Two pulses of diversification across the Isthmus of Tehuantepec in a montane Mexican bird fauna

B. R. Barber^{1,*} and J. Klicka²

¹*Department of Ornithology, American Museum of Natural History, New York, NY 10024, USA*

²*Marjorie Barrick Museum of Natural History, University of Nevada Las Vegas, Las Vegas, NV 89154, USA*

Understanding the evolutionary history of the species in a particular region provides insights into how that fauna was formed. Of particular interest to biogeographers is examining the impact a geographical barrier had in generating temporal genetic diversity among codistributed species. We examined the impact a major New World barrier, the Isthmus of Tehuantepec (IT) in southern Mexico, had on a regional bird fauna. Specifically, genetic data from 10 montane-forest bird taxa were analysed using approximate Bayesian computation (ABC) to test the hypothesis of simultaneous intraspecific diversification at the IT. Because effective population size (N_e) has the greatest impact on coalescent times, thereby affecting tests of divergence among codistributed taxa, we chose priors for both current and ancestral N_e using empirical estimates of theta. The ABC method detected two discrete diversification events. Subsequent analysis with the number of diversification events constrained to two suggests that four taxa diverged in an older event, with the remaining six diverging more recently. Application of a range of mutation rates from 2.0 to 5.0% Myr⁻¹ places both events within the Pleistocene or Late Pliocene, suggesting that fluctuations in montane habitat induced by climate cycles and a late Pliocene seaway may have fractured this montane bird fauna. The results presented here suggest this avian fauna responded in a relatively concerted fashion over the last several million years.

Keywords: approximate Bayesian computation; comparative phylogeography; Isthmus of Tehuantepec; montane birds; simultaneous diversification; Pleistocene

1. INTRODUCTION

A common yet elusive goal in historical biogeography and comparative phylogeography is determining whether codistributed taxa have experienced simultaneous diversification owing to a common barrier (Nelson & Platnick 1981; Avise *et al.* 1998; Bermingham & Moritz 1998; Avise 2000; Edwards & Beerli 2000; Arbogast & Kenagy 2001; Zink 2002). Estimates of the timing of vicariant events can be obtained by applying a molecular clock to species phylogenies. However, such estimates have large variances owing to differences in effective population sizes, mutational rates and mutational rate heterogeneity (Avise 1989; Hudson 1990; Arbogast *et al.* 2002; Rosenberg & Nordburg 2002). Because of this variation, temporally independent events can be difficult to distinguish from one another and may consequently be interpreted as a single event. Conversely, this variation can cause a simultaneous event to appear to have occurred at multiple times (Riddle & Hafner 2006). A method that can reduce or accommodate uncertainty owing to the stochastic nature of the evolutionary process is required if we are to understand the temporal nature of divergence across common barriers (Edwards & Beerli 2000; Knowles 2004).

Statistical phylogeographic methods offer a potential solution because they can account for the stochastic nature of the evolutionary process while allowing tests of simultaneous diversification. These methods can even incorporate biologically realistic scenarios such as different effective population sizes, expanding populations and various levels of gene flow, making them potent tools (Knowles & Maddison 2002; Knowles 2004; DeChaine & Martin 2006; Hickerson *et al.* 2006). In this study, an approximate Bayesian computation (ABC) method implemented in the recently developed statistical phylogeographic program MSBAYES (Hickerson *et al.* 2006, 2007) was used to test for simultaneous intraspecific diversification of montane-forest birds at the Isthmus of Tehuantepec (IT) valley.

The IT (figure 1) in southern Mexico is a major geographical barrier to a variety of taxa (e.g. Parker *et al.* 1996; Peterson *et al.* 1999; García-Moreno *et al.* 2004). A valley approximately 250 m above the sea level and some 200 km wide at its narrowest point characterizes the IT (Barrier *et al.* 1998). The central valley is bounded by three mountain chains, the Sierra Madre Oriental and Sierra Madre del Sur to the northwest and the Chiapas-Guatemala Highlands to the southeast (figure 1). Montane organisms occurring on either side of the IT are currently isolated from one another by the intervening valley. Despite its current configuration, reshuffling of montane forests and their constituent faunas probably occurred multiple times during the last several million years (Wells 1983; Hooghiemstra *et al.* 2006), providing

* Author and address for correspondence: Department of Biology, Brigham Young University, 401 WIDB, Provo, UT 84602, USA (brbarber76@gmail.com).

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Figure 1. Map illustrating the IT region and elevations (shaded in grey) above 900 m.

numerous opportunities for populations to diverge in isolation and for genetic admixture to occur when populations were reconnected. Thus, currently isolated taxon pairs might have originated at different times or perhaps during a single event. Molecular sequence data were used to test the hypothesis of simultaneous intraspecific diversification of 10 montane bird taxa having distributions that span the IT. The results provide an assessment of the temporal nature of intraspecific diversification in montane birds across this key geographical barrier.

2. MATERIAL AND METHODS

(a) *Taxa*

Ten taxa with distributions spanning the IT were examined. Eight study species are Passeriformes, divided into six families: *Cyanocitta stelleri* (Corvidae), *Certhia americana* (Certhiidae), *Piranga flava* (Thraupidae), *Myadestes occidentalis* (Turdidae), *Parula superciliosa*, *Basileuterus belli*, *Myioborus miniatus* (Parulidae) and *Atlapetes albinucha* (Emberizidae). The two remaining taxa include a hummingbird, *Lampornis amethystinus* (Apodiformes: Trochilidae) and a woodpecker, *Picoides villosus* (Piciformes: Picidae). These 10 taxa are found primarily in pine–oak (*Pinus* and *Quercus*) and evergreen forests from 900 to 3500 m in elevation, and all are presumed to be resident taxa (Howell & Webb 1995).

(b) *Molecular data*

New and existing mitochondrial datasets were analysed (table 1: electronic supplementary material, appendix S1). New data were obtained as part of ongoing projects investigating the evolutionary history of montane-bird taxa in the region. Sequence data from the NADH dehydrogenase subunit 2 gene (*ND2*) were obtained from muscle tissues using standard molecular laboratory protocols for mitochondrial data. The primers L5215 (5'-TATC GGGCCCATACCCCGAATAT-3'; Hackett 1996) and H1064 (5'-CTTTGAAGGCCTTCGGTTTA-3'; Drovetski *et al.* 2004) were used in PCR and sequencing. Sequences were aligned and edited using SEQUENCHER 4.7 (Gene Codes, Ann Arbor, MI, USA). Aligned sequences were

checked for internal stop codons and indels to ensure against nuclear copies. Mitochondrial sequences from published sources were obtained from GenBank (table 1; electronic supplementary material, appendix S1). Datasets were grouped into populations occurring east and west of the IT.

(c) *Estimates of the time-to-most-recent-common-ancestor*

To provide an assessment of the variation in gene-tree depths, a Bayesian MCMC analysis was employed to estimate the time-to-most-recent-common-ancestor (TMRCA) and the confidence interval for each taxon. Analyses were conducted in BEAST v1.4.8 (Drummond *et al.* 2002; Drummond & Rambaut 2007). An HKY + G + I substitution model was assumed. Owing to the uncertainty of avian mtDNA substitution rates (Lovette 2004), we employed a range of 2.0–5.0% Myr⁻¹ (Arbogast *et al.* 2002, 2006; Lovette 2004; Weir & Schluter 2008) and an uncorrelated exponential molecular clock (Drummond *et al.* 2006) to covert TMRCA to millions of years ago (Ma). A UPGMA topology was used as a starting tree, all other priors were set to default values. The analyses were run for 20 million generations with the first 25 per cent of the sampled points removed as burn-in. The mean TMRCA and its 95 per cent highest posterior density (HPD) were plotted from trees sampled every 1000 generations using TRACER 1.4 (Rambaut & Drummond 2007).

(d) *Estimates of gene flow*

Gene flow between populations on either side of the IT was estimated using IM (Hey & Nielsen 2004) for two taxa (*M. occidentalis* and *P. superciliosa*) with polyphyletic trees. Each analysis employed 10 heated chains and was run for a minimum of 3×10^7 generations with a burn-in of 5×10^5 . Gene flow was set to be symmetrical between populations. Two independent runs were conducted on each dataset. Convergence was assumed when effective sample sizes (ESSs) were greater than 100 for each parameter (Kuhner & Smith 2007).

(e) *Tests of simultaneous diversification*

An ABC (Beaumont *et al.* 2002) method implemented in the program MSBAYES (Hickerson *et al.* 2006, 2007) was

Table 1. List of taxa included in this study with number of sequences occurring west and east of the IT. Transition/transversion ratio (Ti/Tv) and number of variable sites for each dataset. Tree structure identifies phylogenies that are reciprocally monophyletic (sorted) or with haplotypes shared between populations (polyphyletic). Taxa with superscripts were obtained from GenBank. Localities and GenBank numbers are provided in electronic supplementary material, appendix S1.

taxon	west	east	Ti/Tv	no. variable	reciprocally monophyletic
<i>B. belli</i>	46	15	12.3	86	yes
<i>M. occidentalis</i>	8	12	18	16	no
<i>P. superciliosa</i>	23	3	20	20	no
<i>L. amethystinus</i> ^a	55	14	30	17	yes
<i>P. flava</i>	36	9	14.4	121	yes
<i>C. stelleri</i>	3	4	6.9	39	yes
<i>M. miniatus</i> ^b	4	9	4.7	91	yes
<i>A. albinucha</i>	2	8	3.4	14	yes
<i>P. villosus</i>	6	2	2.6	23	yes
<i>C. americana</i>	17	17	2.1	67	yes

^aCortés-Rodríguez *et al.* (2008).

^bPérez-Emán (2005).

employed to evaluate the relative strength of two hypotheses: simultaneous divergence versus non-simultaneous divergence. This program can simultaneously analyse multiple datasets while allowing each taxon and population to undergo independent demographic histories and has been shown to be effective over a range of conditions with a single locus (Hickerson *et al.* 2006, 2007). In addition, the method performs well with small sample sizes (e.g. one to three samples/population: Hickerson *et al.* 2007). The empirical range of Tajima's (1983) π was used to set the lower and upper limit of the uniform prior for the maximum (current) size of the population $\Theta_{\max}$ (where $\Theta = 4 * N * \mu$ (μ is the per gene mutation rate)). The averaged value of Watterson's (1975) θ (Θ_w) and its ratio to the mean of π (Θ_w/π) was used to set the uniform prior for the maximum ancestral population ($\Theta_{\text{anc-max}}$) to $\Theta_{\max}$. The prior for number of possible maximum number of divergence events ($\mathbb{Y}$) equalled the number of taxa ($\mathbb{Y} = 10$). Posterior distributions and their 95 per cent quantiles for number of divergence events ($\mathbb{Y}$) were constructed using 1000 draws from 500 000 simulated replicates. Because the initial test suggested more than one event (i.e. mode of $\mathbb{Y} > 1.5$ and $\Omega \gg 0$; see below) had occurred, a subsequent analysis was conducted to determine the number of taxa (with mean and 95% quantile) that diverged during each event and the timing ($E(t)$) of each of those events. This analysis was run with $\mathbb{Y}$ constrained to equal the number of divergent events detected in the unconstrained analysis, for example $\mathbb{Y} = 2$. All other priors and settings were identical to the initial-unconstrained analyses. The mode of the mean divergence time across taxa ($E(t)$) was converted to years before the present (YBP) using the formula $t(0.5\Theta_{\max}/\mu)$ and two mutation rates 2.0 and 5.0% Myr⁻¹ (Arbogast *et al.* 2006). For these analyses, the transition to transversion ratio (table 1) was estimated from an equally weighted maximum parsimony tree using the likelihood option, and the HKY model, under tree scores in PAUP* v. 4.0b10 (Swofford 2002). Hickerson *et al.* (2007) recommends using both $\mathbb{Y}$ and Ω ($=\text{Var}(\tau)/E(\tau)$) to evaluate the relative strengths of each hypothesis, where a low value (approx. 0) of Ω and a $\mathbb{Y} = 1$ suggest the data fit a simultaneous model; thus, we used both statistics to evaluate the relative support for each hypothesis.

3. RESULTS

(a) Molecular data

Sequence data from the ND2 gene (1041 bp) were obtained for eight taxa. GenBank numbers, museum numbers and localities are provided in electronic supplementary material, appendix S1. The *L. amethystinus* (Cortés-Rodríguez *et al.* 2008) dataset consisted of 354 base pairs for each individual. Samples sizes ranged from 2 to 55 individuals per population with an average of 14.7 (table 1). The number of variable characters and Ti/Tv ratio averaged 49 (14–121) and 11.4 (2.1–30), respectively (table 1).

(b) Time-to-most-recent-common-ancestor

ESSs were over 500 for nearly all statistics including TMRCA; thus, we assume the trees were well sampled. Mean values in YBP for the TMRCA ranged from 3.1×10^5 for *P. superciliosa* to 3.0×10^6 for *M. miniatus*. In general, there was a considerable variation and overlap in most but not all coalescent times for the 10 taxa (figure 2).

(c) Estimates of gene flow

Gene flow estimates (figure 3), in units of females/generation, between populations east and west of the IT were low for both *M. occidentalis* (0.05) and *P. superciliosa* (0.18); therefore, we assume the lack of unsorted trees in these two taxa is the result of incomplete lineage sorting and not due to ongoing gene flow (Palsbøll *et al.* 2004). Independent runs converged on similar values for all parameters and ESS values for all parameters were greater than 1000; thus convergence upon stationarity was assumed (Hey 2005; Kuhner & Smith 2007).

(d) Test of simultaneous diversification

The ABC method detected two divergence events among the 10 taxa. The upper and lower limits for the uniform prior of $\Theta_{\max}$ were set using the empirical range of π (2.9–33.8). Because the ratio of the means of Θ_w and π was close to one ($13.2/14.2 = 0.93$), we assumed the maximum-ancestral population was equal in size to

Figure 2. Time-of-most-recent-common-ancestor (TMRCA) for 10 species that span the IT. Squares are the mean values, lines are the 95% highest posterior densities (HPD). Scale bar values are in million years before present.

Figure 3. Estimates of the level of ongoing gene flow across the IT for two taxa with unsorted gene trees. The modes for *M. occidentalis* (open circles) and *P. superciliosa* (solid circles) were 0.05 and 0.18 females per generation, respectively.

$\theta_{\max}$; thus $\theta_{\text{anc-max}} = \theta_{\max}$. The initial ABC analysis (figure 4) detected non-simultaneous divergence ($\Psi = 1.8$: 1–4 and $\Omega = 0.23$) among the 10 taxa. The analysis with number of divergence events constrained to two ($\Psi = 2$) suggested six (three to nine) taxa diverged in the more recent of the two events, with the remaining four (one to seven) diverging in the older one. As currently implemented, MSBAYES will not sort taxa into each of the divergence events; instead we used relative depths of TMRCA (figure 2) to rank taxa. Using TMRCA suggests *P. flava*, *L. amethystinus*, *M. miniatus* and *C. stelleri* or *C. americana* split during the older event, with the remaining taxa diverging in the more recent one. The mode divergence times ($E(t)$) for the two events were 0.08 (0–0.21) and 1 (0.42–2.02). When converted to YBP, these values become 130 000 (0–341 000) and 1 623 000 (682 000–3 247 000) YBP using 2.0% Myr⁻¹ and 52 000 (0–136 000) and 649 000 (273 000–1 312 000) using 5.0% Myr⁻¹ (figure 5).

Figure 4. Results of the ABC analysis using MSBAYES. Dashed line is the prior distribution of the number of possible divergence events and was set to equal the number of taxa ($\Psi = 10$). Bold line is the posterior distribution of the number of divergence events (Ψ). The mode for Ψ was 1.8. The upper and lower limits for the uniform prior of the current population size ($\theta_{\max}$) were 33.8 and 2.9, respectively. The maximum of ancestral population size ($\theta_{\text{anc-max}}$) was assumed to be the same as $\theta_{\max}$.

Figure 5. Posterior distribution of the timing ($E(t)$) of two diversification events. The mode divergence times ($E(t)$) for the two events were 0.08 (0–0.21) and 1 (0.42–2.02). When converted to YBP, these values become 130 000 (0–341 000) and 1 623 000 (682 000–3 247 000) YBP using 2% Myr⁻¹ (solid line) and 52 000 (0–136 000) and 649 000 (273 000–1 312 000) using 5% Myr⁻¹ (dashed line).

4. DISCUSSION

Comparative biogeographic studies have usually assumed that differences in coalescence times signal differences in divergence times, thus rejecting simultaneous diversification (Avice 1992; Arbogast & Kenagy 2001). However, coalescent theory predicts a large variation in the depths of gene trees even when multiple codistributed taxa have responded to the same barrier (Hudson 1990). Inspection of TMRCA of the 10 taxa reveals large (as predicted by theory), and mostly overlapping, variation in coalescent times, but with obvious gaps. For example, the 95 per cent HPD of *M. miniatus* and *P. flava* do not overlap with that of *P. superciliosa* and *M. occidentalis*. Thus, variation in TMRCA suggests the possibility of more than one divergence event. The question then becomes whether this variation is the result of a single or multiple divergence events. Examination of TMRCA

alone does not allow the determination of the number of divergence events. We employed a recently developed phylogeographic statistical tool to determine the number of diversification events that occurred among the 10 taxa.

The ABC method used in this study tests for simultaneous diversification among codistributed taxa, while at the same time allowing the population histories (e.g. effective population sizes, population growth, etc.) to vary across taxa. Because effective population size (N_e) has the greatest impact on coalescent times and consequently on tests of divergence times among codistributed taxa, it was important that the prior for N_e approximates empirical values. We chose prior values for current and ancestral N_e using values obtained from the data. In this way, the N_e priors encapsulate the range of observed values. The ABC method detected two pulses of divergence among the 10 montane bird taxa, a result consistent with the variation in TMRCA. Application of a range of mutation rates places the timing of these two events at 130 000 (0–341 000) and 1 623 000 (682 000–3 247 000) YBP using 2.0% Myr⁻¹ and 52 000 (0.0–136 000) and 649 000 (273 000–1 312 000) using 5.0% Myr⁻¹. It is important to note that these dates are the population division and will be more recent than TMRCA estimates. Thus, both events, including their confidence intervals, fall within the Pleistocene and Late Pliocene.

Climate change during the Pleistocene and Late Pliocene had a profound impact on species (Hewitt 2000). Montane forests underwent repeated altitudinal migrations up- and down-slope during this period (Wells 1983; Hooghiemstra *et al.* 2006). It is likely that there existed multiple successive opportunities for populations to diverge in isolation. The amplitude of each climate oscillation, at least for recent cycles, appears to be of similar magnitude (Petit *et al.* 1999; therefore, when forests were reconnected during each successive glacial cycle, evidence of a previous divergence might have been erased (Zink *et al.* 2004). Owing to this fact, we might expect only the last glacial event to leave its genetic signature upon codistributed taxa. These data suggest that an Early Pleistocene or Late Pliocene divergence maintained its evolutionary independence over subsequent glacial cycles.

It is common in biogeographic studies to assume that climate-driven habitat shifts are responsible for shaping genetic patterns when events date to the last two million years (e.g. Weir & Schluter 2004). Although population division owing to habitat shifts is plausible in this system, it is inferred from a casual temporal relationship (as in most studies), and alternatives should be considered. For example, a marine transgression may have crossed the isthmus valley at the Plio–Pleistocene boundary (Campbell 1999; Mulchay *et al.* 2006; but see Miller *et al.* 2005). The postulated timing (approx. 2 Ma) of this seaway falls within the confidence interval of the deepest divergence event (figure 5). Thus, multiple mechanisms (habitat shifts, seaways), operating at different times, may be responsible for fracturing this montane bird community. Vicariance owing to a seaway, rather than habitat shifts, could explain why the oldest divergence event maintained its evolutionary independence over later glacial cycles. Finally, different natural history traits (diet, canopy versus terrestrial foragers, etc.) may

play a role in generating two events. However, uncertainty in the exact number of taxa that were split by each event precludes rigorous analysis.

Several phylogeographic studies of montane taxa exist in the region, providing an opportunity to examine general patterns and processes across taxa. For example, Sullivan *et al.* (2000) conducted a comparative study of two highland rodents spanning the isthmus region and detected phylogeographic patterns indicative of an isthmus vicariant event in both taxa. Overlapping variation in sequence divergence suggests the possibility that both taxa split simultaneously in the Pleistocene or Late Pliocene (Sullivan *et al.* 2000). Estimates of divergence times in two codistributed snakes suggest a Mid-Pliocene event, although confidence intervals overlapped with the earliest event detected in this study (Castoe *et al.* 2009). Finally, additional bird taxa (not included here because they employed different gene regions or occur in different habitat) show phylogeographic breaks at the IT. For example, the common bush-tanager (*Chlorospingus ophthalmicus*) and the emerald toucanet (*Aulacorhynchus prasinus*) show recent phylogeographic breaks (García-Moreno *et al.* 2004; Puebla-Olivares *et al.* 2008) that are roughly consistent with the recent divergence event detected in this study. A deeper and perhaps older genetic division was recovered between populations east and west of the isthmus in the *Buarremon brunneinucha* complex (Cadena *et al.* 2007; Navarro-Sigüenza *et al.* 2008).

5. CONCLUSIONS

Understanding the temporal nature of diversification across a shared barrier is a fundamental yet challenging goal in evolutionary analysis. Documenting the evolutionary divisions within extant faunas will provide insights into how communities have responded to, and been shaped by, historical events (Brooks & McLennan 1991; Zink 2002; Cavender-Bares & Wilczek 2003). ABC suggests that montane bird fauna occurring on both sides of the IT was fragmented during two discrete pulses of divergence. These two events occurred during the Pleistocene or Late Pliocene, implying climate change and possibly a marine transgression were responsible for shaping intraspecific diversity in the region of the isthmus. Finally, we recognize the limitations of using a single locus to test hypotheses of divergence as well as date those events (Edwards & Beerli 2000; Carstens & Knowles 2007). However, these results provide the first comparative genetic-based assessment of the temporal nature of diversification in this montane bird community and a working hypothesis for further study in the IT region.

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REFERENCES

- Arbogast, B. S. & Kenagy, G. J. 2001 Comparative phylogeography as an integrative approach to historical biogeography. *J. Biogeogr.* **28**, 819–825. (doi:10.1046/j.1365-2699.2001.00594.x)
- Arbogast, B. S., Edwards, S. V., Wakeley, J., Beerli, P. & Slowinski, J. B. 2002 Estimating divergence times from molecular data on phylogenetic and populations genetic timescales. *Annu. Rev. Ecol. Syst.* **33**, 707–740. (doi:10.1146/annurev.ecolsys.33.010802.150500)
- Arbogast, B. S., Drovetski, S. V., Curry, R. L., Boag, P., Grant, P., Grant, R., Seutin, G. & Anderson, D. J. 2006 Origin and diversification of Galápagos mockingbirds. *Evolution* **60**, 370–382.
- Avise, J. C. 1989 Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43**, 1192–1208. (doi:10.2307/2409356)
- Avise, J. C. 1992 Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. *Oikos* **63**, 62–76. (doi:10.2307/3545516)
- Avise, J. C. 2000 *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.
- Avise, J. C., Walker, D. & Johns, G. C. 1998 Speciation durations and Pleistocene effects in vertebrate phylogeography. *Proc. R. Soc. Lond. B* **265**, 1707–1712. (doi:10.1098/rspb.1999.0492)
- Barrier, E., Velasquillo, L., Chavez, M. & Gaulon, R. 1998 Neotectonic evolution of the Isthmus of Tehuantepec (southeastern Mexico). *Tectonophysics* **287**, 77–96. (doi:10.1016/S0040-1951(98)80062-0)
- Beaumont, M. A., Zhang, W. & Balding, D. J. 2002 Approximate Bayesian computation in population genetics. *Genetics* **162**, 2025–2035.
- Bermingham, E. & Moritz, C. 1998 Comparative phylogeography: concepts and applications. *Mol. Ecol.* **7**, 367–369. (doi:10.1046/j.1365-294x.1998.00424.x)
- Brooks, D. R. & McLennan, D. A. 1991 *Phylogeny, ecology and behavior*. Chicago, IL: University of Chicago Press.
- Cadena, C. D., Klicka, J. & Ricklefs, R. E. 2007 Evolutionary differentiation in the Neotropical montane region: molecular phylogenetics and phylogeography of *Buarremon* brush-finches (Aves, Emberizidae). *Mol. Phylogenet. Evol.* **44**, 993–1016. (doi:10.1016/j.ympev.2006.12.012)
- Campbell, J. A. 1999 Distribution patterns of amphibians in Middle America. In *Patterns of distribution of amphibians: a global perspective* (ed. W. E. Duellman), pp. 111–210. Baltimore, MD: John Hopkins University Press.
- Carstens, B. C. & Knowles, L. L. 2007 Shifting distributions and speciation: species divergence during rapid climate change. *Mol. Ecol.* **16**, 619–627. (doi:10.1111/j.1365-294X.2006.03167.x)
- Castoe, T. A., Daza, J. M., Smith, E. N., Sasa, M. M., Kuch, U., Campbell, J. A., Chippindale, P. T. & Parkinson, C. L. 2009 Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American highland biogeography. *J. Biogeogr.* **36**, 88–103. (doi:10.1111/j.1365-2699.2008.01991.x)
- Cavender-Bares, J. & Wilczek, A. 2003 Integrating micro- and macroevolutionary processes in community ecology. *Ecology* **84**, 592–597. (doi:10.1890/0012-9658(2003)084[0592:IMAMPI]2.0.CO;2)
- Cortés-Rodríguez, N., Hernández-Baños, B. E., Navarro-Sigüenza, A. G., Peterson, A. T. & García-Moreno, J. 2008 Phylogeography and population genetics of the amethyst-throated hummingbird (*Lampornis amethystinus*). *Mol. Phylogenet. Evol.* **48**, 1–11. (doi:10.1016/j.ympev.2008.02.005)
- DeChaine, R. G. & Martin, A. P. 2006 Using coalescent simulations to test the impact of Quaternary climate cycles on divergence in an alpine plant-insect association. *Evolution* **60**, 1004–1013.
- Drummond, A. J. & Rambaut, A. 2007 BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**, 214. (doi:10.1186/1471-2148-7-214)
- Drummond, A. J., Nicholls, G. K., Rodrigo, A. G. & Solomon, W. 2002 Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. *Genetics* **161**, 1307–1320.
- Drummond, A. J., Ho, S. Y. W., Phillips, M. J. & Rambaut, A. 2006 Relaxed phylogenetics and dating with confidence. *PLoS Biol.* **4**, e88. (doi:10.1371/journal.pbio.0040088)
- Drovetski, S. V., Zink, R. M., Fadeev, I. V., Nesterov, E. V., Koblik, E. A., Red'kin, Y. A. & Rohwer, S. 2004 Mitochondrial phylogeny of *Locustella* and related genera. *J. Avian Biol.* **35**, 105–110. (doi:10.1111/j.0908-8857.2004.03217.x)
- Edwards, S. V. & Beerli, P. 2000 Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution* **54**, 1839–1854.
- García-Moreno, J., Navarro-Sigüenza, A. G., Peterson, A. T. & Sánchez-González, L. A. 2004 Genetic variation coincides with geographic structure in the common bush-tanager (*Chlorospingus ophthalmicus*) complex from Mexico. *Mol. Phylogenet. Evol.* **33**, 186–196. (doi:10.1016/j.ympev.2004.05.007)
- Hackett, S. J. 1996 Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Mol. Phylogenet. Evol.* **5**, 368–382. (doi:10.1006/mpev.1996.0032)
- Hewitt, G. 2000 The genetic legacy of the Quaternary ice ages. *Nature* **405**, 907–913. (doi:10.1038/35016000)
- Hey, J. 2005 On the number of New World founders: a population genetic portrait of the peopling of the Americas. *PLoS Biol.* **3**, e193. (doi:10.1371/journal.pbio.0030193)
- Hey, J. & Nielsen, R. 2004 Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics* **167**, 747–760. (doi:10.1534/genetics.103.024182)
- Hickerson, M. J., Dolman, G. & Moritz, C. 2006 Comparative phylogeographic summary statistics for testing simultaneous vicariance. *Mol. Ecol.* **15**, 209–223. (doi:10.1111/j.1365-294X.2005.02718.x)
- Hickerson, M. J., Stahl, E. & Takebayashi, N. 2007 MSBAYES: a pipeline for testing comparative phylogeographic histories using hierarchical approximate Bayesian computation. *BMC Bioinform.* **8**, 268. (doi:10.1186/1471-2105-8-268)
- Hooghiemstra, H., Wijninga, V. M. & Cleef, A. M. 2006 The paleobotanical record of Colombia: implications for biogeography and biodiversity. *Ann. Mo. Bot.*

- Gard.* **93**, 297–324. (doi:10.3417/0026-6493(2006)93[297:TPROCI]2.0.CO;2)
- Howell, S. N. G. & Webb, S. 1995 *A guide to the birds of Mexico and northern Central America*. Oxford, UK: Oxford University Press.
- Hudson, R. R. 1990 Gene genealogies and the coalescent process. *Oxf. Surv. Evol. Biol.* **7**, 1–44.
- Knowles, L. L. 2004 The burgeoning field of statistical phylogeography. *J. Evol. Biol.* **17**, 1–10. (doi:10.1046/j.1420-9101.2003.00644.x)
- Knowles, L. L. & Maddison, W. P. 2002 Statistical phylogeography. *Mol. Ecol.* **11**, 2623–2635. (doi:10.1046/j.1365-294X.2002.01637.x)
- Kuhner, M. K. & Smith, L. P. 2007 Comparing likelihood and Bayesian coalescent estimation of population parameters. *Genetics* **175**, 155–165. (doi:10.1534/genetics.106.056457)
- Lovette, I. J. 2004 Mitochondrial dating and mixed support for the ‘2% rule’ in birds. *Auk* **121**, 1–6. (doi:10.1642/0004-8038(2004)121[0001:MDAMSF]2.0.CO;2)
- Miller, K. G. *et al.* 2005 The Phanerozoic record of global sea-level change. *Science* **310**, 1293–1298. (doi:10.1126/science.1116412)
- Mulchay, D. G., Morrill, B. H. & Mendelson J. R. 2006 Historical biogeography of lowland species of toads (*Bufo*) across the trans-Mexican neovolcanic belt and the Isthmus of Tehuantepec. *J. Biogeogr.* **33**, 1889–1904.
- Navarro-Sigüenza, A. G., Peterson, A. T., Nyari, A., García-Deras, G. M. & García-Moreno, J. 2008 Phylogeography of the *Buarremon* brush-finch complex (Aves, Emberizidae) in Mesoamerica. *Mol. Phylogenet. Evol.* **47**, 21–35. (doi:10.1016/j.ympev.2007.11.030)
- Nelson, G. & Platnick, N. I. 1981 *Systematics and biogeography: cladistics and Vicariance*. New York, NY: Columbia University Press.
- Palsbøll, P. J., Bérubé, M., Aguilar, A., Notarbartolo-Di-Sciara, G. & Nielsen, R. 2004 Discerning between recurrent gene flow and recent divergence under a finite-site mutation model applied to North Atlantic and Mediterranean Sea fin whale (*Balaenoptera physalus*) populations. *Evolution* **58**, 670–675.
- Parker, T. A., Stotz, D. F. & Fitzpatrick, J. W. 1996 Ecological and distributional databases for Neotropical birds. In *Neotropical birds: ecology and conservation* (eds D. F. Stotz, J. W. Fitzpatrick, T. A. Parker & D. K. Moskovits). Chicago, IL: University of Chicago Press.
- Pérez-Emán, J. L. 2005 Molecular phylogenetics and biogeography of the Neotropical redstarts (*Myioborus*; Aves, Parulidae). *Mol. Phylogenet. Evol.* **37**, 511–528. (doi:10.1016/j.ympev.2005.04.013)
- Peterson, A. T., Soberon, J. & Sanchez-Cordero, V. 1999 Conservatism of ecological niches in evolutionary time. *Science* **285**, 1265–1267. (doi:10.1126/science.285.5431.1265)
- Petit, J. R. *et al.* 1999 Climate and atmospheric history of the past 420000 years from the Vostok ice core, Antarctica. *Nature* **399**, 429–436. (doi:10.1038/20859)
- Puebla-Olivares, F., Bonaccorso, E., Espinosa de los Monteros, A., Omland, K. E., Llorente-Bousquets, J. E., Peterson, A. T. & Navarro-Sigüenza, A. G. 2008 Speciation in the emerald toucanet (*Aulacorhynchus prasinus*) complex. *Auk* **125**, 39–50. (doi:10.1525/auk.2008.125.1.39)
- Rambaut, A. & Drummond, A. J. 2007. *TRACER v1.4*. See <http://beast.bio.ed.ac.uk/Tracer>.
- Riddle, B. R. & Hafner, D. J. 2006 A step-wise approach to integrating phylogeographic and phylogenetic biogeographic perspective on the history of a core North American warm deserts biota. *J. Arid Environ.* **66**, 435–461. (doi:10.1016/j.jaridenv.2006.01.014)
- Rosenberg, N. A. & Nordborg, M. 2002 Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. *Nat. Rev. Genet.* **3**, 380–390. (doi:10.1038/nrg795)
- Sullivan, J., Arellano, E. & Rogers, D. S. 2000 Comparative phylogeography of Mesoamerican highland rodents: concerted versus independent response to past climate fluctuations. *Am. Nat.* **155**, 755–768. (doi:10.1086/303362)
- Swofford, D. S. 2002 *PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sunderland, MA: Sinauer.
- Tajima, F. 1983 Evolutionary relationships of DNA sequences in finite populations. *Genetics* **105**, 437–460.
- Watterson, G. A. 1975 On the number of segregating sites in genetic models without recombination. *Theor. Popul. Biol.* **7**, 256–276. (doi:10.1016/0040-5809(75)90020-9)
- Weir, J. T. & Schluter, D. 2004 Ice sheets promote speciation in boreal birds. *Proc. R. Soc. Lond. B* **271**, 1881–1887. (doi:10.1098/rspb.2004.2803)
- Weir, J. T. & Schluter, D. 2008 Calibrating the avian molecular clock. *Mol. Ecol.* **17**, 2321–2328. (doi:10.1111/j.1365-294X.2008.03742.x)
- Wells, P. V. 1983 Paleobiogeography of montane islands in the Great Basin since the last glaciopluvial. *Ecol. Monogr.* **53**, 342–382.
- Zink, R. M. 2002 Methods in comparative phylogeography, and their application to studying evolution in the North American Aridlands. *Integr. Comp. Biol.* **42**, 953–959. (doi:10.1093/icb/42.5.953)
- Zink, R. M., Klicka, J. & Barber, B. R. 2004 The tempo of avian diversification during the Quaternary. *Phil. Trans. R. Soc. Lond. B* **359**, 215–220. (doi:10.1098/rstb.2003.1392)