ORIGINAL ARTICLE

Coalescent analyses support multiple mainland-to-island dispersals in the evolution of Malagasy *Triaenops* bats (Chiroptera: Hipposideridae)

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ABSTRACT

Aim We investigate the directionality of mainland-to-island dispersals, focusing on a case study of an African-Malagasy bat genus, *Triaenops* (Hipposideridae). Taxa include *T. persicus* from east Africa and three *Triaenops* species from Madagascar (*T. auritus, T. furculus,* and *T. rufus*). The evolution of this bat family considerably post-dated the tectonic division of Madagascar from Africa, excluding vicariance as a viable hypothesis. Therefore, we consider three biogeographical scenarios to explain these species' current ranges: (A) a single dispersal from Africa to Madagascar with subsequent speciation of the Malagasy species; (B) multiple, unidirectional dispersals from Africa to Madagascar resulting in multiple, independent Malagasy lineages; or (C) early dispersal of a proto-species from Africa to Madagascar, with later back-dispersal of a descendant Malagasy taxon to Africa.

Location East Africa, Madagascar, and the Mozambique Channel.

Methods We compare the utility of phylogenetic and coalescent methodologies to address the question of directionality in a mainland-to-island dispersal event for recently diverged taxa. We also emphasize the application of biologically explicit demographic systems, such as the non-equilibrium isolation-with-migration model. Here, these methods are applied to a four-species haploid genetic data set, with simulation analyses being applied to validate this approach.

Results Coalescent simulations favour scenario B: multiple, unidirectional dispersals from Africa to Madagascar resulting in multiple, independent Malagasy bat lineages. From coalescent dating, we estimate that the genus *Triaenops* was still a single taxon approximately 2.25 Ma. The most recent Africa to Madagascar dispersal occurred much more recently (*c.* 660 ka), and led to the formation of the extant Malagasy species, *T. rufus*.

Main conclusions Haploid genetic data from four species of *Triaenops* are statistically most consistent with multiple, unidirectional dispersals from mainland Africa to Madagascar during the late Pleistocene.

Keywords

Chiroptera, coalescent simulations, eastern Africa, island biogeography, isolation-with-migration model, Madagascar, *Triaenops*.

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INTRODUCTION

Biogeographical questions are increasingly amenable to molecular data and associated methods for demographic inference. Traditionally, genetic analyses have relied heavily on phylogenetic approaches. Dated phylogenies can provide working hypotheses for organismal relationships, including the ability to place them within a spatial and temporal framework. This method also has relevance to biogeography; for instance, comparisons of species cladograms and geographical distributions often allow competing dispersal and vicariance hypotheses to be distinguished (Donoghue & Moore, 2003; Near &

Keck, 2005). However, phylogenetic approaches are not always successful, because phylogenies are frequently consistent with more than one biogeographical hypothesis. Furthermore, phylogenetic methods lack power when applied to incipient species or recently diverged taxa. Such cases may be better suited to a coalescent framework, in which the evolutionary vagaries of individual genetic lineages can be traced. Phylogenetic and coalescent approaches have similar theoretical foundations, and they produce identical expectations for some evolutionary parameters (for instance, estimates of autosomal divergence dates) at time intervals greater than $2N_e$ generations, a considerable time depth for taxa with large effective population sizes (N_e). However, phylogenetic approaches cannot address other evolutionary parameters, such as the proportion of an ancestral taxon that diverged into each of two daughter taxa. In the context of comparatively recent timeframes, coalescent methods can provide a complementary approach to more traditional phylogenetic methods, and combined phylogenetic/coalescent analyses allow us to examine a continuum of evolutionary history from ancient divergence events to modern population dynamics.

The directionality of mainland/island dispersals is one biogeographical question that has traditionally been approached from a phylogenetic perspective. Although the most parsimonious explanation is usually dispersal from mainland to island (Yoder *et al.*, 1996, 2003), studies in Madagascar and elsewhere have supported multiple dispersals in both directions (Raxworthy *et al.*, 2002; Dávalos, 2007). When phylogenies contain polytomies or weakly supported bifurcating divisions, the directionality of dispersal often cannot be established from phylogenies alone. Furthermore, multiple biogeographical hypotheses might be consistent with the same phylogenetic topology. Russell *et al.* (2007) encountered this problem in their study of *Triaenops* (Chiroptera: Hipposideridae), a genus of Old World bats (Fig. 1). *Triaenops* contains four extant species, of which three (*T. auritus*,

T. furculus, and *T. rufus*) are distributed across the drier portions of Madagascar, and a fourth (*T. persicus*) ranges over eastern Africa, the Middle East, and south-western Asia (Eger & Mitchell, 2003; Ranivo & Goodman, 2006). The differentiation of the family Hipposideridae is estimated to have taken place 43–37 Ma (Teeling *et al.*, 2005), or *c.* 100 Myr after the separation of Madagascar from the African mainland (165–140 Ma; Storey *et al.*, 1995). Hence, the speciation and dispersal history of the genus *Triaenops* considerably postdates these plate tectonic movements, and vicariance can be excluded as a biogeographical hypothesis for this genus. Consequently, Russell *et al.* (2007) posed three dispersal scenarios to explain the distributions of these species: (A) a single dispersal event from Africa to Madagascar with subsequent speciation of the three Malagasy taxa; (B) two unidirectional dispersals from Africa to Madagascar creating two independent Malagasy lineages; and (C) an early dispersal of the proto-*Triaenops* ancestral species from Africa to Madagascar and a subsequent back-dispersal to Africa of the *T. persicus* ancestor. Only the first of these scenarios is expected to produce a unique phylogenetic signal (Fig. 1a–c).

Phylogenetic analyses (Fig. 1d) rejected the single dispersal hypothesis (scenario A), but could not discriminate between the two alternative models (scenarios B and C) (Yoder *et al.*, 2005; Russell *et al.*, 2007). Employing population genetic analyses, Russell *et al.* (2007) considered factors such as population growth and demic structure: growth is expected to be large, and structure minimal, in recently translocated taxa. The results tentatively supported multiple unidirectional dispersals from Africa to Madagascar (scenario B), but imperfect spatial sampling of the African taxon, *T. persicus*, considerably limited confidence in this conclusion.

Here, we approach the same question from a different perspective. We apply Monte Carlo coalescent simulations to determine the statistical likelihood that each of the three dispersal hypotheses could produce the observed mitochondrial
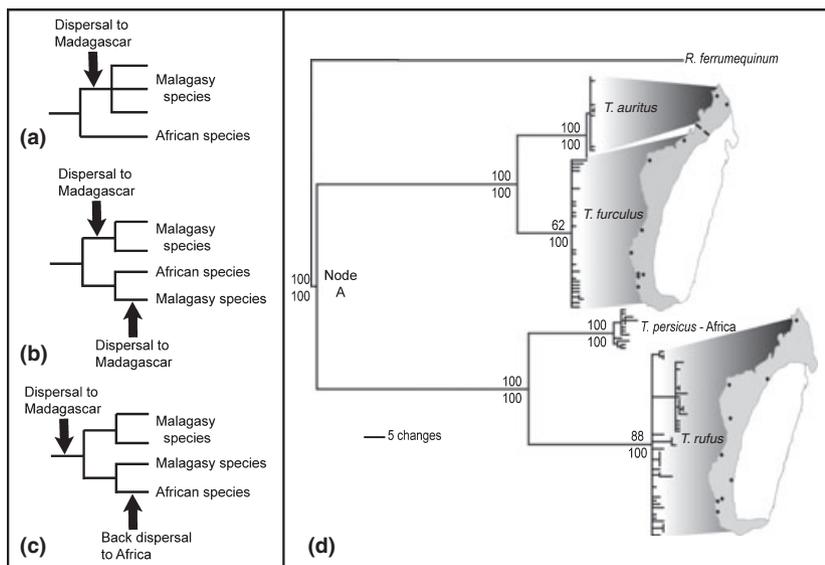

Figure 1 Biogeographical hypotheses and the observed phylogeny for *Triaenops* species: (a) single, unidirectional dispersal; all Malagasy species descend from a common eastward dispersal from Africa; (b) multiple, unidirectional dispersal; Malagasy species descend from two independent eastward dispersals from Africa; (c) multiple, bidirectional dispersal; an early eastward dispersal to Madagascar, followed by a later back-dispersal to Africa. (d) Observed phylogeny from mitochondrial *cytb* sequences (Russell *et al.*, 2007). Bayesian posterior probability support measures below branches; maximum parsimony bootstrap measures above branches.

DNA tree. We adopt Nielsen & Wakeley's (2001) non-equilibrium isolation-with-migration model, which mimics biological communities with more realism than many earlier equilibrium systems, such as island or divergence models. This approach has been applied with some success to recently diverged species (Hey & Nielsen, 2004; Won & Hey, 2005; Nikula *et al.*, 2007), including reciprocally monophyletic taxa (Linnen & Farrell, 2007). We validate the applicability of this isolation-with-migration model to the demographic system studied here using real and simulated genetic data.

The isolation-with-migration model is characterized by seven demographic parameters: effective population size of the ancestral deme (N_A); effective population sizes of the two descendent demes (N_1 and N_2); unidirectional migration between the descendent populations (M_1 and M_2); proportion of the ancestral population founding the first deme (S); and population divergence time (T; Fig. 2). Although the available software infers all seven parameters by default, only the split proportion (S), and more secondarily the divergence time (T) are directly relevant to this study. We assume that a successful cross-strait dispersal, defined in our context as crossing the Mozambique Channel and subsequently establishing a viable population on a new landmass, should be a rare event involving relatively few individuals. Under a model of unidirectional dispersal (scenario B), much of the ancestral population diversity should be retained by the mainland African species, *T. persicus* ($S \to 1$). Under a model of bidirectional dispersal (scenario C), few individuals from a putative ancestral Malagasy range would have crossed the channel to

found African *T. persicus* ($S \to 0$). Less strongly bottlenecked speciation events would produce intermediate signals. This rationale presents a straightforward expectation for S, and this can be tested statistically by coalescent simulations conditioned on the observed genetic data.

METHODS

Sequence data

Our data set comprises mitochondrial DNA (mtDNA) sequences (GenBank accession numbers: DQ005718–DQ005850) from 133 individuals representing four species of hipposiderid bats: *Triaenops rufus* ($n = 54$), *T. persicus* ($n = 12$), *T. auritus* ($n = 24$) and *T. furculus* ($n = 43$) (Russell *et al.*, 2007). The data presented here include only a single population of *T. persicus* from Tanzania, but the remaining taxa were sampled from multiple geographical locations. The 732-bp sequences incorporate most of the cytochrome *b* (*cytb*) gene. Sites characterized by more than two character states, and terminal sites missing from one or more sequences, were excluded from the analysis. Although mitochondrial genomes are not subject to recombination, homoplasy produces signals similar to recombination, thereby preventing the application of infinite-sites modelling. Giving equal weight to sites and haplotypes, we extracted the largest DNA block containing no four-gamete violations from paired-species data sets (Woerner *et al.*, 2007). The *T. persicus*/*T. rufus* data set contained 540 bp (5 sequences from *T. persicus* and 45 sequences from *T. rufus*); the *T. auritus*/*T. rufus* data set contained 652 bp (24 sequences from *T. auritus* and 35 sequences from *T. rufus*); and the *T. furculus*/*T. rufus* data set contained 612 bp (37 sequences from *T. furculus* and 36 sequences from *T. rufus*). We assumed a mutation rate of 4×10^{-8} substitutions/site/year (Hulva *et al.*, 2004), and an average inter-generation interval of 5 years (Russell *et al.*, 2007).

Demographic inference with IM

Seven demographic parameters were inferred under a dual-population isolation-with-migration model: effective size of the ancestral deme (N_A); effective sizes of the two descendent demes (N_1 and N_2); unidirectional migration rates between descendent demes (M_1 and M_2); proportion of the ancestral deme founding the first descendent deme (S), and deme divergence time (T; Fig. 2). All parameters, except S, were inferred as mutation-scaled rates: $\theta_1 = N_1\mu$, $\theta_2 = N_2\mu$, $\theta_A = N_A\mu$, $m_1 = M_1/\mu$, $m_2 = M_2/\mu$ and $t = T\mu$. We emphasize that inference of the splitting parameter, S, is not affected by uncertainty in the mutation rate. Populations were analysed in pairwise combinations using the Markov chain Monte Carlo (MCMC) composite Bayesian/likelihood framework implemented in IM (31 July 2006 version; Hey, 2006). More detailed descriptions, and validation, of this method are presented elsewhere (Nielsen & Wakeley, 2001; Hey & Nielsen, 2004; Hey *et al.*, 2004; Hey, 2005; Won & Hey, 2005).
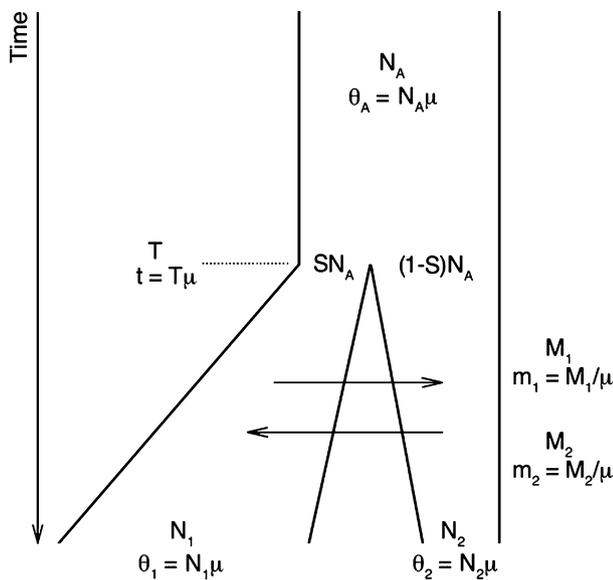

Figure 2 Diagram of the isolation-with-migration model. This model includes seven demographic parameters, namely the effective population size of the ancestral population (N_A), effective population sizes of the two daughter populations (N_1 and N_2), unidirectional migration between the two daughter populations (M_1 and M_2), population divergence time (T), and proportion of the ancestral population founding the first daughter population (S).

We used IM to determine (1) the proportion of the *T. persicus*/*T. rufus* ancestor that founded *T. persicus*, and (2) the divergence time between *T. persicus* and *T. rufus*. Analyses were run on a dispersed-computing grid at the University of Arizona. Data sets were initially parameterized from a single run with bounded uniform priors: $\theta_1, \theta_2, \theta_A \in U(0, 20)$, m_1, $m_2 \in U[0, 10]$, $S \in U(0, 1)$ and $t \in U[0, 20]$. These ranges were raised in subsequent runs to incorporate full marginal posterior probability densities. (We note that IM produces *marginal*, not *joint*, posteriors. Uncertain inference for one parameter is no reflection of uncertain inference for another.) Once bounds accommodating complete posteriors were established, a minimum of six replicate jobs each of five chains were run for a minimum of 10^7 steps. Chain mixing by Metropolis-Hasting coupling, long run times, and multiple independent runs allowed us to identify convergence on the underlying stationary distribution of each parameter.

Additional species pairwise analyses were run for (1) *T. rufus* and *T. furculus*, and (2) *T. rufus* and *T. auritus*, to estimate the time of the deepest divergence in the *Triaenops* tree (Fig. 1d, Node A). Initial prior bounds were applied as above, except $t \in U[0, 864]$, the upper bound being the maximum value estimated for the divergence time between bat families Rhinolophidae and Hipposideridae (equivalent to *c.* 40 Myr; Teeling *et al.*, 2005).

Coalescent validation of IM

Genetic data sets were simulated using a standard coalescent methodology (Hudson, 2002) for a generalized isolation-with-migration model that closely mimics the demography inferred for the *T. rufus*/*T. persicus* species pair (see Results). Coalescent data sets were analysed with the software IM, and marginal posterior distributions for the split parameter, *S*, were examined and compared with model expectations (i.e. $S \rightarrow 0$ or $S \rightarrow 1$).

RESULTS

Evaluation of dispersal hypotheses

We aim to distinguish between two dispersal models for Malagasy bats: multiple unidirectional dispersals from Africa to Madagascar; or a single dispersal from Africa to Madagascar, followed by a subsequent dispersal from Madagascar back to Africa. To this end, we analysed a mtDNA data set comprising four *Triaenops* species under an isolation-with-migration model. As a simple test of this approach, we examined the migration rates between *Triaenops* species inferred across six runs representing 3×10^8 steps of an MCMC state space search over 30 independent chains. Maximum likelihood estimates for migration were consistently zero (see Table 1), as expected for genetic loci showing reciprocal monophyly (see Fig. 1). We note that this lack of migration prevents non-sampled taxa from being possible confounds on subsequent inferences.

Table 1 Demographic parameter estimates for the *Triaenops rufus*/*T. persicus* species pair as inferred by IM. Divergence times in thousands of years; unidirectional migration rates per generation. 95% confidence intervals are listed in parentheses.

Parameter	Maximum likelihood estimate	95% confidence interval
$\theta_{persicus}$	9.50	(3.78, 45.4)
N_e (*T. persicus*)	22,000	(8740, 105,000)
θ_{rufus}	52.4	(30.3, 123)
N_e (*T. rufus*)	121,000	(70,200, 286,000)
t_1	3.20	(1.76, 18.9)
Divergence time	148	(81.6, 874)
t_2	14.2	(1.76, 18.9)
Divergence time	659	(81.6, 874)
S	0.9995	(0.0358, 0.999)
$m_{rufus \rightarrow persicus}$	0	(0, 1.37)
Migration rate (*rufus* $\rightarrow$ *persicus*)	0	(0, 1.48×10^{-4})
$m_{persicus \rightarrow rufus}$	0	(0, 0.187)
Migration rate (*persicus* $\rightarrow$ *rufus*)	0	(0, 2.02×10^{-5})

We also considered the effective sizes of the groups from which our samples were drawn. Maximum likelihood effective population sizes ranged from *c.* 4500 to *c.* 121,000: *c.* 121,000 for *T. rufus*; *c.* 22,000 for *T. persicus*; *c.* 14,700 for *T. furculus*; and *c.* 4500 for *T. auritus*. The true effective size for *T. persicus* is probably much larger, given the wider distribution of this species and the fact that our sample includes only a single population. The compatibility of estimated parameters, such as migration and effective population sizes, with estimates from field-based research (Olsson *et al.*, 2006; Steven M. Goodman, 2007, personal observation) lends confidence to this coalescent approach. (In addition, see validation by simulations in later sections.) Therefore, we considered estimates of the ancestral population splitting parameter, *S*, for the *T. rufus*/*T. persicus* species pair.

Our results support a demographic model with a maximum likelihood estimate for the split proportion approaching 1 [$S = 0.9995$; 95% confidence interval (0.0358, 0.9987); Fig. 3].
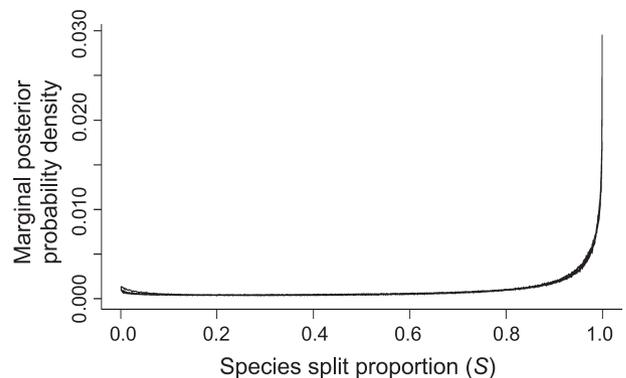

Figure 3 Marginal Bayesian posterior probability densities for the splitting parameter, *S*, between *Triaenops rufus* and *T. persicus* (replicate runs).

Therefore, we infer that *c.* 99.95% of the *T. rufus/T. persicus* ancestral population developed into the *T. persicus* daughter species; that is, most of the ancestral *T. rufus/T. persicus* diversity is retained by *T. persicus*. These estimates are especially robust because we sampled *T. rufus* individuals from many locations across the range of this species, but our *T. persicus* specimens come from only a single population. A priori, we would expect a bias towards observing a higher retention of ancestral diversity in *T. rufus*, but our estimates of *S* fall in the opposite direction. This leads us to support scenario B: two unidirectional dispersals from Africa to Madagascar resulting in the independent evolution of two clades of Malagasy *Triaenops*.

Validating the inference of splitting parameters

To validate our methodology, we tested the power of IM to infer demographic parameters for biological systems that mimic that observed here for *Triaenops*. We focus on the split parameter, *S*, and an evolutionary topology that closely resembles that of the *T. rufus/T. persicus* species pair, both of which are central to our primary argument.

Briefly, we modelled a haploid locus (i.e. mtDNA) of 500 bp with a mutation rate, μ, of 4×10^{-8} bp^{-1} yr^{-1} and a generation interval of 5 years. An ancestral deme with effective size $N_A = 5 \times 10^4$ was split into two demes 5×10^5 yr BP (1×10^5 generations ago). To mimic the evolutionary processes involved with incipient species, migration rates were set to zero following the population split. The first deme represents an effective continuance of the ancestral population and retains much of the ancestral diversity ($S = 0.9995$), whereas

the second deme represents a new population founded with only 25 individuals ($S = 0.0005$). This parameter value is the same as that inferred for the *T. rufus/T. persicus* demography. Both populations were allowed to grow exponentially to their modern effective sizes, $N_1 = N_2 = 5 \times 10^4$, and individuals ($n = 25$) were sampled from each species during the current generation. In accordance with the observed *T. rufus/ T. persicus* data, all resulting data sets were reciprocally monophyletic.

Coalescent data sets ($n = 20$) were generated under this demographic model and analysed with the software IM. We note that IM is extremely intensive computationally: each data set required a runtime well in excess of 24 h. Traditional power analyses (e.g. with the number of data sets in the order of 10^4–10^6) are not therefore feasible. However, the statistical approach applied here has been used previously to validate other parameters inferred by the IM software (e.g. Hey & Nielsen, 2004). The marginal posterior distribution generated by IM for the split parameter, *S*, was examined for all 20 coalescent data sets, and our simulation model dictates that this value should be large (i.e. where *S* is *c.* 0.9995).

Thirteen data sets (65%) contained enough evolutionary information to produce informative posterior distributions. L-shaped posteriors (45% of data sets; Fig. 4a) or S-shaped posteriors (20% of data sets; Fig. 4b), which favour a specific value of *S* over all other values, characterize each one of these cases. In all 13 instances, the maximum likelihood estimate of *S* was correctly inferred as exactly $S = 0.9995$. Confidence intervals were routinely of the same order as those calculated for our empirical data. Because we found no informative posteriors for $S \rightarrow 0$ (indeed, *S* was always 0.9995), our error
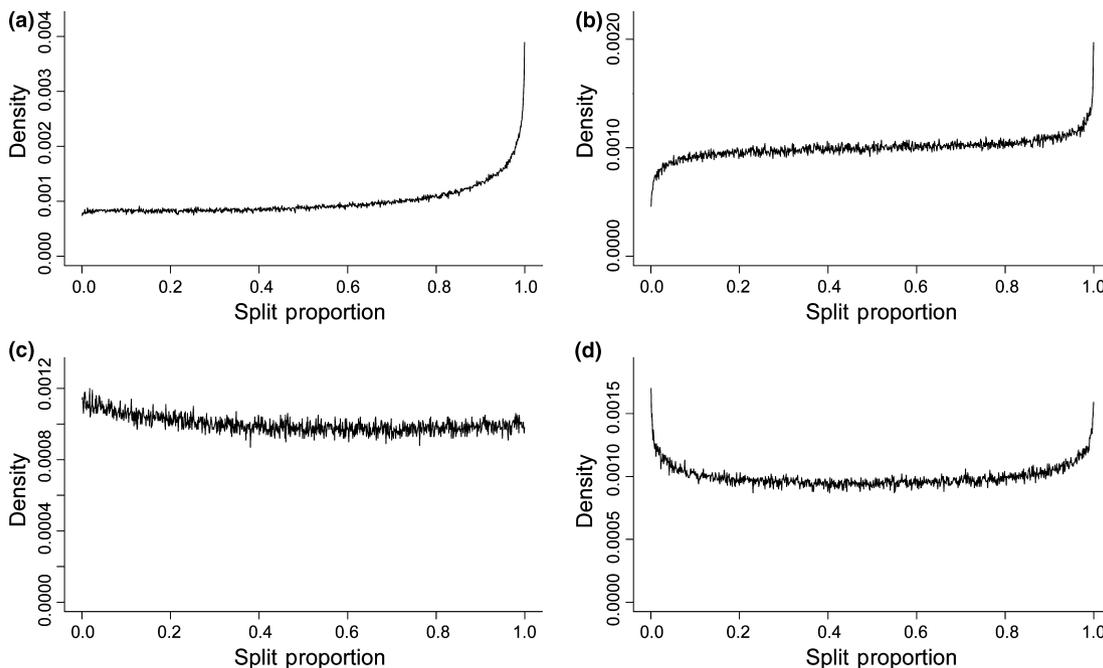

Figure 4 Representative examples of informative and uninformative marginal posterior distributions for the split parameter, *S*, as generated by IM. Informative posterior distributions are either (a) L-shaped or (b) S-shaped, whereas uninformative posterior distributions are either (c) I-shaped or (d) U-shaped.
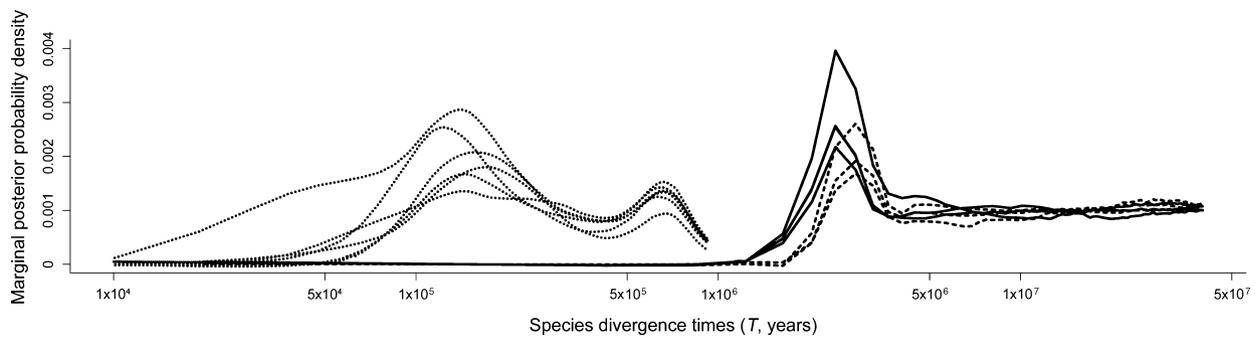
Figure 5 Local regression of marginal Bayesian posterior probability densities for the demic divergence time, *T*, between *Triaenops rufus* and *T. persicus* (dotted), *T. rufus* and *T. furculus* (solid), and *T. rufus* and *T. auritus* (dashed).

rate is estimated to be less than 1/13 (i.e. a false positive rate, α, < 0.077). Finally, we note that all IM runs on our empirical *T. rufus*/*T. persicus* data set produced informative L-shaped posteriors for the split parameter, *S* (Fig. 3); that is, they were identical to the informative L-shaped posterior shown here in Fig. 4a.

Seven data sets (35%) did not contain enough information to produce informative posterior distributions. These were characterized by I-shaped posteriors (no information for any value of *S*; 10% of data sets; Fig. 4c) or U-shaped posteriors (inability to distinguish $S = 0$ or $S = 1$; 25% of data sets; Fig. 4d). Note that not all data sets are necessarily informative for the splitting parameter, *S*, but that uninformative distributions are readily apparent as such. Together with the low false error rate for informative estimates of *S*, this finding suggests that the split parameter inferred for our observed data genuinely reflects the taxon splitting proportion of the *T. rufus*/*T. persicus* species pair. We further note that this validation study shows that the split parameter can be inferred robustly with the data available, namely *c.* 500 bp of sequence from a single haploid locus. Although we strongly advocate the use of multiple loci (Garrigan *et al.*, 2007), such data prove unnecessary to address the primary question considered here.

Timing of dispersal events

A natural following question is: when did these dispersal events occur? There is a paucity of published dates for the divergence of bat taxa below the family level, and most are directly reliant on a sparse fossil record (Teeling *et al.*, 2005). However, the isolation-with-migration approach applied here is, *sensu lato*, independent of direct palaeontological dating (the exception being the derivation of mutation rate estimates). We began by inferring the time of the deepest division in the *Triaenops* tree (Fig. 1), as defined by the *T. rufus*/*T. furculus* and *T. rufus*/*T. auritus* comparisons (Fig. 5). Maximum likelihood values are consistently estimated at 2.25 Ma [95% confidence intervals (1.7 Ma, 3.5 Ma)] for the date at which *Triaenops* still formed a single ancestral population. We note that uncertainty in the mutation rate will influence the exact dates presented

here, but that the proportional chronology of divergence dates is unaffected by this uncertainty.

Dating is less clear for the dispersal of individuals from the *T. rufus*/*T. persicus* ancestral population to Madagascar, which resulted in the incipient Malagasy species, *T. rufus* (Fig. 5). Our data give bimodal support to a demic split occurring over two broad time periods: *c.* 148 ka and *c.* 660 ka [95% confidence interval (82 ka, 874 ka)].

Coalescent likelihood of the *T. rufus*/*T. persicus* divergence time

To distinguish between these two estimates of the divergence date, we used a model that exactly represents the demography inferred for the *T. rufus*/*T. persicus* species pair. Our two competing hypotheses were (1) a young divergence time (148 ka), or (2) an old divergence time (660 ka). Genetic data sets were simulated using a coalescent methodology (Hudson, 2002) for a generalized isolation-with-migration model (see previous section). Coalescent data sets ($n = 10^6$) were generated for each model, and the distributions of segregating sites, a summary statistic correlated with the total depth of a phylogenetic tree, were compared with the number of segregating sites (*SS*) observed in the empirical data. The likelihood of finding a number of segregating sites equal to or greater than that observed in the real data set (*SS* = 65) was calculated for each model, and the best model was chosen on the basis of the Akaike information criterion (AIC; Akaike, 1974).

Simulated distributions of segregating sites are plotted in Fig. 6. The young divergence model produced an average of 24 segregating sites [95% confidence interval (14, 37)]. The number of segregating sites (*SS* = 65) surveyed for the empirical *T. rufus*/*T. persicus* data set is an outlier under this distribution; $P(SS \geq 65 \mid 148 \text{ ka}) = 7 \times 10^{-6}$ (AIC = 25.7). By contrast, the old divergence model produced an average of 54 segregating sites [95% confidence interval (38, 71)]. The number of segregating sites observed for the empirical data set is consistent with this distribution; $P(SS \geq 65 \mid 660 \text{ ka}) = 0.11$ (AIC = 6.42). We therefore suggest that the number of sites segregating in the empirical *T. rufus*/*T. persicus* data set is
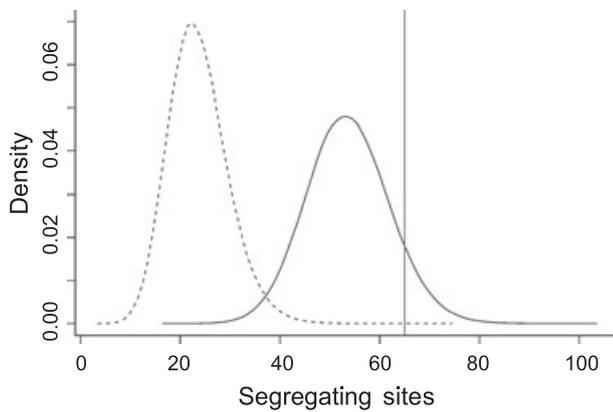
Figure 6 Distributions of segregating sites under coalescent models with young (148 ka, dashed) and old (660 ka, solid) dates for the divergence between *Triaenops rufus* and *T. persicus*. The vertical line indicates the number of segregating sites (*SS* = 65) observed in the empirical *T. rufus/T. persicus* data set.

consistent with the older divergence time (*c.* 660 ka, smaller AIC), but does not fit expectations under the younger divergence model (*c.* 148 ka).

CONCLUSIONS

We show that at least one question of animal dispersal and vicariance that cannot be addressed using phylogenetic approaches is amenable to coalescent methodologies. We do not suggest that this will be true for all evolutionary systems, organisms, or data sets. However, we do see coalescent theory playing an increasingly important role in the study of incipient species or recently diverged taxa (Russell *et al.*, 2008). Here, we extend this framework to test an explicit biogeographical question: we address the role of mainland-to-island dispersals in the evolutionary history of a genus of Malagasy bats.

Our analyses focus on two scenarios: multiple, unidirectional dispersals from Africa to Madagascar forming independent Malagasy bat lineages of *Triaenops* (Fig. 1b); or an early dispersal of the *Triaenops* ancestral species from Africa to Madagascar, with later back-dispersal to Africa of the *T. persicus* ancestor (Fig. 1c). Previous population genetic research has tentatively favoured multiple, unidirectional dispersals for two reasons (Russell *et al.*, 2007). First, *T. rufus* exhibits a strong signal of population growth, which is absent from other *Triaenops* species. Second, no evidence of population structuring was found in *T. rufus* over its large range (spanning the entire western half of Madagascar). Although these patterns are consistent with population growth from a relatively small founder event in *T. rufus* and subsequent range expansion, they do not definitively support one of the biogeographical models over the other.

Our coalescent analyses under an isolation-with-migration model more strongly support the scenario of multiple, unidirectional dispersals, in which *T. rufus* and the *T. furcu-*

lus/T. auritus clade each resulted from separate dispersal events from mainland Africa to Madagascar. Estimates of the ancestral population splitting proportion, *S*, suggest that very few individuals from the *T. rufus/T. persicus* ancestral population were recruited into the daughter species *T. rufus*. Assuming that successful long-distance dispersal and subsequent colonization is a process involving relatively few individuals, our split proportion estimate (*S* = 0.9995) favours an origin of Malagasy *T. rufus* by means of dispersal from Africa rather than an origin of African *T. persicus* from an immediate Malagasy source. Furthermore, the estimate of *S* → 1 supports our initial assumption that this dispersal event involved a comparatively small proportion of the ancestral population (a few tens of individuals at most, given inferred effective population sizes).

Through validation studies and comparison of simulated with empirical demographic estimates, we conclude that IM has sufficient power to determine the split parameter, *S*, for approximately 65% of data sets with an evolutionary history resembling that of the empirical *T. rufus/T. persicus* data set. Data sets that are not amenable to this approach produce distinctively uninformative marginal posterior distributions (I-shaped or U-shaped) rather than producing ambiguously false estimates of *S*. These uninformative distributions do not resemble the informative, L-shaped, posteriors obtained for our empirical *T. rufus/T. persicus* data set. We therefore suggest that the *S* parameter for the *T. rufus/T. persicus* species pair is reliably close to *S* = 0.9995 with an error rate not likely to exceed $\alpha \approx 0.077$. We emphasize that not all data sets (simulated or empirical) will necessarily be amenable to coalescent methods; we claim only that some data sets, including this particular *T. rufus/T. persicus* data set, are informative using this approach.

As a secondary question, times for the differentiation of the genus *Triaenops* and the dispersal of the *T. rufus* ancestors from Africa to Madagascar were also inferred. The deepest split in the *Triaenops* phylogeny occurred *c.* 2.25 Ma, when *Triaenops* still comprised a single ancestral population. The timing of the later *T. rufus* dispersal to Madagascar is less clear; its probability distribution is bimodal at *c.* 148 ka and *c.* 660 ka. Despite ambiguity in the posterior probability distribution for this parameter, coalescent simulations based on the inferred *T. rufus/T. persicus* demography firmly support the earlier date (660 ka). Although estimates of divergence time (in years) may suffer from inaccuracies in the mutation rate, the relative ages of the two nodes are strictly proportional. Our support for a much younger divergence time for *T. rufus* and *T. persicus* relative to the origin of the genus *Triaenops* remains statistically robust.

Of course, the history of dispersal and speciation of *Triaenops* is probably more complex than represented here by extant fauna. For instance, an extinct species of *Triaenops* has recently been named from the Late Pleistocene of western Madagascar (Samonds, 2007). Based on cranial characteristics (Ranivo & Goodman, 2006), this species is a member of the *T. furculus/T. auritus* group. This implies that patterns of

speciation in this species complex involve at least one other taxon that has gone extinct in the recent geological past. Furthermore, an undescribed member of this genus allied to the *T. furculus/T. auritus* group will soon be named from the Aldabra Atoll in the Seychelles (Goodman & Ranivo, in press), located about 480 km to the north-east of the northern tip of Madagascar. The highest point on Aldabra is 8 m above sea level, and all of the currently existing exposed landmass was under water during the Quaternary. Hence, given this example and the Late Pleistocene fossil on Madagascar, movements of founding populations of *Triaenops* across considerable stretches of sea appear to have occurred on numerous occasions, and more often in recent geological time than current extant fauna would seem to indicate.

We conclude that the evolutionary history of living Malagasy members of the genus *Triaenops* probably involved two dispersal events from Africa to Madagascar. The directionality of dispersal thus supported by these analyses fits well with most studies of other Malagasy mammals (Yoder *et al.*, 1996; Douady *et al.*, 2002; Yoder *et al.*, 2003; reviewed in Yoder & Nowak, 2006). We suggest that the *Triaenops* genus of Madagascar proves no exception to this model, in which islands act as sinks to mammalian dispersals from continental sources.

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