TEST FOR SIMULTANEOUS DIVERGENCE USING APPROXIMATE BAYESIAN COMPUTATION

MICHAEL J. HICKERSON,[1,2] ELI A. STAHL,[3,4] AND H. A. LESSIOS[5,6]

[1]*Museum of Vertebrate Zoology, University of California, 3101 Valley Life Sciences Building, Berkeley, California 94720-3160*
[3]*Department of Biology, University of Massachusetts–Dartmouth, 285 Old Westport Road,
North Dartmouth, Massachusetts 02747*
[4]*E-mail: estahl@umassd.edu*
[5]*Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Republic of Panama*
[6]*E-mail: Lessiosh@stri.org*

Abstract.—Comparative phylogeographic studies often reveal disparate levels of sequence divergence between lineages spanning a common geographic barrier, leading to the conclusion that isolation was nonsynchronous. However, only rarely do researchers account for the expected variance associated with ancestral coalescence and among-taxon variation in demographic history. We introduce a flexible approximate Bayesian computational (ABC) framework that can test for simultaneous divergence (TSD) using a hierarchical model that incorporates idiosyncratic differences in demographic history across taxon pairs. The method is tested across a range of conditions and is shown to be accurate even with single-locus mitochondrial DNA (mtDNA) data. We apply this method to a landmark dataset of putative simultaneous vicariance, eight geminate echinoid taxon pairs thought to have been split by the Isthmus of Panama 3.1 million years ago. The ABC posterior estimates are not consistent with a history of simultaneous vicariance given these data. Subsequent ABC estimates under a constrained model that assumes two divergence times across the eight taxon pairs suggests simultaneous divergence 3.1 million years ago in seven of the taxon pairs and a more recent divergence in the remaining taxon pair. These ABC estimates on the simultaneous divergence of the seven taxon pairs correspond to a DNA substitution rate of approximately 1.59% per lineage per million years at the mtDNA cytochrome oxidase I gene. This ABC framework can easily be modified to analyze single taxon-pair datasets and/or be expanded to include multiple loci, migration, recombination, and other idiosyncratic demographic histories. The flexible aspect of ABC and its built-in evaluation of estimator bias and statistical power has the potential to greatly enhance statistical rigor in phylogeographic studies.

Key words.—Approximate Bayesian computation, coalescent, comparative phylogeography, echinoid, hierarchical model, meta-analysis, vicariance.

The division of freely mixing populations into geographic isolates can have profound influences on population history, population genetic architecture, and the potential for speciation (Mayr 1954; Avise et al. 1998; Coyne and Orr 2004; Gavrilets 2004). Therefore, it is no surprise that the timing of divergence has been a critical parameter in population genetics, phylogenetics, and evolutionary biology (Arbogast et al. 2002; Thorne and Kishino 2002; Hey and Nielsen 2004). Similarly, divergence time estimates have assumed great importance in community ecology and biogeography, because of their potential for testing deterministic and stochastic models of community composition, range delineation, and the effect of climatic changes on speciation and extinction patterns (Cunningham and Collins 1998; Moritz et al. 2000; Hubbell 2001; Ricklefs 2003).

Estimating divergence times across different taxon pairs that span the same historical barrier to gene flow is of central importance in the emerging field of comparative phylogeography (Bermingham and Moritz 1998; Avise 2000; Arbogast and Kenagy 2001). For example, tests of alternative models of speciation across sister taxon pairs rely on estimates of population divergence times, which can in turn be used to test alternative climate-driven and ecologically deterministic community diversification and distribution models (Hugall

et al. 2002; Graham et al. 2004; Carstens et al. 2005). Given the importance of divergence-time estimates in comparative phylogeographic studies, rigorous methods that incorporate the coalescent variation among and within lineages are needed to prevent misleading inferences about community history (Arbogast et al. 2002).

Previous studies have generally found an elevated amount of variation in the pairwise genetic divergences between sister taxa that are hypothesized to have arisen simultaneously from the same emergent biogeographical barrier. This variation is usually concluded to have arisen from variance in mutation rates, divergence times, or unobserved extinctions, without considering a null model that incorporates intrinsic sources of variance (Knowlton and Weigt 1998; Schneider et al. 1998; Lessios et al. 2001; Marko 2002). Given a null model derived from coalescent theory (Tajima 1983), we should expect a great deal of variation in genetic divergence among sister pairs that diverged at the same time and experience equal mutation rates. Such variation is expected given the variance in the time of the mitochondrial most recent common ancestor (TMRCA; see Fig. 1) and the variance in mutations given a Poisson mutational process (Gillespie 1984; Takahata 1986; Edwards and Beerli 2000; Hickerson et al. 2003). Therefore, comparative phylogeographic inferences should be based on models that account for such sources of variance while being flexible enough to incorporate biological differences among taxa that affect the data, such as ancestral population size differences. Although tools exist
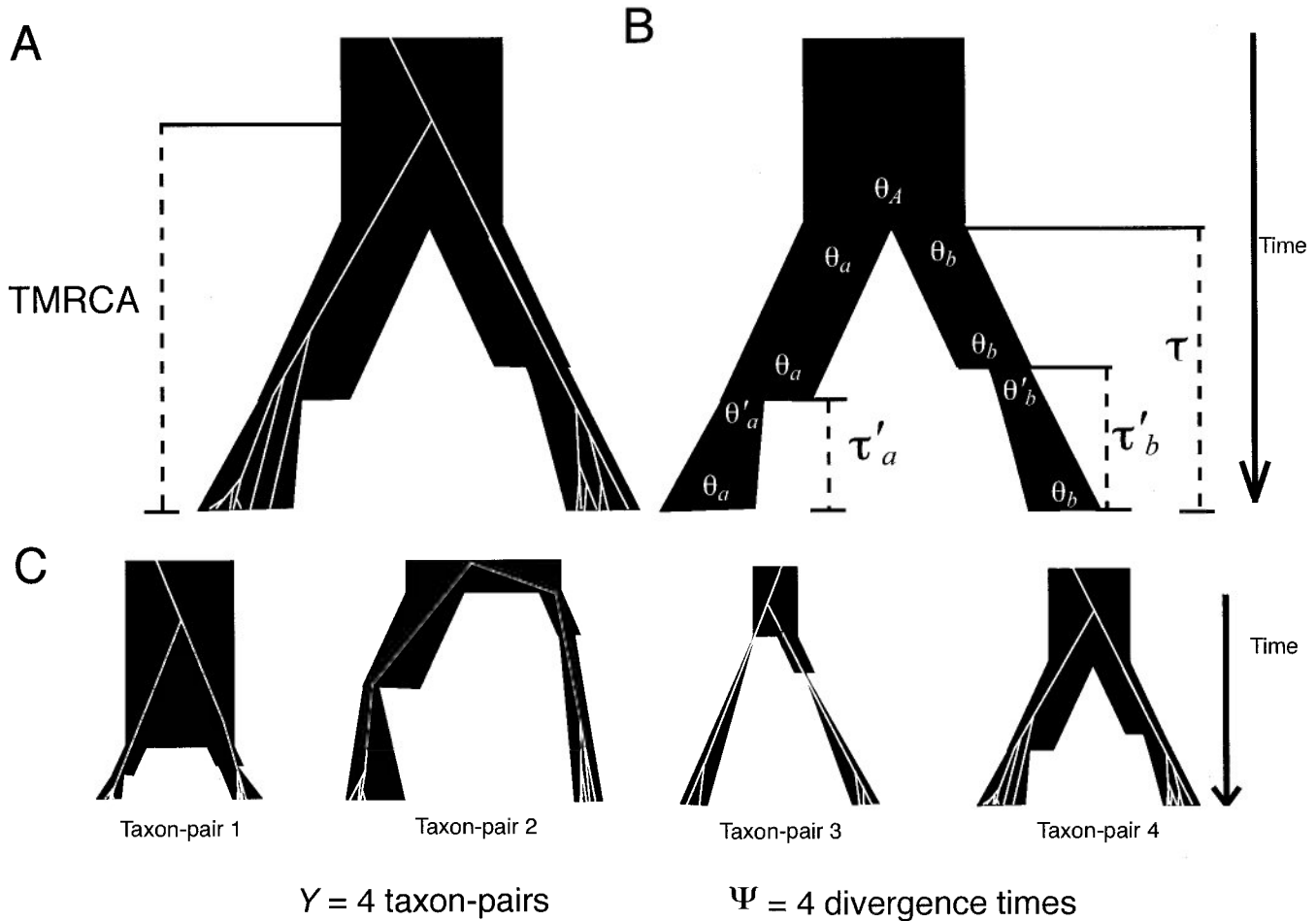

FIG. 1.   Depiction of the multiple taxon-pair divergence model used for the approximate Bayesian computation estimates. (A) The time to the mitochondrial DNA genealogically most recent common ancestor (TMRCA). (B) Parameters in the multiple taxon-pair divergence model. (C) Example of a simulated four-taxon-pair comparative phylogeographic dataset where parameters in (B) are drawn from uniform priors (see Table 1).

for the analysis of a single taxon splitting into daughter populations (Wakeley and Hey 1997; Nielsen and Wakeley 2001; Hey and Nielsen 2004), they are not designed to simultaneously test hypotheses involving multiple taxa with variable demographic parameters. Furthermore, such methods prohibit rapid diagnosis of accuracy, bias, and sensitivity with independent simulations.

Here we develop an approximate Bayesian computation (ABC) framework that estimates parameters characterizing multiple phylogeographic datasets in a single analysis, with the specific aim of quantifying the probability of simultaneous divergence. Approximate likelihood and approximate Bayesian methods are an attractive option for such data, because their flexible simulation-based approach allows complex comparative biogeographic hypotheses to be tested without having to explicitly express and calculate the likelihood of the model. Therefore, there are no particular constraints on incorporating the complexity of a priori idiosyncratic biological realism into the model when using approximate methods (Beaumont et al. 2002; Beaumont 2004), although the lack of analytical expressions can also be a disadvantage if explicit transparency of the model is lost. A second im-

portant benefit of ABC is that the estimator's bias, model sensitivity, and statistical power can be obtained using independent simulations at a computational cost that is orders of magnitude less than those of likelihood/Bayesian methods using Markov chain-Monte Carlo (MCMC; Excoffier et al. 2005). ABC methods have been developed for testing hypotheses related to single-taxon demographic histories (Estoup et al. 2004; Tallmon et al. 2004; Excoffier et al. 2005), and phylogenetic questions (Plagnol and Tavare 2002). Until now, these approaches have not been applied to multiple taxa that span the same putative biogeographic barrier.

After describing and testing the ABC framework, we use it to test for simultaneous divergence (TSD) in eight genera of tropical echinoids that span the Isthmus of Panama. This barrier to marine species is thought to have arisen approximately 3.1 million years ago (Coates et al. 1992). Jordan (1908) coined the term "geminate species" for sister species separated by the same barrier. Past comparative phylogeographic studies involving the isthmus have usually found elevated variation in mitochondrial divergences among sister taxon pairs that were thought to have been simultaneously split by this barrier (Bermingham et al. 1997; Marko 2002),

most often leading to conclusions involving ecologically deterministic divergence (Knowlton and Weigt 1998) or admixture subsequent to the main split in a subset of taxa (Lessios et al. 2001). The methodology introduced in this study will better quantify such comparative phylogeographic inferences (Bermingham and Moritz 1998; Knowles and Maddison 2002) and thereby better inform questions related to community ecology, speciation, phylogenetics, biogeography, and historical ecology.

MATERIALS AND METHODS

Hierarchical Approximate Bayesian Computation

The simulation-based summary statistic approach of approximate likelihood or Bayesian methods allow for greater model flexibility and relax the need for calculating the likelihood expression (Fu and Li 1997; Weiss and von Haeseler 1998; Pritchard et al. 1999; Beaumont et al. 2002; Plagnol and Tavare 2002; Beaumont and Rannala 2004). Although approximate methods sacrifice a degree of model transparency afforded by the analytical expressions of the likelihood function and also lose some of the information content from the data by using summary statistics, the complexity inherent in comparative phylogeographic models make approximate methods particularly attractive. Instead of explicitly calculating the likelihood function, or using MCMC or importance sampling, ABC methods obtain estimates by simulating data and their summary statistics under the model in order to sample from the posterior distribution. Comparative phylogeographic models are inherently parameter rich and therefore we make use of a hierarchical Bayesian model in our implementation of ABC. This facilitates testing comparative phylogeographic hypotheses by allowing estimation of across-taxon-pair parameters (i.e., hyperparameters) and estimation or incorporation of uncertainty in within taxon-pair parameters. Our hyperparameters describe differences in divergence times across taxon pairs, while our within-taxon-pair parameters include the vector of actual divergence times as well as other important demographic parameters that can differ across taxon pairs (e.g., population sizes).

To this end, we extend the ABC method to estimate the variability in divergence times across taxon pairs by simulating observations from this joint posterior distribution under a hierarchical model (Beaumont et al. 2002; Gelman et al. 2004). This meta-analysis is accomplished by implementing a hierarchical Bayesian model in which the distributions of within-taxon-pair parameters (Φ) are conditional on hyperparameters (ϕ) that describe the comparative phylogeographic dataset (multiple taxon pairs). For example, divergence times (Φ) can freely vary across a set of taxon pairs conditional on a set of hyperparameters (ϕ) that are drawn from their hyperprior distribution. These hyperparameters could be the number of divergence times across the set of taxon pairs as well as the mean and variance in these divergence times.

Although we could estimate every divergence time separately, use of a hierarchical model allows us to set up a clear hypothesis test from posterior distributions of hyperparameters. Furthermore, the borrowing strength gained from using a hierarchical model makes hypothesis testing and pa-

rameter estimation more efficient. By pooling information across taxon pairs without assuming they evolved under identical sets of parameter values, this borrowing strength results in reducing the standard error of each within-taxon-pair parameter estimate while also obtaining estimates of hyperparameters (James and Stein 1960; Gelman et al. 1995; Beaumont and Rannala 2004). In this case, we estimate the hyperparameters while allowing the demographic differences among taxon pairs to be treated as nuisance parameters that incorporate uncertainty (i.e., uninformative prior distributions of within-taxon-pair parameters).

Under this implementation of ABC, data are generated from a hierarchical model M determined by the joint parameter set ϕ and Φ (consisting of both hyperparameters and within-taxon-pair parameters). These have a joint prior distribution $P(\phi)P(\Phi \mid \phi) = f(\phi, \Phi)$, and the data are summarized in a summary statistic vector $\mathbf{D}$. The joint posterior distribution is then $f(\phi, \Phi \mid \mathbf{D}) \propto P(\phi, \Phi)P(\mathbf{D} \mid \phi, \Phi)$ (Gelman et al. 2004), which is the conditional density that can be calculated by first estimating the joint density $P(\mathbf{D}, \phi, \Phi)$ and dividing by an estimate of the marginal density $P(\mathbf{D})$ in the limit of $\mathbf{D}$ being its observed value ($\mathbf{D}^*$). Our method for generating random observations from the joint posterior $f(\phi_i, \Phi_i \mid \mathbf{D}_i)$ uses a rejection/acceptance algorithm (Fu and Li 1997; Weiss and von Haeseler 1998; Plagnol and Tavare 2002) followed by a weighted local rejection step (Beaumont et al. 2002; Tallmon et al. 2004; Excoffier et al. 2005). This is based on the idea that the parameter sets for which $\|\mathbf{D}_i - \mathbf{D}^*\|$ is small comprise an approximate posterior random sample.

In practice, the ABC approach involves three steps detailed in Beaumont et al. (2002) and Figure 2. First, a dataset equal in size to the observed dataset (same number of individuals and base pairs) is repeatedly simulated by randomly drawing parameters from prior distributions (defined in Table 1). The second step involves comparing the simulated data to the observed data using summary statistics and accepting an arbitrary proportion of simulated datasets and their corresponding parameters, those that are closest to the observed datasets with respect to Euclidian distance. The third step involves estimating the hyperparameters by performing local weighted linear regression on the accepted summary statistics and their corresponding parameters.

Explicitly, we randomly draw the joint parameter set (ϕ_i, Φ_j) from the joint prior distribution $f(\phi, \Phi)$ specified by hierarchical model M to simulate the data and calculate a summary statistic vector $\mathbf{D}_i$. The summary statistics within vector $\mathbf{D}_i$ are scaled to have unit variance and the simulations are repeated K times. Each simulated $\mathbf{D}_i$ is compared to the observed $\mathbf{D}^*$ and the joint posterior distribution is obtained from the proportion of simulations that yielded summary statistics closest to the observed $\mathbf{D}^*$. Our method samples this joint posterior distribution using a two-step process: rejection and weighted local regression on the accepted values. In the first step, a set of simulated parameters and their associated summary statistic vector $\mathbf{D}_i$ are accepted when the Euclidian distance $\|\mathbf{D}_i - \mathbf{D}^*\| = d$ is $<\varepsilon$, where ε is an arbitrary critical value. Each value of ε controls the tolerance, which is the proportion of values closest to $\mathbf{D}^*$ that are accepted. In the second step, each accepted parameter set is given a weight between zero and one that declines quadratically as $\|\mathbf{D}_i - $
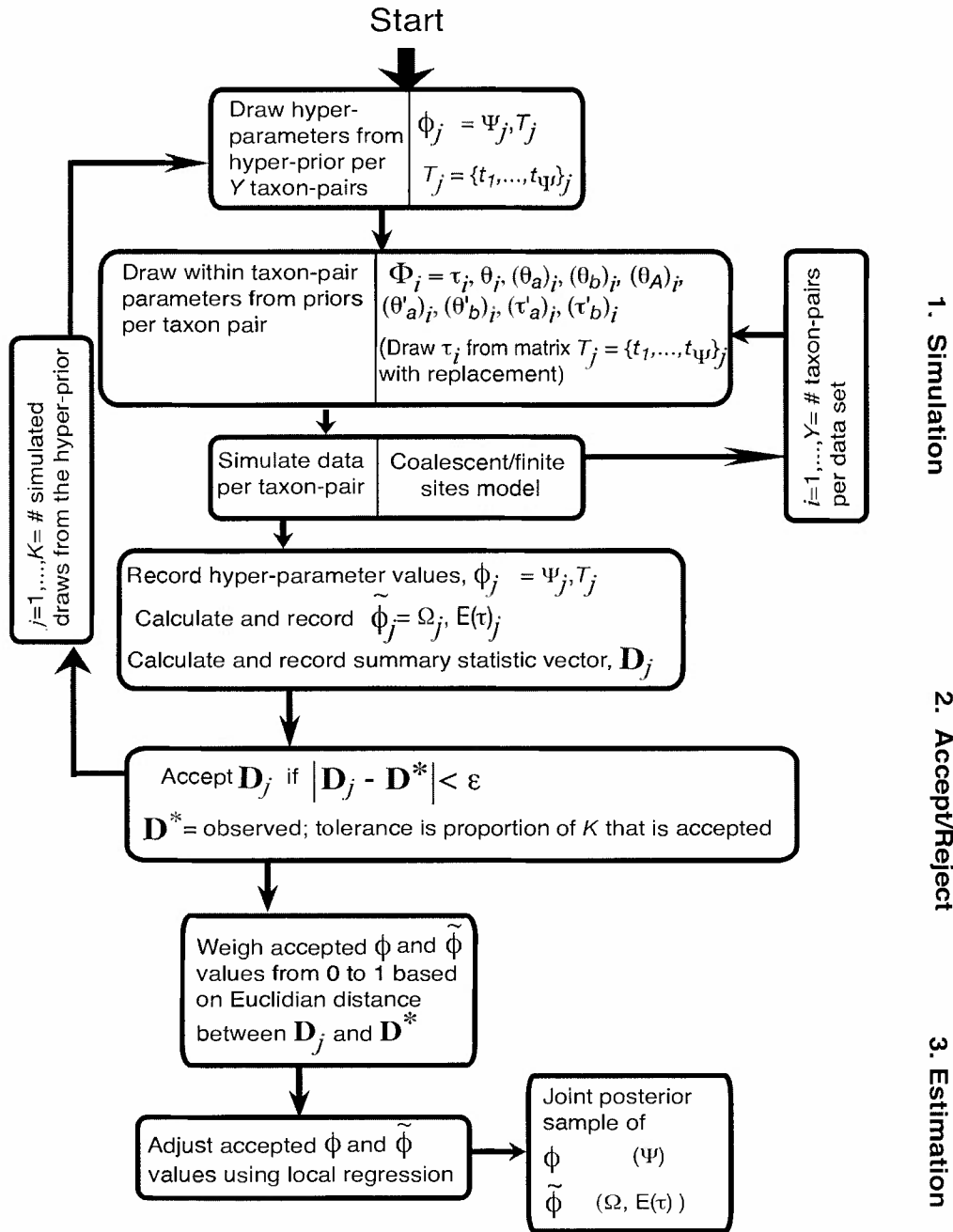
Start

FIG. 2. Flow chart representing the generalized approximate Bayesian computation procedure for comparative phylogeographic inference for Y taxon pairs. See Table 1 for definition of parameters.

$\mathbf{D}^*\|$ approaches ε. In this case, a weight of one is assigned when $\|\mathbf{D}_i - \mathbf{D}^*\| = 0$ and a weight of zero is assigned when $\|\mathbf{D}_i - \mathbf{D}^*\| = \varepsilon$. Given that $d = \|\mathbf{D}_i - \mathbf{D}^*\|$ for a chosen ε, these weights are assigned using the Epanechnikov kernel function $K_\varepsilon(d)$,

$$K_\varepsilon(d) = \begin{cases} c\varepsilon^{-1}[1 - (d/\varepsilon)^2] & d \le \varepsilon \\ 0 & d > \varepsilon, \end{cases} \quad (1)$$

where c is a normalizing constant. Subsequently, weighted linear regression is used to adjust each accepted parameter value following the methods of Beaumont et al. (2002) and

Tallmon et al. (2004), such that these adjusted parameter values are assumed to be random samples from the joint posterior distribution $f(\phi, \Phi \mid \mathbf{D})$. The accuracy of obtaining this joint posterior by approximate means depends on how strongly the chosen summary statistic vector $\mathbf{D}$ is a function of the parameters ϕ and Φ. We investigate two factors that could affect the bias of the estimator. This includes tolerance (determined by ε), and the number of summary statistics within the data vector $\mathbf{D}$. An overall schematic of our implementation of the ABC approach for comparative phylogeographic inference is depicted in Figure 2.

TABLE 1. Parameters and their prior distributions. Hyperparameters (ϕ) are randomly drawn once per Y taxon pairs. Within-taxon parameters (Φ) are randomly drawn once per ith taxon pair. Hyperparameters Ω and $E(\tau)$ ($\tilde{\phi}$) are calculated once per Y taxon pairs. The per generation per gene DNA mutation rate (μ) is uniform across all taxa. Each divergence time is scaled by μ, such that dividing by μ yields the divergence time (t) in generations. Because Ω and $E(\tau)$ are essentially properties of the Y divergence times within T_Y, they are not true hyperparameters and are therefore delineated as $\tilde{\phi}$.

Hyperparameters (ϕ)	Description	Prior distribution
μ	the per gene per generation mutation rate	assumed to be uniform if estimating divergence time
ψ	number of possible divergence times across Y taxon pairs	discrete uniform $(1, Y)$
$T = \{t_1, \cdots, t_\psi\}$	matrix of ψ possible divergence times (t) among Y taxon pairs	each t within T drawn from uniform $(0, \tau_{max})$
$T_Y = \{\tau_1, \cdots, \tau_Y\}$	matrix of Y divergence times among Y taxon pairs	each τ within T_Y randomly drawn with replacement from T matrix
$E(\tau)$	the mean τ across Y taxon pairs calculated from $\tau_1, \cdots, \tau_Y$ taxon pairs; in units of μ, such that $\tau = 10.0$ would be $10.0/\mu$ in generations	determined by τ_{max}, ψ, Y
Ω	$Var(\tau)/E(\tau)$, the variance of τ, divided by the mean of τ across Y taxon pairs calculated from $\tau_1, \cdots, \tau_Y$; in units of $\mu/2\theta_{AVE}$, where $2\theta_{AVE}$ is a constant determined by the parametric expected value of θ	determined by τ_{max}, Ψ, Y
Within-taxon parameters (Φ)		
$\tau_i, i = 1, \cdots, Y$	each (ith) taxon pair's divergence time drawn randomly (with replacement) from Ψ divergence times within matrix $T = \{t_1, \cdots, t_\psi\}$	uniform $(0, \tau_{max})$
$\theta_i, i = 1, \cdots, Y$	total population mutation parameter of each taxon pair, where $\theta_i = 2N_i\mu$	uniform $(0.5\,\theta_{max})$
$(\theta_a)_i, i = 1, \cdots, Y$	population mutation parameters for daughter	$(\theta_a)_i =$ uniform $(0.0, \theta_i)$
$(\theta_b)_i, i = 1, \cdots, Y$	populations a and b, $i = 1, \cdots, Y$, $\theta_i = (\theta_a + \theta_b)_i$	$(\theta_b)_i =$ uniform $(0.0, \theta_i \mid (\theta_b)_i = \theta_i - (\theta_a)_i)$
$(\theta_A)_i, i = 1, \cdots, Y$	population mutation parameter for the ancestral population size of the ith taxon pair	uniform $(0.01, (\theta_A)_{max})$
$(\theta'_a)_i, i = 1, \cdots, Y$	coefficient of population bottleneck magnitude	uniform $(0.01, \theta_i)$
$(\theta'_b)_i, i = 1, \cdots, Y$	in daughter populations a and b at beginning of population bottleneck (τ'_a and τ'_b before the present)	
$(\tau'_a)_i, i = 1, \cdots, Y$	length of time between beginning of bottleneck	uniform $(0.0, \tau_i)$
$(\tau'_b)_i, i = 1, \cdots, Y$	in daughter populations a and b and the present time	

Hierarchical Model of Divergence Across Taxon Pairs

Our comparative phylogeographic model is a hierarchical Bayesian model consisting of jointly distributed within-taxon-pair parameters and hyperparameters (ϕ) describing the distribution of divergence times. This involves Y ancestral populations that each split into a taxon pair at times $T_Y = \{\tau, \ldots, \tau_Y\}$ in the past. The primary hyperparameter is, Ψ the number of different possible divergence times across Y taxon pairs and is drawn from the discrete uniform distribution $(1, Y)$. After randomly drawing Ψ, each of the Ψ divergence times $T = \{t, \ldots, t_\Psi\}$ are randomly assigned values from a continuous uniform prior distribution $(0, \tau_{max})$, the maximum possible divergence time. Then, each of the Y taxon pairs randomly chooses with replacement from the $\{t, \ldots, t_\Psi\}$ divergence times. In addition to using Ψ for estimating the degree of variability in τ across Y taxon pairs, we make use of two other hyperparameters including $E(\tau)$, the mean divergence time across T_Y, and $\Omega = Var(\tau)/E(\tau)$, the ratio of the variance to the mean of divergence times across T_Y. Because $E(\tau)$ and Ω are not explicitly drawn from the hyperprior but instead are calculated from T_Y after Ψ and $T = \{t, \ldots, t_\Psi\}$ are drawn, we denote $E(\tau)$ and Ω as $\tilde{\phi}$ instead of ϕ. Although this is some abuse of notation, $E(\tau)$ and Ω can be estimated as hyperparameters and their hyperprior

distributions can be explicitly characterized via simulation (Fig. 3).

The divergence times τ are scaled by the per gene per generation mutation rate, μ, such that $\tau = t\mu$, where t is the divergence time in generations. Our estimates are based on the assumption that μ is constant across taxa, with the differences among taxa being parameterized by the composite population mutation parameter, $\theta = 2N\mu$ (where N is the female effective population size given mtDNA data). Given nuclear data, $\theta = 4N\mu$ and N would be the diploid effective population size.

In addition to Y divergence times, our hierarchical model incorporates other lower-level parameters describing other aspects of the Y demographic histories. These are treated as nuisance parameters and are allowed to freely vary among Y taxon pairs by using continuous prior distributions (Table 1). The θ of each taxon pair is drawn from a uniform prior distribution, such that the ith taxon pair's θ_i is independently drawn from a uniform distribution bounded by $(0.05\,\theta_{max})$ and the two θ values for each pair of respective daughter population, $(\theta_a)_i$ and $(\theta_b)_i$, are drawn from uniform distributions bounded by $(0.01\theta, 1.0\theta_i)$ conditional on $(\theta_a)_i = \theta - (\theta_b)_i$. The ith taxon pair's ancestral mutation parameter $(\theta_A)_i$, is independent of the corresponding θ_i and is drawn
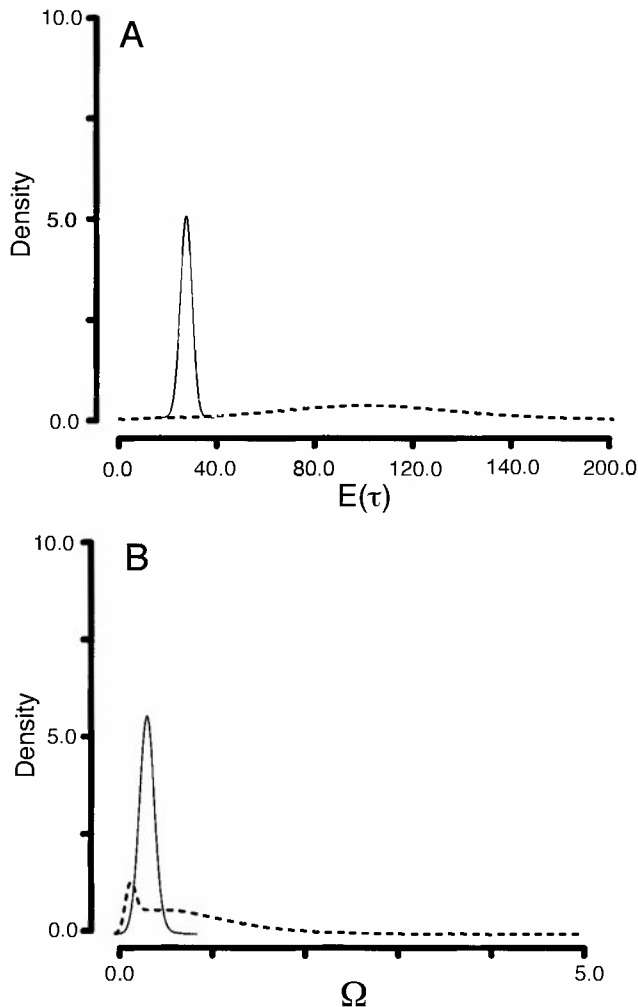
FIG. 3. Prior (dashed lines) and posterior (solid lines) distributions of $E(\tau)$ (A) and Ω (B). The degree of uniformity in τ across Y taxon pairs is characterized by Ω, the ratio between variance and mean of τ. The prior distributions are not analytically characterized but rather are empirically derived from simulating the model $K = 500{,}000$ times given $Y = 8$ taxon pairs and $\tau_{max} = 200.0$.

from the uniform distribution $(0.05, \theta_{max})$. In practice, this uniform prior for θ spans all of the empirical estimates of θ from the comparative phylogeographic dataset using either Tajima's (1983) or Watterson's (1975) estimator of θ. While $E(\tau)$ is calculated from the scaled divergence times $\{\tau, \ldots, \tau_Y\}$, Ω is calculated from divergence times scaled by μ/θ_{AVE}, where θ_{AVE} is a constant determined by the parametric expected value of θ given its prior distribution, $(0.05, \theta_{max})$.

To allow for demographic expansion, we use an exponential bottleneck model specified by the duration of each bottleneck in the ith pair of each ancestor's two descendent daughter populations from the time of each bottleneck's beginning to the present, $(\tau'_a)_i$ and $(\tau'_b)_i$ (Fig. 1). Exponential growth occurs from the beginning of each bottleneck, where the size of the daughter populations a and b are free parameters $(\theta'_a)_i$ and $(\theta'_b)_i$ at the beginning of the bottleneck phase. Each exponential phase continues for the duration of the bottleneck, where $(\theta'_a)_i$ and $(\theta'_b)_i$ grow to be sizes $(\theta_a)_i$ and $(\theta_b)_i$ (Fig. 1). These four bottleneck parameters are drawn from

uniform distributions specified in Table 1. We assume that evolution follows the coalescent under the Wright-Fisher and finite sites models. In our analysis, we used an HKY model of DNA evolution, which is robust and commonly used for mtDNA regions (Hasegawa et al. 1985), but one could use most other models of DNA sequence evolution that best fit the data (Cunningham et al. 1998).

Summary Statistics

For ABC to work well, it is best to use summary statistics showing a strong correspondence with parameter values. Pairwise Euclidian distances between the summary statistics generated under identical parameter values should be minimized. To implement ABC, the summary statistic vector $\mathbf{D}$ consists of a specified number of summary statistics that are collected from all taxon pairs in a comparative phylogeographic dataset. The vector $\mathbf{D}$ includes summary statistics that have been demonstrated to contain relevant information for testing simultaneous vicariance across taxon pairs (Hickerson et al. 2006). Briefly, π is the average number of pairwise differences among all sequences within a single phylogeographic dataset (a single taxon pair), whereas π_w and π_b are the average number of pairwise differences partitioned within and between each taxon pair, respectively (Tajima 1983; Takahata and Nei 1985). The number of segregating sites normalized for sample size is θ_W (Watterson 1975), and $\mathrm{Var}(\pi - \theta_W)$ is the denominator of Tajima's D (Tajima 1989, eq. 38). These summary statistics in total make up the components of two composite summary statistics that are positively correlated with divergence time, π_{net} and Tajima's D (Hickerson et al. 2006). Nei and Li's corrected nucleotide divergence is the difference $\pi_b - \pi_w = \pi_{net}$ (Nei and Li 1979). Tajima's D (Tajima 1989) is a function of π, θ_W, and $\mathrm{Var}(\pi - \theta_W)$ and has been demonstrated to have the least amount of correlation with these other summary statistics with respect to divergence time (Hickerson et al. 2006).

The vector $\mathbf{D}$ is made up of a two-dimensional array where the number of columns correspond to the classes of summary statistics and the number of rows correspond to the number of taxon pairs (Y) per comparative phylogeographic dataset. We use up to four classes of summary statistics including π, π_{net}, θ_W, and $\mathrm{Var}(\pi - \theta_W)$. Given these four classes of summary statistics collected per taxon pair and Y taxon pairs, the summary statistic vector

$$\mathbf{D} = \left\{ \begin{array}{cccc} (\pi_{net})_1 & \pi_1 & (\theta_W)_1 & \mathrm{Var}(\pi - \theta_W)_1 \\ \vdots & \vdots & \vdots & \vdots \\ (\pi_{net})_Y & \pi_Y & (\theta_W)_Y & \mathrm{Var}(\pi - \theta_W)_Y \end{array} \right\} \quad (2)$$

would include $4Y$ summary statistics. One of the underlying principles of ABC is to calculate $\mathbf{D}$ consistently (empirical and simulated data). Following this, we must choose a way of ordering the rows within $\mathbf{D}$ consistently across all datasets. Because ABC will work most efficiently when there is a high correlation between $\Delta\phi$ and $\Delta\mathbf{D}$, where Δ is Euclidian distance, ordering by sample size (number of individuals per taxon pair) would have resulted in an inefficient ABC esti-

TABLE 2. Taxon pairs thought to have arisen from ancestral species splitting into descendent Pacific–Atlantic pairs. Values in parentheses are the number of COI sequences used from each taxon.

Genus	Pacific taxon	Atlantic taxon
Diadema	*mexicanum* (13)	*antillarum b* (2)
Arbacia	*spatuligera* (16)	*lixula* (74)
Echinometra	*vanbrunti* (14)	*lucunter* (43)
Eucidaris	*galapagensis* (14)	*clavata-tribuloides* (58)
Lytechinus	*semituburculatus* (5)	*variegatus-williamsi* (82)
Tripneustes	*depressus-gratilla* (164)	*ventricosus*(Western Atlantic) (48)
Astropyga	*pulvinata* (4)	*magnifica* (2)
Meoma	*ventricosa grandis* (4)	*ventricosa* (4)

mator. For example, the distribution of π_{net} across the Y rows in $\mathbf{D}$ would be random under $\Psi = 1$ such that $\Delta\mathbf{D}$ among datasets simulated under $\Psi = 1$ would be similar to $\Delta\mathbf{D}$ between these simultaneous divergence datasets and those datasets simulated under $\Psi > 1$ because these latter datasets would also yield a random distribution of π_{net} across the Y rows in $\mathbf{D}$ if ordering was done by sample size.

Instead of ordering by sample size, we increase the efficiency of the ABC procedure by ordering rows 1 though Y within each column by the ascending values of each taxon pair's net divergence (π_{net}), the metric that is strongly correlated with divergence time (Takahata and Nei 1985; Hickerson et al. 2006). For example, each row within $\mathbf{D}$ would contain summary statistics collected from the same taxon pair, and $(\theta_W)_1$ within $\mathbf{D}$ would be from the taxon pair with the lowest π_{net} and $(\theta_W)_Y$ would be from the taxon pair with the highest π_{net}. This ordering is based on the *exchangeability* of the Y rows within $\mathbf{D}$, $(\mathbf{D}_1, \ldots, \mathbf{D}_Y)$. If τ_i and $\mathbf{D}_i$ are invariant to the permutations of the indexes $(1, \ldots, Y)$ and each taxon pair's sample size is unrelated to the expectation of its τ or $\mathbf{D}$, there is exchangeability in our model (Gelman et al. 2004).

Simulations

To estimate Ω and $E(\tau)$, we generated K simulated datasets to sample from the joint prior distribution $f(\phi, \Phi)$ under the model M of population divergence. The proportion (tolerance) of K values accepted as samples of the joint posterior distribution $f(\phi, \Phi \mid \mathbf{D})$ is set by using a particular value of ε.

Three C programs (msprior, msDQH, and msStatsVector) were glued together by a Perl shell (which we call msBayes) to: (1) sample from the joint prior $f(\phi, \Phi)$; (2) generate the finite sites DNA sequence data; and (3) calculate the summary statistic vector $\mathbf{D}$. The second of these three programs (msDQH) was a modified version of Hudson's (2002) coalescent simulator (ms) that produced data according to an arbitrary finite sites model and arbitrary population history, and the third program (msStatsVector) was a modified version of Hudson's sumstats (Hudson 2002). The parameter estimates by ABC regression and the corresponding joint posterior surfaces were calculated using the density estimation method implemented in the R statistical package (Loader 1996; R Development Core Team 2004) with a script kindly provided by M. Beaumont. Because Ω and $E(\tau)$ are bounded by zero, accepted values can end up having adjusted values outside parameter space (negative) during the regression step. Therefore, we transform negative values to zero before re-

porting and summarizing the posterior densities. In general, the regression step is achieved in under a minute, while simulating 500,000 draws from the joint prior takes approximately 5 h per taxon pair on a Linux computer (4-GB RAM, 2-GHz dual processor). All code and scripts are available from the first author upon request.

Empirical Application

We estimated Ω from a mtDNA dataset of eight genera of tropical echinoids (Table 2). Each of the eight genera contains geminate taxon pairs and thereby consists of Atlantic and Pacific taxa that were possibly descended from an ancestral population split by the rise of the Isthmus of Panama approximately 3.1 million years ago (Mayr 1954; Chesher 1972; Coates et al. 1992). These eight geminate taxon pairs include taxa in the genera *Eucidaris* (Lessios et al. 1999), *Diadema* (Lessios et al. 2001), *Echinometra* (McCartney et al. 2000), *Tripneustes* (Lessios et al. 2003), *Lytechinus* (Zigler and Lessios 2004), *Arbacia* (Lessios et al. 2001), *Astropyga*, and *Meoma* (K. S. Zigler and H. A. Lessios, unpubl. data). These data consist of DNA fragments 632–639 bases long from the cytochrome oxidase I (COI) gene collected from multiple individuals per taxon pair. Although these eight divergences geographically correspond to the rise of the Isthmus of Panama, some of the descendent sister taxa subsequently split into new species within either ocean after the isthmian separation.

To be conservative, we jointly estimated Ω and $E(\tau)$ across the eight echinoid taxon pairs using two independent replicates of $K = 500,000$ draws from the joint prior distribution and repeated this using three different upper bounds for the prior of θ_A (40.0, 20.0, and 10.0) because of θ_A's strong expected affect on ancestral coalescence. Specific prior values are listed in Table 1 with the upper bounds for θ and τ being $\theta_{max} = 40.0$, and $\tau_{max} = 200.0$. The uniform priors for θ and θ_A encompass estimates of θ based on Tajima's (1989) and Watterson's (1975) estimate of this parameter in the echinoid data in which the maximum empirical values of π and θ_W were 31.73 and 28.03, respectively, per taxon pair and 13.04 and 12.30, respectively, per daughter population. We report joint posterior estimates based on a summary statistic vector $\mathbf{D}$ that included the 32 summary statistics (π_{net}, π, θ_W, and $Var[\pi - \theta_W]$ per taxon pair) and a tolerance of 0.002 thereby yielding estimates based on 1000 draws from the joint posterior given $K = 500,000$ simulated draws from the joint prior. Additionally, we report estimates based on $K =$

Rejection sampling only
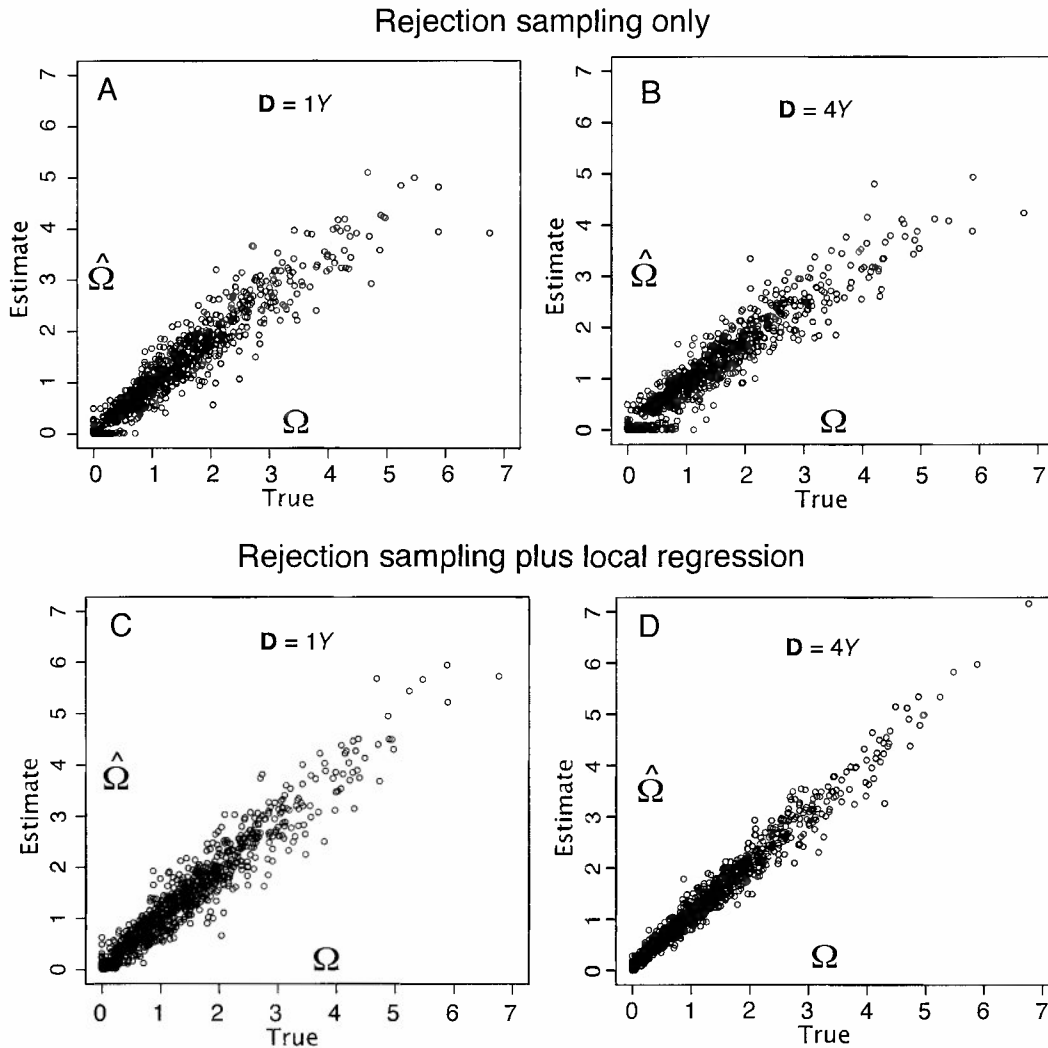

Rejection sampling plus local regression

FIG. 4.   Panels (A–H) each plot 1000 true values of Ω or $E(\tau)$ against their approximate Bayesian computation (ABC) estimates. Each true parameter value is randomly drawn from the hyperprior distribution. Panels (A–D) depict ABC estimates of Ω, whereas panels (E–H) depict ABC estimates of $E(\tau)$. Results using simple rejection sampling are depicted in panels (A), (B), (E), and (F), and results using rejection sampling plus local regression are depicted in panels (C), (D), (G), and (H). All estimates are based on a tolerance of 0.002 and K = 500,000 draws from the hyperprior. Estimates in panels (A), (C), (E), and (G) are obtained using one summary statistic per taxon pair ($\mathbf{D}$ = π_{net} from each taxon pair), and the estimates in panels (B), (D), (F), and (H) are obtained using four summary statistics per taxon pair ($\mathbf{D}$ = $\{\pi, \pi_{net}, \theta_W, \mathrm{Var}(\pi - \theta_W)\}$).

800,000 draws from the joint prior (1600 draws from the joint posterior).

Performance of Estimator

To evaluate the performance of our estimator, we used our ABC technique on six sets of 1000 pseudo-observed (simulated) datasets. Each set of 1000 pseudo-observed datasets was simulated under different fixed values of Ω and $E(\tau)$, and an ABC estimate of Ω and $E(\tau)$ was obtained from each pseudo-observed dataset. Every simulated dataset consisted of eight taxon pairs that were identical to the eight echinoid datasets with respect to numbers of base pairs (632–639) and numbers of individuals within each taxon pair (Table 2). Within-taxon-pair parameters of the pseudo-observed datasets were fixed across all simulations and were initially set

to a single random draw from the per taxon pair priors (Table 2). The summary statistics were identically computed in all simulated datasets and then used as pseudo-observed summary statistics from which the ABC estimates of Ω and $E(\tau)$ are obtained. While drawing from the joint prior K = 500,000 times by simulation can take about 5 h per taxon pair (4-GB RAM, 2-GHz dual processor), the estimation step takes seconds to minutes. Therefore, by reusing the K = 500,000 draws from the joint prior, 1000 ABC estimates from each set of 1000 simulated datasets can be completed with relatively little computational cost (Excoffier et al. 2005).

We quantified performance by reporting how accurate and/ or biased the ABC estimator is given the fixed true values of Ω and $E(\tau)$. Over each set of 1000 datasets, we report the median estimates, mean estimates, and 95% coverage (pro-

Rejection sampling only
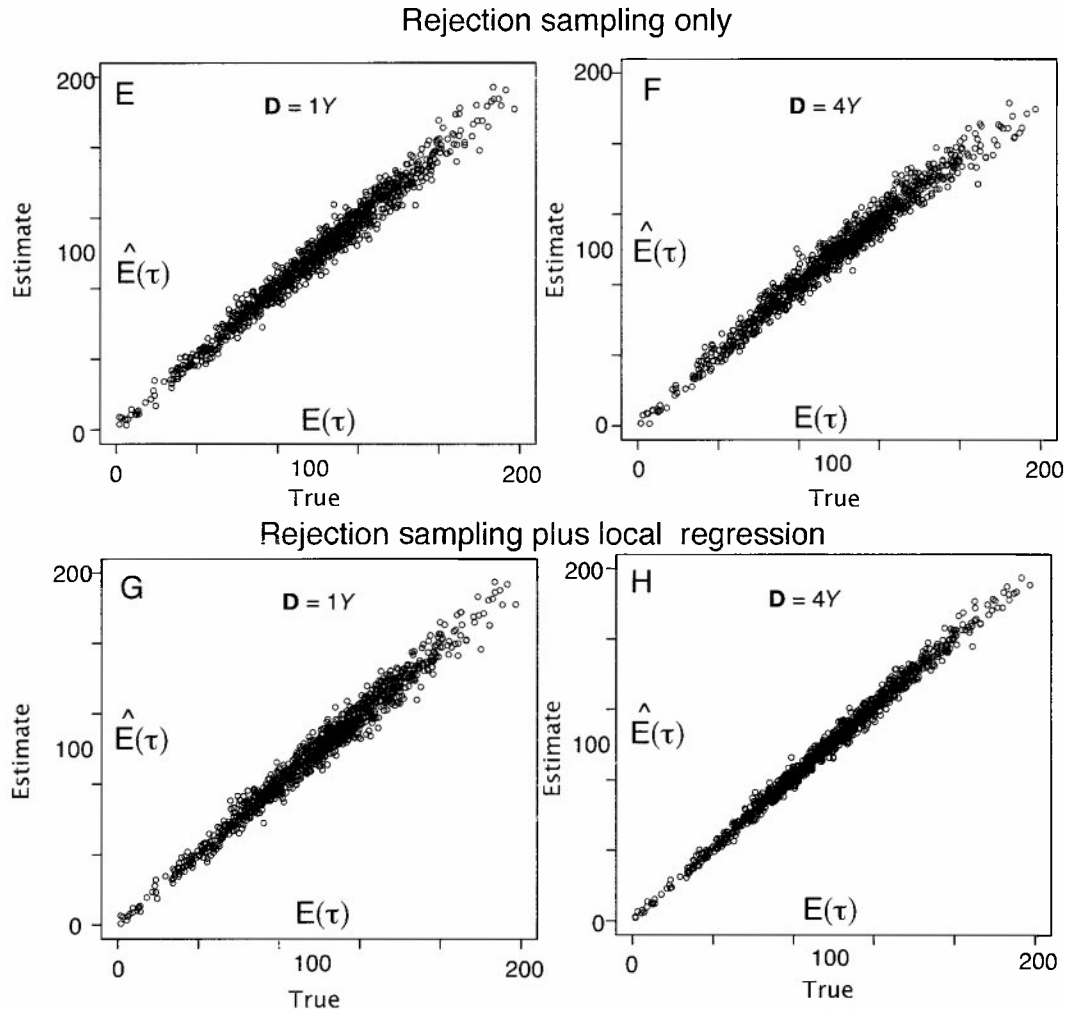

Rejection sampling plus local regression

FIG. 4. Continued.

portion of times the true value is within the equal-tailed 95% credibility intervals around the point estimate). We explored the performance given two tolerances (0.002 and 0.01 of accepted values) and two different sets of summary statistics within vector $\mathbf{D}$ ($\mathbf{D} = 8\{\pi_{net}\}$ and $\mathbf{D} = 32\{\pi_{net}, \pi, \theta_W, \mathrm{Var}(\pi - \theta_W)\}$). Each estimate was based on the same $K = 500{,}000$ draws from the joint prior. To gauge a more general evaluation of the estimator, we also estimated Ω and $E(\tau)$ on 1000 datasets simulated from parameter sets randomly drawn from the joint prior distribution.

To further assess our ABC method, we compared its performance to the performance of the MCMC method employed by isolation with migration (IM; Hey and Nielsen 2004), which is designed to estimate a divergence time between two populations. To be consistent between these two methods, we scaled down our ABC method to estimate a single divergence time between a single pair of populations given a single mtDNA locus of 639 base pairs evolving under the HKY model of evolution. For this comparison, we obtained three independent estimates given the same simulated dataset for both the ABC and MCMC methods (IM), where the simulated taxon pair has 72 individuals (58 and 14, respectively,

from the two descendent populations). Each of the three independent ABC estimates of divergence time was from an independent $K = 2{,}000{,}000$ draws from the prior and corresponding 4000 draws from the posterior (tolerance = 0.002). For IM, each of the three independent estimates of the divergence time involved running the program with the prior for migration set to zero. We used IM with 10 MCMC coupled chains with a burn-in time of 500,000 steps, and we adjusted the heating values so that the initial update rates for divergence time were >40% and that swap rates between adjacent chains were at least 80%.

RESULTS

Performance of Estimator

We found our ABC method to successfully estimate $E(\tau)$ with notable accuracy and little bias across all of the parameter space and conditions we explored, while success in estimating Ω improved as $E(\tau)$ became older (see Fig. 4; Table 2). For both $E(\tau)$ and Ω, the 95% coverage values were commonly as large as 100% such that the true values of Ω were within the 95% credibility intervals 100% of the time. The

One taxon pair dataset simulated with a divergence time of τ = 24.0
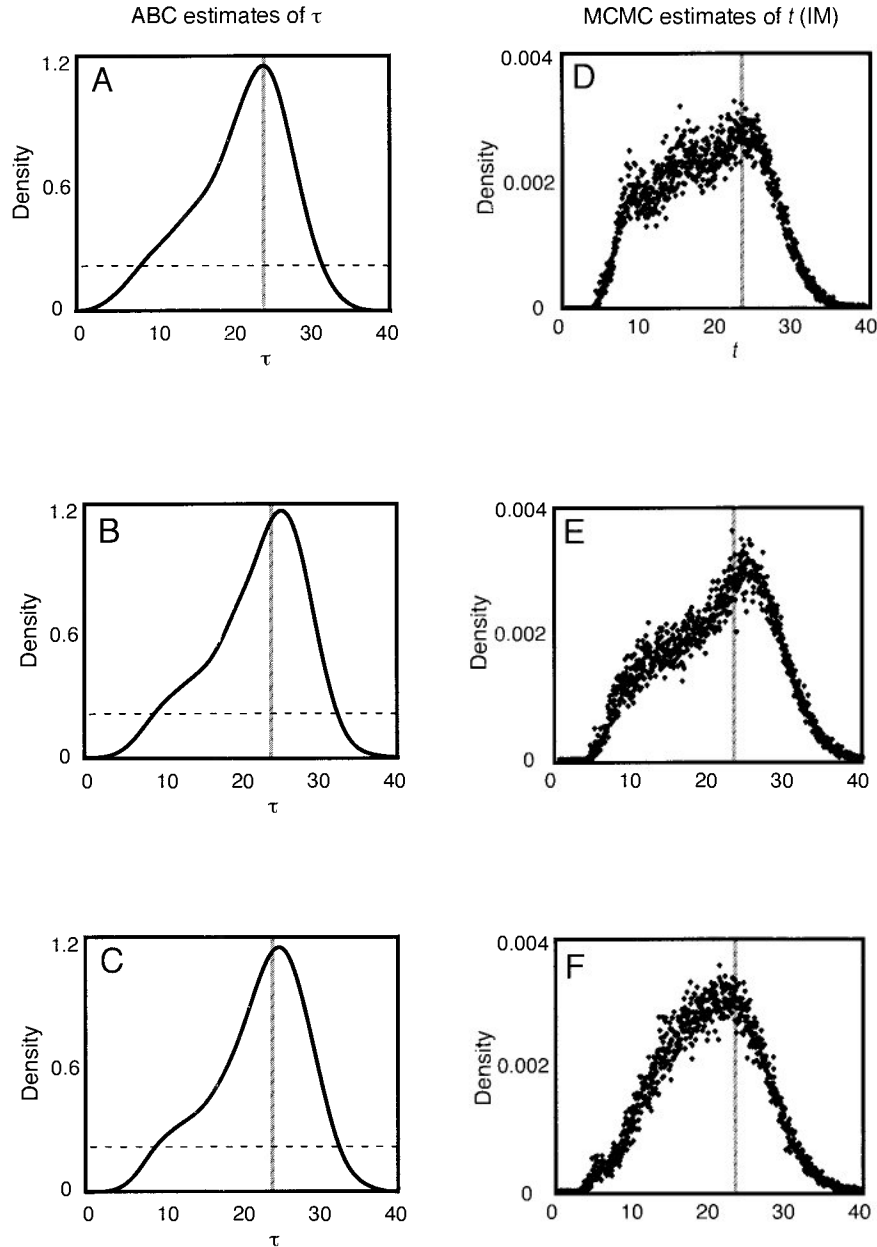
ABC estimates of τ   MCMC estimates of *t* (IM)

Fig. 5. Panels (A–C) depict three independent approximate Bayesian computation estimates of τ given a single taxon-pair dataset that were simulated with a divergence time of τ = 24.0. Panels (D–F) are three independent isolation with migration (IM) estimates of τ given the same simulated dataset. The true value of τ is represented by a gray line. The simulated dataset consisted of 72 individuals with 58 and 14 individuals, respectively, in each population, and it consisted of a single haploid mitochondrial DNA locus of 639 base pairs.

exception to this was estimating Ω when $E(\tau)$ was recent (2.0) and Ω = 0.2 (history B), indicating that distinguishing between variable and simultaneous divergence histories will be most challenging for this recent time scale given single-locus data. Although our tests of estimator performance could have been heavily dependent on the fixed values of within-taxon-pair parameters we used (θ and θ_A), we found similar performance when the per taxon parameters were random

draws from the prior across all pseudo-observed datasets (not shown).

Tolerance and number of summary statistics within vector **D** were found to only slightly affect estimates under the conditions we explored. Although estimates of Ω were more biased when using the larger summary statistic vector (**D** = 32), doing so often led to higher coverage than when using the smaller summary statistic vector that only contained π_{net}
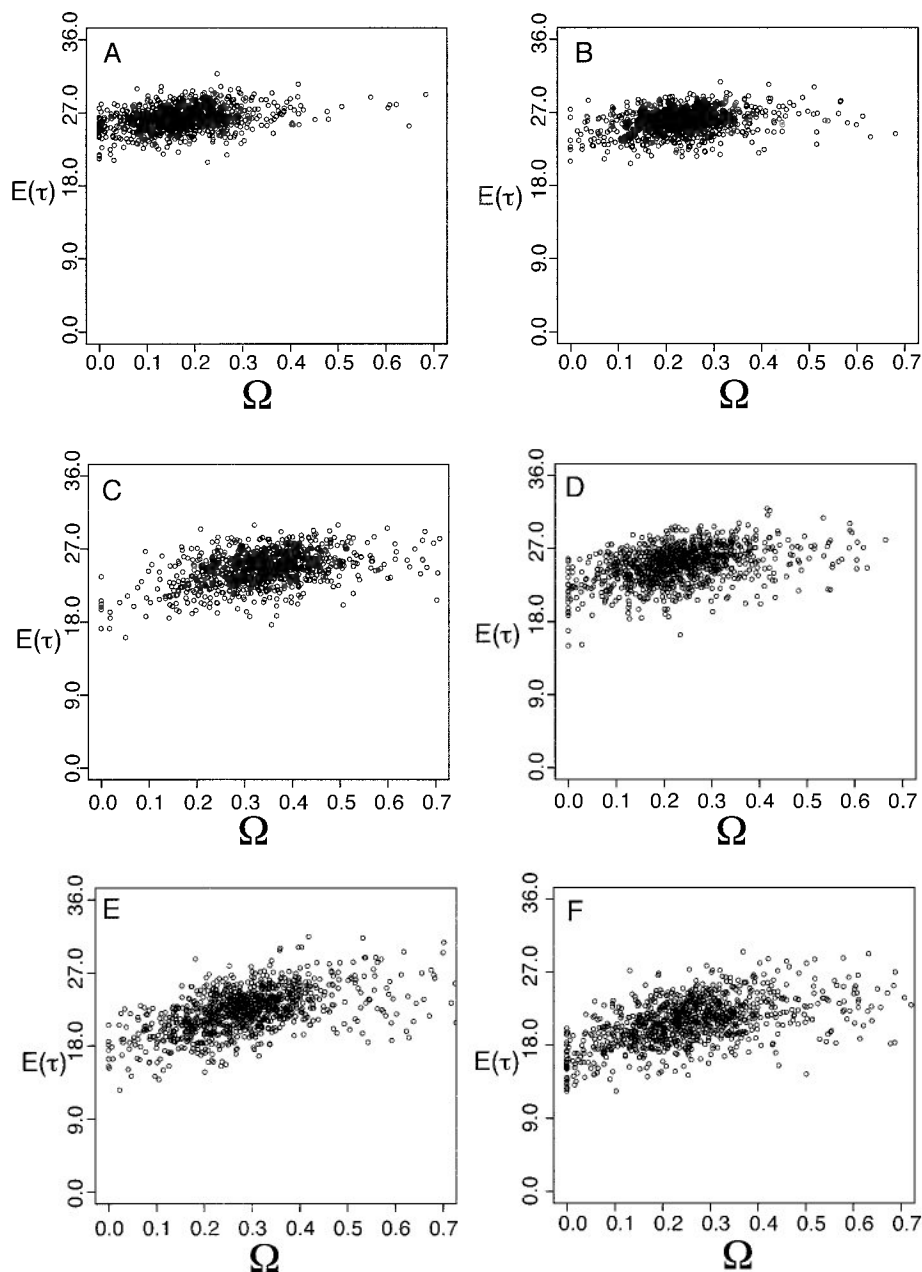
FIG. 6. Six independent joint posterior probability densities for $E(\tau)$ and Ω using the approximate Bayesian computation estimator, with a tolerance of 0.002, four summary statistics per taxon pair in vector $\mathbf{D}$ (π_{net}, π, θ_W, $\text{Var}(\pi - \theta_W)$), $\tau_{max} = 10.0$, and six independent K = 500,000 draws from the prior. The uniform prior for θ_A was bounded by 0.01 and 10.0 (in A and B), by 0.01 and 20.0 (C and D), and by 0.01 and 40.0 (E and F).

from each taxon pair. Although a tolerance of 0.002 yields only 1000 accepted values (given K = 500,000 draws from the joint prior) and thereby could have resulted in substantial noise in the regression fit given 32 summary statistics, our simulation study suggests that 1000 values are sufficient for accurate estimates as well as for characterizing the posterior density using distributional summaries and density estimates (Beaumont et al. 2002). Although estimator performance could have potentially improved from using >500,000 draws from the joint prior, convergence is likely to be close because

replicate estimates based on separate K = 500,000 draws from the joint prior resulted in nearly identical estimates. In empirical applications of our ABC method, it would be prudent to base conclusions on multiple estimates based on replicate draws from the prior and base final estimates using the number of draws from the prior that are computationally feasible.

The comparison between our ABC method and the MCMC method of Hey and Nielsen (2004; IM) was generally consistent when making the independent estimates of a single
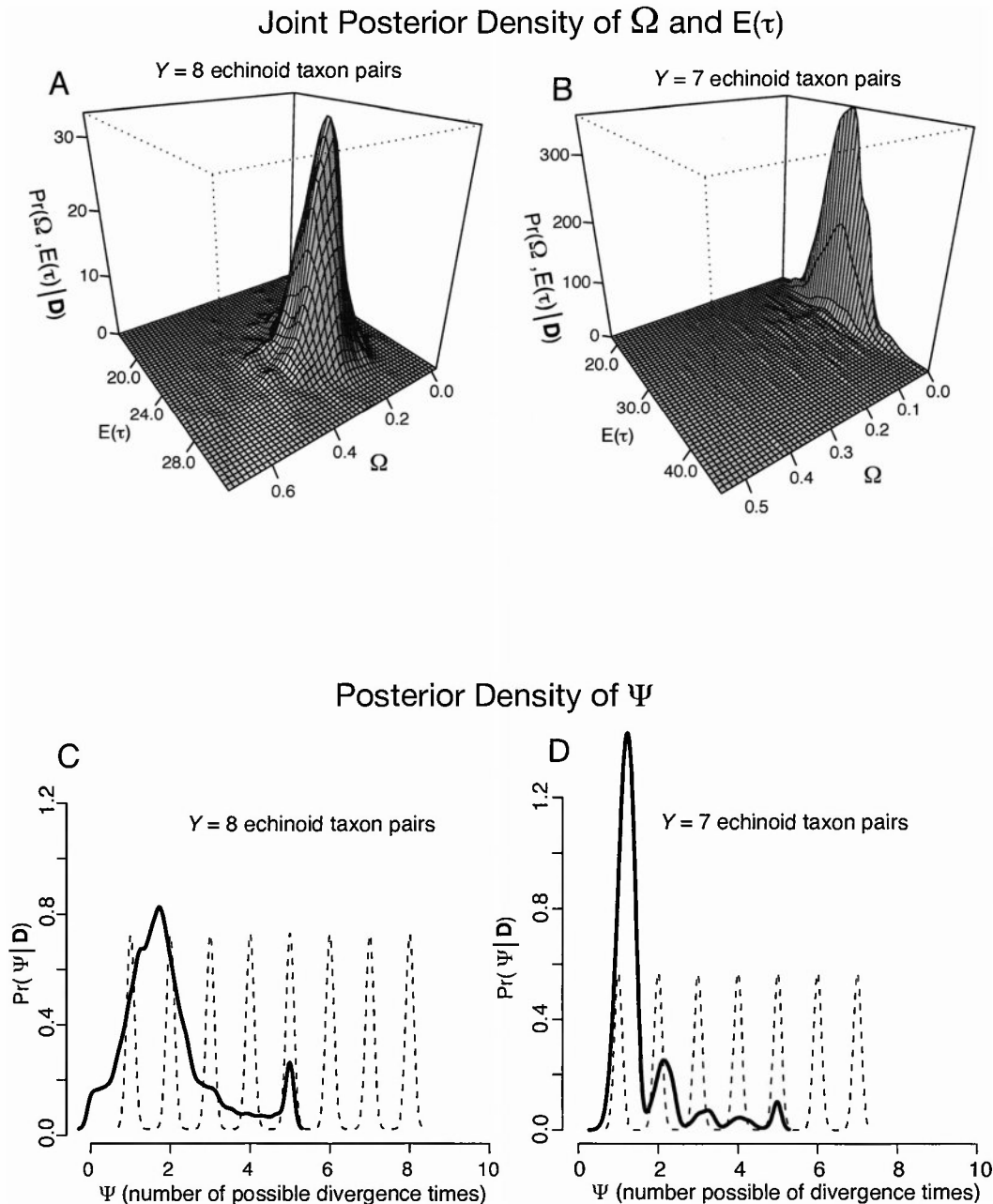
Joint Posterior Density of Ω and $E(\tau)$

Posterior Density of Ψ

Fig. 7. (A, B) Three-dimensional joint posterior probability densities for $E(\tau)$ and Ω. (C, D) Posterior probability densities for Ψ, the number of divergence times given Y taxon pairs. Estimates in (A) and (C) are based on data from all eight echinoid taxon pairs, whereas estimates in panels (B) and (D) are based on a dataset in which *Diadema* was not included. These estimates use the same uniform prior for θ_A bounded by 0.01 and 20.0 and are based on $K = 800{,}000$ draws from the joint hyperprior and 1600 draws from the joint posterior. In panels (C) and (D), the dotted line is the prior for Ψ and the solid line is the posterior for Ψ.

divergence time for a single simulated taxon pair given single-locus mtDNA data. Although estimates from both methods were generally close to the true value of divergence time ($\tau = 24.0$; Fig. 5), the ABC posterior estimates obtained with much lower CPU cost. Each ABC estimate took 20 h, whereas the MCMC estimates each took 120 h with the same Linux computer (4-GB RAM, 2-GHz dual processor). In reality, each ABC estimate was completed in 5 h of real time by simulating the prior on parallel computers with subsequent concatenations.

Divergence in Transisthmian Echinoids

Estimates of Ω and Ψ do not strongly support a history of simultaneous divergence in all eight echinoid taxon pairs. The estimates of Ω were robust to differences in the upper bound of the prior for θ_A and replicates of $K = 500{,}000$ draws from the joint prior (Fig. 6). Additionally, the conclusion supporting variable divergence held when making estimates of Ω and Ψ from $K = 800{,}000$ draws from the joint prior (Figs. 7A,C). The estimate of $E(\tau)$ defined by the mode of

TABLE 3. Performance of approximate Bayesian computation (ABC) estimators of Ω and $E(\tau)$ given data from eight taxon pairs simulated 1000 times under fixed histories A through F. Estimation procedure was repeated using two different summary statistic vectors ($\mathbf{D}$ = $1Y,\{\pi_{net}\}$ and $\mathbf{D}$ = $4Y,\{\pi_{net}, \pi, \theta_W, Var(\pi - \theta_W)\}$) and two tolerance levels (0.002 and 0.01). Each ABC estimate was based on K = 500,000 observations drawn from the joint prior distribution, $f(\phi,\Phi)$. Each simulated dataset consists of eight taxon pairs matching the eight echinoid datasets with respect to numbers of base pairs (632–639) and numbers of individuals. Coverage refers to the proportion of times the true value is within the equal-tailed 95% credibility intervals around the posterior mode estimate. Histories B, D, and F are variable τ histories and have the following fixed divergence times: history B, T_Y = $\{0.4, 0.4, 0.4, 0.4, 0.4, 0.4, 6.4, 6.4\}$; history D, T_Y = $\{8.0, 8.0, 29.4, 29.4, 29.4, 29.4, 29.4, 29.4\}$; and history F, T_Y = $\{51.0, 51.0, 51.0, 51.0, 51.0, 51.0, 84.0, 84.0\}$. The per taxon parameters for θ and θ_A are as follows: θ = $\{5.2, 9.6, 14.0, 18.4, 22.4, 26.4, 31.6, 36.0\}$; and θ_A = $\{4.6, 2.4, 3.5, 9.0, 7.9, 6.8, 1.3, 5.7\}$.

Tolerance	$\mathbf{D}$	True Ω	Mean estimate Ω	Median estimate Ω	95% coverage	True $E(\tau)$	Mean estimate $\hat{E}(\tau)$	Median estimate $\hat{E}(\tau)$	95% coverage
History A
0.002	4Y	0.00	0.02	0.00	1.00	2.00	2.27	2.25	0.87
0.002	1Y	0.00	0.02	0.00	1.00	2.00	2.26	2.07	0.88
0.01	4Y	0.00	0.04	0.00	1.00	2.00	2.47	2.67	0.87
0.01	1Y	0.00	0.02	0.00	1.00	2.00	2.44	2.03	0.87
History B
0.002	4Y	0.20	0.05	0.04	0.11	2.00	1.22	1.68	0.89
0.002	1Y	0.20	0.03	0.01	0.16	2.00	1.25	1.46	0.87
0.01	4Y	0.20	0.09	0.07	0.82	2.00	2.29	2.44	0.87
0.01	1Y	0.20	0.04	0.03	0.82	2.00	2.47	2.28	0.87
History C
0.002	4Y	0.00	0.05	0.02	1.00	24.00	24.41	24.46	0.97
0.002	1Y	0.00	0.02	0.00	1.00	24.00	24.09	24.23	0.98
0.01	4Y	0.00	0.05	0.02	1.00	24.00	24.69	24.64	0.99
0.01	1Y	0.00	0.03	0.01	1.00	24.00	23.81	24.86	0.99
History D
0.002	4Y	0.20	0.28	0.27	0.99	24.00	25.01	24.82	0.99
0.002	1Y	0.20	0.21	0.21	0.95	24.00	25.09	24.88	0.99
0.01	4Y	0.20	0.30	0.29	0.99	24.00	24.05	24.53	1.00
0.01	1Y	0.20	0.23	0.21	1.00	24.00	24.05	24.43	0.99
History E
0.002	4Y	0.00	0.06	0.01	1.00	59.20	60.22	59.80	1.00
0.002	1Y	0.00	0.01	0.00	1.00	59.20	59.09	59.23	1.00
0.01	4Y	0.00	0.07	0.01	1.00	59.20	61.42	61.69	1.00
0.01	1Y	0.00	0.03	0.01	1.00	59.20	59.47	59.47	1.00
History F
0.002	4Y	0.18	0.34	0.27	1.00	59.20	60.61	59.46	1.00
0.002	1Y	0.18	0.20	0.16	1.00	59.20	59.81	59.20	1.00
0.01	4Y	0.18	0.36	0.34	1.00	59.20	61.64	60.03	1.00
0.01	1Y	0.18	0.33	0.28	1.00	59.20	59.80	59.48	1.00

the joint posterior ranged from 22.3 to 24.9 and the estimate of Ω ranged from 0.14 to 0.27.

Hypothesis Testing

Although our simulation study demonstrates the ABC estimator of Ω to be well suited for distinguishing variable divergence from simultaneous divergence (Table 2), the ABC framework also allows a posteriori hypothesis tests. The posterior density mode estimates of Ω do not strongly support an equal τ history for the geminate echinoids of Panama, yet the posterior does cover values of $\Omega < 0.05$ (Fig. 6). To further evaluate a history of simultaneous vicariance, we use two hypothesis test procedures. First, we use the Bayes factor; second, we use a frequentist procedure by simulating histories of simultaneous divergence and calculating the probability of rejecting simultaneous divergence (Type I error).

The use of Bayes factors provides an alternative to the classical hypothesis testing, and by specifying an equal τ history and a variable τ history as two competing models

(M_{λ_1} and M_{λ_2}), we can the Bayes factor $B(\lambda_1, \lambda_2)$ to compare these models by averaging the posterior probabilities over the parameters (Kass and Raftery 1995). Given that $f(M_{\lambda_1})$ is the prior probability of the model specifying an equal τ history and $f(M_{\lambda_2})$ is the prior probability of the model specifying a variable τ history, then the Bayes factor comparing these two models is

$$B(\lambda_1, \lambda_2) = \frac{f(M_{\lambda_1} \mid \mathbf{D})/f(M_{\lambda_2} \mid \mathbf{D})}{f(M_{\lambda_1})/f(M_{\lambda_2})}, \quad (3)$$

where $f(M_{\lambda_1} \mid \mathbf{D})$ and $f(M_{\lambda_2} \mid \mathbf{D})$ are the posterior probabilities of these two models given a summary statistic vector $\mathbf{D}$. To calculate a Bayes factor, we must specify exactly how these two models are defined given our paramerization. We therefore delineate the two models using an arbitrary threshold for Ω, and define M_{λ_1} to be specified by draws from the posterior of Ω that are below this threshold and M_{λ_2} specified by draws from the posterior of Ω higher than this threshold. Using this approach, support for a variable divergence model
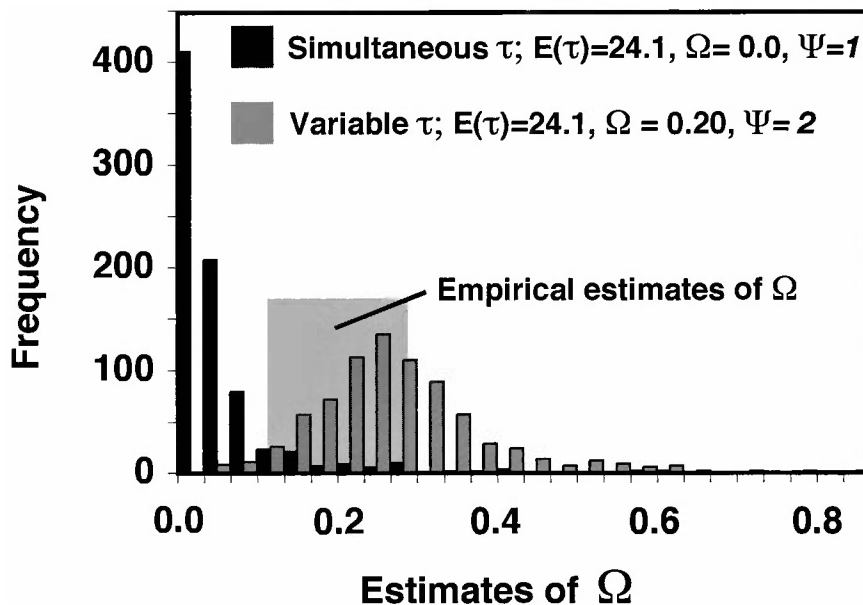
FIG. 8. Two frequency distributions of 1000 posterior mode estimates on data simulated using fixed values for $E(\tau)$ and Ω. The 1000 simulated simultaneous τ histories (black bars) were simulated with the eight divergences equal to the mean empirical estimate for $E(\tau)$ (24.1). The 1000 simulated variable τ histories (gray bars) were simulated given the means of both estimates for $E(\tau)$ and Ω. For this second variable divergence history, the eight divergence times were $T_Y = \{8.0, 8.0, 29.4, 29.4, 29.4, 29.4, 29.4, 29.4\}$.

(M_{λ_2}) was strong $(B[\lambda_1, \lambda_2] < 0.10)$ if using $\Omega = 0.01$ as a threshold and weak $(B[\lambda_1, \lambda_2] > 1.0)$ if using $\Omega = 0.1$ as a threshold (Jeffreys 1961), and this was relatively insensitive to prior for θ_A or particular replicate (Table 3). Because even $\Omega = 0.02$ is consistent with marked discordance in τ, we consider the threshold of 0.01 as signifying strong support for temporal discordance to be most relevant.

To assess the probability of making a Type I error (falsely rejecting an equal τ history) given our empirical estimates of Ω, we made 1000 estimates of Ω on 1000 datasets simulated with Ω fixed at 0.0 and $E(\tau)$ fixed to the average of our empirical estimates (24.3). Only 5.5% of these 1000 mode estimates were $\Omega > 0.1$, while 62% were $\Omega > 0.01$ (Fig. 8). In these 1000 simulated datasets, the average proportion of accepted points greater than $\Omega = 0.1$ was 0.11%, whereas this ranged from 0.89% to 0.95% in the empirical estimates. Although the average proportion of accepted points greater than $\Omega = 0.01$ and 0.05 reached 0.56% and 0.25% respectively, these were also much higher in the empirical estimates, which ranged from 0.91% to 1.00%.

TABLE 4. Bayesian factors comparing hypotheses of simultaneous and variable divergence histories under three different thresholds of Ω to delineate these two hypotheses. Data are from the eight sea urchin taxon pairs.

Threshold of Ω	Prior for ancestral θ_A		
	Uniform (0.01,10.0)	Uniform (0.01,20.0)	Uniform (0.01,40.0)
0.01	0.05	0.04	0.03
	0.11	0.07	0.13
0.05	0.21	0.18	0.18
	0.29	0.21	0.29
0.10	0.73	0.68	0.57
	0.60	0.74	0.81

To further determine confidence in our empirical estimates, we also simulated the data 1000 times by fixing both Ω and $E(\tau)$ to their average estimate (0.21 and 24.3, respectively). This allowed us to investigate the power in rejecting an equal τ history if the real history matches our empirical estimate of Ω. To this end, we estimated Ω from these 1000 datasets and recorded the proportion of estimates that were greater than our chosen thresholds of Ω (0.01 and 0.1). Although fixed values of Ω and $E(\tau)$ could be consistent with many different sets of eight values of τ and Ψ (the number of divergence times), we chose to simulate under the simplest history where $\Psi = 2$ and the divergence time vector $T_Y = \{8.0, 8.0, 29.4, 29.4, 29.4, 29.4, 29.4, 29.4\}$. The distribution of Ω estimates given data simulated under this variable τ history was markedly different than the distribution of Ω estimates given data simulated under the simultaneous τ history (Fig. 8). Although only 73.2% of the mode estimates of Ω were greater than 0.1, 99.7% of the estimates of Ω were greater than 0.01. If we were to use 0.01 as a criterion for rejecting a simultaneous τ history, than there would be a nearly perfect statistical power in rejecting this history given the simulated variable τ history ($\Omega = 0.21$; $E[\tau] = 24.3$).

DISCUSSION

It is encouraging that our ABC method can obtain fairly reasonable estimates of Ω and therefore be able to distinguish variable and simultaneous divergence histories given single-locus mtDNA data. In spite of using a complex and parameter-rich model that allows demography to vary across taxon pairs, and in spite of reducing the data to a mere four summary statistics per taxon pair, our simulation study demonstrates that our ABC estimates can distinguish simultaneous divergence from variable divergence histories with only 500,000
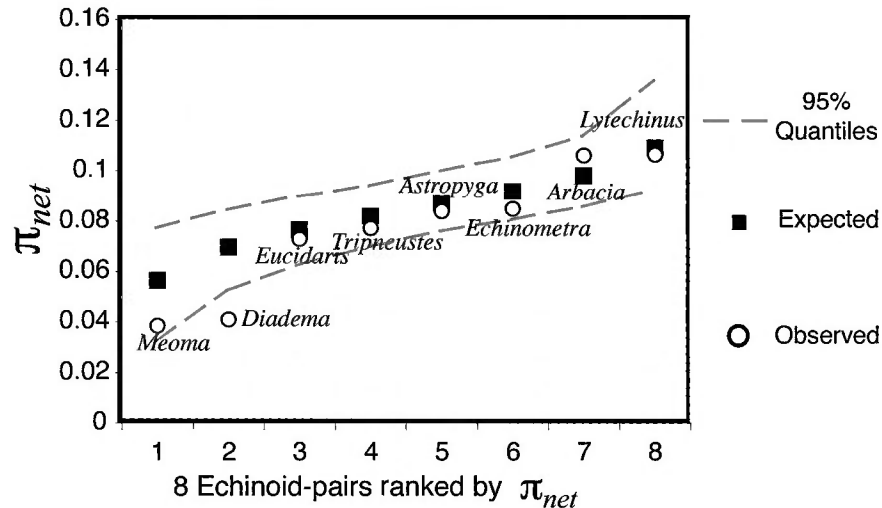
FIG. 9.   The eight ranked values of pairwise net nucleotide divergence (π_{net}) observed in eight echinoid sister taxon pairs thought to have arisen through allopatric isolation when the Isthmus of Panama emerged approximately 3.1 million years ago. The expected values are the mean values determined by simulating the data 10,000 times under a simultaneous vicariance history with E(τ) fixed at 24.1. The dashed lines delineates the upper and lower 95% credibility boundaries of these ranked π_{net} values. The data were simulated using the taxon pairings and sample sizes in Table 2A.

draws from the joint prior and only 1000 approximate draws from the joint posterior (tolerance = 0.002). However, the simulations also suggest that this is more difficult when the average divergence time is recent (Table 4). This is not unexpected given that ancestral coalescent variance has a proportionally larger affect on the total genetic divergence when divergence times are more recent (Edwards and Beerli 2000; Arbogast et al. 2002). Although the simulation testing we present might be beyond the scope of most empirical studies, such studies can and should be expected to conduct an overall test of estimator bias, as we report in Figure 4, especially given that doing so is computationally inexpensive.

Perhaps the demonstrated accuracy of our Ω estimator is counter-intuitive given that a simultaneous (Ψ, Ω) = (1, 0.0) history yielding data with high variance in π_{net} across taxon pairs can be accommodated by large θ_A values. Although these parameter sets of large θ_A values can be drawn from a uniform prior conditional on the hyperprior being Ψ = 1, a larger proportion of draws from the hyperprior conditional on Ψ = 2 will have sets of τ_1, τ_2, and θ_A values that accommodate this same observed high variance in π_{net}. In this simple case, the posterior would likely favor a Ψ = 2 history over a Ψ = 1 history and this would emerge from the corresponding Bayes factor. The degree to which this posterior supports a Ψ = 2 history given this observed high variance in π_{net} across taxon pairs depends on the upper bound for the uniform prior for θ_A. This is illustrated in the extreme case of this uniform for θ_A being (0.0, 0.001). Here, no portion of the posterior would correspond to Ψ = 1 because the lack of ancestral coalescence would force τ to be about equal to the TMRCA and therefore the high variability in π_{net} is only accommodated by histories where Ψ > 1.

The choice of the prior for θ_A should be informed by geology and ecology and from the intrapopulation genetic data. By examining the ranges in observed pairwise differences within populations, π_w, one can get an idea of a plausible

range in θ_A. For example, if the maximum value of π_w is 8.3, then it could be reasonable to use 10.0 as the upper bound for θ_A. If geological information suggests that an emerging barrier to gene flow bisected the range of all ancestral populations, then it would be reasonable to use 20.0 as this upper bound of this prior for θ_A instead of 10.0. However, as we demonstrate in this study, it is best to gauge robustness by using a range of priors for this parameter.

Although our ABC estimator performed well under a wide range of conditions, there are some parameter combinations and conditions (such as variable mutation rates) that will make such inferences inherently difficult without also collecting many unlinked nuclear loci (Felsenstein 2006; Hickerson et al. 2006). Investigating all possible conditions that could influence the estimator performance is beyond the scope of this study, yet there were some conditions that proved to hinder the estimator's performance. This included using other subsets of summary statistics within $\mathbf{D}$, other tolerances (0.1 and 0.0002), as well as other ordering schemes for the summary statistics within $\mathbf{D}$ (not shown). A fuller investigation of estimator bias and model sensitivity will be presented in a future study. In the empirical application of this ABC method, a hypothesis of simultaneous vicariance in the natural experiment of transisthmian echinoids is not well supported. How do we interpret this result in light of biology, population genetics, and geology? Our independent estimates of Ω were all well over 0.1 (Fig. 6), and the Ψ estimates were $\approx$2.0 (Fig. 7C), and therefore most consistent with a history of more than one divergence time (or mutation rate). Although the main isthmus closure is thought to have occurred 3.1 million years ago (Coates et al. 1992), subsequent admixture could have occurred in a subset of the genera if sea levels rose sufficiently to breach the isthmus dramatically approximately 2 million years ago (Cronin and Dowsett 1996).

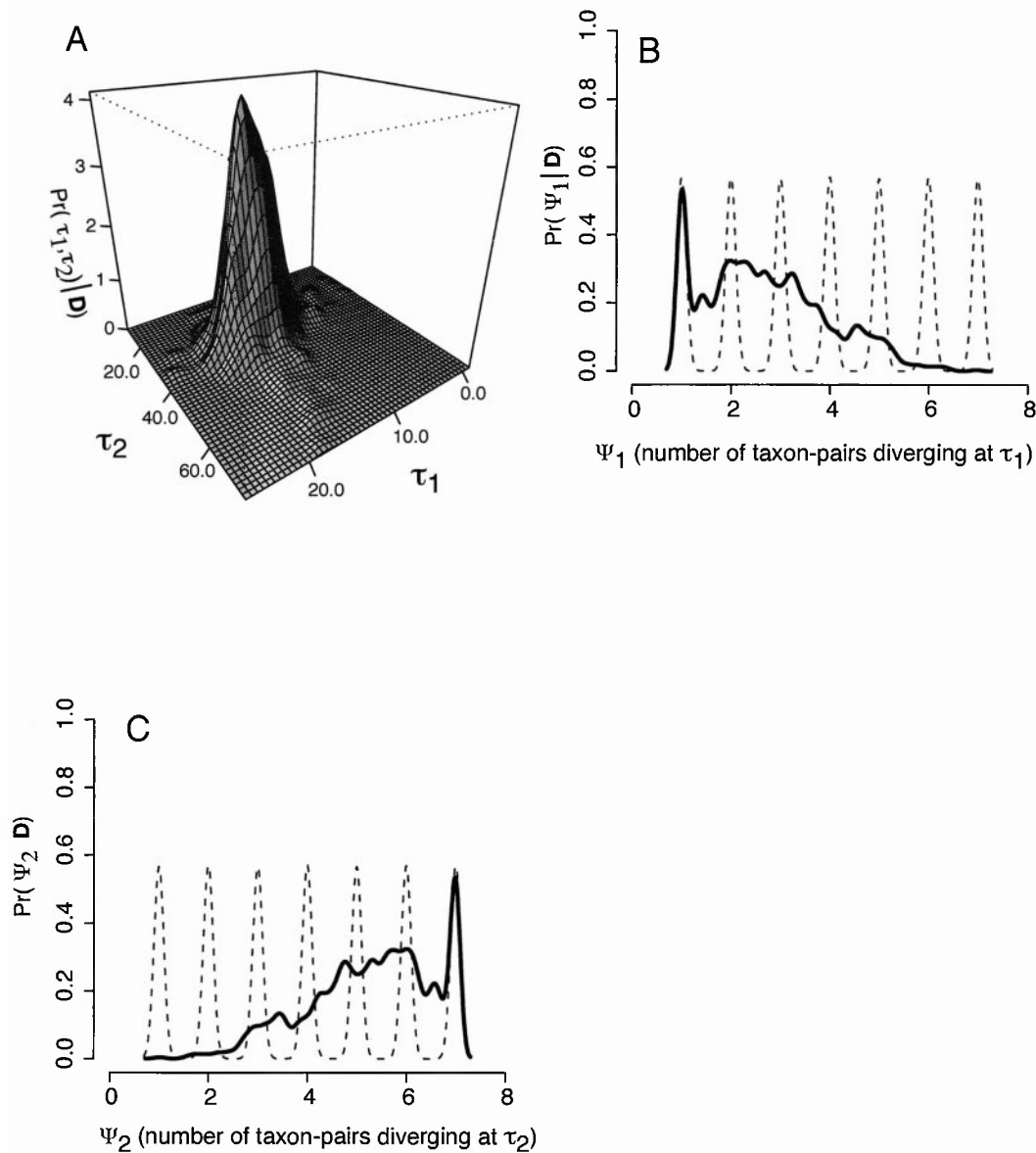To investigate the disparity in divergence times, we first

Y = 8 echinoid taxon pairs; Conditional on Ψ = 2

FIG. 10.   (A) Three-dimensional joint posterior probability density for τ_1 and $_2$ under the model where the number of divergence times (Ψ) is constrained to be 2. (B, C) The posterior probabilities of Ψ_1 and Ψ_2, the number of taxon pairs that diverged at times τ_1 and τ_2, respectively.

simulated the data under a simultaneous vicariance history constrained by the mean empirical estimate of $E(\tau)$ = 24.1, which was demonstrated to be accurately estimated under most divergence times (Table 2). When comparing the observed ranked values of π_{net} to the expected (mean) ranked values of π_{net} under the history of simultaneous divergence, one of the taxon pairs (*Diadema*) falls outside the 95% credibility intervals, whereas the other taxon pairs are within the values expected under simultaneous divergence (see Fig. 9). If we obtain ABC estimates on these seven genera (excluding *Diadema*), the posterior estimates of Ω and $E(\tau)$ become 0.0 and 31.42, respectively (Fig. 7B), and the posterior estimate

of becomes 1.12 (Fig. 7D). However, this approach is heuristic and somewhat biased from the estimate of $E(\tau)$ being conditional on the full model of unconstrained Ψ.

To investigate the disparity in divergence times more formally, we take advantage of the flexibility of ABC by simulating the hyperprior conditional on there being two divergence times across the eight taxon pairs (Ψ = 2; K = 500,000). In so doing, we obtained ABC estimates (tolerance = 0.002) on four additional hyperparameters: τ_1, the recent divergence time; τ_2, the more ancient divergence time; Ψ_1, the number of taxon pairs diverging at τ_1; and Ψ_2, the number of taxon pairs diverging at τ_2. These additional estimates

conditional on $\Psi = 2$ were highly consistent with *Diadema* diverging more recently (Fig. 10). The two divergence times τ_1 and τ_2 were 14.6 and 31.8, respectively, with the highest posterior support for only one urchin taxon pair diverging at τ_1 and the rest diverging at τ_2. This result is strongly consistent with a history of simultaneous divergence in seven of the eight taxon pairs (Figs. 7, 8). Given the older estimate for these seven taxon pairs, an assumed simultaneous rise of the land bridge 3.1 million years ago, and generation time of 2 years (Lessios 1979), the mtDNA COI substitution rate would be approximately 1.59% per million years (divergence rate of 3.17% per million years), an estimate consistent with other estimates of this commonly used locus (DeSalle et al. 1987; Hickerson et al. 2003).

Two different divergence times is a result congruent with previous conclusions from these echinoid data (Lessios et al. 2001). Lessios et al. speculated that the disparity in genetic divergences were the result of two separate isolation events. Isozyme data in *Diadema* also show an order of magnitude less divergence than the other genera (Lessios 1979), suggesting that total isolation in *Diadema* populations occurred later than in the other echinoid genera. This could possibly be due to high sea level stands that may have occurred 2 million years ago and thereby provided an opportunity for Atlantic–Pacific gene flow subsequent to the rise in the Isthmus of Panama 3.1 million years ago in this species (Cronin and Dowsett 1996). Although *Diadema* lies outside the 95% credibility intervals under the constrained history of $E(\tau) = 24.1$ and $\Psi = 1$ (Fig. 9), *Meoma* has nearly the same π_{net}. However, by only removing *Diadema* we yield an estimate of $\Omega = 0.0$ (Figs. 7C, D). Perhaps the small sample size of *Meoma* could explain how we cannot fully reject the hypothesis that *Meoma* was separated 3.1 million years ago.

Conclusion

The ABC method we introduce here provides a means for quantitative treatment of comparative phylogeographic datasets. The TSD procedure we demonstrate allows the statistical assessment of disparity in genetic divergences between members of species pairs that span a biogeographic barrier in common and thereby brings the study of comparative phylogeography further into the realm of statistical phylogeography (Bermingham and Moritz 1998; Knowles and Maddison 2002). Most important, the simple and flexible ABC framework that we present here demonstrates that complex comparative phylogeographic hypotheses can be robustly tested by estimating the hyper parameters that characterize the degree of uniformity among taxa. A second important feature that distinguishes this approach is that we can verify these estimates with simulations under many different conditions without excessive additional computational cost (Excoffier et al. 2005). Although we show that there is some uncertainty and bias with respect to testing for simultaneous vicariance, collecting more data from nuclear loci should increase statistical power in making such inferences (Felsenstein 2006; Hickerson et al. 2006) as well as better inform researchers on the genomic nature of divergence and reproductive isolation (Fitzpatrick 2002). Further development of this flexible simulation-based method will allow incorpora-

tion of more parameters (i.e., migration and recombination) and thereby allow testing of even more complicated comparative phylogeographic hypotheses that are implicitly represented by specific prior distributions and/or models. Ultimately, comparative phylogeographic data will be integrated within a framework that can incorporate various informational sources (Arbogast and Kenagy 2001; Hugall et al. 2002), and the hierarchical ABC framework we demonstrate here is well suited for this endeavor (Beaumont and Rannala 2004).

LITERATURE CITED

Arbogast, B. S., and G. J. Kenagy. 2001. Comparative phylogeography as an integrative approach to historical biogeography. J. Biogeogr. 28:819–825.

Arbogast, B. S., S. V. Edwards, J. Wakeley, P. Beerli, and J. B. Slowinski. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annu. Rev. Ecol. and Sys. 33:707–740.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge, MA.

Avise, J. C., D. Walker, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. R. Soc. Lond. B 265:1707–1712.

Beaumont, B. A. 2004. Recent developments in genetic data analysis: What can they tell us about human demographic history? Heredity 92:365–379.

Beaumont, B. A., and B. Rannala. 2004. The Bayesian revolution in genetics. Nat. Rev. Genet. 5:251–261.

Beaumont, M. A., W. Zhang, and D. J. Balding. 2002. Approximate Bayesian computation in population genetics. Genetics 162: 2025–2035.

Bermingham, E., and C. Moritz. 1998. Comparative phylogeography: concepts and applications. Mol. Ecol. 7:367–369.

Bermingham, E., S. S. McCafferty, and A. P. Martin. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. Pp. 113–128 *in* T. D. Kocher and C. A. Stepien, eds. Molecular systematics of fishes. Academic Press, San Diego.

Carstens, B. C., S. J. Brunsfeld, J. R. Demboski, J. D. Good, and J. Sullivan. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: hypothesis testing within a comparative phylogeographic framework. Evolution 59: 1639–1652.

Chesher, P. H. 1972. The status of knowledge of Panamainian echinoids. Bull. Biol. Soc. Wash 2:139–158.

Coates, A. G., J. B. C. Jackson, L. S. Collins, T. M. Cronin, H. J. Dowsett, L. M. Bybell, P. Jung, and J. A. Obando. 1992. Closure of the Isthmus of Panama: the near-shore marine record of Costa Rica and western Panama. Geol. Soc. Am. Bull. 104:814–828.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer Associates, Sunderland, MA.

Cronin, T. M., and T. M. Dowsett. 1996. Biotic and oceanographic

response to the Pliocene closing of the central American Isthmus. Pp. 76–104 *in* J. B. C. Jackson, A. F. Budd, and A. G. Coates, eds. Evolution and environment in tropical America. Univ. of Chicago Press, Chicago.

Cunningham, C. W., and T. M. Collins. 1998. Beyond area relationships: extinction and recolonization in molecular marine biogeography. Pp. 297–322 *in* B. Schierwater, B. Streit, G. Wagner, and R. DeSalle, eds. Molecular ecology and evolution: approaches and applications. Birkhauser Verlag, Basel, Switzerland.

Cunningham, C. W., H. Zhu, and D. M. Hillis. 1998. Best-fit maximum likelihood models for phylogenetic inference: empirical tests with known phylogenies. Evolution 52:978–987.

DeSalle, R., T. Freedman, E. M. Prager, and A. C. Wilson. 1987. Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. J. Mol. Evol. 26:157–164.

Edwards, S. V., and P. Beerli. 2000. Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54:1839–1854.

Estoup, A., B. A. Beaumont, F. Sennedot, C. Moritz, and J.-M. Cornuet. 2004. Genetic analysis of complex demographic scenarios: spatially expanding populations of the cane toad, *Bufo marinus*. Evolution 58:2021–2036.

Excoffier, L., A. Estoup, and J.-M. Cornuet. 2005. Bayesian analysis of an admixture model with mutations and arbitrarily linked markers. Genetics 169:1727–1738.

Felsenstein, J. 2006. Accuracy of coalescent likelihood estimates: Do we need more sites, more sequences, or more loci? Mol. Biol. Evol. 23:691–700.

Fitzpatrick, B. M. 2002. Molecular correlates of reproductive isolation. Evolution 56:191–198.

Fu, Y.-X., and W.-H. Li. 1997. Estimating the common ancestor of a sample of DNA sequences. Mol. Biol. Evol. 14:195–199.

Gavrilets, S. 2004. Models of speciation: What have we learned in 40 years? Evolution 57:2197–2215.

Gelman, A., J. B. Carlin, H. S. Stern, and D. B. Rubin. 1995. Bayesian data analysis. Chapman and Hall/CRC, London.

———. 2004. Bayesian data analysis. 2nd ed. Chapman and Hall/CRC, Boca Raton, FL.

Gillespie, J. H. 1984. Molecular evolution over the mutational landscape. Evolution 38:1116–1129.

Graham, C. H., S. R. Ron, J. C. Santos, C. J. Schneider, and C. Moritz. 2004. Integrating phylogeographic and environmental niche models to explore speciation mechanisms in dendrobatid frogs. Evolution 58:1781–1793.

Hasegawa, M., H. Kishino, and T. A. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.

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., M. A. Gilchrist, and N. Takebayashi. 2003. Calibrating a molecular clock from phylogeographic data: moments and likelihood estimators. Evolution 57:2216–2225.

Hickerson, M. J., G. Dolman, and C. Moritz. 2006. Comparative phylogeographic summary statistics for testing simultaneous vicariance across taxon-pairs. Mol. Ecol. 15:209–224.

Hubbell, S. P. 2001. The unified neutral theory of biodiversity and biogeography. Princeton Univ. Press, Princeton, NJ.

Hudson, R. R. 2002. ms: a program for generating samples under neutral models. Bioinformatics 18:337–338.

Hugall, A., C. Moritz, A. Moussalli, and J. Stanisic. 2002. Reconciling paleodistribution models and comparative phylogeography in the wet tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). Proc. Natl. Acad. Sci. USA 99: 6112–6117.

James, W., and C. Stein. 1960. Estimation with quadradic loss. Proceedings of the fourth Berkeley symposium on mathematical statistics and probability, Berkeley, CA.

Jeffreys, H. 1961. Theory of probability. Clarendon Press, Oxford, U.K.

Jordan, D. S. 1908. The law of geminate species. Am. Nat. 42: 73–80.

Kass, R. E., and A. Raftery. 1995. Bayesian factors. J. Am. Stat. Assoc. 90:773–795.

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

Knowlton, N., and L. A. Weigt. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc. R. Soc. Lond. B 265:2257–2263.

Lessios, H. A. 1979. Use of Panamanian sea-urchins to test the molecular clock. Nature 280:599–601.

Lessios, H. A., B. D. Kessing, D. R. Robertson, and G. Paulay. 1999. Phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents. Evolution 53: 806–817.

Lessios, H. A., B. D. Kessing, and J. S. Pearse. 2001. Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. Evolution 55:955–975.

Lessios, H. A., J. Kane, and D. R. Robertson. 2003. Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. Evolution 57: 2026–2036.

Loader, C. 1996. Local likelihood density estimation. Ann. Stat. 24:1602–1618.

Marko, P. B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. Mol. Biol. Evol. 19:2005–2021.

Mayr, E. 1954. Geographic speciation in tropical echinoids. Evolution 8:1–18.

McCartney, M. A., G. Keller, and H. A. Lessios. 2000. Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. Mol. Ecol. 9: 1391–1400.

Moritz, C., J. L. Patton, C. J. Schneider, and T. B. Smith. 2000. Diversification of rainforest faunas: an integrated molecular approach. Annu. Rev. Ecol. Syst. 31:533–563.

Nei, M., and W. Li. 1979. Mathematical model for studying variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

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

Plagnol, V., and S. Tavare. 2002. Approximate Bayesian computation and MCMC. Pp. 99–114 *in* H. Neiderreiter, ed. Monte Carlo and quasi-Monte Carlo methods. Springer Verlag, Berlin.

Pritchard, J. K., M. T. Seielstad, A. Perez-Lezaun, and M. W. Feldman. 1999. Population growth of human Y chromosomes: a study of Y chromosome microsatellites. Mole. Biol. Evol. 16: 1791–1798.

R Development Core Team. 2004. R: a language and environment for statistical computing. R Development Core Team, Vienna. Available via http://www.R-project.org

Ricklefs, R. E. 2003. Genetics, evolution, and ecological communities. Ecology 84:185–192.

Schneider, C. J., M. Cunningham, and C. Moritz. 1998. Comparative phylogeography and the history of endemic vertebrates in the wet tropics rainforests of Australia. Mol. Ecol. 7:487–498.

Tajima, F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105:437–460.

———. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Takahata, N. 1986. An attempt to estimate the effective size of the ancestral species common to two extant species from which homologous genes are sequenced. Genet. Res. 48:187–190.

Takahata, N., and M. Nei. 1985. Gene genealogy and variance of intrapopulational nucleotide differences. Genetics 110:325–344.

Tallmon, D. A., G. Luikart, and B. A. Beaumont. 2004. Comparative evaluation of a new effective population size estimator based on approximate Bayesian computation. Genetics 167:977–988.

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

Wakeley, J., and J. Hey. 1997. Estimating ancestral population pa-
    rameters. Genetics 145:847–855.
Watterson, G. A. 1975. On the number of segregating sites in ge-
    netic models without recombination. Theor. Popul. Biol. 7:
    256–276.
Weiss, G., and A. von Haeseler. 1998. Inference of population
    history using a likelihood approach. Genetics 149:1539–1546.

Zigler, K. S., and H. A. Lessios. 2004. Speciation on the coasts of
    the New World: phylogeography, reproductive isolation, and the
    evolution of bindin in the sea urchin genus *Lytechinus*. Evolution
    58:1225–1241.

Corresponding Editor: K. Crandall