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Phylogeography of the mountain chickadee (*Poecile gambeli*): diversification, introgression, and expansion in response to Quaternary climate change

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Abstract

Since the late 1990s, molecular techniques have fuelled debate about the role of Pleistocene glacial cycles in structuring contemporary avian diversity in North America. The debate is still heated; however, there is widespread agreement that the Pleistocene glacial cycles forced the repeated contraction, fragmentation, and expansion of the North American biota. These demographic processes should leave genetic 'footprints' in modern descendants, suggesting that detailed population genetic studies of contemporary species provide the key to elucidating the impact of the late Quaternary (late Pleistocene–Holocene). We present an analysis of mitochondrial DNA (mtDNA) variation in the mountain chickadee (*Poecile gambeli*) in an attempt to examine the genetic evidence of the impact of the late Quaternary glacial cycles. Phylogenetic analyses reveal two strongly supported clades of *P. gambeli*: an Eastern Clade (Rocky Mountains and Great Basin) and a Western Clade (Sierra Nevada and Cascades). Post-glacial introgression is apparent between these two clades in the Mono Lake region of Central California. Within the Eastern Clade there is evidence of isolation-by-distance in the Rocky Mountain populations, and of limited gene flow into and around the Great Basin. Coalescent analysis of genetic variation in the Western Clade indicates that northern (Sierra Nevada/Cascades) and southern (Transverse/Peninsular Ranges) populations have been isolated and evolving independently for nearly 60 000 years.

Keywords: divergence times, gene flow, incomplete lineage sorting, phylogeography, Poecile, Quaternary

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Introduction

The biotic consequences of Quaternary climate change have been an intensely studied topic in evolutionary biology for decades (e.g. Anderson 1948, 1949, 1953; Mengel 1964; Remington 1968; Hubbard 1973; Hewitt 1996, 1999, 2000, 2004; Klicka & Zink 1997; Willis & Whittaker 2000; Lascoux *et al*. 2004). The fact that the Pleistocene glacial cycles resulted in profound shifts in the ranges of temperate species, including the repeated contraction, fragmentation, and subsequent expansion of temperate biotas, is evidenced in fossil and pollen records (Bennett 1998; Williams *et al*. 1998; Graham 1999). These demographic processes have left genetic 'footprints' in modern descendent species and biotas. Through the application of modern molecular techniques and advances in molecular data analysis, including phylogeography (Avise 2000, 2004; Knowles & Maddison 2002) and coalescent theory based analyses (Edwards & Beerli 2000; Zink *et al*. 2000; Knowles & Maddison 2002; Avise 2004), studies have begun to reveal the genetic consequences of Quaternary climate change. In Europe, studies of genetic variation across a myriad of taxa have led to the development of well-supported hypotheses for the Quaternary biogeography of this continental biota (Hewitt 1996, 2000, 2004). On the other hand, studies of

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North American taxa have indicated that although common biogeographic patterns do exist, these tend to be habitat specific and in many cases temporally idiosyncratic (aridlands, Riddle *et al*. 2000; Zink *et al*. 2001; Pacific northwest mesic forests, Brunsfeld *et al*. 2002; Carstens *et al*. 2005; California floristic region, Calsbeek *et al*. 2003; alpine meadows, DeChaine & Martin 2005). Thus, the detailed phylogeographic study of organisms occupying specific habitats remains critical to the collective understanding of the genetic consequences of Quaternary climate change in North America.

The fauna that inhabit the xeric coniferous forests of western North America remain a group that has received little phylogeographic study. Palynologic (Graham 1999) and fossil (Williams *et al*. 1999) data indicate that the coniferous forests of western North America were dramatically displaced by the climatic oscillations of the Quaternary. There also exists phylogeographic data from the xeric coniferous forests of western North America suggesting that they were fragmented during the Quaternary climatic oscillations. In a study of genetic variation in ponderosa pine (*Pinus ponderosa*, one of the dominant tree species in western North American xeric coniferous forests), Latta & Mitton (1999) described the historical isolation of Sierra Nevada/Cascade (western) populations of ponderosa pine from Rocky Mountain (eastern) populations and recent contact and introgression of the historical isolates in Montana. They hypothesized that the genetic divergence observed in ponderosa pine populations was due to isolation of populations in refugia during the Quaternary glacial advances. Although there is considerable evidence western North American coniferous forest habitats were dramatically impacted by Quaternary events, the impact these events had on the population structure of the resident fauna remains poorly understood. In a phylogeographic study of the blue grouse (*Dendragopus obscurus*), a common resident of western coniferous forests, Barrowclough *et al*. (2004) demonstrated that the genetic consequences of Quaternary climate change were likely twofold. First, three distinct phylogroups (a group of haplotypes that are more closely related to each other than they are to any haplotype outside the group, Avise 2000) of blue grouse distributed in the Sierra Nevada/Cascade Mountains, northern Rocky Mountains, and southern Rocky Mountains, respectively, most likely diverged from one another during the Quaternary. The origination of divergence among these distinct phylogroups was attributed to glacier-induced fragmentation that originally isolated populations in the Sierra Nevada and Cascade mountain ranges from those in the Rocky Mountains, as first described by Miller (1951), Mengel (1964), and Remington (1968). Second, the distinct phylogroups have recently (Holocene) come into contact, resulting in the introgression of these groups; however, the introgression appears to be limited and localized to the forests of eastern Washington. Whether the pattern of genetic variation observed in blue grouse is shared among other western montane fauna in North America remains to be seen.

In this study, we use phylogeographic methods to infer the genetic structure and population history of the mountain chickadee (*Poecile gambeli*). The mountain chickadee is a common permanent resident of the xeric coniferous forests of western North America. It is well suited for phylogeographic study because it exhibits extreme philopatry, limited altitudinal migration during winter, a patchy distribution, and marked geographic variation in morphology. Mountain chickadees are cavity-nesting birds and thus require mature woodland for breeding (McCallum *et al*. 1999). They also cache conifer seeds as they become available, and the defense of these caches requires the formation of tight social groups normally comprised of several breeding pairs (McCallum 1990; Ekman 1989; Matthysen 1990). Social groups often dissolve during winter as individuals move from higher to lower elevations, where they can be found in pinyon-juniper and low elevation riparian habitats on their native mountain ranges (McCallum *et al*. 1999). However, seasonal migrants return to their same social groups in spring and mate within these groups for the duration of their life (McCallum *et al*. 1999). In mountain chickadees, natal dispersal is believed to be limited to adjacent social groups; however, a full characterization of natal dispersal has yet to be conducted (McCallum *et al*. 1999). These life history traits suggest that the mountain chickadee may be employed in a comparative phylogeographic framework to assess the generality of the blue grouse pattern for understanding the influences of Pleistocene climate changes on fauna associated with coniferous forests (Barrowclough *et al*. 2004).

Although the taxonomic status of the mountain chickadee itself has not been called into question, the validity of the number of subspecies occupying its broad geographic distribution has. The 1957 American Ornithologists' Union (AOU) Check-list of North American Birds officially recognized the following six subspecies of mountain chickadee: *P. g. gambeli* (MT, WY, eastern UT, CO, AZ, NM, TX), *P. g. grinnelli* (BC, WA, OR, ID), *P. g. inyonensis* (southern ID, NV, western UT, southeastern CA), *P. g. abbreviatus* (central and northern CA, OR, western NV), *P. g. baileyi* (southern CA), and *P. g. atratus* (northern Baja CA). These subspecies were based primarily on clinal variation in plumage colouration (buff in the east grading to grey in the west) and bill shape (Behle 1956). Behle (1956) argued that to more accurately capture the geographic variation observed in mountain chickadees, the seven subspecies should be condensed into three groups (Fig. 1): *gambeli* (Rocky Mountains), *baileyi* (southern CA, Sierra Nevada, Cascades), and *inyoensis* (Great Basin). He believed that the morphological differences observed between these three

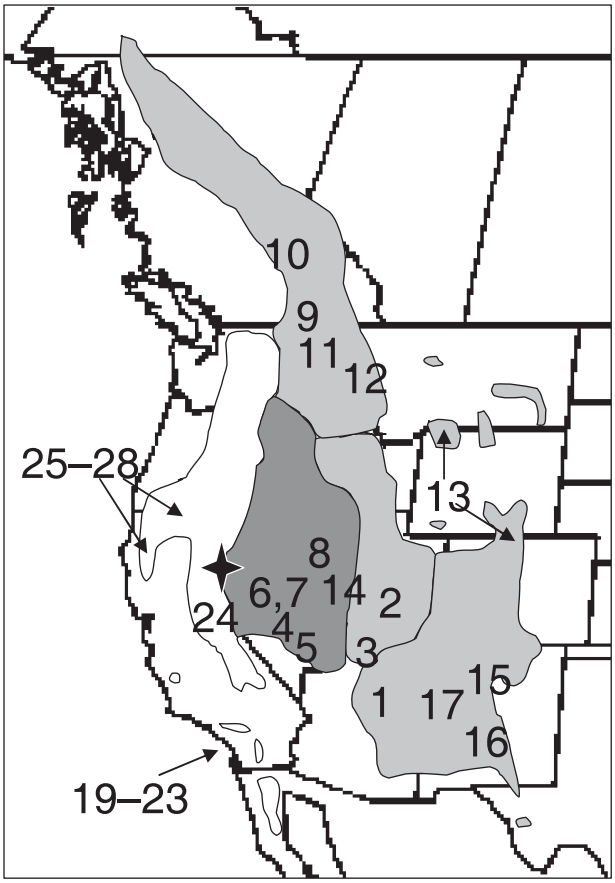

Fig. 1 Geographic distribution of the mountain chickadee and sampling localities. Only sampling localities with sample sizes ≥ 9 are shown. The shaded areas correspond to the three major morphological groups described by Behle (1956): *gambeli* — light grey, *inyoensis* — dark grey, and *baileyi* — white. The divisions illustrated in the light grey area mark the subspecies described within the Gambeli group. The numbers on the map mark the sampling localities listed on Table 1. The four pointed star corresponds to the Mono Lake, CA population where introgression between the two main clades was detected.

groups could only be explained by prolonged isolation. There is some evidence that these morphologic differences may be corroborated by genetic data. Gill *et al*. (1993, 2005) discovered that individuals from southern AZ and southern CA are approximately 2.6%–4.5% divergent from one another in terms of their mtDNA. However, the geographic distribution of these two lineages and full extent of the genetic variation within mountain chickadees remains unexplored.

We analysed mitochondrially encoded ND2 sequences from sampled populations from throughout the distribution of the mountain chickadee. These data were used to evaluate the population structure and evolutionary history of the species and make inferences regarding the impact of Quaternary events on its genetic 'footprint'.

Methods

Samples and laboratory techniques

Tissue samples were obtained for 320 individuals from 31 different localities and represented all but one (*P. g. atratus*; northern Baja California) of the recognized subspecies of mountain chickadee (see Table S1, Supplementary material, for complete details). Whenever possible we sampled 10 individuals from each location, because in coalescence theory variances do not decrease when sample sizes of over 10 are used (Harding 1996). Samples of *P. atricapilla* and *P. sclateri* were obtained from the Marjorie Barrick Museum (MBM) as outgroup taxa, following Gill *et al*. (1993, 2005). Total genomic DNA was extracted from all specimens using either a DNeasy tissue extraction kit (Qiagen, Valencia, CA) following the manufacturer's protocol, or a phenol-chloroform procedure. We amplified the nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 2 (ND2) gene using the primers L5215 (Hackett 1996) and H6313 (Johnson & Sorenson 1998), and sequenced using L5215 and Hgambeli (this project). The ND2 gene evolves rapidly and has proven useful for uncovering intraspecific genetic structure in Passeriform birds (Drovetski *et al*. 2004; Zink *et al*. 2006). All fragments were amplified in 12.5 μL reactions under the following conditions: denaturation at 94 °C, followed by 40 cycles of 94 °C for 30 s, 54 °C for 45 s, and 72 °C for 1 minute. This was followed by a 10 minute extension at 72 °C and a 4 °C soak. Products were purified using a Qiagen PCR Purification Kit or Exosap-IT (USB Corporation) purification, following the manufacturer's protocols. We performed 20 μL BigDye (ABI) sequencing reactions using 20–40 ng of purified and concentrated PCR product following standard ABI protocols. Sequencing reactions were purified using a magnetic bead clean-up procedure designed by Agencourt Biosciences and run on an ABI 3100-*Avant* automated sequencer. Complementary strands of each gene were unambiguously aligned using Sequencher 4.2 (Gene Codes Corporation). All sequences were translated and compared to the *Gallus* ND2 sequence (Desjardins & Morais 1990) to confirm the correct reading frame and to check for the presence of stop codons.

Phylogenetic methods

Maximum parsimony (MP) and maximum likelihood (ML) phylogenetic analyses were used to identify major clades and evaluate relationships among haplotypes. All MP analyses were performed in PAUP* 4.10b (Swofford 2001) using a heuristic search with 10 random sequence repetitions and tree-bisection-reconnection (TBR) branch-swapping. ML analyses were performed using TREEFINDER (Jobb *et al*. 2004; Ver. October 2005). MODELTEST 3.06 (Posada & Crandall 1998) and the AIC model selection criterion

(Shapiro *et al*. 2006), with the outgroup sequence removed, were used to select a model of sequence evolution to be used for ML phylogeny reconstruction. This model was then set as the model of sequence evolution for the TREEFINDER reconstructions. Non-parametric bootstrapping (100 replicates; Felsenstein 1985) performed in the programs PAUP* (MP) and TREEFINDER (ML) was used to evaluate nodal support among clades, with 70% or greater considered to provide strong support (Hillis & Bull 1993). Once clades were identified, we used the program NETWORK (Bandel *et al*. 1999) to construct median joining networks to visualize relationships among haplotypes within these clades.

To determine when the divergences between major clades occurred and whether or not they corresponded best with Quaternary events, divergence times between clades were estimated using the program MDIV (Nielsen & Wakeley 2001). MDIV uses a Bayesian approach to simultaneously approximate the posterior distribution of three parameters: divergence time between populations ($T = t_{div}/2N_e$), the migration rate between populations since divergence ($M = 2N_e m$), and the population parameter theta ($\theta = 2N_e\mu$, where μ is the per locus mutation rate). The program was first run using default search settings and default priors (for theta and divergence time). Following this data exploration, we set our prior for T to equal 10. MDIV analyses were run for 5 000 000 generations following a burn-in period of 500 000 generations, and repeated three times to ensure convergence upon the same posterior distributions for each of the parameter estimates. Estimates of T were converted to real time assuming a range of neutral mutation rate estimates (because a direct rate estimate for the ND2 gene is not available) for mtDNA in birds: 1.0×10^{-8}–2.5×10^{-8} substitutions/site/year (for reviews see Arbogast *et al*. 2002; Lovette 2004). These rates were multiplied by the number of sequenced nucleotides per individual [for example; $(2.5 \times 10^{-8}) \times 694 = 1.73 \times 10^{-5}$ substitutions/locus/year] to obtain our estimate of μ for the conversions.

Inter- and Intrapopulation analyses

Inter- and intrapopulation level analyses were performed for populations with sample sizes of nine or more. Ultimately, this led to the exclusion of 20 samples from six populations and only represented two unique haplotypes. Genetic diversity within populations was characterized by the number of unique haplotypes per population and the number of private haplotypes per population. We estimated nucleotide diversity (π; Nei 1987) along with its 95% confidence interval (1000 replicates) using the program ARLEQUIN 2.0 (Schneider *et al*. 2000). ARLEQUIN was also used to compute mismatch distributions (Slatkin & Hudson 1991) and evaluate their significance (1000 replicates). Tajima's D (Tajima 1989a, b) and Fu's F_s (Fu 1997) were calculated using DNASP (Rozas & Rozas 1999) and their significance was tested using coalescent simulations. To evaluate population structure, analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was performed with the program ARLEQUIN. To explore whether there existed significant genetic variation at multiple geographic levels, nested AMOVAS were performed with sequences grouped by region and then by individual population within each region. Eastern samples (Great Basin and Rocky Mountains) were partitioned into two regions: Rocky Mountains and Great Basin mountain ranges. The Rocky Mountain region included populations 1, 2, 9–17 (Fig. 1 and Table 1), and the Great Basin region included populations 3–8 and 14. Western samples were also partitioned into two regions: Sierra Nevada/Cascades and Peninsular/Transverse Ranges. The Sierra Nevada/Cascades region included populations 24–28, and the Peninsular/Transverse region included populations 19–23. We used ARLEQUIN to calculate the Mantel correlation coefficient (Mantel 1967) between matrices of geographic and genetic distance $[F_{ST}/(1 - F_{ST})]$ to determine if the data fit a pattern of isolation by distance. The significance of the Mantel correlation was assessed using 1000 random permutations of matrices.

MIGRATE version 1.7.6 (Beerli & Felsenstein 2001) was used to estimate gene flow among populations on the mountain ranges (sky-islands) of the Great Basin and the Rocky Mountains, because the geography of these populations best fits the model implemented in the program. In order to reduce the number of parameters and degrees of freedom in our gene flow analyses, we used a stepping-stone model of population structure (Kimura & Weiss 1964). Thus, gene flow was only estimated between populations that occurred adjacent to one another. In MIGRATE, we used the ML based estimation and a Metropolis coupled-Monte Carlo-Markov chain (MCMCMC) procedure. Runs consisted of 10 short chains of 100 000 steps followed by one long chain of 10 000 000 steps; each chain had a burn-in period of 100 000 steps. All chains were sampled every 100 steps. Adaptive heating was used with four chains and initial temperatures of 1, 1.3, 1.5, and 2. Starting values of theta and gene flow were estimated using F_{ST}. The F84 model of sequence evolution was used with base frequencies estimated from the data. We ran the program two times with different random seed numbers to examine the robustness of our estimates.

We applied the program IM (Hey & Nielsen 2004) to the samples from our western populations only (Sierra/Cascade vs. Peninsular/Transverse; see AMOVA regions above) to determine if the observed pattern of genetic variation was a result of historical divergence or limited contemporary migration. IM was used in this instance instead of MDIV because it allows for asymmetric rates of gene flow and different effective populaton sizes of the source populations. IM uses a MCMCMC approach to simultaneously

Table 1 Genetic diversity within populations. The values in the columns correspond to sample size (*N*), unique haplotypes (*H*), private haplotypes (Pri), nucleotide diversity (π) and its 95% confidence interval, significance of the mismatch distribution (MM; ns = not significantly different from the expectation under exponential growth), Tajima's *D*, and Fu's F_s (an asterisk denotes statistical significance). The numbers to the left of the populations from the Eastern Clade correspond to the populations shown in Fig. 4

		N	*H*	Pri	π	π C.I.	MM	*D*	F_s
Eastern Clade									
1	Coconino, AZ	10	6	4	0.002245	0.001651	NS	–1.57*	–2.52*
2	Wasatch Range, UT	10	2	0	0.000513	0.000612	NS	0.014	0.416
3	North Rim, AZ	10	3	2	0.001058	0.000967	NS	0.12	–0.101
4	Test Site, NV	10	8	6	0.00279	0.001952	NS	–0.926	–5.219*
5	Spring Mts, NV	10	5	3	0.002341	0.001704	NS	–0.972	–1.083
6	Toiyabe, NV	10	7	3	0.002405	0.00174	NS	0.686	–3.89*
7	Monitor, NV	10	7	3	0.002982	0.002058	NS	–0.697	–3.167*
8	Ruby, NV	10	3	0	0.001539	0.001251	NS	1.641	0.602
9	Merritt, BC	9	3	1	0.001122	0.001018	NS	0.195	–0.107
10	Fraser, BC	11	5	0	0.001994	0.001495	NS	–0.73	–1.265
11	Kootenai, ID	11	5	1	0.001574	0.001259	NS	–0.736	–1.844*
12	Missoula, MT	11	5	2	0.001941	0.001466	NS	–0.054	–1.329
13	N Central Rockies	16	8	6	0.001936	0.001418	NS	–1.855*	–4.448*
14	Snake Range, NV	10	5	3	0.009396	0.005491	< 0.01	1.445	2.293
15	San Miguel, NM	10	3	0	0.00109	0.000986	NS	–1.034	–0.046
16	Sacramento, NM	17	5	2	0.001804	0.001341	NS	–1.668*	–0.774
17	Valencia, NM	12	3	2	0.000481	0.000579	NS	–1.451	–1.324*
Western Clade									
19	San Diego, CA	14	4	3	0.001726	0.001316	NS	–0.157	–0.023
20	Riverside, CA	11	5	3	0.001441	0.001146	NS	–1.108	–1.426
21	San Bernardino, CA	13	4	2	0.001108	0.000972	NS	–0.645	–1.079
22	Los Angeles, CA	10	3	2	0.002882	0.002002	< 0.01	0.527	1.982
23	Ventura, CA	10	4	2	0.001313	0.001119	NS	–0.506	–1.071
24	Carson Range, NV	10	3	3	0.001185	0.001043	NS	0.525	0.108
25	Siskiyou, CA	15	6	1	0.003376	0.002187	NS	–0.575	–0.4
26	Glenn, CA	10	1	1	NA	NA	NA	NA	NA
27	Mendocino, CA	10	2	0	0.000288	0.000439	NS	–1.111	–0.339
28	Trinity, CA	10	2	1	0.000288	0.000439	NS	–1.111	–0.339

estimate the following parameters: θ_1 (effective number of females in population 1), θ_2 (effective number of females in population 2), θ_a (effective number of females in the ancestral population), m_1 (number of migrants from population 1 to population 2), m_2 (number of migrants from population 2 to population 1), and *t* (time of divergence). The program was run for 50 000 000 steps using five chains, following a 500 000 step burn-in. To ensure the program was performing well, we ran the program three times with different random seeds. The program produced very similar parameter estimates from each run (all parameter estimates from each run fell within the 95% highest posterior densities, HPD, for the parameter estimates from the other runs). We report the mode and the 95% HPD for each parameter estimate from the run that produced the highest effective sample sizes (ESS; Hey & Nielsen 2004) for all the parameter estimates. Estimates of *t* (time since divergence scaled to the neutral mutation rate) were converted to actual values of time using the equation $t = t/u$ (Hey & Nielsen 2004), where *u* is the locus specific neutral mutation rate and *t* is the estimate provided by IM. We assumed a range of neutral mutation rates of 1.0×10^{-8}–2.5×10^{-8} substitutions/site/year, the same rates used for the MDIV analyses. These rates multiplied by the number of sequenced nucleotides per individual were used as our estimates of *u* for the conversions. The effective number of female migrants between populations was calculated using $M_1 = (\theta_1 m)/2$ and $M_2 = (\theta_2 m)/2$ (Hey & Nielsen 2004).

Results

Phylogenetic analyses

We sequenced 694 base pairs of the ND2 gene for each individual. These sequences yielded 97 variable sites (44 parsimony informative), identifying 84 haplotypes (Fig. 2; haplotpyes sequences were deposited in GenBank under accession numbers DQ989022–DQ989105). Unweighted MP analysis produced 4985 equally parsimonious trees (score = 218). MODELTEST indicated the TIM + G (R matrix

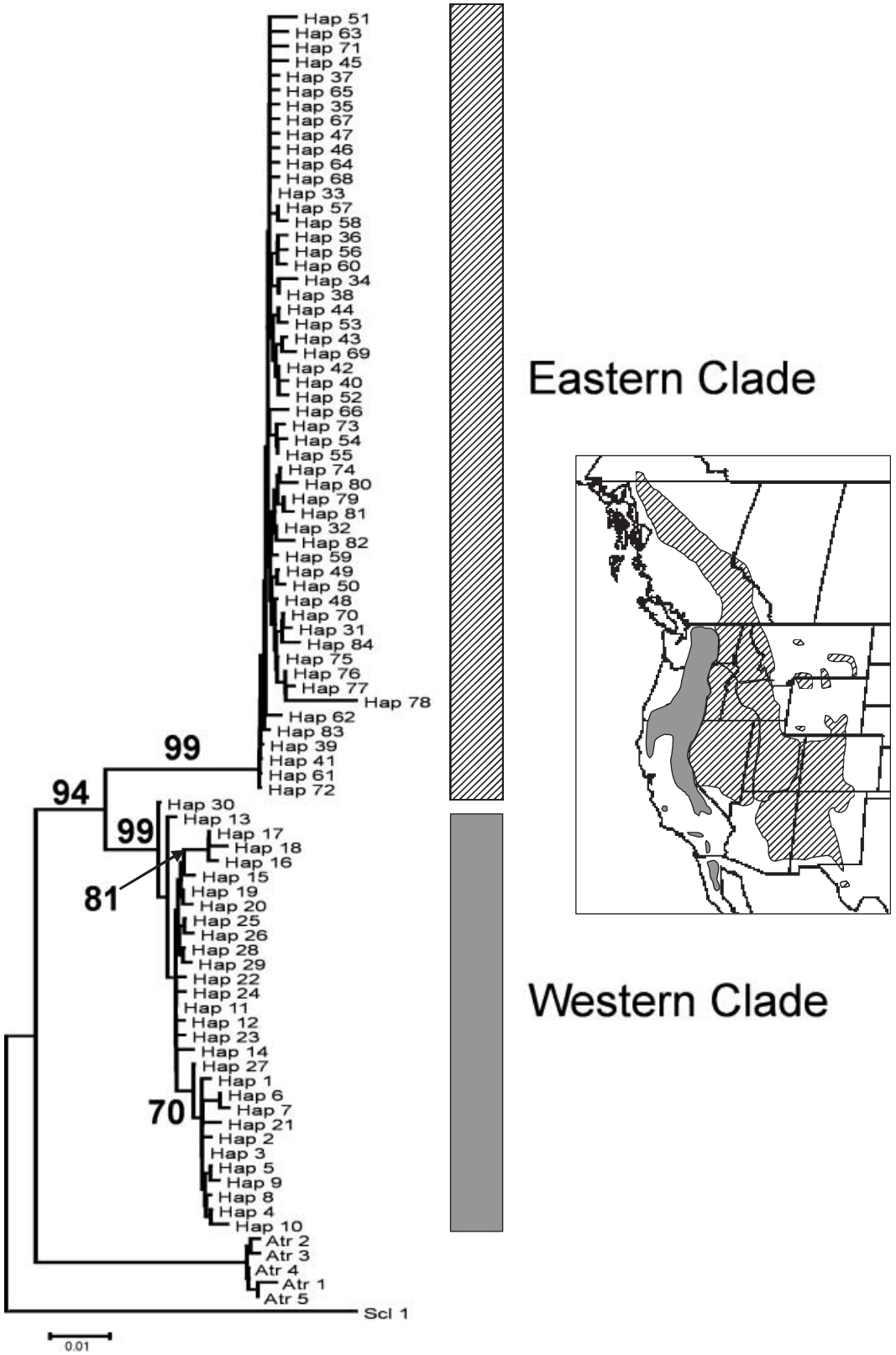

Fig. 2 Maximum likelihood phylogeny of all unique haplotypes. The values above the branches are maximum likelihood bootstrap values (> 70% support). The geographic distribution of the two clades is portrayed on the inset map. The abbreviations atri and scl correspond to the outgroup species, *Poecile atricapilla* and *P. sclateri*, respectively, used for phylogenetic analysis.

1.0, 16.08, 2.96, 2.96, 8.19, 1.0; $\alpha = 0.2905$) as a best fit for our data. ML analyses using this model of sequence evolution produced a single most likely tree (–ln L = 2331.4963; Fig. 2). Both MP and ML indicated the data formed two well-supported clades. Thus, we show only the ML phylogeny (Fig. 2). The ML and MP trees differed from one another in the placement of haplotypes within the two main clades; however, the branches leading to these haplotypes lacked bootstrap support.

The ML tree depicts two well-supported clades within *P. gambeli* (Fig. 2). The geographic distribution of haplotypes within these clades showed very little overlap. The 'Eastern Clade' corresponds directly with the Rocky Mountains, Great Basin mountain ranges, and the mountain ranges of eastern Oregon and eastern Washington (Fig. 2). The 'Western Clade' corresponds directly with the Sierra Nevada, Cascade, Peninsular, and Transverse mountain ranges (Fig. 2). The average uncorrected pairwise sequence divergence between clades was 0.044 (s.d. = 0.007). There was only one sampling locale (Mono Lake region in central California) that contained individuals with haplotypes from both clades. At this site, there were three individuals sampled (MBM 12162, 12167, 12180; Table S1) and each possessed a unique and private haplotype (Haps: 30, 31, 41). Two of these haplotypes were associated with the Eastern Clade (31 and 41) and one was associated with the Western Clade (30; Fig. 2).

Within clades, phylogenetic structure was generally shallow. There was a complete lack of bootstrap support for relationships among haplotypes within the Eastern Clade (Fig. 2). Within the Western Clade, there were only two well-supported branches. The first of these supported (81% bootstrap) the close association of three haplotypes (16–18) found only in the Siskiyou population of northern California. The second branch supported (70% bootstrap) the close association of haplotypes (1–10, 21) found only in populations from the Peninsular and Transverse Ranges of southern California.

The divergence time estimate for the split between the Eastern and Western Clades of the mountain chickadee provided by MDIV was $T = 2.52$ (95% HPD $T = 0.8–6.04$). Using our estimates of μ to convert the MDIV estimate to real time, we inferred the Eastern and Western Clades diverged from one another approximately 610 000 and 1 530 000 years ago.

Inter- and intrapopulation analyses: Eastern Clade

Genetic diversity within populations varied substantially (Table 1). There were three widespread haplotypes (33, 48, 70; Table S1). Haplotype 33 was widespread throughout the Rocky Mountains (from New Mexico north to British Columbia); however, in the Great Basin populations the frequency of haplotype 33 declined and was replaced in frequency by haplotypes 48 and 70. Thirteen of the 17 populations examined within the Eastern Clade possessed private haplotypes (Table 1). The Great Basin populations exhibited a high number of private haplotypes per population (five of six populations with three or more private haplotypes, Table 1: test site, Spring Mts, Toiyabe, Snake, Monitor). Nucleotide diversity was low throughout and ranged from 0.000481 in Valencia Co., New Mexico to 0.009396 in the Snake Range, Nevada. There was no relationship between nucleotide diversity and latitude. Only one population (Snake Range, Nevada) demonstrated a significant deviation from a mismatch distribution expected under a model of exponential population growth (Table 1). Tajima's *D* differed significantly from the expectation under neutrality in three populations (Coconino, north-central Rockies, and Sacramento). Fu's F_s differed significantly from the expectation under neutrality in seven populations (Coconino, north-centralentral Rockies, Valencia, Kootenai, test site, Toiyabe, and Monitor; Table 1, Fig. 1). Deviations from the expectation under neutrality for both Tajima's *D* and Fu's F_s for geographically constrained samples are often attributed to recent population expansion (Tajima 1989a; Fu 1997).

The Snake Range population was an outlier for most intrapopulation statistics. This population exhibited a significantly bimodal mismatch distribution, large positive values of *D* and F_s, and nucleotide diversity nearly four-times greater than any other population in the Eastern Clade. The cause of this was the presence of a divergent haplotype (78) found only in three individuals within the Snake Range (Fig. 2, Table S1). This divergent haplotype was five-base changes different than its closest relative, but appeared to be in a derived position on the ML phylogeny. When included in the interpopulation analyses this population behaved poorly, resulting in unrealistically high estimates of θ and irreproducible results. Because of these complications, this population was removed from the final interpopulation analyses.

Hierarchical analysis of molecular variance (AMOVA) of populations in the Eastern Clade indicated that there was significant genetic structure at multiple levels (Table 2). Most of the variation was found within populations (Table 2). There was also significant variation between the populations in the Great Basin vs. the Rocky Mountain regions, and significant genetic variation among populations within the Great Basin and Rocky Mountain regions. A Mantel test failed to find a correlation between geographic distance and genetic distance among all populations of the Eastern Clade ($r = 0.0032$, $P = 0.97$; Fig. 3). However, because we observed significant genetic structure between the Rocky Mountain and Great Basin populations, we ran an additional Mantel test that only included populations from the Rocky Mountains (excluding the isolated Great Basin populations). The results of this test suggested that

Table 2 Analysis of molecular variance (AMOVA). Analyses were performed separately for populations in each clade

Clade	Category description	% Var.	Statistic	*P*
Eastern	Among regions (Rockies vs. Great Basin)	18.77	$\Phi_{SC} = 0.201$	< 0.001
	Among populations in regions	16.30	$\Phi_{FT} = 0.351$	< 0.001
	Within populations	64.93	$\Phi_{CT} = 0.188$	< 0.001
Western	Among regions (Sierra/Cascades vs. Transverse/Peninsular Ranges)	42.87	$\Phi_{SC} = 0.528$	< 0.001
	Among populations in regions	30.15	$\Phi_{FT} = 0.731$	< 0.001
	Within populations	26.98	$\Phi_{CT} = 0.429$	< 0.001

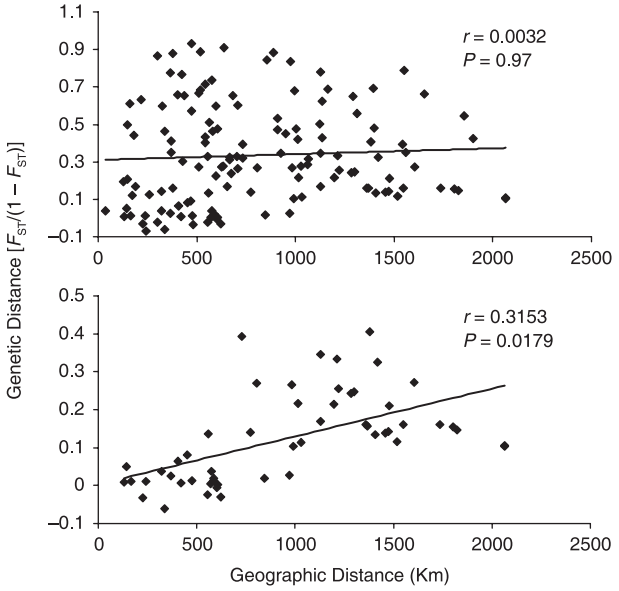

Fig. 3 Scatter-plots of genetic differentiation and geographical distance showing the results of the Mantel tests in the Eastern Clade. Top: all populations included. Bottom: Great Basin populations removed.

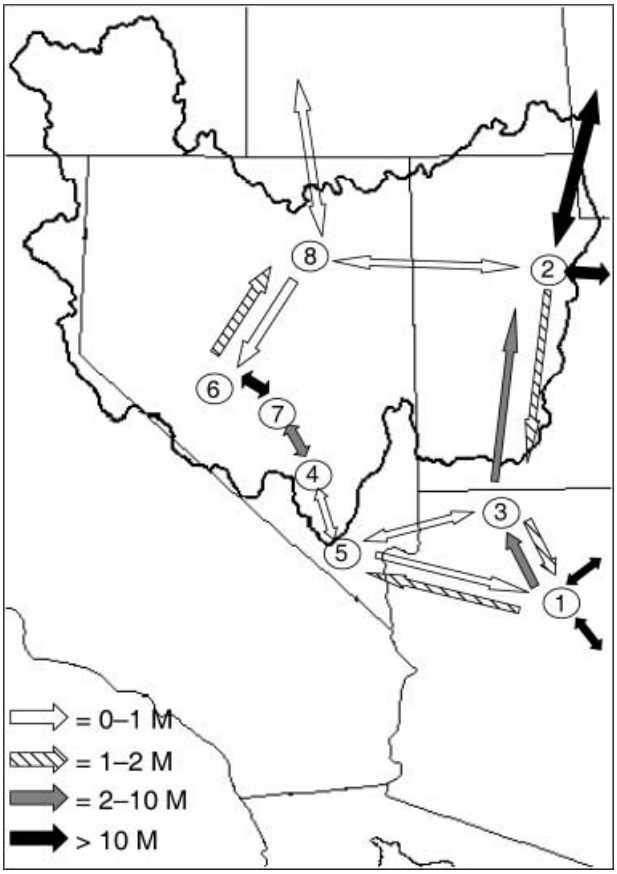

Fig. 4 Gene flow estimates in the Great Basin. The outlined area within the map portrays the hydrological limits of the Great Basin. The numbers correspond to the numbered populations in Table 1. The colour or pattern of the arrows corresponds to the number of female migrants per generation as determined by the program MIGRATE and detailed in the legend in the lower left-hand corner of the map.

mountain chickadee populations in the Rocky Mountains do show a significant isolation-by-distance pattern ($r = 0.3153$, $P = 0.0179$; Fig. 3).

The mountain chickadee populations on Great Basin mountain ranges exhibited many characteristics of insular populations: high frequency of private haplotypes, genetically dissimilar to the Rocky Mountain populations (AMOVA), and genetically dissimilar to one another (AMOVA). To assess how genetically isolated these populations are, we used MIGRATE to calculate the amount and direction of gene flow observed among populations within the Great Basin and their nearest neighbouring populations in the western and northern Rocky Mountains (Fig. 4). The results of the MIGRATE analyses indicated very low levels of gene flow (0–2 females per generation; Fig. 4) from the western and northern Rocky Mountains (populations 1–3) into the Great Basin (populations 5, 8), and vice versa. Rates of gene flow among populations within the Great Basin varied (Fig. 4). Gene flow between the Spring Mountains (population 5) and the Ruby Mountains (population 8) and their nearest Great Basin neighbour were low (0–2 females per generation; Fig. 4). Conversely, the rates of gene flow among the Monitor Range, Nevada test site, and Toiyabe Range populations (populations 4, 6, 7) were moderate to

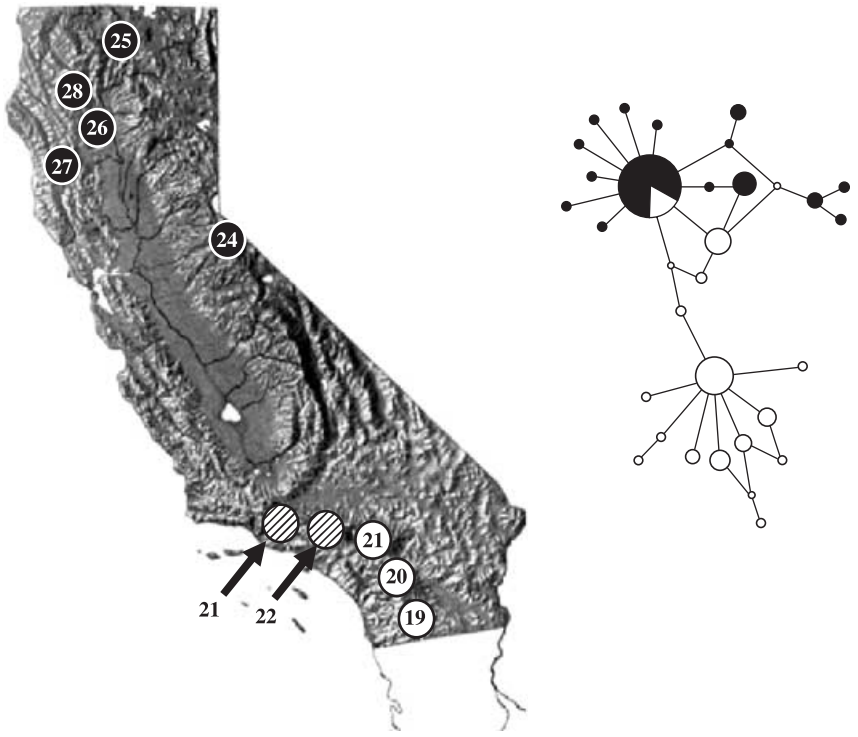

Fig. 5 Population sampling and genetic variation in the Western Clade. The map shows the location of all populations sampled with sample sizes of $N \geq 10$. The black and white circles show the general geographical distribution of haplotypes in the network. The populations with the black and white pattern show the southern populations that share a haplotype with the northern populations. On the right, median joining network of haplotypes in the Western Clade: black = northern populations, white = southern populations.

high (> 2 females/generation; Fig. 4). High to moderate rates of gene flow were also observed among the populations of the Rocky Mountains (> 2 females/generation; Fig. 4).

Inter- and intrapopulation analyses: Western Clade

As in the Eastern Clade, within-population genetic diversity in the Western Clade varied substantially. Genetic variation in the Western Clade appears to be partitioned between the southern populations from the Tranverse and Peninsular Ranges and the northern populations from the Sierra Nevada and Cascades. This result was expected because the phylogeny supported (70% bootstrap) the close relationship of a clade of haplotypes found only in populations from the Transverse and Peninsular Ranges of southern California (Fig. 2). The median joining network and map in Fig. 5 show the relationships amongst haplotypes in the Western Clade and the geographic distribution of those haplotypes between northern and southern California. There were two widespread haplotypes (haplotypes 3 and 11; Figs 2 and 5). Haplotype 3 was found only in the populations from the Transverse and Peninsular Ranges of southern California (Los Angeles, San Bernardino, Riverside, and San Diego; Fig. 5). In contrast, haplotype 11 was widespread and very common in the northern populations of the Sierra Nevada and Cascades, but also found in low frequency in two populations from the Transverse Range (Fig. 5). Nucleotide diversity was low in all populations (π = 0.000288–0.003376; Table 1), and showed no relationship with latitude (data not shown). Only the Los Angeles population deviated significantly from the expected mismatch distribution under a model of exponential growth (Table 1). No populations deviated from the expectations of a neutral model of evolution (D and F_s; Table 1).

AMOVA analysis indicated that there was significant genetic structure at many levels within the Western Clade (Table 2). Most of the genetic variation can be explained at the regional level (Table 2), when the populations were grouped as southern Transverse/Peninsular populations and northern Sierra Nevada/Cascade populations. However, there was also a considerable amount of variation among populations within regions and within populations (Table 2).

The partitioning of genetic variation between southern Transverse/Peninsular populations and northern Sierra Nevada/Cascade populations in the Western Clade can be explained by either incomplete lineage sorting or recent gene flow between the two regions. These two phenomena are often difficult to distinguish from one another, because they result in similar patterns of genetic variation (Nielsen & Wakeley 2001). However, new coalescent-model based methods (IM and MDIV) have proven efficient at inferring the relative effects that lineage sorting and gene flow have had on the structuring of genetic variation between naturally occurring and simulated populations (Nielsen & Wakeley 2001; Hey & Nielsen 2004). We applied the isolation-with-migration model to our data in the Western Clade. The

populations were grouped as follows (see Fig. 4): south (Transverse/Peninsular populations) and north (Sierra Nevada/Cascade populations). Each parameter estimate possessed a strongly unimodal distribution (data not shown). The modes for the θ (effective population sizes scaled to the neutral mutation rate) estimates of both populations were very similar: θ_{south} = 25.16 (95% HPD 12.07–47.21) and θ_{north} = 27.23 (95% HPD 14.54–48.05). Ancestral θ, however, was much smaller (θ_A = 4.93, 95% HPD 0.77–43.09). These estimates of theta suggested that both northern and southern populations have experienced recent population growth, and that the extent of this growth was effectively the same in both areas. These results were consistent with the results of the mismatch distribution analyses (Table 1), thus suggesting that mountain chickadee populations in the western clade have grown substantially.

Gene flow and divergence estimates (from the IM analyses) between northern and southern groups of mountain chickadees in the Western Clade were consistent with independent recent histories. The posterior distributions for gene flow from north to south and south to north both peaked at m = 0.005, and the 95% HPD intervals include zero (95% $HPD_{north\text{-}south}$ 0.0–0.47 and 95% $HPD_{south\text{-}north}$ 0.0–1.02). When converted to real estimates of gene flow, less than a single female migrant per generation (M = 0.61; 95% HPD 0–13) has moved between the northern and southern populations since their separation. The mode for our estimate for the divergence time (scaled by the neutral mutation rate) between northern and southern populations was t = 0.885 (95% HPD 0.585–7.79), which suggests that these populations diverged from each other approximately 51 000 and 130 000 years ago.

Discussion

Broad-scale phylogeographic patterns and concordance with codistributed taxa

Overall, our data agree with the twofold response to Quaternary climate change suggested by Barrowclough *et al*. (2004). There are two widespread phylogroups of mountain chickadee that can be separated from each other on a north/south axis along the eastern face of the Sierra Nevada and Cascade mountain ranges (Fig. 2). This east/west split corroborates previous findings (Gill *et al*. 1993, 2005) of a genetic break in mountain chickadees; however, due to our dense geographic sampling, this is the first time that the distribution of these two phylogroups has been described. Also, the two clades are remarkably consistent with patterns of morphological variation within mountain chickadees (baileyi groups in the west and gambeli groups in the east), which supports the hypothesis of the long-term isolation of mountain chickadee populations in the Sierra Nevada and Rocky Mountains (Behle 1956).

Considerable evidence exists in the pollen (Graham 1999) and fossil (Williams *et al*. 1999) records that montane coniferous forests were isolated in eastern (Rocky Mountain) and western (Sierra Nevada) refugia at the southern edge of their current distribution during the glacial advances of the Pleistocene epoch (12 000–1 800 000 years ago). If the fauna that inhabit these forests were similarly fragmented as they tracked their preferred habitat into these isolated glacial refugia, then we would expect genetic divergences between Rocky Mountain and Sierra Nevada populations to correspond temporally with the Pleistocene. The range of divergence time estimates (610 000–1 530 000 years ago) between phylogroups of mountain chickadees falls entirely within the Pleistocene. Therefore, the divergence between clades of mountain chickadee is concordant with a Pleistocene glacial refugia hypothesis. Similar patterns of divergence have now been observed in blue grouse (Barrowclough *et al*. 2004), White-breasted nuthatches (G. M. Spellman & J. Klicka, unpublished data), flying squirrels (Arbogast 1999), and tree squirrels (Arbogast & Kenagy 2001). However, the timing of divergence in these taxa varies substantially (for example 30 000 years in blue grouse, 300 000–600 000 years in white-breasted nuthatches, and 700 000–1 300 000 years in flying squirrels), and often these estimated divergence times do not overlap. Because climate change throughout the Quaternary was cyclical, glacial refugia would have occurred episodically throughout the epoch during periods of glacial maxima. The differences in divergence time between Eastern and Western Clades in these codistributed taxa could be due to the fact that these taxa were originally isolated into these refugia during different glacial maxima. On the other hand, we also cannot exclude the possibility that the differences in divergence time between these taxa are simply due to coalescent stochasticity (Edwards & Beerli 2000) or poor estimates of the rate of molecular evolution for the mtDNA genes used in these studies (for review see Arbogast *et al*. 2002; Lovette 2004). Regardless of whether these divergences all correspond to the same isolating event or to multiple events, there is a growing body of evidence from phylogeographic studies that the Pleistocene climatic cycles and glacial refugia played an integral role in the evolution of western North American montane taxa (Arbogast 1999; Barrowclough *et al*. 2004; G. M. Spellman & J. Klicka, unpublished data).

The two phylogroups of mountain chickadee have also recently come into contact. Our data identified a single sampling location, near Mono Lake and Mono Crater in central and eastern California, with a population that contained mtDNA from both Eastern and Western Clades. The extent of introgressive hybridization occurring within the site is difficult to infer, because we were only able to obtain three individuals from this site. Nonetheless, we suggest that the frequency of individuals with mtDNA from either

clade is relatively equal given that even with the small sample size we identified both clades within this population. The location of the contact zone between Eastern and Western Clades of mountain chickadees does not directly correspond to the contact zones observed in other co-distributed taxa. In a study of mtDNA in fox sparrows (*Passerella iliaca*), Zink (1994) discovered a contact zone between Sierra Nevada and Rocky Mountain/Great Basin forms in the White Mountains of California. The White Mountains are located approximately 50 miles southeast of Mono Lake and Mono Crater. Unfortunately, we were not able to obtain mountain chickadee samples from the White Mountains to contrast directly with the fox sparrow study, but the position of this mountain range between the Sierra Nevada and Great Basin (similar to Mono Lake) suggests that it would be an important area for further study of the mountain chickadee contact zone. Alternatively, in blue grouse and tree squirrels, contact zones between Eastern and Western Clades were observed in the coniferous forests of eastern Washington and eastern Oregon, respectively (Arbogast 1999; Barrowclough *et al*. 2004). Interestingly, all three of these contact zones appear to be narrow or extremely localized, and correspond directly to areas emphasized by Remington (1968) as 'suture zones' between the biotas of the Rocky Mountains and Sierra Nevada/Cascades. It is likely the contact zones observed in mountain chickadees, fox sparrows, blue grouse and tree squirrels are geographically restricted because the phylogroups have only recently come into contact in these areas. However, a thorough genetic characterization of the populations in these areas is necessary to truly tease apart the nature of the zones of introgression.

Phylogeography and population structure: Eastern Clade

Genetic structure in the Eastern Clade reveals three distinct patterns. First, genetic structure throughout the clade is shallow and only a single population (Snake Range, NV) exhibits a mismatch distribution that deviates from the expectation under a model of exponential population growth (Table 1). Population genealogies with evolutionary patterns consistent with rapid population growth and expansion are indeed the norm for North American temperate taxa (Zink 1994, 1997; Mila *et al*. 2000; Lessa *et al*. 2003; Zink *et al*. 2000; Peters *et al*. 2005). Evidently the mountain chickadee is no exception to this rule, as our data suggests that populations in the Rocky Mountains and Great Basin have experienced a period of explosive population growth and expansion. However, there is no direct evidence in our data to infer the direction of population expansion. Several studies on the genetic consequences of Quaternary climate change have demonstrated a pattern of decreasing genetic diversity with increasing latitude in temperate taxa (Hewitt 1996; Lessa *et al*. 2003; Mila *et al*. 2000); a pattern consistent with the expansion of populations from southern refugia. Nucleotide diversity (δ) is low throughout the distribution of the Eastern Clade of the mountain chickadee, and does not show a pattern of decreasing genetic diversity with increasing latitude. The behavioural life history traits of mountain chickadees can also explain the pattern of low nucleotide diversity throughout the Eastern Clade. In mountain chickadees, the caching of conifer seeds promotes the formation of tight social groups (McCallum *et al*. 1999). Individuals normally breed within these groups for the duration of their life and natal dispersal from these groups is restricted to neighbouring groups (McCallum *et al*. 1999). Thus, the evolutionary effective size of local mountain chickadee populations is likely very small and would not be able to harbour as much genetic diversity as nonsocial species. Low levels of nucleotide diversity may simply be the norm in mountain chickadee populations, regardless of how widespread they appear to be.

Second, there is a significant amount of genetic variation between populations of mountain chickadees in the Great Basin vs. those in the Rocky Mountains (Table 2). The mountain ranges of the Great Basin harbour relatively small patches of coniferous forest that are isolated from one another and the larger forests of the Rocky Mountains and Sierra Nevada by broad valleys of sage-brush steppe or desert scrub habitats. Thus, these forests have been deemed 'sky-islands' and the diversity and distribution of the resident fauna on these mountain ranges has played an integral role in the discussion of the application of the island biogeographic theory (MacArthur & Wilson 1963) to continental 'sky-island' systems (Johnson 1975, 1978; Brown 1978). The mountain chickadee was one of the original bird species used to study patterns of diversity on the Great Basin mountain ranges (Johnson 1975, 1978; Brown 1978). In these papers, it was generally concluded that bird diversity was not determined by an equilibrium between island size and distance from a continental source of migrants (i.e. Rocky Mountains or Sierra Nevada), as hypothesized by the traditional island biogeographic theory. Instead, it was suggested that migration (gene flow) into the Great Basin mountain ranges was too frequent and they were not behaving like true 'islands' (Brown 1978), or that bird diversity on these mountain ranges was determined by ecological complexity within the ranges (Johnson 1975, 1978). Although, we cannot directly test the second of these hypotheses (Johnson 1975, 1978), we can assess the patterns of gene flow within the Great Basin and between the Great Basin and the Rocky Mountains. Our estimates of gene flow into and around the Great Basin suggest mixed support for the hypothesis of frequent migration. The rate of gene flow into the Great Basin from the Rocky Mountain populations is less than two females per generation and appears to be restricted to the southern populations (Fig. 4). Rates of gene flow among the populations in the Great

Basin vary substantially. The populations on the periphery of the Great Basin exchange very few female migrants with those on the central ranges and the central ranges exchange many female migrants per generation (Fig. 4). These patterns of gene flow among populations of mountain chickadees in the Great Basin and between these populations and the Rocky Mountains suggest that many of the mountain ranges are behaving like 'islands' contrary to the frequent migration hypothesis (Brown 1978). However, it is possible that male mediated gene flow is frequent between Great Basin mountain ranges, and we are missing it because of the maternal inheritance of mtDNA. Further study of gene flow among Great Basin populations of mountain chickadees using multiple unlinked nuclear loci would help elucidate the true insularity (or lack of insularity) of the populations on these isolated mountain ranges.

Lastly, mountain chickadee populations in the Rocky Mountains show a pattern of isolation by distance (Fig. 3). Once again, the behavioural life history traits of mountain chickadees are likely responsible for this pattern. As stated before, the formation of tight, lifelong social groups in mountain chickadees coupled with limited natal dispersal would restrict gene flow to neighbouring groups (McCallum *et al*. 1999). Thus, even though mountain chickadees are widely distributed, gene flow is limited. This lack of long distance dispersal would severely curtail gene flow over great distances allowing for the genetic divergence of populations at the ends of the mountain chickadee's broad distribution.

Phylogeography and populations structure: Western Clade

Our data indicate, through multiple analyses (e.g. AMOVA and IM), that mountain chickadee populations in the Transverse and Peninsular Ranges of southern California, and in the Sierra Nevada and Cascade Ranges, have experienced recent independent evolutionary histories. The results of the IM analysis suggest these populations were separated within the last 130 000 years and have exchanged less than a single female migrant per generation since their separation. There is also the possibility that the incipient divergence observed between the northern and southern California populations in our study is due to isolation by distance. The observation of a strong pattern of isolation by distance in the Rocky Mountain populations indicates that mountain chickadees may be prone to local population isolation. The genetic break between northern and southern California populations may simply be a genetic cline across the large gap in our sampling between the two areas. However, recent phylogeographic studies of other bird species distributed across this area, suggest that species that occupy chaparral and coniferous forest habitats commonly exhibit a shallow phylogenetic break between northern and southern California (Cicero 1996; Sgariglia & Burns 2003; Burns & Barhoum 2006). Phylogenetic or phylogeographic breaks between the Peninsular/Tranverse and the Sierra Nevada/Cascades mountain ranges are also often observed in many nonavian taxa that inhabit the California Floristic Province, but the depth of divergence between populations is normally deeper in these groups (Calsbeek *et al*. 2003). The rapid uplift of the Transverse and Peninsular mountain ranges in the Pliocene (Axelrod 1958; Graham 1999) is often invoked as the vicariant event responsible for these phylogenetic splits; however, an event of Pliocene age is far too old to account for the splits observed in the avian studies conducted to date. Instead, the recent separation of avian populations in the Transverse and Peninsular Ranges is most likely in response to shifts in habitat distribution due to recent climate change (Axelrod 1979; Betancourt & VanDevender 1990; Graham 1999). Within the last 50 000 years, Sonoran and Mojave xeric vegetation has expanded dramatically in southern California (Axelrod 1979; Betancourt & VanDevender 1990). This expansion of desert vegetation into the valleys between southern California mountain ranges has led to the fragmentation and isolation of chaparral, forest, and woodland habitats. These relatively recent habitat shifts appear to have dramatically reduced or halted gene flow between the avifaunas of the Transverse/Peninsular Ranges of Southern California and the Sierra Nevada, at least in the few taxa that have been examined so far. Further phylogeographic research on additional resident birds of the Transverse and Peninsular mountain ranges is necessary to determine how common this pattern is.

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Supplementary material

The supplementary material is available from http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3199/MEC3199sm.htm

Table S1 Specimen date. The H column refers to the haplotype number in Fig. 1. Population numbers correspond to numbers in Table 1 and Fig. 2.

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