic variation, our survey detected either lower or higher levels of estimated variation than other avian species. We first compared our estimated levels of AFLP heterozygosity with those found in other vertebrate species (Table 7). Although some of our populations likely have low variation due to small sample size (e.g., *cassini*), we find that the levels of heterozygosity and the proportion of polymorphic loci for house finches generally are considerably lower than in other taxa, including the geographically less widespread willow flycatcher (*Empidonax traillietimus*). Low heterozygosity has been a character of avian populations surveyed by similar methods (e.g., Nusser et al. 1996), but many other studies have detected considerable intrapopulation and intraspecific variation in recent years for both nuclear and mitochondrial genes using DNA sequencing (Baker et al. 1995; Friesen et al. 1997). The dominant nature of AFLP markers may underestimate heterozygosities revealed in house finch populations. It has been suggested that birds exhibit less DNA variability than many other taxa (Shields and Helm-Bychowski 1988), but some studies suggest otherwise (Baker et al. 1995). Mindell et al. (1996) discussed evidence for a rate slowdown at the DNA level and suggested that a higher avian body temperature might be responsible. However, avian enzymatic loci are by and large as variable as those of other vertebrates (Crochet 2000). It may be premature to ascribe the low AFLP variation to the techniques or estimation procedures, and future studies are warranted.

By contrast, if instead we focus on a per nucleotide estimate of genetic variability (π), we find that our results are comparable to and often higher than values derived from sequence data of nuclear loci in avian and other genomes (see Table 8). But there are few similar studies in avian populations with which to compare results. Bird mitochondrial DNA control region studies suggest that nucleotide diversity is quite comparable to our nuclear results (data not shown). There are several reasons that nucleotide diversity from AFLP dataset may be overestimated. First, the AFLP markers occur across the entire nuclear genome, including coding and noncoding regions and repetitive sequences. The latter two subgenomes evolve faster than coding regions, and so AFLPs may exhibit high diversity to the extent to which they occur in noncoding regions. For example, for single

TABLE 7. Average heterozygosity and proportion of polymorphic loci for amplified fragment length polymorphism markers in this and other studies.

Species	Average heterozygosity (*H*)	Proportion of polymorphic loci (*P*)	Individual numbers studied	Sample area	References
Willow flycatcher	0.221–0.348	52.6–89.7	3–34	southwestern US	Busch et al. (2000)
Anolis oculatus	not estimated	61.9	4	central Lesser Antilles	Ogden et al. (2002)
Anolisroquet	not estimated	62.9	15	central Lesser Antilles	Ogden et al. (2002)
Warblers	not estimated	37.7–63.6	15	western Pyrenees	Bensch et al. (2002)
Hawaiian crickets	not estimated	28–43	6–10	Hawaiian Islands	Parsons et al. (2001)
Herring gull assemblage	0.10–0.19	20–68	3–10	western Palearctic	De Knijff et al. (2001)
House finch (*frontalis* subspecies)	0.08–0.11	27.8–32.5	156	entire range	this study
House finch (*griscomi* subspecies)	0.08	22.9	10	southern Mexico	this study
Purple finch	0.10	25.62	3	WA	this study
Cassin's finch	0.06	16.80	6	WA	this study

anonymous noncoding loci in the pied flycatcher (*Ficedula hypoleuca*) the average nucleotide diversity ($\times 1000$) is 1.8×10^3 and 2.5×10^3 in the collared flycatcher (*F. albicollis*). For microsatellites, diversity is 4.5×10^3 in pied flycatcher and 5.7×10^3 in collared flycatcher; and for random clones is 2.9×10^3 in pied flycatcher and 2.3×10^3 in collared flycatcher (Primmer et al. 2002). Second, the algorithm employed in this method may overestimate the nucleotide diversity. DNA sequence data will always provide a more direct estimate of nucleotide diversity than will AFLP or other DNA fragment techniques. To verify this pattern and the suggestion of higher diversity in house finches, controlled studies employing both AFLPs and direct sequencing need to be done.

The fluorescent, semi-automated form of AFLP (fluorescent amplified fragment length polymorphism, FAFLP), permits analysis using internal size standards and direct comparison of AFLP patterns between samples on the same or other gels, thus increasing accuracy. It is also possible to increase efficiency by running up to three different AFLP amplifications in one lane. The digital nature of the data makes it suitable for rapid electronic dissemination, manipulation, interlaboratory comparison, and storage in databases. Genome-sequence-based FAFLP analysis of *Escherichia coli* shows accurate fragment size (± 1 bp), reproducibility and high discriminatory power (Arnold et al. 1999), and several studies have now demonstrated homology at the sequence level of comigrating AFLP bands (Rouppe van der Voort et al. 1997; Parsons and Shaw 2001). Finally, AFLPs are not strongly subject to ascertainment biases, because both variant and invariant fragments are scored (Brumfield et al. 2003). Thus, AFLPs are a handy way to scale up in genomewide scans of variation.

The avian genome is thought to contain less noncoding DNA than, for example, most mammals (Primmer et al. 1997; Waltari and Edwards 2002). Under these conditions AFLP surveys may interrogate a correspondingly higher fraction of coding than noncoding DNA in birds versus mammals and hence recover a lower proportion of overall diversity of birds. Thus, we can expect that genomic features, as well as historical and demographic processes, will likely contribute to the levels of variation detected by AFLP in different vertebrate groups.

ACKNOWLEDGMENTS

Samples from the house finch Washington population as well outgroup materials were provided by Burke Museum genetic resources collection, University of Washington. The Mexico population materials were provided by Museum of Zoology, University of Michigan. AL and MI samples were collected by GEH. All others are from ROM. The amplified fragment length polymorphism nucleotide diversity software was provided by S. Larson (Utah State University). We are grateful to P. Beardsley for his assistance in AFLP techniques and software. We thank Edwards' lab members for much help during the research and C. Hess and three anonymous reviewers for comments on the manuscript. This work was supported by National Science Foundation grants (DEB IRCEB 0077804) to GEH, SVE, and S. Roberts, and IBN9722171 to GEH.

TABLE 8. Estimates of nucleotide diversity for surveys of avian autosomes.

Species	Nucleotide diversity (π or θ)	Assay method[1]	References
House finches	0.0057–0.0086	AFLP markers	this study
Pied flycatcher	0.0018–0.0045	nuclear SNPs	Primmer et al. (2002)
Collared flycatcher	0.0025–0.0057	nuclear SNPs	Primmer et al. (2002)
Marbled murrelet	0.00007–0.0046	SSCPs and direct sequence of nuclear introns	Congdon et al. (2000)
Least auklet	0.0038	SSCPs and direct sequence of nuclear introns	Walsh and Friesen (2003)
Crested auklet	0.013		

[1] AFLP, amplified fragment length polymorphism; SNP, single nucleotide polymorphism; SSCP, single stranded conformation polymorphism.

Literature Cited

Ajmone-Marsan, P., A. Valentini, M. Cassandro, G. Vecchiotti Antaldi, G. Bertoni, and M. Kuiper. 1997. AFLP markers for DNA fingerprinting in cattle. Animal Genetics 28:418–426.

Albertson, R. C., J. A. Market, P. D. Danley, and T. D. Kocher. 1999. Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. Proc. Natl. Acad. Sci. USA 96: 5107–5110.

Aldrich, J. W. 1982. Rapid evolution in the house finch (*Carpodacus mexicanus*). J. Yamashina Inst. Ornith 14:1979–1986.

Aldrich, J. W., and J. S. Weske. 1978. Origin and evolution of the eastern house finch population. Auk 95:528–536.

American Ornithologists' Union. 1983. Checklist of North American birds. 6th edition. Allen Press, Lawrence, KS.

Arnold, C., L. Metherell, G. Willshaw, A. Maggs, and J. Stanley. 1999. Predictive fluorescent amplified-fragment length polymorphism analysis of *Eschericchia coli*: high-resolution typing method with phylogenetic significance. J. Clin. Microbiol. 37: 1274–1279.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Avise, J. C., R. M. Ball, and J. Arnold. 1988. Current versus historical population sizes in vertebrate species with high gene flow: a comparison based on mitochondrial DNA lineages and inbreeding theory for neutral mutations. Mol. Biol. Evol. 5: 331–344.

Badyaev, A. V., and G. E. Hill. 2000. The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. Evolution 54:1784–1794.

Badyaev, A. V., G. E. Hill, A. M. Stoehr, P. M. Nolan, and K. J. McGraw. 2000. The evolution of sexual dimorphism in the house finch. II. Population divergence in relation to local selection. Evolution 54:2134–2144.

Badyaev, A. V., G. E. Hill, M. L. Beck, A. A. Dervan, R. A. Duckworth, K. J. McGraw, P. M. Nolan, and L. A. Whittingham. 2002. Sex-biased hatching order and adaptive population divergence in a passerine bird. Science 295:316–318.

Baker, A. J., and A. Moeed. 1987. Rapid genetic differentiation and founder effect in colonizing populations of common mynas (*Acridotheres tristis*). Evolution 41:525–538.

Baker, A. J., C. H. Daugherty, R. Colbourne, and J. L. Mclennan. 1995. Flightless brown kiwis of New Zealand posess extremely subdivided population structure and cryptic species like small mammals. Proc. Natl. Acad. Sci. USA 92:8254–8258.

Barton, N., and A. Clark. 1990. Population structure and process in evolution. Pp. 115–173 *in* K. Wohrmann and S. K. Jain, eds. Population biology: ecological and evolutionary viewpoints. Springer-Verlag, Berlin.

Benham, J., J. U. Jeung, M. Jasieniuk, V. Kanazin, and T. Blake. 1999. Genographer: a graphical tool for automated AFLP and microsatellite analysis. J. Agric. Genomics 4:Article 4.

Benner, W. L. 1991. Mitochondrial DNA variation in the house finch (*Carpodacus mexicanus*). M.Sc. thesis, Cornell University, Ithaca, NY.

Bensch, S., A. J. Helbig, M. Salomon, and I. Seibold. 2002. Amplified fragment length polymorphism analysis identifies hybrids between two subspecies of warblers. Mol. Ecol. 11:473–481.

Black, W. C. I. 1996. RAPDPLOT 2.4. Dept. of Microbiology, Colorado State Univ., Fort Collins, CO.

Bock, C. E., and L. W. Lepthien. 1976. Growth in the eastern house finch population, 1962–1971. Am. Birds 30:791–792.

Brumfield, R. T., P. Beerli, D. A. Nickerson, and S. V. Edwards. 2003. The utility of single nucleotide polymorphism (SNPs) in inferences of population history. Trends Ecol. Evol. 18:249–256.

Busch, J. D., M. P. Miller, E. H. Paxton, M. K. Sogge, and P. Keim. 2000. Genetic variation in the endangered southwestern willow flycatcher. Auk 117:586–595.

Clegg, S. M., S. M. Degnan, J. Kikkawa, C. Moritz, A. Estoup, and I. P. Owens. 2002. Genetic consequences of sequential founder events by an island-colonizing bird. Proc. Natl. Acad. Sci. USA 99:8127–8132.

Congdon, B. C., J. F. Piatt, K. Martin, and V. L. Friesen. 2000. Mechanism of population differentiation in marbled murrelets: historical versus contemporary process. Evolution 54:974–986.

Crochet, P. A. 2000. Genetic structure of avian populations: allozymes revisited. Mol. Ecol. 9:1463–1469.

De Knijff, P., F. Denker, N. D. van Swelm, and M. Kuiper. 2001. Genetic affinities within the herring gull *Larus argentatus* assemblage revealed by AFLP genotyping. J. Mol. Evol. 52:85–93.

Dice, L. R. 1945. Measures of the amount of ecological association between species. Ecology 26:297–302.

Efron, B. 1982. The jackknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia, PA.

Elliot, J. J., and R. S. Arbib. 1953. Origin and status of the house finch in the eastern United States. Auk 70:31–37.

Emerson, B. C., E. Paradis, and C. Thebaud. 2001. Revealing the demographic histories of species using DNA sequences. Trends Ecol. Evol. 16:707–716.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791.

Foster, S. A., R. J. Scott, and W. A. Cresko. 1998. Nested biological variation and speciation. Proc. R. Soc. Lond. B 353:207–218.

Friesen, V. L., B. C. Congdon, H. E. Walsh, and T. P. Birt. 1997. Intron variation in marbled murrelets detected using analysis of single stranded conformational polymorphisms. Mol. Ecol. 6: 1047–1058.

Giannasi, N., R. S. Thorpe, and A. Malhotra. 2001. The use of amplified fragment length polymorphism in determining species trees at fine taxonomic levels: analysis of a medically important snake, *Trimeresurus albolabris*. Mol. Ecol. 10:419–426.

Grinnell, J. 1911. The linnet of the Hawaiian Islands: a problem in speciation. Univ. Calif. Publ. Zool. 7:79–95.

Hill, G. E. 1994. Geographic variation in male ornamentation and female mate preference in the house finches: a comparative test of models of sexual selection. Behav. Ecol. 5:64–73.

———. 1996. Subadult plumage in the house finch and tests of models for the evolution of delayed plumage maturation. Auk 113:858–874.

———. 2002. A red bird in a brown bag: the function and evolution of colorful plumage in the house finch. Oxford Univ. Press, New York.

Hochachka, W. M., and A. A. Dhondt. 2000. Density-dependent decline of host abundance resulting from a new infectious disease. Proc. Natl. Acad. Sci. USA 99:5303–5306.

Holsinger, K. E., P. O. Lewis, and D. K. Dey. 2002. A Bayesian approach to inferring population structure from dominant markers. Mol. Ecol. 11:1157–1164.

Innan, H., R. Terauchi, G. Kahl, and F. Tajima. 1999. A method for estimating nucleotide diversity from AFLP data. Genetics 151:1157–1164.

Kempenaers, B., B. Congdon, P. Boag, and R. J. Robertson. 1999. Extrapair paternity and egg hatchability in tree swallows: evidence for the genetic compability hypothesis? Behav. Ecol. 10: 304–311.

Kuhner, M. K., J. Yamato, and J. Felsenstein. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429–434.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Martin, J. A., and N. K. Johnson. 1986. Genetic relationships of North American cardueline finches. Condor 88:409–420.

Miller, M. P. 1997a. Tools for population genetic analysis (TFPGA). Ver. 1.3. A windows program for the analysis of allozyme and molecular population genetic data. Dept. of Biological Sciences, Northern Arizona Univ., Flagstaff, AZ.

———. 1997b. AMOVA-PREP: a program for the preparation of input files for use with WINAMOVA. Dept. of Biological Sciences, Northern Arizona Univ., Flagstaff, AZ.

Mindell, D. P., A. Knight, C. Baer, and C. J. Huddleston. 1996. Slow rates of molecular evolution in birds and the metabolic

rate and body temperature hypotheses. Mol. Biol. Evol. 13: 422–426.
Moore, R. T. 1939. A review of the house finches of the subgenus *Burrica*. Condor 33:177–205.
Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70:3321–3323.
Nei, M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.
Nei, M., and J. C. Miller. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction site data. Genetics 125:873–879.
Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variation in populations. Evolution 29:1–10.
Nusser, J. A., R. M. Goto, D. B. Ledig, R. C. Fleischer, and M. M. Miller. 1996. RAPD analysis reveals low genetic variability in the endangered light-footed clapper rail. Mol. Ecol. 5:463–472.
Ogden, R., and R. S. Thorpe. 2002. The usefulness of amplified fragment length polymorphism markers for taxon discrimination across graduated fine evolutionary levels in Caribbean *Anolis* lizards. Mol. Ecol. 11:437–445.
Otsen, M., M. D. Bieman, and M. T. R. Kuiper. 1996. Use of AFLP markers for gene mapping and QTL detection in the rat. Genomics 37:289–294.
Page, R. D. M. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput. Appl. Biosci. 12: 357–358.
Parsons, Y. M., and L. Shaw. 2001. Species boundaries and genetic diversity among Hawaiian crickets of the genus *Laupala* identified using amplified fragment length polymorphism. Mol. Ecol. 10:1765–1772.
Polanski, A., M. Kimmel, and R. Chakraborty. 1998. Application of a time-dependent coalescence process for inferring the history of population size changes from DNA sequence data. Proc. Natl. Acad. Sci. USA 95:5456–5461.
Power, D. M. 1979. Evolution in peripheral isolated populations of *Carpodacus* finches on the California Islands. Evolution 33: 834–847.
Primmer, C. R., T. Raudsepp, B. P. M. Chowdhary, A. P. Møller, and H. Ellegren. 1997. Low frequency of microsatellites in the avian genome. Genome Research 7:471–482.
Primmer, C. R., T. Borge, J. Lindell, and G. P. Saetre. 2002. Single-nucleotide polymorphism characterization in species with limited available sequence information: high nucleotide diversity revealed in the avian genome. Mol. Ecol. 11:603–612.
Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.
Questiau, S., M. C. Eybert, and P. Taberlet. 1999. Amplified fragment length polymorphism (AFLP) markers reveal extra-pair parentage in a bird species: the bluethroat (*Luscinia svecica*). Mol. Ecol. 8:1331–1339.
Roberts, S. R., P. M. Nolan, L. H. Lauerman, L. Q. Li, and G. E. Hill. 2001. Characterization of the mycoplasmal conjunctivitis epizootic in a house finch population in the Southeastern USA. J. Wildl. Dis. 37:82–88.
Rosenberg, N. A., E. Woolf, J. K. Pritchard, T. Schaap, D. Gefel, I. Shpirer, U. Lavi, B. Bonne-Tamir, J. Hillel, and M. W. Feldman. 2001a. Distinctive genetic signatures in the Libyan Jews. Proc. Natl. Acad. Sci. USA 98:858–863.
Rosenberg, N. A., T. Burke, K. Elo, M. W. Feldman, P. J. Freidlin, M. A. M. Groenen, J. Hillel, A. Maki-Tanila, M. Tixier-Boichard, A. Vignal, K. Wimmers, and S. Weigend. 2001b. Empirical evaluation of genetic clustering methods using multilocus genotypes from 20 chicken breeds. Genetics 159:699–713.
Rouppe van der Voort, J. N. A. M., P. van Zandvoort, H. J. van Eck, R. T. Folkertsma, R. C. B. Hutten, J. Draaistra, F. J. Gommers, E. Jacobsen, J. Helder, and J. Bakker. 1997. Use of allele specificity of comigrating AFLP markers to align genetic maps from different potato genotypes. Mol. Gen. Genet. 255:438–447.
Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. Can. J. Zool. 69: 82–90.
Shields, G. F., and K. M. Helm-Bychowski. 1988. Mitochondrial DNA of birds. Pp. 273–295 *in* R. F. Johnston, eds. Current ornithology. Plenum Press, New York.
Slatkin, M. 1994. Gene flow and population structure. Pp. 3–17 *in* L. A. Real, eds. Ecological genetics. Princeton Univ. Press, Princeton, NJ.
Sprenkle, J. M., and C. R. Blem. 1984. Metabolism and food selection of eastern house finches. Wilson Bull. 96:184–195.
Stangel, P. W. 1985. Incomplete first prebasic molt of Massachusetts house finches. J. Field Ornithol. 56:1–8.
Stewart, C. N., and L. Excoffier. 1996. Assessing population genetic structure and variability with RAPD data: application to *Vaccinium macrocarpon* (American cranberry). J. Evol. Biol. 9: 153–171.
Storfar, A. 1996. Quantitative genetics: a promising approach for the assessment of genetic variation in endangered species. Trends Ecol. Evol. 11:343–348.
Sunnucks, P. 2000. Efficient genetic markers for population biology. Trends Ecol. Evol. 15:199–203.
Swofford, D. L. 2001. PAUP*: phylogenetic analysis using parsimony (* and other methods). Ver. 4. Sinauer Associates, Sunderland, MA.
Szmidt, A. E., X. R. Wang, and M. Z. Lu. 1996. Empirical assessment of allozyme and RAPD variation in *Pinus sylvestris* (L.) using haploid tissue analysis. Heredity 76:412–420.
Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7:381–397.
Travis, S. E., J. Maschinski, and P. Keim. 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically endangered plant, using AFLP markers. Mol. Ecol. 5:735–745.
Vasquez-Phillips, M. A. 1992. Population differentiation of the house finch in North America and the Hawaii Islands. M.S. thesis, University of Toronto, Toronto, Canada.
Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, and M. Kuiper. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.
Walsh, H. E. and V. L. Friesen. 2003. A comparison of Intra-specific patterns of DNA sequence variation in mitochondrial DNA α-enolase, and MHC class II B loci in auklets (Charadriiformes: Alcidae.). J. Mol. Evol. *In press*.
Waltari, E., and S. V. Edwards. 2002. The evolutionary dynamics of intron size, genome size, and physiological correlates in archosaurs. Am. Nat. 160:539–552.
Weiss, G., and A. von Haesler. 1998. Inference of population history using likehood approach. Genetics 149:1539–1546.
Wright, S. 1951. The genetical structure of populations. Ann. Eugenics 15:323–354.
Wootton, J. T. 1986. Clutch size difference in western and introduced eastern populations of house finches: patterns and hypotheses. Wilson Bull. 98:459–462.
Yeh, F. C., and T. J. B. Boyle. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belg. J. Bot. 129:157.

Corresponding Editor: T. Smith