Syst. Biol. 57(4):544–561, 2008
Copyright © Society of Systematic Biologists
ISSN: 1063-5157 print / 1076-836X online
DOI: 10.1080/10635150802304761

A Likelihood Framework for Estimating Phylogeographic History on a Continuous Landscape

ALAN R. LEMMON AND EMILY MORIARTY LEMMON

Section of Integrative Biology, University of Texas–Austin, 1 University Station, No. C0930, Austin, Texas 78712, USA; and Section of Evolution and Ecology, University of California–Davis, One Shields Avenue, Davis, California 95616; USA; E-mail: alemmon@evotutor.org (A.R.L.); emlemmon@ucdavis.edu (E.M.L.)

Abstract.— Due to lack of an adequate statistical framework, biologists studying phylogeography are abandoning traditional methods of estimating phylogeographic history in favor of statistical methods designed to test a priori hypotheses. These new methods may, however, have limited descriptive utility. Here, we develop a new statistical framework that can be used to both test a priori hypotheses and estimate phylogeographic history of a gene (and the statistical confidence in that history) in the absence of such hypotheses. The statistical approach concentrates on estimation of geographic locations of the ancestors of a set of sampled organisms. Assuming a spatially explicit random walk model of migration, we derive an equation describing the likelihood of the geographic coordinates of the individuals represented by internal nodes on a tree (the parameters of interest) and the mean per-generation dispersal distance (which can be estimated as a nuisance parameter), given the geographic coordinates of the individuals represented by tips on the tree (topology and branch lengths are assumed to be known). Using a maximum likelihood approach, which is implemented in the new program PhyloMapper, we apply this statistical framework to a 246-taxon mitochondrial genealogy of North American chorus frogs, focusing in detail on one of these species. We demonstrate three lines of evidence for recent northward expansion of the mitochondrion of the coastal clade of *Pseudacris feriarum*: higher per-generation dispersal distance in the recently colonized region, a noncentral ancestral location, and directional migration. After illustrating one method of accommodating phylogenetic uncertainty, we conclude by discussing how extensions of this framework could function to incorporate a priori ecological and geological information into phylogeographic analyses. [Maximum likelihood; phylogeography; PhyloMapper; *Pseudacris*; random walk; statistical phylogeography; landscape.]

Understanding the processes that drive the formation of new species is one of the most compelling challenges of evolutionary biology. Speciation is typically thought to occur when biotic and abiotic factors cause geographic isolation of populations, which inhibits migration and allows for genetic divergence (Mayr, 1942). One of the primary aims of phylogeography is to reconstruct the history of migration within species and identify the specific factors that shaped this history, especially those factors contributing to incipient speciation. Attaining this goal will require integration of diverse types of data within a common statistical framework (Knowles and Maddison, 2002; Knowles, 2004).

The field of phylogeography has experienced a rapid shift from descriptive analyses to tests of explicit a priori hypotheses (Knowles, 2004). Earlier phylogeographic work typically involved analyses of mitochondrial data using nested clade analysis (Templeton, 1998, 2004; Posada et al., 2006). Nested clade analysis enjoyed such popularity because it could be applied to virtually any data set and because it provided an estimate of phylogeographic history that did not require a priori assumptions about potential historical scenarios. One limitation of nested clade analysis is that estimates of phylogeographic history (i.e., inferences) are not based on a statistical model. Consequently, the uncertainty of an estimated history cannot be known, alternative hypotheses cannot be tested with statistical rigor (Panchal and Beaumont, 2007), and additional data (e.g., ecological information) cannot be directly incorporated into analyses.

Phylogeography is entering a new phase of development because of recent advances in the fields of landscape genetics and coalescent theory, the development of tools for generating ecological niche and paleoclimate models, and the increasing availability of nuclear genetic markers. At fine geographic scales, advances in landscape genetics allow researchers to study in detail how local ecological factors affect gene flow and result in geographic structure of populations (Manel et al., 2003; Guillot et al., 2005; Spear et al., 2005; Holderegger and Wagner, 2006; Corander et al., 2007; Storfer et al., 2007). For example, environmental data can be used within a GIS framework to identify the most probable geographic paths of gene flow among populations as well as potential barriers to migration (Storfer et al., 2007).

Researchers are also addressing broad-scale phylogeographic questions using recent advances in coalescent theory (e.g., Kuhner et al., 1998; Beerli and Felsenstein, 1999, 2001; Nielsen and Wakeley, 2001). Developments include the use of coalescent simulations to test simple a priori hypotheses (Carstens et al., 2005; Steele and Storfer, 2006), to examine concordance of divergence times among species pairs (Hickerson et al., 2006b, 2007), and to test alternative scenarios of population expansion and colonization on a continuous landscape (Currat et al., 2004; Currat and Excoffier, 2005; Ray et al., 2005). One new approach involves a two-step process in which alternative hypotheses regarding patterns of population differentiation are first generated by projecting ecological niche data onto paleoclimate models and then evaluated statistically using coalescent simulations (Carstens and Richards, 2007; Knowles et al., 2007; Richards et al., 2007). Although these new approaches have brought statistical rigor to phylogeography, their scope may be limited to systems in which simple a priori hypotheses can be generated. Whereas use of this methodology to distinguish among simple historical scenarios may be straightforward, application to systems with more complex phylogeographic histories may be very difficult.

These recent developments have resulted in a class of methods that are focused heavily on hypothesis testing but are not particularly useful descriptive tools. Many empirical systems would benefit from a statistical framework that could be used to both test a priori hypotheses and estimate phylogeographic history in the absence of such hypotheses. These two approaches are not mutually exclusive. In the field of likelihood-based phylogenetics, for instance, relationships among species (and the statistical confidence in the estimate) are often estimated without a priori hypotheses in mind (Felsenstein, 2004). The majority of biologists would agree that this is a worthy endeavor, even if hypotheses are not explicitly tested. Nonetheless, the relative merits of alternative hypotheses are sometimes tested in the same statistical framework (e.g., using a parametric bootstrapping approach; Goldman et al., 2000). The same type of flexibility would greatly benefit the field of phylogeography. We argue that the ideal phylogeographic framework would have the flexibility and descriptive power of nested clade analysis *and* the statistical rigor necessary to test hypotheses and assess statistical confidence in parameter estimates.

In this paper, we introduce a statistical framework that can be used to test explicit a priori hypotheses when available, or estimate phylogeographic history in the absence of such hypotheses. We focus on estimating the geographic locations of ancestors of sampled individuals, rather than population genetic parameters such as population size and mutation rate. In order to accommodate any type of geographic sampling (i.e., from discrete populations or continuously distributed samples), we assume that individuals occupy continuous geographic space. We also simplify the analyses by assuming that the gene tree relating the samples is known, a common assumption for many types of evolutionary methods (e.g., Felsenstein, 1985; Schluter et al., 1997; Pagel, 1999; Sanderson, 2002; O'Meara et al., 2006). Though we introduce this new framework using a simplified model in this paper, we will relax the simplifying assumptions in future work by incorporating additional information (see Assessing Phylogenetic Uncertainty and Discussion).

We begin by developing a method that utilizes a gene tree and the geographic coordinates of individuals at the tips of the tree to estimate the geographic coordinates of the ancestors at the internal nodes of the tree. After developing the statistical model, we demonstrate the flexibility of this approach by analyzing a 246-taxon data set of the North American chorus frogs (Lemmon et al., 2007b). We then introduce four novel statistical tests that can be used to test explicit a priori hypotheses regarding the phylogeographic history of the gene. After suggesting one possible method of accounting for gene tree uncertainty, we compare our framework to existing methods and conclude by discussing ways in which this new framework could be used to integrate geological, climatic, and ecological data.

MODEL

Here we develop a model-based method of estimating the geographic location of an ancestor for a set of extant taxa. After laying out the notation and assumptions, we describe a random-walk model of migration and outline how this model can be used in a maximum likelihood framework to estimate key parameters of phylogeographic history. We conclude this section by considering the null expectation for the location of the ancestor, discussing how the parameter estimates should be interpreted, and outlining some conditions that may lead to biased estimates.

Notation and Assumptions

Our model describes the migration of individuals in continuous geographic space. The data used in the model are a rooted genealogy with branch lengths and the geographic coordinates (latitude and longitude) of each individual represented by a tip of the genealogy. Let T, $\mathbf{b}$, $\boldsymbol{\phi}_{\mathrm{o}}$, and $\boldsymbol{\lambda}_{\mathrm{o}}$ represent the tree, the vector of branch lengths, the vector of observed latitudes, and the vector of observed longitudes, respectively. The primary aim is to estimate the vectors of latitudes and longitudes, $\boldsymbol{\phi}_{\mathrm{e}}$ and $\boldsymbol{\lambda}_{\mathrm{e}}$, of individuals represented by internal nodes on the genealogy. This model is analogous to reconstructing continuous character states on a phylogeny (e.g., Schluter et al., 1997), where the geographic coordinates are the trait data.

We assume the genealogy is known without error. In Empirical Application, we suggest one way of relaxing this assumption. The branch lengths are assumed to be proportional to time. Although our model requires the tips of the genealogy to be contemporaneous, an absolute time scale is not necessary. Therefore, a genealogy can be made ultrametric (e.g., by rate-smoothing; Sanderson, 1997) assuming either a known age or an arbitrary age for the root node. In each generation, individuals are assumed to migrate in a random direction and distance. Offspring are assumed to migrate in a manner that is independent of their parents and their siblings.

Our model is general in several respects. First, it makes no assumptions regarding population identity (i.e., that samples come from discrete populations), life history (e.g., nonoverlapping generations), mutation rate, or mating system. Second, the model allows for any type of geographic sampling (but see Parameter Interpretation below), though uniform sampling is expected to yield the most informative results. Third, the model accommodates a large class of dispersal functions, such as the negative exponential, in which the probability of dispersal to a point decreases rapidly with increasing distance. Problematic dispersal functions include those with infinite moments (such as the Couchy distribution, which has fat tails that may represent many long distance dispersers). Lastly, the model can easily be extended to allow for variation in the dispersal distance across the genealogy (see Variation in Dispersal Distance).

The Model

Model of migration.—The migration model developed here is most similar to that of Neigel et al. (1991). Assume that individuals migrate in a direction, θ, that is distributed as f_θ and a distance, δ, that is distributed as f_δ and independent of direction. We also assume that θ is uniformly distributed over (0,2π), such that $f_\theta = 1/(2\pi)$. Let $\{\phi_i, \lambda_i\}$ be the geographic coordinates (latitude and longitude, respectively) of some ancestor i and $\{\phi_j, \lambda_j\}$ be the coordinates of some descendant j of the ancestor. The coordinates of the descendant relative to the ancestor are $\{\Delta\phi_j, \Delta\lambda_j\}$, where $\Delta\phi_j = \phi_j - \phi_i$ and $\Delta\lambda_j = \lambda_j - \lambda_i$.

Ancestor-descendant distance.—Because dispersal is assumed to be radially symmetric, we can define $\sigma_\chi^2 = \sigma_\phi^2 = \sigma_\lambda^2$ to be the variance in descendant position along any arbitrary axis (passing through the ancestral coordinates) after one generation. The position of a descendant relative to its ancestor is distributed as $f_{\Delta\phi,\Delta\lambda}(\sigma_g^2)$, where σ_g^2 is the variance in descendant position after g generations. By the Central Limit Theorem, as $g \to \infty$, $f_{\Delta\phi,\Delta\lambda}$ approaches a bivariate normal distribution with mean equal to zero and variance equal to $\sigma_g^2 = g\sigma_\chi^2$ along any arbitrary axis. This approximation holds for any dispersal kernel f_δ with finite moments. Preliminary simulations suggest that the normal approximation is accurate when g is on the order of 100 generations or longer (see online Figure S1 at www.systematicbiology.org).

The random walk model becomes the diffusion model (i.e., Brownian motion model) in the limit when the generation time and dispersal distance approach zero. The diffusion model has been applied to a broad range of evolutionary questions that can be answered by estimating the ancestral state of a biological parameter (e.g., Felsenstein, 1985; Schluter et al., 1997). We do not make the diffusion approximation here because we wish to keep the dispersal parameter in units of distance *per-generation* (instead of rate per time), so that estimates of this parameter may more easily be compared to estimates from field studies.

If g is not known, but the length of the branch connecting the ancestor and descendant (b, proportional to generations) is known, we can substitute $g = b/\gamma$, where γ is the average generation time (time per generation, where time is on the same scale as b). With this substitution, the density function $f_{\Delta\phi,\Delta\lambda}$ is

$$f_{\Delta\phi,\Delta\lambda} = \left(\frac{1}{\sqrt{2\pi\sigma_\chi^2 b/\gamma}} e^{-\Delta\lambda^2/(2\sigma_\chi^2 b/\gamma)}\right) \times \left(\frac{1}{\sqrt{2\pi\sigma_\chi^2 b/\gamma}} e^{-\Delta\phi^2/(2\sigma_\chi^2 b/\gamma)}\right)$$

$$= \frac{1}{2\pi\sigma_\chi^2 b/\gamma} e^{-(\Delta\lambda^2\Delta\phi^2)/(2\sigma_\chi^2 b/\gamma)} \tag{1}$$

Equation (1) is equivalent to the density of a bivariate normal distribution with means equal to zero and variances equal to $\sigma_\chi^2 b/\gamma$. Because γ may be unknown, it will be convenient to define $\psi^2 = \sigma_\chi^2/\gamma$, which is the variance in descendant position scaled by the generation time. We refer to ψ hereafter as the scaled dispersal parameter. For clarity, we assume the value of this parameter is the same for all branches but discuss how the assumption can be relaxed. Also, because $f_{\Delta\phi,\Delta\lambda}$ is radially symmetric, we can rewrite (1) in terms of δ_b, the distance between an ancestor and descendant separated by a branch of length b. This can be accomplished by substituting δ^2 for $\Delta\lambda^2 + \Delta\phi^2$ and noting that the area of the ring delineated by $\delta \pm \varepsilon$ becomes $2\pi\delta$ as $\varepsilon \to 0$. With these substitutions we have

$$\begin{aligned}
f_{\delta_b} &= 2\pi\delta_b f_{\lambda,\phi}\\
&= 2\pi\delta_b \frac{1}{2\pi\sigma_\chi^2 b/\gamma} e^{-(\Delta\lambda^2+\Delta\phi^2)/\left(2\sigma_\chi^2 b/\gamma\right)}\\
&= \frac{\delta_b}{\sigma_\chi^2 b/\gamma} e^{-(\Delta\lambda^2+\Delta\phi^2)/\left(2\sigma_\chi^2 b/\gamma\right)}\\
&= \frac{\delta_b}{\sigma_\chi^2 b/\gamma} e^{-\delta_b^2/\left(2\sigma_\chi^2 b/\gamma\right)}\\
&= \frac{\delta_b}{b\psi^2} e^{-\delta_b^2/(2b\psi^2)}
\end{aligned} \tag{2}$$

Equation (2) is the probability density function describing the ancestor-descendant distances as a function of the length of the branch separating them. When the geographic scale is sufficiently small, the distance δ_i between two individuals, j and k, at opposite ends of branch i can be approximated as the straight-line distance between two points:

$$\delta_i = \sqrt{(\phi_j - \phi_k)^2 + (\lambda_j - \lambda_k)^2}. \tag{3}$$

When the geographic scale is larger, the great-circle distance is more appropriate:

$$\delta_i = 2\,R \arcsin \times \left[\sqrt{\sin^2\left(\frac{\phi_k^r - \phi_j^r}{2}\right) + \cos(\phi_j^r)\cos(\phi_k^r)\sin^2\left(\frac{\lambda_k^r - \lambda_j^r}{2}\right)}\right], \tag{4}$$

where the superscript r denotes that the latitude or longitude has been converted to radians and R is the quadratic mean radius of the earth (Sinnott, 1984). The great circle distance takes into account the fact that organisms are migrating on a sphere, instead of a plane. In principle, a hypothesis testing approach could be used to determine whether (4) provides a significantly better fit than (3) for a particular data set.

Likelihood function.—Now we use (2) to derive the likelihood of the ancestral geographic coordinates and the value of the scaled dispersal parameter, given the observed geographic coordinates (assuming known topology and branch lengths). Under our assumption that individuals migrate independently, the full likelihood can be obtained by taking the product of f_{δ_b} over all branches:

$$L(\boldsymbol{\phi}_{\mathrm{e}}, \boldsymbol{\lambda}_{\mathrm{e}}, \psi | \boldsymbol{\phi}_{\mathrm{o}}, \boldsymbol{\lambda}_{\mathrm{o}}) = \prod \left(\frac{\delta_i}{b_i \psi^2} e^{-\delta_i^2/(2b_i\psi^2)} \right). \tag{5}$$

Note that although this equation allows for polytomies, the polytomies are assumed to be hard.

Maximum likelihood estimation of ψ.—The simplicity of (5) allows us to solve for $\hat{\psi}$, the maximum likelihood estimate of the scaled dispersal parameter, by taking the derivative of (5) with respect to ψ, setting it equal to zero, and solving for ψ. The derivative of (5) with respect to ψ is

$$\frac{\partial}{\partial\psi} L(\boldsymbol{\phi}_{\mathrm{e}}, \boldsymbol{\lambda}_{\mathrm{e}}, \psi | \boldsymbol{\phi}_{\mathrm{o}}, \boldsymbol{\lambda}_{\mathrm{o}}) = \frac{(\xi - 2)e^{-\frac{1}{2}\xi}}{\psi^{2n+1}} \prod \frac{\delta_i}{b_i}, \tag{6}$$

where

$$\xi = \frac{1}{\psi^2} \sum \frac{\delta_i^2}{b_i}, \tag{7}$$

After setting the derivative equal to zero and solving for ψ, we have

$$\hat{\psi} = \frac{1}{2n} \sum \frac{\delta_i^2}{b_i}, \tag{7}$$

which is proportional to the standard deviation of the position of descendants relative to their ancestors, scaled by the length of the branch between them. Unfortunately, equations simultaneously describing the maximum likelihood locations of all ancestors ($\hat{\boldsymbol{\phi}}_{\mathrm{e}}$ and $\hat{\boldsymbol{\lambda}}_{\mathrm{e}}$) are not tractable.

Variation in ψ across branches.—Thus far we have assumed that ψ does not vary across branches. This assumption can easily be relaxed by defining $\boldsymbol{\psi}$ to be a vector containing the values of ψ for d dispersal classes. Each branch on the genealogy can then be assigned to one of the dispersal classes. An equation similar to (5) can be used to calculate the likelihood for the branches in each class and the full likelihood can be calculated by multiplying the likelihood across dispersal classes. As we will see below in Empirical Application, maximum likelihood scores for different class assignments can be used to test for variation in dispersal across clades or geographic space.

Parameter optimization.—Because an exact solution is not tractable analytically, we use an iterative optimization approach to estimate the maximum likelihood values of $\boldsymbol{\phi}_{\mathrm{e}}$ and $\boldsymbol{\lambda}_{\mathrm{e}}$. The optimization routine consists of the following steps: (i) $\boldsymbol{\phi}_{\mathrm{e}}$ and $\boldsymbol{\lambda}_{\mathrm{e}}$ are initialized with random values (uniformly distributed) within the range of $\boldsymbol{\phi}_{\mathrm{o}}$ and $\boldsymbol{\lambda}_{\mathrm{o}}$, respectively; (ii) each value in $\hat{\boldsymbol{\psi}}$ is computed using (7); (iii) values of $\hat{\boldsymbol{\phi}}_{\mathrm{e}}$ and $\hat{\boldsymbol{\lambda}}_{\mathrm{e}}$ are estimated using (5) and the Newton-Raphson (Ypma, 1995) optimization technique (elements of $\hat{\boldsymbol{\phi}}_{\mathrm{e}}$ and $\hat{\boldsymbol{\lambda}}_{\mathrm{e}}$ are optimized in a random order); (iv) steps ii and iii are repeated until the change in each of the parameters between iterations is less than 10^{-12}, and (v) final values in $\hat{\boldsymbol{\psi}}$ are computed using (7). The above routine was implemented in the Java software PhyloMapper and is available at http://www.evotutor.org/PhyloMapper.

The method of parameter optimization described above performs well, though entrapment in a local optimum is possible for some random starting points. Preliminary analyses suggest that precise estimates of ancestral latitude and longitude, as well as the scaled dispersal parameter, are easily obtainable given 100 or more replicate analyses with randomized starting locations (see online Figure S2 at www.systematicbiology.org). This suggests that the global optimum can be reliably found. The method is also fast: optimization for a 246-taxon genealogy with 200 starting replicates (see Empirical Application below) typically requires less than 20 min of CPU time on a 2.13-GHz Intel Pentium IV PC. The speed of this optimization allows one to ascertain the effect of phylogenetic uncertainty or evaluate the likelihood surface for an ancestral location (see below).

Null Expectation

When a population is at a stable equilibrium, what is the expected location of the most recent common ancestor of the extant individuals? Kirkpatrick and Barton (1997) presented a seminal study of the interaction of migration and selection at the edge of a species' range. The model that they developed suggests that the edge of the species range should act as a sink because individuals that migrate towards the edge are maladapted (García-Ramos and Kirkpatrick, 1997; Kirkpatrick and Barton, 1997). This model gives us insight into the null expectation for the location of the most recent common ancestor of the extant individuals: the center of the range. This phenomenon has been demonstrated in coalescent simulations (Wilkins and Wakeley, 2002).

The center of the range, however, is only the *expected* geographic location of the ancestor. Because of stochastic processes, such as migration and reproduction, the actual location of the ancestor could have been some distance away from the center of the range. The degree of deviation from the center is a function of the distance individuals migrate, the densities of the populations across the range, and other properties of the species. In the extreme case where the dispersal distance is very high (on the order of the width of the range), there should be no association between the geographic location of two sampled individuals and their genetic distance (Wright, 1943; Barton and Wilson, 1995). In this case, we expect the deviation of the ancestral location from the null expectation (the center of the range) to be large. In the other extreme case (where dispersal distance $\ll$ range width), we expect the deviation to be much smaller and a phylogeographic association (i.e., isolation by distance; Wright, 1943) may exist.

Parameter Interpretation

Care must be taken when interpreting estimates of ϕ_i and λ_i, the geographic coordinates of the ancestor represented by the internal node i. An internal node represents the most recent common ancestor of the *sampled* descendants of that individual. If one wishes to estimate the geographic location of the most recent common ancestor of a particular species, for example, one needs to sample individuals from across the entire range of the species. Samples taken from only part of the range can only be used to estimate the location of the ancestor of individuals in that part of the range. Also note that because a single gene is used, then what is actually being estimated is the geographic location of the individual in which the sampled genes coalesced. For human mitochondrial data, for example, this is the "mitochondrial eve" (Cann et al., 1987; Ingman et al., 2000). Multiple nuclear genes could be used to estimate patterns of migration that are representative of the entire nuclear genome (see Discussion).

The scaled dispersal parameter (ψ) of a species may evolve through the evolution of the per-generation dispersal distance (δ) or the generation time (γ). The former case has been demonstrated in cane toads, for example, where Phillips et al. (2006) found that the dispersal distance has evolved at the advancing edge of the species range. More research is needed to determine the extent to which generation time may evolve within species.

Estimates of the scaled dispersal parameter may be biased by processes that violate the assumptions of our model. The most obvious process is recent expansion, which is expected to result in positively biased estimates. When populations are expanding into a new geographic region, for example, we might expect that individuals at the edge of the current range are more likely to migrate in the direction of the new region (due to lower competition). Because individuals sampled in the newly colonized region are likely to be descended from individuals that invaded this new region, the directions that ancestors along this branch migrated are expected to be nonrandom. This process will positively bias estimates of the scaled dispersal parameter and may also affect estimates of the ancestral locations. The strength of this effect is expected to diminish as time since the expansion event increases.

EMPIRICAL APPLICATION

Background

In this section we test a series of hypotheses regarding the phylogeographic history of the trilling chorus frogs by applying the statistical framework developed above to the 246-taxon genealogy estimated by Lemmon et al. (2007b). The trilling chorus frogs form a clade within the North American genus *Pseudacris*. This clade is geographically widespread across the continent, ranging from the East Coast to the Rocky Mountains and from northern Mexico to northern Canada (Conant and Collins, 1998). Species within this group congregate to breed in temporary pools in late winter or early spring, and then disperse to terrestrial habitats for the remainder of the year. *Pseudacris* show high fidelity to ponds of origin, suggesting relatively low dispersal rates (Spencer, 1964). Given their geographic distributions, it is likely that these taxa have been affected by a battery of different climatological and geological processes, which have left their footprint in the genetic structure of populations (Lemmon et al., 2007a).

We focus in particular on one species, *Pseudacris feriarum*, that contains two major clades: a coastal clade, distributed from Georgia to Pennsylvania primarily east of the Appalachian Mountains, and an inland clade, distributed from Florida to Kentucky west of the Appalachians. Both of these clades currently occupy a warm mixed forest biome (see Williams et al., 2004). Climate change models of the last 10,000 years indicate that the position of this biome has shifted northward on the eastern side of the Appalachians but has remained relatively stable on the western side (Williams et al., 2004). This suggests that members of the coastal clade may have recently expanded northward along the East Coast of the United States, whereas members of the inland clade have not recently expanded.

Using the statistical framework outlined in the previous section, we test for an association between phylogenetic relatedness and geographic proximity (isolation by distance) in 10 major clades within the trilling chorus frogs. We then test three predictions of recent expansion in the coastal and inland *Pseudacris feriarum* clades. The predictions are first, the estimated scaled dispersal parameter ($\hat{\psi}$) should be higher in the recently invaded area. Second, the location for the ancestor of the clade should differ significantly from the null expectation (center of range). Third, migration within the clade should tend to be in the predicted direction (north). For each test, we first describe the rationale and general methods, then we describe how each test was applied to the empirical data set, and finally, we interpret the test results. We conclude this section by illustrating one way of assessing the effect of phylogenetic uncertainty on estimates of phylogeographic history.

Rate Smoothing the Gene Tree

The analyses presented below utilize the Lemmon et al. (2007b) genealogy and the divergence times estimated for a subset of these taxa by Lemmon et al. (2007a). In the latter study, divergence times of eight nodes within the trilling frogs were estimated using fossil calibrations and penalized likelihood (Sanderson, 2002). The divergence times of three of these nodes were also corroborated using an independent coalescent approach (see Lemmon et al., 2007a, for details). In order to construct a chronogram of the full 246 taxa, we employed nonparametric rate smoothing (Sanderson, 1997), while constraining the ages of the eight dated nodes. The resulting chronogram of the entire trilling frog clade is presented online in Figure S3 (www.systematicbiology.org) and the chronogram of the focal clade, *Pseudacris feriarum*, is presented in Figure 1. Note that results do not change qualitatively if a single arbitrary age is used for the root of the trilling frogs, regardless of the age chosen. Nonparametric rate smoothing was implemented in PhyloMapper because it can be easily automated. Future versions of the software may also incorporate penalized likelihood (Sanderson, 2002).

Phylogeographic Association

General methods.—The ability to infer the phylogeographic history of a clade depends on the strength of the association between the phylogenetic and geographic distances among individuals. Therefore, it is reasonable to begin by assessing the degree of phylogeographic association for the clade of interest. Here we demonstrate a novel test for this association. Specifically, we use $\hat{\psi}$, the estimate of the scaled dispersal parameter, as an indicator of phylogeographic association, where a lower value indicates a relatively stronger association. Use of this parameter as a test statistic makes biological sense because the amount of phylogeographic association is a function of the dispersal variance, σ_χ^2, and the distance between samples. Because $\psi = \sigma_\chi/\sqrt{\gamma}$, a relatively large value of ψ suggests that the per-generation dispersal variance is high and thus that there is low phylogeographic association (assuming the generation time is held constant). A smaller value of ψ, conversely, suggests that there may be evidence for phylogeographic structure (i.e., isolation by distance).

How small must $\hat{\psi}$ be before one can conclude that a phylogeographic association exists? Under the null hypothesis that there is no association (correlation) between geographic distance and genetic distance, the two sister taxa are just as likely to be far apart geographically as two taxa that are phylogenetically distant. Thus, one can estimate the null distribution of $\hat{\psi}$ by holding the genealogy and the sampling localities constant, but randomizing the relationship between the two. Specifically, one can generate the null distribution by repeating the following three steps: (1) randomize the assignment of geographic location (ϕ_i and λ_i) to the tips of the genealogy; (2) estimate maximum likelihood values of ψ, ϕ_e, and $\boldsymbol{\lambda}_e$; and (3) store the value of $\hat{\psi}$. To test for significance, the null distribution is compared to the value of $\hat{\psi}$ obtained when the relationship between the genealogy and sampling localities are not randomized.

Tests with Pseudacris.—We tested for phylogeographic association within each species, within the coastal *P. feriarum* clade, and within the northern coastal *P. feriarum* clade (Table 1, Fig. 1). For each test, we noted the test statistic after optimizing all parameters, then generated the null distribution by performing 10,000 randomizations as described in steps 1 to 3 above.

TABLE 1. Randomization tests for phylogeographic association in 10 clades of the trilling chorus frogs. The test statistic is the estimate of the dispersal parameter ($\hat{\psi}$) given a genealogy and the geographic coordinates of the individuals represented by the tips. Note that the per-generation dispersal distance estimate ($\hat{\delta}$, in meters per generation) is equal to $\hat{\psi}$ under the assumptions that the generation time is one year (Green, 1964; Caldwell, 1987; Smith, 1987) and individuals disperse according to a negative exponential dispersal kernel (in which case $\bar{\delta} = \sigma_\chi$). The P-value indicates the proportion of samples from the null distribution (see text) that is less than the test statistic. Asterisks indicate significance at the $\alpha = 0.05$ significance level. Maximum known dispersal distances (meters in one generation) from empirical studies are shown for taxa for which these data are available and are taken from the following studies: [a]Green (1952), [b]Spencer (1964), and [c,d]Kramer (1973, 1974). Note that here we compare estimated *mean* dispersal distances to *maximum* observed dispersal distances.

Clade	Taxa	$\hat{\psi}$	P-value	Max dispersal
P. brimleyi	8	73.36	0.0062*	—
P. brachyphona	21	92.59	<0.0001*	610[a]
P. maculata/clarkii	61	370.12	<0.0001*	686[b]
P. nigrita	17	131.31	0.0016*	—
P. fouquettei	28	194.24	0.0214*	—
P. kalmi	10	55.59	0.1572	—
P. triseriata	25	135.52	<0.0001*	213[c], 180[d]
P. feriarum	60	144.59	<0.0001*	—
P. feriarum coastal	28	172.60	<0.0001*	—
P. feriarum coastal N	19	195.70	0.0023*	—

Results.—We found evidence for phylogeographic association in 9 of the 10 clades tested (Table 1). The one exception is *Pseudacris kalmi*, which has the smallest range of the trilling chorus frogs (encompassing only New Jersey and the Delmarva Peninsula, which includes parts of Delaware, Maryland, and Virginia). This result is not surprising given the limited number of samples available (10) and the small geographic distribution of the species (see Lemmon et al., 2007b). One interesting result is that the degree of phylogeographic association in the *P. maculata/clarkii* clade was found to be significant, despite very low genetic variation within the clade (Lemmon et al., 2007b). This association may have been detected because of the large number of samples included (61) and/or the large geographic scale at which the samples were taken (see Lemmon et al., 2007b).

Variation in Dispersal Distance

General methods.—The first prediction of recent geographic expansion is that the estimated dispersal parameter should be higher in the area hypothesized to be recently invaded. We expect $\hat{\psi}$ to be higher in the recently invaded area because the individuals sampled in this region are descended from ancestors that migrated in a non-random direction (in the direction of the expansion). Directional migration results in a positively biased estimate of $\hat{\psi}$ because individuals separated by a set amount of time are expected to be further apart geographically when migration is non-random with respect to direction.

To test the prediction of greater dispersal distance, one must identify the clade containing the tips that exist in the area hypothesized to be recently invaded (an alternative test should be constructed if the individuals

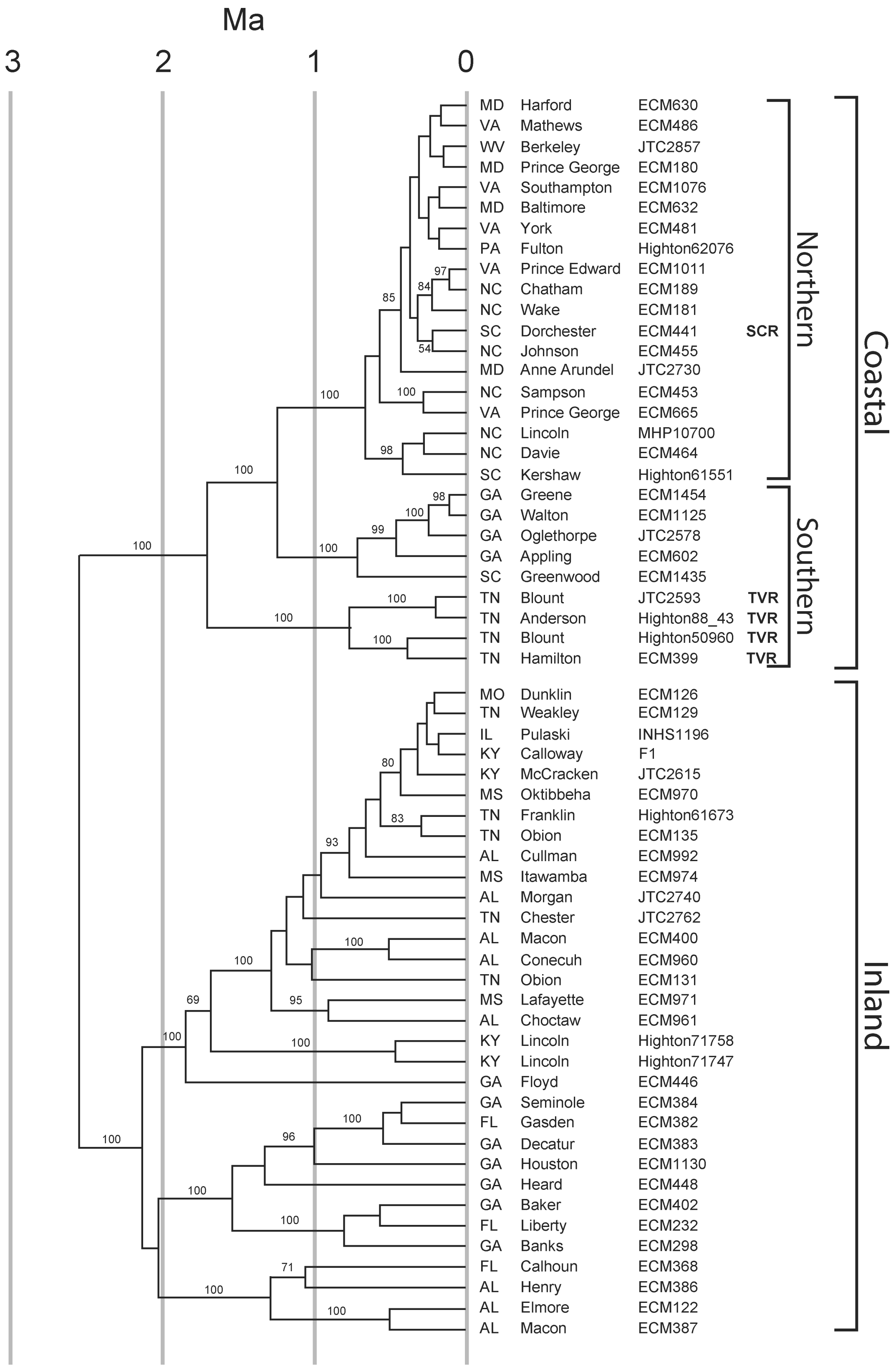

FIGURE 1. Chronogram of *Pseudacris feriarum*. Brackets indicate major groups discussed in the text. This chronogram was taken from the trilling frog chronogram (Fig. S3), which was estimated by applying nonparametric rate smoothing to the genealogy estimated by Lemmon et al. (2007b), and node ages estimated by Lemmon et al. (2007a). Support values on the branches indicate posterior probabilities greater than 50 (see Lemmon et al., 2007b, for details of the Bayesian phylogenetic analysis). Samples taken from the proposed South Carolina refugium and Tennessee Valley refugium are denoted SCR and TVR, respectively.

existing in this area are not monophyletic). All branches in this clade are assigned to set A and all branches outside this clade are assigned to set B. Under the null hypothesis, $\psi_A = \psi_B$, whereas under the alternative hypothesis, $\psi_A > \psi_B$. Two analyses are then performed, one in which all branches are constrained to have the same value for ψ (compatible only with the null hypothesis), and a second in which branches in sets A and B are allowed to have different values of ψ (compatible with the alternative hypothesis). Let L_1 and L_2 be the maximum likelihood scores from the first and second analyses, respectively. Significance can then be assessed with a likelihood-ratio test, using the test statistic $\chi_s^2 = 2 * (L_2/L_1)$ with one degree of freedom. A significant test indicates that the scaled dispersal parameter is greater in the recently invaded area and provides evidence for recent expansion. Note, however, that a significant result for this test alone is not sufficient evidence for expansion, because other histories (recent bottleneck), evolution of the dispersal distance, evolution of the generation time, or evolution of the rate of molecular evolution should produce the same pattern. Also note that one could test for significant variation in the scaled dispersal parameter across three or more sets of branches by specifying a more complicated alternative hypothesis (e.g., $\psi_1 \neq \psi_2$ or $\psi_1 \neq \psi_3$ or $\psi_2 \neq \psi_3$).

Tests with Pseudacris.—We tested for variation in dispersal distance at three levels in the *Pseudacris* genealogy: across species, within a species, and within a clade. For the across-species test, we used the entire trilling frog chronogram. We then compared the maximum likelihood score obtained when all branches found within species were assigned a single dispersal parameter to the score obtained when each species was assigned its own parameter. To ensure that the two hypotheses were nested, branches outside species-level clades were assigned an additional parameter in each analysis. Significance was assessed using a likelihood-ratio test with seven degrees of freedom (the difference in the number of dispersal distance classes between the null and alternative models).

For the within-species test, we focused on *Pseudacris feriarum*. We compared the maximum likelihood score obtained with a single dispersal parameter for all branches in *P. feriarum* to the score obtained with two dispersal parameters, one assigned to the coastal clade and one assigned to the other branches ($df = 1$).

The within-clade test focused on the coastal *Pseudacris feriarum* clade, which contains two southern clades and one northern clade (see Fig. 1). We compared the maximum likelihood score from an analysis assuming a single dispersal class to the score from a second analysis assuming two dispersal classes (class 1 contained only branches in the northern clade). Again, significance was determined using a likelihood-ratio test with one degree of freedom.

Results.—We found evidence for significant variation in dispersal distance at all three levels tested (Table 2). In the across-species test, the *Pseudacris kalmi* clade was found to have the smallest estimate of the per-generation dispersal distance ($\hat{\delta} = 57.38$ m). The estimate for *P. maculata/clarkii*, in contrast, was found to be the largest ($\hat{\delta} = 373.37$ m). This result is consistent with the findings of Lemmon et al. (2007a), who suggested that recent expansion occurred in this clade. Note that despite morphological conservatism in the trilling frogs as a whole, *P. maculata* has the shortest tibia length relative to body size and thus is expected to have a smaller dispersal distance (Lemmon et al., 2008). Also note that there is some empirical evidence that *P. maculata* may have a longer lifespan than other trilling frogs (Spencer, 1964), though the average generation time has not been estimated. Failure to account for this (i.e., assuming a generation time of 1 year, as we did) would have the effect of negatively biasing the dispersal distance estimate.

In the within-species test, the coastal *P. feriarum* clade was found to have a significantly larger estimate of the per-generation dispersal distance ($\hat{\delta} = 174.31$ m) than the inland *P. feriarum* clade ($\hat{\delta} = 113.50$ m). These results suggest that the distribution of the inland clade has been more stable than the distribution of the coastal clade. The final test, which compared the northern and southern coastal *P. feriarum* clades, supports the hypothesis that the coastal *P. feriarum* clade recently expanded northward. In this test, the scaled dispersal parameter of the northern clade was found to be nearly three times that of the southern clades.

Centers of Origin

General methods.—The second prediction of recent geographic expansion is a noncentral location for the ancestor of the clade that expanded. Recall that at equilibrium (no recent expansion), genes tend to flow from the center of the species' range to the range borders (Kirkpatrick and Barton, 1997). During expansion, conversely, individuals begin in one geographic region (a refugium, perhaps) and migrate into and fill a new region. Under this scenario, the ancestor of the extant species probably did not exist in the center of the range, but instead may have existed in the center of the region inhabited just before the expansion event.

Once the center of the sampled distribution has been established, one can conduct a likelihood-ratio test for significant noncentrality. The likelihood under the null hypothesis can be obtained by constraining the ancestor to be at the estimated center. The likelihood of the alternative hypothesis can be obtained through an unconstrained analysis. The test statistic is simply the ratio of the likelihoods of the unconstrained and constrained models. The degrees of freedom for this test are equal to two because two parameters (latitude and longitude for the ancestor) are fixed in the constrained analysis. A significant test statistic indicates that the ancestor of the sampled individuals did not exist at the point assumed to be the center. Note that a similar approach could be used to test any point (e.g., a hypothesized refugium).

One potential complicating factor is that the genealogy may not have been constructed using uniformly sampled individuals. To control for geographic sampling bias, one

TABLE 2. Likelihood-ratio tests of dispersal distance variation. Tests were performed at three levels: across species (Trilling), within species (*P. feriarum*), and within clades (*P. feriarum* coastal). Two analyses were performed at each level (number of dispersal classes differed between analyses). The b superscript indicates the dispersal class to which the basal branches were assigned (in each case, the outcome was the same when the basal branches were assigned to the other class). Asterisks indicate significance in a likelihood-ratio test (where χ^2 is the test statistic and $\alpha = 0.05$), where H_O denotes the null model and H_A denotes the alternative model. Other symbols given are the same as in Table 1. The degrees of freedom for each of the three tests is equal to the difference between the null and alternative models in the number of dispersal classes (7, 1, and 1, respectively).

Clade	Subclade	$\hat{\psi}$	Dispersal classes	Model	lnL	χ^2	*P*-value
Trilling		251.85	2	H_O	−2627.11	—	—
	(basal branches)	1.08					
	P. brimleyi	74.82	9	H_A	−2503.93	246.36	<0.0001*
	P. brachyphona	93.37					
	P. maculata/clarkii	373.37					
	P. nigrita	134.05					
	P. fouquettei	196.39					
	P. kalmi	57.38					
	P. triseriata	136.48					
	P. feriarum	147.57					
	(basal branches)	135.54					
P. feriarum		145.99	1	H_O	−603.47	—	—
	P. feriarum inland^b	113.50	2	H_A	−595.49	15.4	<0.0001*
	P. feriarum coastal	174.31					
P. feriarum coastal		174.14	1	H_O	−274.96	—	—
	P. feriarum coastal S^b	77.80	2	H_A	−266.01	17.91	<0.0001*
	P. feriarum coastal N	213.25					

can estimate the center of the sampled range using the following steps: (i) randomize the assignment of geographic location to the tips of the genealogy (as done above to test for phylogeographic association); (ii) optimize all parameters and note the maximum likelihood location of the ancestor of the clade; (iii) repeat the first two steps many times to generate a null distribution for the center of the range, given the locations sampled; and (iv) compute the center as the mean latitude and longitude of this null distribution.

Tests with Pseudacris.—We tested for a noncentral ancestral location in two clades: the coastal and inland *P. feriarum* clades. We estimated the geographic center of each clade using the procedures outlined above with 10,000 randomizations. For each clade, a likelihood-ratio test was performed using the maximum likelihood score from an analysis in which the location of the ancestor was constrained to be at the estimated center and the score from an analysis in which the location of the ancestor was unconstrained.

We also tested two potential refugia for coastal *Pseudacris feriarum*. The first refugium, delineated S in Figure 2b, is a disjunct population located in South Carolina. The second refugium, delineated T in Figure 2b, is the Tennessee Valley (eastern TN), which is located in the southwestern part of the clade's range. For each test we represented the proposed refugium as the ancestral location within the refugium that maximized the likelihood. Note that using this point minimizes the probability of rejecting the null hypothesis that the ancestor existed in the proposed refugium.

Results.—As shown in Table 3 and Figure 2, tests involving coastal *Pseudacris feriarum* indicate that the ancestor of this clade was not located in the center of the range ($P = 0.0060$). In the coastal *P. feriarum* refugia tests, we were able to reject the South Carolina refugium ($P = 0.0107$) but not the Tennessee Valley refugium ($P = 0.1308$). Taken together, these results suggest that it is more likely that the ancestor of coastal *P. feriarum* existed in the Tennessee Valley refugium than the South Carolina refugium. The estimated location of the ancestor of inland *P. feriarum*, in contrast, was not significantly different than the center of the range ($P = 0.7194$).

Directional Migration

General methods.—The third prediction of recent expansion is that migration occurred in a non-random direction. This prediction is critical because it most directly reflects the process of interest: directional migration. Here, we are interested not in the locations of the ancestors themselves, but rather in the distribution of directions of migration across the genealogy. Because migration is assumed to be independent across branches, we can determine this distribution by considering, for each branch, the location of the descendant node relative to the location of the ancestor node. The arrow pointing from the ancestor location to the descendant location indicates the maximum likelihood estimate for the average direction of migration along the branch.

We present two methods for testing for directional migration. The first test, referred to hereafter as the *overall directionality test*, addresses the question: Did individuals tend to migrate in a non-random direction? Many statistical tests exist for directional data (Jammalamadaka and SenGupta, 2001). For example, Rayleigh's test is often used to test for a nonuniform distribution of samples around a circle (Zar, 1999). Unfortunately, the null distribution of directions in our case is not uniform, but instead depends on the range of the species and the distribution of geographic locations sampled. We can, however, estimate this null distribution using the

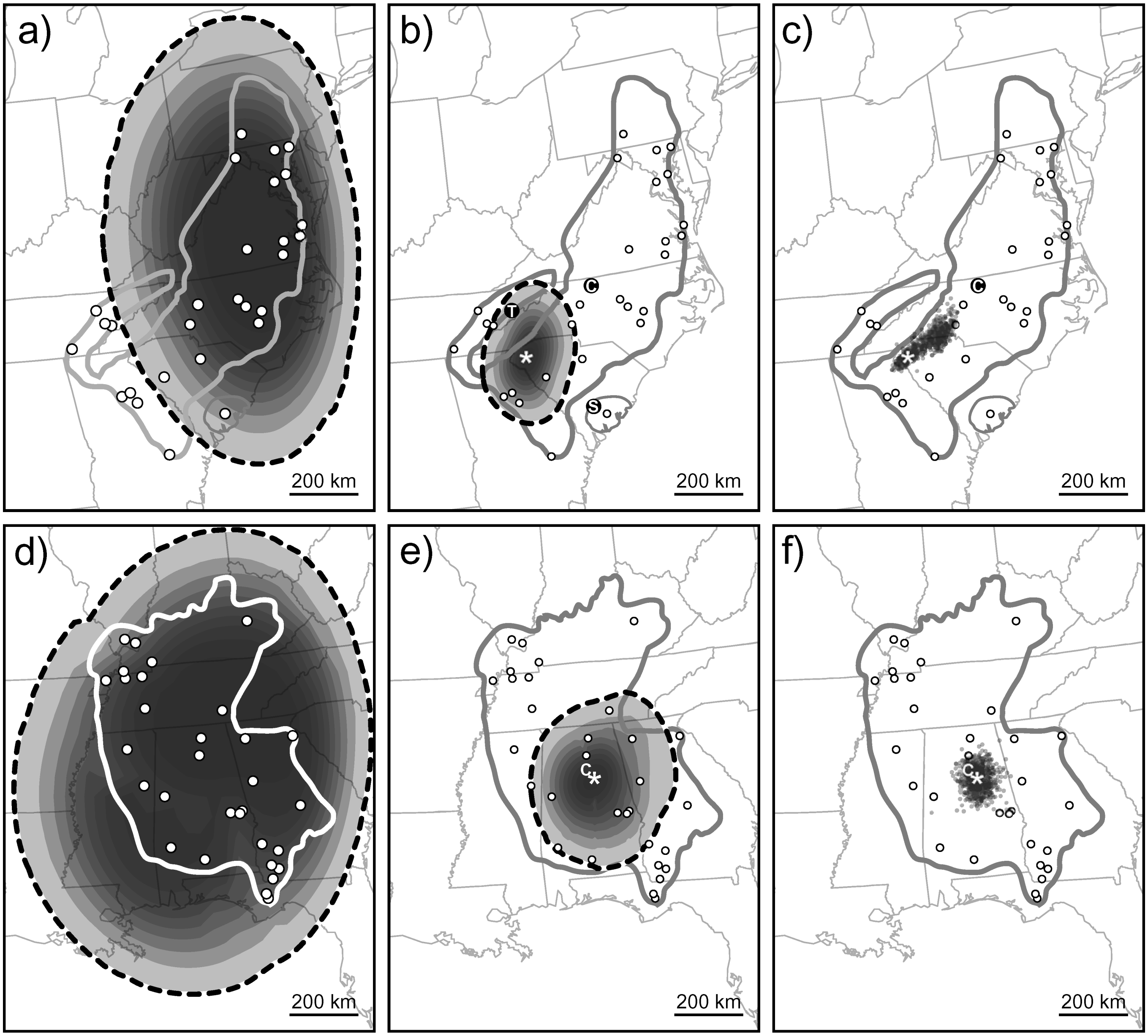

FIGURE 2. Estimates of the geographic locations for the ancestors of coastal (a–c) and inland (d–f) *Pseudacris feriarum*. Small open circles indicate the sampling locations. In a and d, we present an expected likelihood surface based on the sampling localities and an assumption of no phylogeographic association. To obtain this null expectation, we randomized the assignment of geographic location to the tips of the genealogy. The likelihood surface is represented by a series of filled contours, where the darkness of a contour corresponds to the likelihood obtained when the ancestor was constrained to be at a location inside the contour (with all other parameters optimized). The dashed oval delineates the 95% confidence envelope (all locations outside this envelope are significantly worse than the maximum likelihood location in a likelihood-ratio test). Other contours delineate the 85% confidence envelope, 75% confidence envelope, etc. In b and e, we present the likelihood surfaces for the observed phylogeographic association (assignment of geographic locations *not* randomized). Stars indicate the maximum likelihood estimate for the location of the basal ancestor of each clade. The points labeled C, S, and T indicate the estimated center of the range, the South Carolina refugium, and the Tennessee Valley refugium, respectively. In c and f, we present the effect of phylogenetic uncertainty on the estimates of the ancestral geographic location. Each black point indicates the maximum likelihood ancestral location estimated using 1 of 1000 trees sampled from the posterior probability distribution (Lemmon et al., 2007b).

following steps: (i) randomize the assignment of geographic location to the tips of the genealogy (as in the test for phylogeographic association); (ii) optimize all of the parameters; (iii) compute the expected net dispersal, $\Delta\delta_e = \sqrt{\overline{\Delta\phi}^2 + \overline{\Delta\lambda}^2}$, where $\overline{\Delta\phi}$ is the average ancestor-descendant latitudinal change and $\overline{\Delta\lambda}$ is the average ancestor-descendant longitudinal change; and (iv) repeat the above steps numerous times to generate the null distribution of $\Delta\delta_\mathrm{e}$. Performing steps ii to iii with the phylogeographic association intact (assignment

TABLE 3. Likelihood-ratio tests for geographic location. Tests were performed in two clades, inland *Pseudacris feriarum* (Inland) and coastal *P. feriarum* (Coastal). For each test, the likelihood score obtained when the ancestor of the clade was constrained to be at the proposed location (refugium or center) was compared to the score obtained when the ancestor was at the unconstrained, maximum likelihood (ML) location. The locations labeled S and T indicate the locations assumed for the South Carolina and Tennessee Valley refugia, respectively. Asterisks indicate a significant likelihood-ratio test with $\alpha = 0.05$ and $df = 2$. Other symbols given are the same as in Table 1.

Clade	Test	Location	Latitude	Longitude	Model	lnL	χ^2	P-value
Inland		ML	33.5527	−86.4123	H_A	−309.54	—	—
	Center	Center	33.8931	−86.6530	H_O	−309.87	0.659	0.7194
Coastal		ML	34.7652	−82.7998	H_A	−266.01	—	—
	Center	Center	36.3214	−79.9908	H_O	−271.12	10.237	0.0060*
	Refugium	S	33.4113	−80.5763	H_O	−270.54	9.069	0.0107*
	Refugium	T	36.0479	−83.1147	H_O	−268.04	4.068	0.1308

of locations not randomized) produces the test statistic $\Delta\delta_o$, the observed net dispersal. If the test statistic is greater than 95% of the samples from the null distribution, the null hypothesis of random directionality can be rejected.

The second test, referred to hereafter as the *a priori directionality test*, can be used to test whether individuals tended to move in a specific direction that was predicted prior to the data collection. For example, if northern expansion is predicted, the test can be used to answer the question: Did individuals in this clade tend to migrate northward? The null distribution for this test can be generated with the following steps: (i) randomize the assignment of geographic location to the tips of the genealogy; (ii) optimize all of the parameters; (iii) compute the angle between each arrow (pointing from an ancestor to its descendant) and the arrow pointing in the hypothesized direction; (iv) take the average of the angles over all of the branches; and (v) repeat the above steps to generate the null distribution. The test statistic, which is computed using steps ii to iv above, measures the average deviation between the observed directions and the hypothesized direction.

The above procedure can also be used to generate a graph depicting the directional tendency (if any) of the clade of interest. To generate the graph, first repeat the test stated above numerous times, each time using a different "predicted" direction (from 0 to 2π). The graph consists of a large number of lines, radiating out from a single point. Each line depicts the P-value (where a longer line represents a smaller P-value) for the "predicted" direction in which the line is drawn. Note, however, that this graph can serve only as a visualization tool and cannot be used to assess significance or generate a hypothesized direction to be tested with the same data set.

Tests with Pseudacris.—We tested for directional migration in the inland and coastal *P. feriarum* clades, performing the two tests described above on each clade. The hypothesized direction for the second test was north, based on models of vegetation migration since the last glacial maximum (Williams et al., 2000, 2004). After performing the hypothesis tests, we generated graphs depicting the directional tendencies of the coastal and inland clades.

Results.—Results from the overall directionality and a priori directionality tests support the hypothesis that coastal *Pseudacris feriarum* expanded northward (Table 4). The direction of inland *P. feriarum* migration, however, was not found to be significantly different than the null expectation. These contrasting results can be seen in Figure 3, in which we have plotted the observed (estimated) paths of migration for the two clades. This figure also shows an expected path under the null hypothesis, where there is no phylogeographic association. The directional tendency of migration for the two clades can be seen in Figure 4. In this graph, it is clear that migration in the coastal *P. feriarum* clade tended to occur in the northeastern direction. This pattern contrasts with the pattern seen for inland *P. feriarum*, in which we unexpectedly observed evidence for southward migration. Additional data will be needed to confirm this finding.

Assessing Phylogenetic Uncertainty

Thus far, we have assumed that the genealogy is known without error. Here, we briefly present one way of assessing the effect of phylogenetic uncertainty on estimates of ancestral geographic location. Suppose that instead of an exact estimate of the genealogy, a posterior probability distribution of trees is available from a Bayesian phylogenetic analysis. This distribution represents an estimate of phylogenetic uncertainty. In order to take this uncertainty into account, one could randomly draw and rate smooth trees from the posterior distribution, and then estimate phylogeographic history assuming each tree. The outcome of this procedure would be a distribution of phylogeographic histories, sampled in proportion to the posterior probability of the assumed tree. The distribution of geographic locations estimated for a particular node can then be used to assess the effect

TABLE 4. Randomization tests for directionality. Two types of tests were performed for each clade (see text for details). Asterisks indicate significance at the $\alpha = 0.05$ level. A significant result for the overall directionality test suggests that individuals migrated in a non-random direction. For the a priori test, north was chosen as the hypothesized direction. In this test, a significant result indicates that individuals migrated in the predicted direction more often than is expected by chance.

Clade	Test	Test statistics	P-value
Inland	Overall	88.079	<0.0001*
	A priori	1.576	0.9689
Coastal	Overall	84.931	<0.0001*
	A priori	1.485	0.0018*

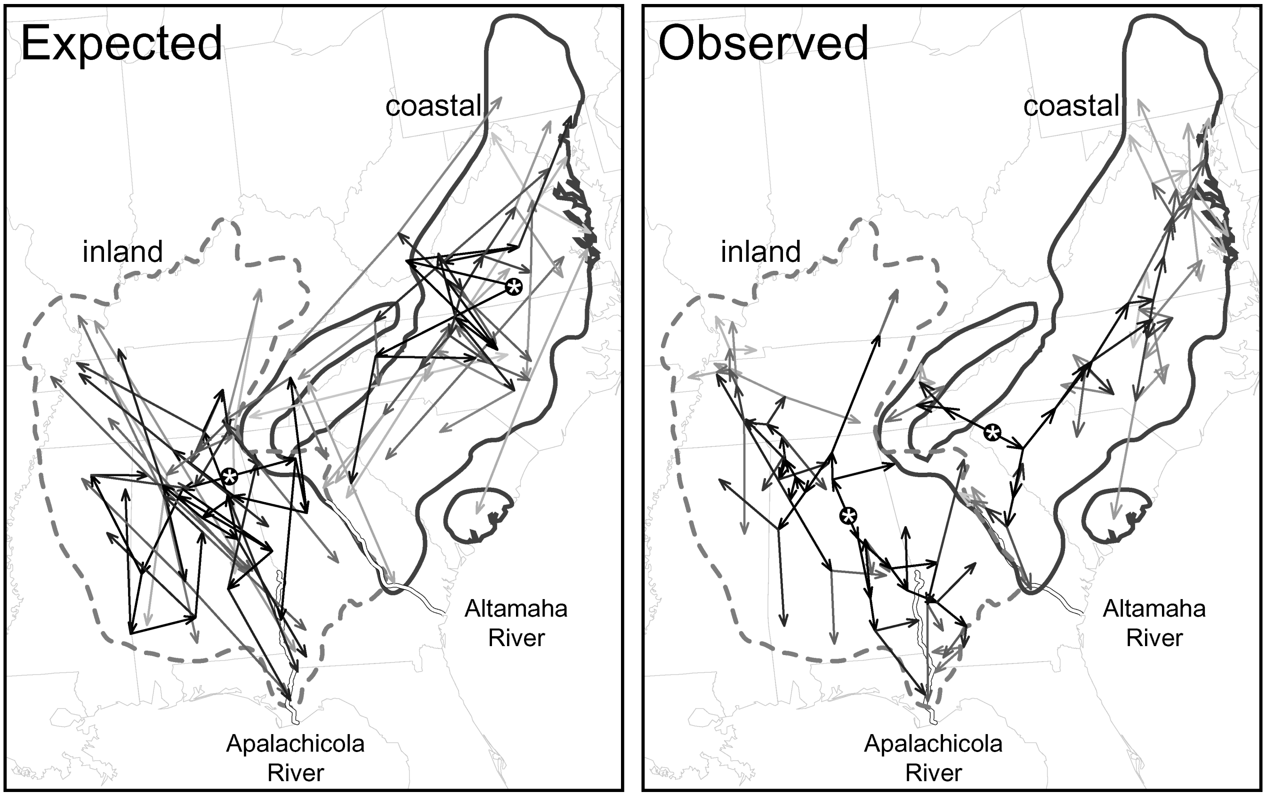

FIGURE 3. Estimated migration routes for ancestors of inland and coastal *Pseudacris feriarum*. Each arrow points from the estimated location of one ancestor (represented by an internal node on the genealogy) to the estimated location of one of its descendants. Arrows corresponding to basal branches are darker than derived branches. Stars denote the location of the most basal ancestor for each clade. The panel labeled "Expected" presents an expected pattern of migration under the assumption of no phylogeographic association. The expected ancestral migration patterns were estimated after randomizing the assignment of geographic location to the tips of the genealogy. Note that we present the results of one random replicate (e.g., location of star varies across random replicates). The panel labeled "Observed" presents the maximum-likelihood migration routes given the observed phylogeographic association (assignment of location was not randomized). Note that coastal *P. feriarum* shows a pattern of northward migration.

of phylogenetic uncertainty on the maximum likelihood estimate of the geographic location of that node.

We performed the above procedure for inland and coastal *Pseudacris feriarum* clades. Figure 2c and f present the distributions of estimated ancestral geographic locations for the most recent common ancestors of the coastal and inland clades, respectively. Two interesting patterns emerge. The first pattern is that phylogenetic uncertainty has an unexpectedly small effect on the estimate of phylogeographic history: the cluster of points representing the uncertainty in the estimated location of the ancestor is small relative to both the phylogeographic uncertainty (seen in Fig. 2b and e) and the size of the clade's range. The second pattern is that the estimates tend to fall between the maximum likelihood estimate (indicated by a star) and the center of the clade's range (indicated by a C). This pattern is not surprising because the genealogy used to estimate the ancestral location is the majority-rule consensus tree constructed using the posterior probability distribution (see Lemmon et al., 2007b, for more details). A tree drawn from the posterior distribution is expected to be closer to random (i.e., have a lower likelihood) than the majority-rule tree on average. Because use of a random tree would on average produce an estimate for the location that is near the center of the range, we should expect that trees drawn from the posterior distribution should produce an estimate for the location that is between the ML estimate and the center.

DISCUSSION

We have introduced a new statistical framework for studying the phylogeographic history of a gene using genetic data taken from across a clade's geographic range. This framework can be used to test explicit a priori hypotheses and/or estimate phylogeographic history in the absence of a priori hypotheses. To demonstrate the utility of the new approach, we have estimated the phylogeographic history of the mitochondrion of two chorus frog clades: coastal and inland *Pseudacris feriarum*. These two clades provide ideal test cases because environmental data suggest that the range of first has expanded northward, whereas the range of the second has not. The estimated phylogeographic history, as well as results from a priori hypothesis tests (variation in dispersal distances, center of origin, and directional migration),

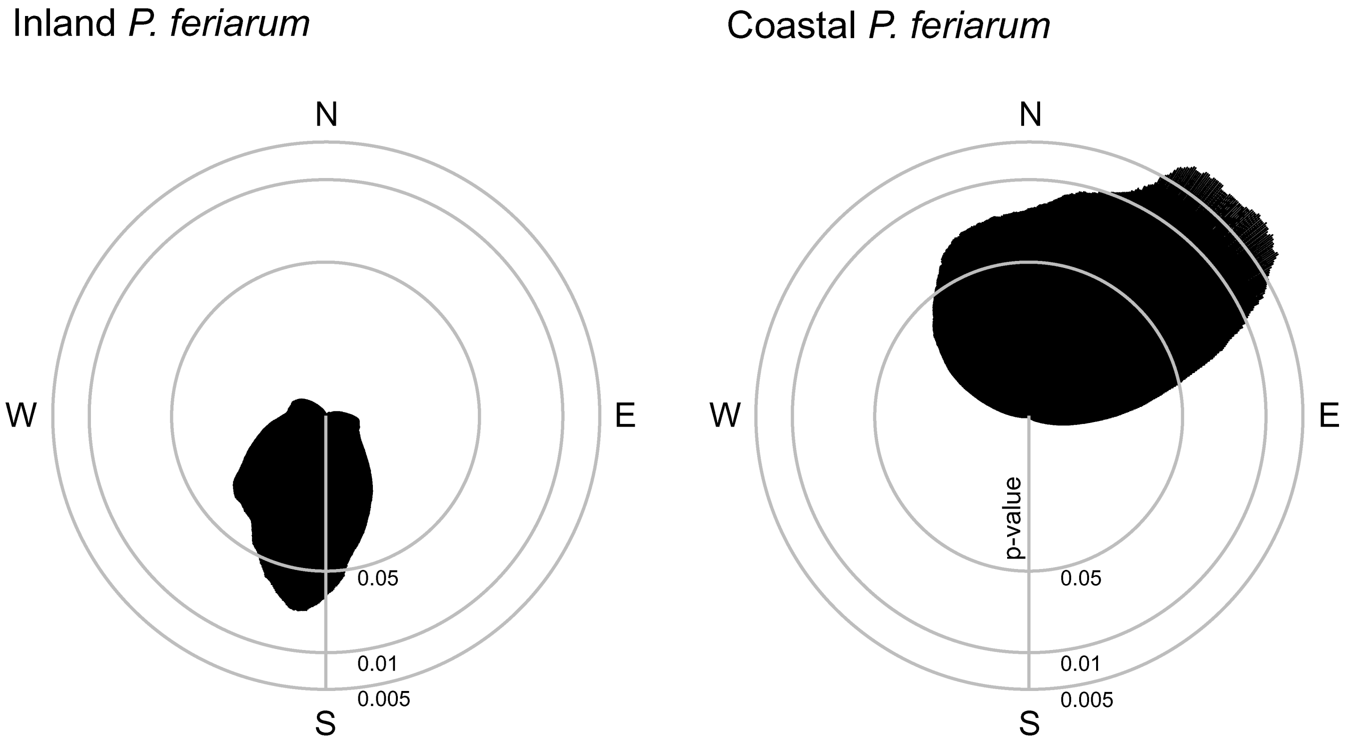

FIGURE 4. Directional tendency of migration in inland and coastal *Pseudacris feriarum*. Each graph was constructed by drawing a large number of lines, radiating out from a central point. The direction of each line corresponds to a potential hypothesized direction of expansion. The length of each line corresponds to the P-value that would be obtained if the hypothesized direction corresponding to that line was chosen a priori. Note that a longer line represents a smaller (more significant) P-value.

provides strong support for the hypothesis that the mitochondrion of coastal *P. feriarum* has recently expanded northward. Though this result is consistent with recent range expansion of the species, analysis of additional genes will be necessary to distinguish this history from a selective sweep operating on the mitochondrion.

We have also estimated the per-generation dispersal distance of each species within the trilling chorus frogs. These estimates pertain to female dispersal because mitochondrial sequences were used. With respect to the three species for which external corroboration is possible (*Pseudacris brachyphona*, *P. maculata*, and *P. triseriata*), our estimates of *mean* dispersal distances are all less than the *maximum* observed dispersal distances from empirical studies (see Table 1; Green, 1952; Spencer, 1964; Kramer, 1973, 1974). In addition, the estimated mean dispersal distance of *P. triseriata* (95.83 m) is corroborated by observations by Kramer (1974), who studied dispersal over a 4-month period (see Fig. S4 for details; www.systematicbiology.org). This result is particularly encouraging because several factors are expected to affect accuracy of the estimate (see Sources of Error below). Additional corroboration involving other species will be needed to fully evaluate the accuracy and precision of this estimator.

We should note that our method is very similar to that of Neigel et al. (1991), who proposed the use of pairwise genetic and geographic distances to estimate per-generation dispersal distances. One of the main difficulties with the approach of Neigel et al. (1991) is that when *pairwise* genetic distances are used, the deep branches in the tree contribute disproportionately to the dispersal distance estimate since the majority of pairs are connected by long, deep branches. This property is expected to render estimates of dispersal distance inaccurate because deeper branches contain larger amounts of uncertainty (Felsenstein, 1985; Barton and Wilson, 1995). Our method, in contrast, utilizes each branch once and should produce more accurate dispersal distance estimates since the shallow branches (which contain less uncertainty) are more numerous than the deep branches.

Method Comparison

Focus of estimation.—When comparing the strengths and weaknesses of different methods, it is important to understand clearly the parameters that each method is able to estimate. For nested clade analysis, the parameters of estimation are the relative degrees of geographic dispersion of related haplotypes (quantified as D_c and D_n, see Posada et al., 2006). For the coalescent-based approaches that are currently being applied to phylogeography, the primary parameters estimated are population sizes, migration rates, divergence times, and mutation rates. For the approach we present here, in contrast, the primary parameters of interest are the geographic locations of ancestors and the per-generation dispersal distance (note the difference between migration *rate* and dispersal *distance*).

Because these methods are designed to estimate different parameters, they are useful for answering different types of questions. Nested clade analysis is useful for addressing questions regarding geographic heterogeneity in levels of haplotype dispersion. The high false-positive rate and lack of a model-based statistical inference framework, however, greatly limits the utility of nested clade analysis in addressing some questions about phylogeographic history (Knowles and Maddison, 2002; Petit and Grivet, 2002; Panchal and Beaumont, 2007; Petit, 2008). Coalescent-based methods are useful for answering questions about population divergence times and demography (e.g., changes in population size and number through time), as well as rates of migration among discrete populations (Kuhner et al., 1998; Beerli and Felsenstein, 1999, 2001; Nielsen and Wakeley, 2001; Hey and Nielsen, 2004). The majority of phylogeographic analyses utilizing a coalescent framework, however, are not spatially explicit (i.e., the statistical model does not directly incorporate the geographic locations of populations/individuals or the distances among them), although spatially explicit ecological niche and paleoclimate models have been used to generate a priori predictions that can be tested in a coalescent framework (Carstens and Richards, 2007; Knowles et al., 2007; Richards et al., 2007). The method we present here can be useful for addressing questions about the historical locations of the ancestors of sampled individuals and changes in the per-generation dispersal distance. These questions can be addressed through either a priori hypothesis tests or estimates of phylogeographic history in the absence of a priori hypotheses.

Assumptions.—Models of phylogeographic history are necessarily oversimplistic, and different approaches require different assumptions to reduce model complexity to a tractable level. One explicit assumption of nested clade analysis, for example, is that interior clades of an intraspecific cladogram are older and more frequent than tip clades (Posada et al., 2007). What limits the utility of nested clade analysis, however, is the implicit assumption that there is a one-to-one match between the patterns of observed haplotype dispersion and the population histories presented in Templeton's inference key (Templeton, 2004). This assumption is certainly violated since multiple phylogeographic histories can often produce the same pattern of haplotype dispersion (Panchal and Beaumont, 2007).

One restrictive assumption of coalescent-based methods is panmixia within populations/demes (Kuhner et al., 1998; Beerli and Felsenstein, 1999, 2001; Nielsen and Wakeley, 2001; Kuhner, 2006). This assumption will be violated when the geographic range of the assumed population is much larger than the distance that individuals tend to disperse in one generation. Geographic structure within populations is known to affect estimates of effective population size (Wright, 1943), migration rates, and divergence times (Wakeley, 2000). A second assumption is that migration among sampled and unsampled sets of populations is negligible (i.e., that all relevant populations have been sampled; see Slatkin, 2005). This assumption is also expected to be violated as the geographic scale of analysis increases, because sampling numerous populations across a large geographic scale can be difficult. Perhaps the most restrictive assumption typical of coalescent-based analyses is that a simple population history involving few populations is an adequate description of the true phylogeographic history. This assumption is expected to be increasingly unrealistic as the geographic and temporal scale of analysis increases and as the number of loci increases (see Multilocus Phylogeography below).

The method we propose here also makes several fairly restrictive assumptions. The most apparent assumption is that individuals are uniformly distributed across geographic space. We expect this assumption to be violated when landscape heterogeneity exists on the same geographic scale as that of the sampled individuals. Our method would perform poorly, for instance, on data taken from an island archipelago. Note that this is the type of situation in which coalescent methods may be most useful because populations can be appropriately defined and all populations can be sampled. The second assumption is that the gene tree is known without error. This assumption may only be reasonable for genes evolving at a rate that is appropriate for the geographic scale of interest, although uncertainty due to low gene tree resolution can be accommodated (Fig. 2). The third assumption pertains to estimates of the per-generation dispersal distance. Estimation of this parameter requires that the temporal scale of the assumed chronogram is known, either through use of external calibrations (Thorne et al., 1998; Kishino et al., 2001; Thorne and Kishino, 2002; Sanderson, 2002, 2003; Drummond et al., 2006; Drummond and Rambaut, 2007), molecular clock estimates (Zuckerkandl and Pauling, 1962, 1965), or divergence time estimates based on coalescent analyses (Nielsen and Wakeley, 2001). Note, however, that the ancestral locations and *relative* dispersal distances (among clades) can still be estimated in the absence of a time-calibrated chronogram.

Sources of error.—When interpreting the results of any analysis, it is especially important to be aware of the sources contributing to error and to be clear about the types of error for which the method accounts. For the sake of brevity, we focus on the sources of error that pertain to the framework introduced in this paper. The procedure required by the method we introduce involves several steps, including sequence alignment, gene tree estimation, chronogram estimation, gene phylogeographic history estimation (ancestral location estimation), and dispersal distance estimation. An estimate of phylogeographic history, therefore, will be affected by error contributed at all of these levels. Although some types of error can be minimized fairly easily, other types may contribute substantially to the estimates.

We expect four types of error to have significant effects on our estimates of ancestral locations, including error resulting from (1) gene tree uncertainty (both topology and branch lengths), (2) a finite number of sampled individuals, (3) environmental heterogeneity (improper model assumption), and (4) biased geographic sampling. The first type of error can be examined by plotting the ancestral locations estimated using gene trees randomly drawn from a Bayesian posterior distribution (Fig. 2c, f). Although this error is relatively small for the mitochondrial example we present, the error is expected to be more substantial for genes evolving at much slower rates (and therefore containing less phylogenetic information). The second type of error can be examined by observing the likelihood surface for an ancestral geographic location (Fig. 2b, e). Though this type of error is more substantial in our example than the error from gene tree uncertainty, we are still able to reject several null hypotheses regarding the location of this ancestor. Additional sampling is expected to reduce this error. The third type of error, which we did not address here, is that due to environmental heterogeneity. This error undoubtedly affects our estimates since we know from museum records that *Pseudacris feriarum* are rarely found above 400 meters elevation (Fig. S5; www.systematicbiology.org), and therefore gene flow across the Appalachian Mountains is likely to be limited in this species. Future versions of PhyloMapper will reduce this error through the integration of ecological niche data into analyses (see Future Extensions). Finally, the fourth type of error, also not examined here, is that due to biased geographic sampling. Although efforts were made to sample fairly uniformly across the geographic range of species, some areas may have been underrepresented. We expect that our ability to reject the South Carolina refugium, for example, may be influenced by the fact that we obtained only a single sample from that region. Additional sampling will be necessary to determine whether our conclusions regarding this refugium are robust.

With respect to our estimates of the per-generation dispersal distance, two types of error should be considered. The first is the error associated with the temporal scale estimate on the genealogy. We based the temporal scale of our analyses on published estimates of the age of the several nodes within the trilling chorus frogs (Lemmon et al., 2007a). In order to assess the impact of uncertainty in the temporal scale estimate, we computed the dispersal distance estimates based on the lower and upper 95% confidence interval for each node age, in addition to the estimates based on the maximum likelihood estimates (see online Table S1 at www.systematicbiology.org). Based on this information, the bounds of the 95% confidence interval for each dispersal distance estimate are within ∼20% of the maximum likelihood estimate. Note that error in the temporal scale estimate is expected to have a negligible effect on estimates of ancestral location.

The second source of error that is expected to affect our dispersal distance estimates is the error due to violation of the assumption that individuals disperse uniformly with respect to direction. This assumption is certainly violated to some degree due to recent range expansion, as well as environmental heterogeneity (especially near large geographic barriers). These two factors are expected to positively and negatively bias estimates of dispersal distance, respectively. Future integration of ecological niche models and geological data should help alleviate this second factor.

Challenges.—Significant challenges face each of the three approaches discussed above. For nested clade analysis, the primary challenge is to determine and quantify the reliability of the inference key (see Panchal and Beaumont, 2007). For coalescent-based analyses, challenges include (1) scaling up statistical models that were developed for fine scale analyses of very few populations to geographic scales containing hundreds or thousands of populations; 2) incorporating uncertainty due to unsampled populations and geographic structure within populations; 3) accommodating complex demographic and spatial histories, especially when different genes follow different histories (see Multilocus Phylogeography below); and (4) applying the methods to systems for which a priori hypotheses are not available. Challenges facing the approach we propose here include (1) finding nuclear genes that evolve rapidly enough to produce accurate within-species gene trees; (2) incorporating external data to relax the uniform landscape assumption; and (3) comparing numerous phylogeographic history estimates across genes (see Multilocus Phylogeography below). Clearly, much work is needed to overcome the challenges facing each of these approaches.

Need for simulations.—The phylogeography literature is surprisingly depauperate in the kind of simulation studies necessary to test the accuracy of the various approaches, especially compared to other fields such as phylogenetic inference (e.g., Huelsenbeck and Hillis, 1993; Swofford et al., 2001; Lemmon and Moriarty, 2004; Brown and Lemmon, 2007). The few existing studies have addressed the performance of various summary statistics used to test for simultaneous vicariance in comparative phylogeography (Hickerson et al., 2006a) and the accuracy of nested clade analysis (Knowles and Maddison, 2002; Templeton, 2004; Panchal and Beaumont, 2007). Additional simulation studies will be needed to determine how the accuracy of the parameters estimated in each framework is affected by the following factors: the number of individuals, the number of loci, the sampling strategy, the complexity of history, and the degree of environmental heterogeneity. In addition, these studies should evaluate the sensitivity of each method to assumption violations as well as determine the temporal and geographic scale at which each approach is accurate. Although success of this endeavor will require full automation of the various methods (e.g., Panchal and Beaumont, 2007) and a framework for simulating complex phylogeographic histories, confidence in empirical inferences generated from these approaches depends on a careful analysis of their scope of application.

The Future of Phylogeography

Data integration.—One key to the success of the field of phylogeography is the ability to integrate data from diverse fields. Fortunately, the types of data needed for comprehensive estimation of phylogeographic history are becoming available for an increasing number of systems. Types of data include ecological (e.g., Carstens and Richards, 2007), paleoclimatic (e.g., Ruegg et al., 2006), geological (e.g., Waters et al., 2001), landscape genetic (e.g., Spear et al., 2005), multilocus nuclear (e.g., Dolman and Moritz, 2006), divergence time (e.g., Lemmon et al., 2007a), and multispecies data (e.g., Sunnucks et al., 2006; Bell et al., 2007). The success of each approach to the study of phylogeography will depend largely upon how effectively the approach can integrate data from these fields into a cohesive statistical framework.

Multilocus phylogeography.—The increasing availability of unlinked nuclear markers calls for a shift in the way that biologists think about phylogeographic history. When many loci from across the genome are being analyzed, it may be unrealistic to assume that all genes have followed the same phylogeographic history because different genes may be affected by different processes. Some processes, such as selective sweeps, are only expected to leave a signature on the patterns of genetic variation on a few genes in the genome. Other processes, such as range expansions or bottlenecks, are expected to leave signatures on the patterns of genetic variation on all genes in the genome. A complete understanding of the phylogeographic history of the species, therefore, will require comparisons among the estimated histories of many genes sampled from across the genome.

Unfortunately, multiple genes cannot be incorporated by simply constraining histories of unlinked genes to be identical, either by concatenating sequences or by constraining genes to have the same phylogeographic history. Such an approach would lead to increasingly confusing results as an increasing number of loci were sampled (because genes may have different histories). A similar problem is now recognized in the phylogenetics literature and has been termed the gene tree/species tree problem (Fitch, 1970; Maddison, 1997). In this instance, gene trees derived from the same species tree may differ due to incomplete lineage sorting, hybridization, or gene duplication. The former process can be accommodated fairly easily by estimating the species tree that would most likely produce the set of independently estimated gene trees (Edwards et al., 2007), whereas the latter two processes will be more difficult to accommodate (but see Reeves and Richards, 2007). For phylogeographic analyses, the situation is even more difficult since genes typically have less within-species than between-species information and the geographic history must also be estimated.

Given that different genes are likely to coalesce in different geographic locations, and sometimes even in different populations, perhaps the overarching goal should be to estimate the *genome phylogeographic history*, instead of the population history. We define the genome phylogeographic history to be the set of phylogeographic histories for all genes in the genome. A complete understanding of the genome phylogeographic history will allow inference of the processes affecting all genes (e.g., range expansion), as well as those that affect subsets of the genome (e.g., selection). A comparison of the gene histories can then be used to infer the population history, as well as the barriers that have allowed the evolution of reproductive isolation.

An example will illustrate the need to view phylogeographic history in this way. Suppose that two geographically isolated subspecies evolve differences through drift or selection. Upon secondary contact and hybridization, genes in the genome will introgress at different rates, depending on the strength of selection operating on those genes (Barton and Hewitt, 1985; Lemmon, 2007). Genes involved in pre- and postzygotic isolation, for example, will flow very slowly across the hybrid zone and thus will retain the signature of allopatric separation for a longer period of time. Other genes will coalesce on one or the other side of the hybrid zone (the side in which the favored allele evolved before it swept through the other side). If one were to estimate the phylogeographic history of each gene separately, he/she would find that these histories would fall into at least three categories, one reflecting an invasion of the gene from one side of the barrier, one reflecting an invasion of the gene from the other side of the barrier, and one reflecting a deep divergence. In this example, estimating a single phylogeographic history (read population history) for all genes will certainly result in confusion. A better approach may be to use a statistical framework to determine which categories exist and which genes belong to each category.

Future extensions.—A number of extensions will greatly enhance the power of our framework to infer phylogeographic history at the genic and genomic level. The first extension is to implement our statistical model in a Bayesian framework. A Bayesian framework will allow gene tree uncertainty to be more easily integrated into the analysis. This extension will also allow additional data, such as that from ecological niche and paleoclimate models, to be incorporated directly into the statistical analysis. A raster layer of environmental suitability, for example, could be used as a prior on the locations of the ancestors. This will help to relax the assumption of uniform geographic space and allow for more accurate estimates of ancestral location and dispersal distance. The second extension is to allow ancestors to be constrained to user-defined geographic areas. This will allow more complex a priori hypotheses to be tested, such as the hypothesis that different genes coalesce in different geographic areas or populations.

In conclusion, we present a novel approach for estimating phylogeographic history using a maximum-likelihood model of dispersal across a continuous landscape. Our approach differs from previous methods in that it focuses on estimating the geographic locations of ancestors rather than on demographic parameters or measures of haplotype dispersion. One unique feature of our approach is that it can be used as either a descriptive tool or a hypothesis-testing tool. Future extensions will integrate diverse types of ecological, genetic, and geographic data into a single statistical framework, thereby allowing researchers to more accurately estimate phylogeographic history.

ACKNOWLEDGMENTS

The authors thank Fredrik Ronquist for advice concerning the statistical model and for discussions regarding future directions. We are also grateful to Santiago Ron, Greg Pauly, Scott Solomon, and Jeremy Brown for comments on a previous version of the manuscript. Both authors were supported by an NSF IGERT Fellowship in Computational Phylogenetics and Applications to Biology at the University of Texas at Austin (DGE-0114387).

REFERENCES

Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. Ann. Rev. Ecol. Syst. 16:113–148.

Barton, N. H., and I. Wilson. 1995. Genealogies and geography. Phil. Trans. R. Soc. Lond. B 349:49–59.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763–773.

Beerli, P., and J. Felsenstein. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 98:4563–4568.

Bell, K. L., C. Moritz, A. Moussalli, and D. K. Yeates. 2007. Comparative phylogeography and speciation of dung beetles from the Australian Wet Tropics rainforest. Mol. Ecol. 16:4984–4998.

Brown, J. M., and A. R. Lemmon. 2007. The importance of data partitioning and the utility of Bayes factors in Bayesian phylogenetics. Syst. Biol. 56:643–655.

Caldwell, J. P. 1987. Demography and life history of two species of chorus frogs (Anura: Hylidae) in South Carolina. Copeia 1987:114–127.

Cann, R. L., M. Stoneking, and A. C. Wilson. 1987. Mitochondrial DNA and human evolution. Nature 1:31–36.

Carstens, B. C., J. D. Degenhardt, A. L. Stevenson, and J. Sullivan. 2005b. Accounting for coalescent stochasticity in testing phylogeographic hypotheses: Modeling Pleistocene population structure in the Idaho giant salamander *Dicamptodon aterrimus*. Mol. Ecol. 14:255–265.

Carstens, B. C., and C. L. Richards. 2007. Integrating coalescent and ecological niche modeling in comparative phylogeography. Evolution 61:1439–1454.

Conant, R., and J. T. Collins. 1998. A field guide to reptiles and amphibians of eastern and central North America. Houghton-Mifflin, Boston.

Corander, J., J. Sirén, and E. Arjas. 2007. Bayesian spatial modeling of genetic population structure. Computation. Stat. 23:111–129.

Currat, M., and L. Excoffier. 2005. The effect of the Neolithic expansion on European molecular diversity. Proc. R. Soc. Lond. B Biol. Sci. 272:679–688.

Currat, M., N. Ray, and L. Excoffier. 2004. SPLATCHE: A program to simulate genetic diversity taking into account environmental heterogeneity. Mol. Ecol. Notes 4:139–142.

Dolman, G., and C. Moritz. 2006. A multilocus perspective on refugial isolation and divergence in rainforest skinks (*Carlia*). Evolution 60:573–582.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:e88.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Edwards, S. V., L. Liu, and D. K. Pearl. 2007. High-resolution species trees without concatenation. Proc. Natl. Acad. Sci. USA 101:13820–13825.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Felsenstein, J. 2004. Inferring Phylogenies. Sinauer Associates, Sunderland, Massachusetts.

Fitch, W. M. 1970. Distinguishing homologous from analogous proteins. Syst. Zool. 19:99–113.

García-Ramos, G., and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51:21–28.

Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49:652–670.

Green, N. B. 1952. A study of the life history of *Pseudacris brachyphona* (Cope) in West Virginia with special reference to behavior and growth of marked individuals. PhD thesis. The Ohio State University Microfilm. Ann Arbor, Michigan (Dissertation Abstract 17:3135–3136).

Green, N. B. 1964. Postmetamorphic growth in the Mountain Chorus Frog (*Pseudacris brachyphona*) Cope. Proc. West Virginia Acad. Sci. 36:34–38.

Guillot, G., A. Estoup, F. Mortier, and J. F. Cosson. 2005. A spatial statistical model for landscape genetics. Genetics 170:1261–1280.

Hey, J., and R. Nielsen. 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.

Hickerson, M. J., G. Dolman, and C. Moritz. 2006a. Comparative phylogeographic summary statistics for testing simultaneous vicariance. Mol. Ecol. 15:209–223.

Hickerson, M. J., E. A. Stahl, and H. A. Lessios. 2006b. Test for simultaneous divergence using approximate Bayesian computation. Evolution 60:2435–2453.

Hickerson, M. J., E. Stahl, and N. Takebayashi. 2007. msBayes: Pipeline for testing comparative phylogeographic histories using hierarchical approximate Bayesian computation. BMC Bioinformatics 8:268.

Holderegger, R., and H. H. Wagner. 2006. A brief guide to landscape genetics. Landscape Ecol. 21:793–796.

Huelsenbeck, J. P., and D. M. Hillis. 1993. Success of phylogenetic methods in the four-taxon case. Syst. Biol. 42:247–264.

Ingman, M., H. Kaessmann, S. Pääbo, and U. Gyllensten. 2000. Mitochondrial genome variation and the origin of modern humans. Nature 408:708–713.

Jammalamadaka, S. R., and A. SenGupta. 2001. Topics in circular statistics. World Scientific Publishing, New Jersey.

Kirkpatrick, M., and N. H. Barton. 1997. Evolution of a species' range. Am. Nat. 150:1–23.

Kishino, H., J. L. Thorne, and W. J. Bruno. 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. Mol. Biol. Evol. 18:352–361.

Knowles, L. L. 2004. The burgeoning field of statistical phylogeography. J. Evol. Biol. 17:1–10.

Knowles, L. L., B. C. Carstens, and M. L. Keat. 2007. Coupling genetic and ecological-niche models to examine how past population distributions contribute to divergence. Curr. Biol. 17:940–946.

Knowles, L. L., and W. P. Maddison. 2002. Statistical phylogeography. Mol. Ecol. 11:2623–2635.

Kramer, D. C. 1973. Movements of western chorus frogs *Pseudacris triseriata triseriata* tagged with Co^{60}. J. Herp. 7:231–235.

Kramer, D. C. 1974. Home range of the western chorus frog *Pseudacris triseriata triseriata*. J. Herp. 8:245–246.

Kuhner, M. K. 2006. LAMARC 2.0: Maximum likelihood and Bayesian estimation of population parameters. Bioinformatics 22:768–770.

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

Lemmon, A. R. 2007. Analytical, computational, and statistical approaches to studying speciation. PhD thesis. University of Texas at Austin.

Lemmon, A. R., and E. C. Moriarty. 2004. The importance of proper model assumption in Bayesian phylogenetics. Syst. Biol. 53:265–277.

Lemmon, E. M., A. R. Lemmon, and D. C. Cannatella. 2007a. Geological and climatic forces driving speciation in the continentally distributed trilling chorus frogs (*Pseudacris*). Evolution 61:2086–2103.

Lemmon, E. M., A. R. Lemmon, J. T. Collins, and D. C. Cannatella. 2008. A new North American chorus frog species (Amphibia: Hylidae: *Pseudacris*) from the south-central United States. Zootaxa 1675:1–30.

Lemmon, E. M., A. R. Lemmon, J. T. Collins, J. A. Lee-Yaw, and D. C. Cannatella. 2007b. Phylogeny-based delimitation of species boundaries and contact zones in the trilling chorus frogs (*Pseudacris*). Mol. Phylogenet. Evol. 44:1068–1082.

Maddison, W. P. 1997. Gene trees in species trees. Syst. Biol. 46:523–536.

Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: Combining landscape ecology and population genetics. Trends Ecol. Evol. 18:189–197.

Mayr, E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

Neigel, J. E., R. M. Ball Jr., and J. C. Avise. 1991. Estimation of single generation migration distances from geographic variation in animal mitochondrial DNA. Evolution 45:423–432.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: A Markov Chain Monte Carlo approach. Genetics 158:885–896.

O'Meara, B. C., C. Ané, M. J. Sanderson, and P. C. Wainwright. 2006. Testing for different rates of continuous trait evolution using likelihood. Evolution 60:922–933.

Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48:612–622.

Panchal, M., and M. A. Beaumont. 2007. The automation and evaluation of nested clade phylogeographic analysis. Evolution 61:1466–1480.

Petit, R. J. 2008. The coup de grâce for the nested clade phylogeographic analysis? Mol. Ecol. 17:516–518.

Petit, R. J., and D. Grivet. 2002. Optimal randomization strategies when testing the existence of a phylogeographic structure. Genetics 161:469–471.

Phillips, B. L., G. P. Brown, J. K. Webb, and R. Shine. 2006. Invasion and the evolution of speed in toads. Nature 439:803.

Posada, D., K. A. Crandall, and A. R. Templeton. 2006. Nested clade analysis statistics. Mol. Ecol. Notes 6:590–593.

Ray, N., M. Currat, P. Berthier, and L. Excoffier. 2005. Recovering the geographic origin of early modern humans by realistic and spatially explicit simulations. Genome Res. 15:1161–1167.

Reeves, P. A., and C. M. Richards. 2007. Distinguishing terminal monophyletic groups from reticulate taxa: Performance of phenetic, tree-based, and network procedures. Syst. Biol. 56:302–320.

Richards, C. L., B. C. Carstens, and L. L. Knowles. 2007. Distribution modelling and statistical phylogeography: An integrative framework for generating and testing alternative biogeographical hypotheses. J. Biogeogr. 34:1833–1845.

Ruegg, K. C., R. J. Hijmans, and C. Moritz. 2006. Climate change and the origin of migratory pathways in the Swainson's thrush, *Catharus ustulatus*. J. Biogeogr. 33:1172–1182.

Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14:1218–1231.

Sanderson, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. Mol. Biol. Evol. 19:101–109.

Sanderson, M. J. 2003. r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301–302.

Schluter, D., T. Price, A. O. Mooers, and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51:1699–1711.

Sinnott, R. W. 1984. Virtues of the haversine. Sky Telescope 68:159.

Slatkin, M. 2005. Seeing ghosts: The effect of unsampled populations on migration rates estimated for sampled populations. Mol. Ecol. 14:67–73.

Smith, D. C. 1987. Adult recruitment in chorus frogs: Effects of size and date at metamorphosis. Ecology 68:344–350.

Spear, S. F., C. R. Peterson, M. D. Matocq, and A. Storfer. 2005. Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). Mol. Ecol. 14:2553–2564.

Spencer, A. W. 1964. The relationship of dispersal and migration to gene flow in the boreal chorus frog. PhD thesis. Colorado State University.

Steele, C. A., and A. Storfer. 2006. Coalescent-based hypothesis testing supports multiple Pleistocene refugia in the Pacific Northwest for the Pacific giant salamander (*Dicamptodon tenebrosus*). Mol. Ecol. 15:2477–2487.

Storfer, A., M. A. Murphy, J. S. Evans, C. S. Goldberg, S. Robinson, S. F. Spear, R. Dezzani, E. Delmelle, L. Vierling, and L. P. Waits. 2007. Putting the 'landscape' in landscape genetics. Heredity 98:128–142.

Sunnucks, R., M. J. Blacket, J. M. Taylor, C. J. Sands, S. A. Ciavaglia, R. C. Garrick, N. N. Tait, D. M. Rowell, and A. Pavlova. 2006. A tale of two flatties: Different responses of two terrestrial flatworms to past environmental climatic fluctuations at Tallaganda in montane southeastern Australia. Mol. Ecol. 15:4513–4531.

Swofford, D. L., P. J. Waddell, J. P. Huelsenbeck, P. G. Foster, P. O. Lewis, and J. S. Rogers. 2001. Bias in phylogenetic estimation and its relevance to the choice between parsimony and likelihood methods. Syst. Biol. 50:525–539.

Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: Testing hypotheses about gene flow and population history. Mol. Ecol. 7:381–397.

Templeton, A. R. 2004. Statistical phylogeography: Methods of evaluating and minimizing inference errors. Mol. Ecol. 13:789–809.

Thorne, J. L., and H. Kishino. 2002. Divergence time and evolutionary rate estimation with multilocus data. Syst. Biol. 51:689–702.

Thorne, J. L., H. Kishino, and I. S. Painter. 1998. Estimating the rate of evolution of the rate of molecular evolution. Mol. Biol. Evol. 15:1647–1657.

Wakeley, J. 2000. The effects of subdivision on the genetic divergence of populations and species. Evolution 54:1092–1101.

Waters, J. M., D. Craw, J. H. Youngson, and G. P. Wallis. 2001. Genes meet geology: Fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. Evolution 55:1844–1851.

Wilkins, J. F., and J. Wakeley. 2002. The coalescent in a continuous, finite, linear population. Genetics 161:873–888.

Williams, J. W., B. N. Shuman, T. Webb III, P. J. Bartlein, and P. L. Leduc. 2004. Late-Quaternary vegetation dynamics in North America: Scaling from taxa to biomes. Ecol. Monogr. 74:309–334.

Williams, J. W., T. Webb III, P. H. Richard, and P. Newby. 2000. Late Quaternary biomes of Canada and the eastern United States. J. Biogeogr. 27:585–607.

Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

Ypma, T. J. 1995. Historical development of the Newton-Raphson method. SIAM Rev. 37:531–551.

Zar, J. H., 1999. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey.

Zuckerkandl, E., and L. Pauling. 1962. Molecular disease, evolution and genic heterogeneity. Pages 189–225 *in* Horizons in biochemistry (M. Kasha and B. Pullman, eds.). Academic Press, New York.

Zuckerkandl, E., and L. Pauling. 1965. Evolutionary divergence and convergence in proteins. Pages 97–166 *in* Evolving genes and proteins (V. Bryson and H. J. Vogel, eds.). Academic Press, New York.

First submitted 25 May 2007; reviews returned 3 August 2007; final acceptance 23 April 2008

Associate Editor: Elizabeth Jockusch