

Species-specific responses to island connectivity cycles: refined models for testing phylogeographic concordance across a Mediterranean Pleistocene Aggregate Island Complex

ANNA PAPADOPOULOU and L. LACEY KNOWLES

Department of Ecology and Evolutionary Biology, Museum of Zoology, University of Michigan, 1109 Geddes Ave., Ann Arbor, MI 48109-1079, USA

Abstract

The contribution of Pleistocene sea level changes to diversification patterns in archipelagos around the world, and specifically whether the repeated cycles of island connectivity and isolation acted as a 'species pump' is debated. The debate has been perpetuated in part because of the type of evidence used to evaluate the species-pump hypothesis. Specifically, existing tests of the 'Pleistocene Aggregate Island Complex' (PAIC) model of diversification interpret the lack of concordant divergence times among multiple codistributed taxa as a rejection of the PAIC model. However, the null expectation of concordance disregards taxon-specific ecological traits and geographic characteristics that may affect population persistence and gene flow among islands. Here, we study the factors affecting population divergence in thirteen flightless darkling beetle species (Coleoptera: Tenebrionidae) across the PAIC system of the Cycladic plateau in the Aegean archipelago. Based on isolation-by-resistance analyses, hierarchical AMOVA and the degree of genealogical sorting on individual islands, we identify a major effect of bathymetry and habitat stability on the levels of genetic divergence across the PAIC, with island size and body size playing a secondary role as well. We subsequently use bathymetric maps and habitat association to generate predictions about the set of islands and group of taxa expected to show phylogeographic concordance. We test these predictions using hierarchical approximate Bayesian computation and show how our interpretations regarding the role of PAICs as drivers of divergence change when relying on a null expectation of concordance compared to a refined model that takes geography and ecological traits into account.

Keywords: Aegean, bathymetry, hABC, habitat stability, sea level, Tenebrionidae

Received 8 April 2015; revision received 21 June 2015; accepted 24 June 2015

Introduction

There is an increasing interest in assessing the genetic consequences of climate change and evaluating scenarios of climate-driven diversification in temperate and tropical continental biota (Soltis *et al.* 2006; Carnaval *et al.* 2009, 2014; Morgan *et al.* 2011; Bell *et al.* 2012), while less attention has been paid to the responses of

island communities to sea level changes during Pleistocene climatic oscillations (Jordan *et al.* 2005; Salvi *et al.* 2014). In an insular setting, the climate itself may play a secondary role in comparison with the predominant influence of the sea element. Nevertheless, the potential contribution of sea level fluctuations to diversity patterns in archipelagos around the globe remains largely unexplored. Specifically, the proposed 'species-pump' action of rising and falling sea levels under the Pleistocene Aggregate Island Complex (PAIC) model of diversification (Heaney 1986; Brown & Diesmos 2009)

Correspondence: Anna Papadopoulou, Fax: +1 734 763 4080; E-mail: apapadop@umich.edu

has found mixed support based on species distribution patterns (e.g. data on the Philippines; Brown *et al.* 2013), while the results from genetic analyses (Gorog *et al.* 2004; Roberts 2006; Esselstyn & Brown 2009; Siler *et al.* 2010; Hosner *et al.* 2014) have been contradictory and case specific, suggesting that alternative processes may also be operating.

A specific expectation derives from the 'species-pump' model within a comparative phylogeographic framework: if island connectivity cycles drive diversification, then divergence times across a PAIC system should be temporally clustered and coincide with high sea level periods (Oaks *et al.* 2013). Therefore, tests to identify the role of dynamic sea level changes in driving diversification have involved statistical evaluation of the concordance in divergence times among multiple codistributed taxa. Specifically, the lack of evidence for temporally clustered divergence times across 22 taxon pairs from the Philippines PAICs based on hierarchical approximate Bayesian computation (hABC) analyses (Oaks *et al.* 2013; Oaks 2014) has been interpreted as a rejection of the PAIC model. However, this lack of concordance is not entirely surprising, given that the 22 focal taxon pairs spanned a wide range of taxonomic groups (including mammals, squamates and anuran lineages) with different ecological traits and dispersal capabilities and that the taxa were sampled from different pairs of islands across the Philippines. In other words, differences in the timing of divergence can be expected due to species-specific traits and geographic characteristics, suggesting that a refinement of the expectations for concordance is needed, if concordance itself is a meaningful metric for evaluating dynamic models of diversification like the 'species-pump' model. Specifically, refinements of the 'species-pump' model should consider the main processes involved in population divergence (Futuyma 1987; Dynesius & Jansson 2014) across a PAIC system, such as local population persistence on individual islands and population connectivity (gene flow) among islands. These processes will be influenced by a range of geographic factors (e.g. island size, duration of island connections, size and suitability of corridors formed among islands) as mediated by each species' ecological traits (e.g. area requirements, dispersal ability and habitat association). That is, predictions for temporally clustered divergences should be informed by geography and target ecologically similar taxa (see also Heaney *et al.* 2005; Massatti & Knowles 2014).

Even with refined hypotheses for evaluating predictions about concordance, the challenges with conducting such tests need to be recognized, and especially the potential for biased conclusions about the prevalence of concordance. In particular, special caution is required

when interpreting inferences of simultaneous divergence from hierarchical approximate Bayesian computation analyses (hABC; Hickerson *et al.* 2006b; Huang *et al.* 2011). Such implementations are biased towards supporting clustered divergences (Oaks *et al.* 2013, 2014) due to improper prior distributions on demographic and divergence-time parameters (Oaks *et al.* 2014) especially affecting analyses of large number of taxa. Despite suggestions that using narrow empirically informed prior distributions can correct for the observed biases (Hickerson *et al.* 2014), only by replacing the uniform priors with more appropriate flexible and broad distributions that do not favour disproportionately models with small numbers of divergence events (Oaks 2014) can such tests be unbiased. We here use the example of a Mediterranean PAIC system to show how the results from hABC analyses using appropriate prior distributions suggest considerable uncertainty and variation in divergence times when applied on a set of taxa of different ecological traits, while simultaneous divergence across the PAIC is supported when targeting the appropriate spatial scale and set of taxon characteristics expected to show phylogeographic congruence.

The Cycladic plateau in the central Aegean archipelago provides an ideal PAIC system to assess the importance of refining the expectations of concordance: it comprises islands that are fairly uniform in terms of current climate and habitat diversity, but they differ significantly in size, and the complex topography/bathymetry of the region results in great variation in the timing of island connections and shape of corridors formed among the islands (Fig. 1). The focal darkling beetle species (Coleoptera: Tenebrionidae) occupy comparable climatic and trophic niches (adapted to warm and dry climate, soil dwelling and detritivores) and have similar inherent dispersal capabilities (flightless, lack of wings). However, they differ in soil-type preference (and thus in habitat association), which affects population persistence across the Aegean archipelago (Papadopoulou *et al.* 2009). Specifically, sand-obligate (psammophilous) species are associated with disturbed and relatively ephemeral sandy coastal habitats, while geophilous (hereafter referred to as 'soil') taxa are associated with comparatively stable inner habitats (mostly 'phrygana' shrublands and maquis). The targeted species for this study also differ in body size (Fig. 2; Table S1, Supporting information), which is positively correlated with travelling distance in flightless insects (Peters 1983; Gutierrez & Menéndez 1997) as well as with area requirement (Biedermann 2003), so both migration rate through interisland corridors and local extinction rate on small islands/habitat patches may differ among taxa. We first conduct a set of descriptive

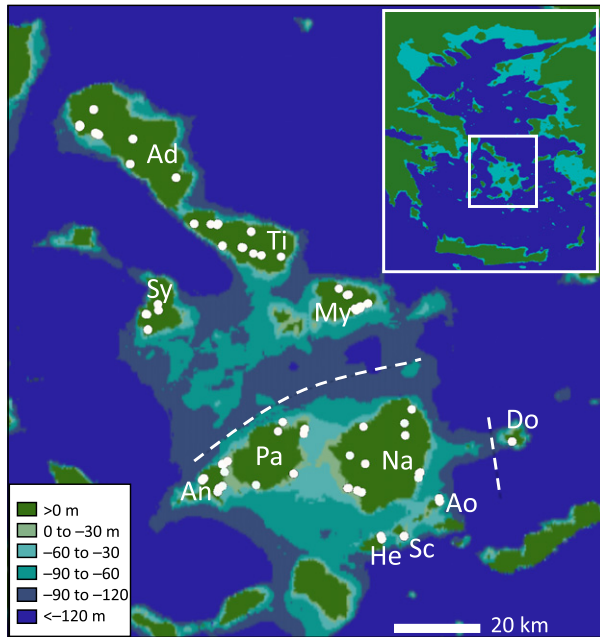


Fig. 1 Map of the Cycladic plateau in the central Aegean archipelago with bathymetric data. Sixty sampling localities are shown (white dots) on 11 islands (Ad, Andros; Ti, Tinos; My, Mykonos; Sy, Syros; Na, Naxos; Pa, Paros; An, Antiparos; He, Herakleia; Sc, Schinoussa; Ao, Ano Koufonissi; Do, Donoussa). White dashed lines indicate the two major bathymetric splits (<-90 m) across the PAIC. Inset map on the top right shows the position of the Cycladic plateau within the Aegean archipelago.

analyses to investigate the relative importance of the above geographic factors (bathymetry, island size) and species-specific traits (soil-type preference, body size) on population divergence of thirteen darkling beetle species across the Cycladic plateau, and then, we use hABC to assess how our interpretations regarding the role of PAICs as drivers of divergence change when we rely on a null expectation of concordance in divergence times that does not distinguish among different classes of geographic and species-specific characteristics compared to a refined model that takes these factors into account.

Methods

Study taxa and genetic data

We focus on 13 flightless darkling beetle species, which are broadly distributed across the Cycladic plateau and represent different soil-type preferences (six 'soil', five sand-obligate and two generalist species) and a wide range of body sizes (length 0.3–2 cm, width 0.05–1 cm; Fig. 2). Samples were collected from 60 localities on 11 present-day islands (Fig. 1; Table S2, Supporting infor-

mation), including seven major Cyclades (35–428 km²) and four smaller ones (3.8–17.5 km²; from now on referred to as 'islets'). Four of the species were collected from all 11 islands, seven species were sampled from 7 to 10 islands, and the remaining two species were only encountered in a smaller subset of 4–5 islands. The lack of samples from certain islands was mostly (in 64% of the cases) due to species absence (i.e. the species has not been recorded on the respective island based on existing literature), while the remaining cases were due to incomplete sampling (see Table S3, Supporting information). The genetic data set includes 403 mitochondrial cytochrome oxidase I (*Cox1*) sequences and 273 nuclear muscular protein 20 (*Mp20*) sequences (Table S3, Supporting information). Although two independent loci are a small number for phylogeographic analyses when the goal is to estimate specific demographic parameters, the hABC analyses in contrast derive information contained across multiple species and thus gain statistical 'borrowing strength' (Hickerson & Meyer 2008). Therefore in this sense, our sampling is not atypical (see for example Stone *et al.* 2012; Hope *et al.* 2013; Smith *et al.* 2014). A total of 643 sequences are from Papadopoulou *et al.* (2008, 2009, 2010, 2011), while 33 new sequences have been submitted to the European Nucleotide Archive (Accession nos LN835313–LN835345; see Table S7, Supporting information). For details about the sampling, the laboratory protocols, sequence editing and alignment and haplotype reconstruction, see Papadopoulou *et al.* (2009, 2011).

Linking bathymetry with population connectivity and genetic differentiation

Based on bathymetric data from digital terrain models for the Aegean region (EMODnet Bathymetry portal <http://www.emodnet-bathymetry.eu>; at 0.25-arc-min resolution), we identified major bathymetric barriers across the Cycladic plateau (i.e. with a minimum depth of 90 m separating the present-day islands; Fig. 1) and performed analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) to assess how genetic differentiation is structured across these major bathymetric splits. AMOVA for each species was conducted in ARLEQUIN v3.5.1.3 (Excoffier & Lischer 2010) to estimate the proportion of genetic variance attributable to different hierarchical levels: among groups of islands on either side of the inferred bathymetric split (Φ_{CT}), among islands within such island groups (Φ_{SC}) and within islands (Φ_{ST}). The significance of the fixation indices was assessed with 1000 permutations. Given the unequal sampling between the two loci, we conducted (i) separate analyses using the *Cox1* locus and a more inclusive

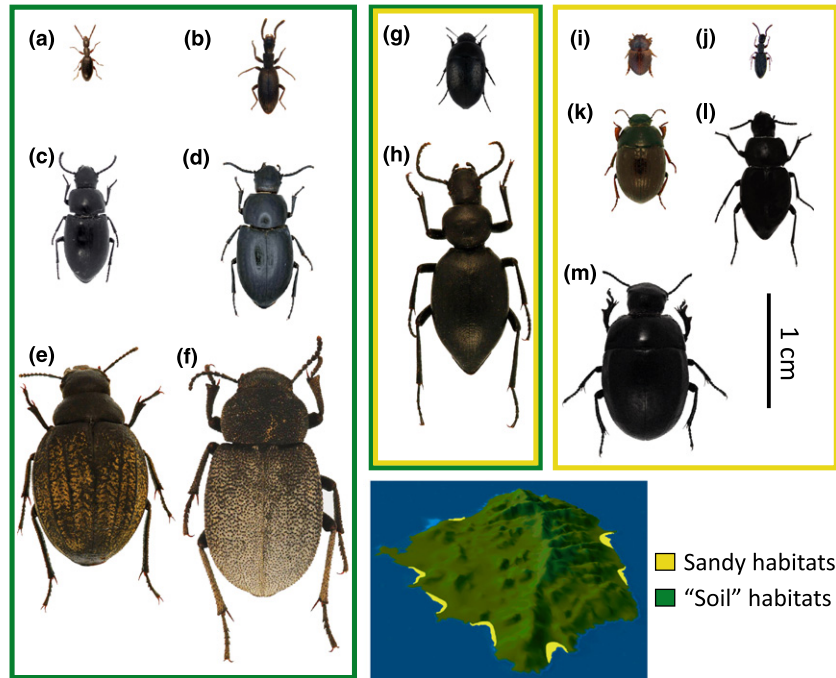


Fig. 2 Photographs of the 13 focal darkling beetle taxa (drawn to scale) and their respective soil-type preference (Photograph credit: Chris Georgiadis). (a) *Eutagenia* sp., (b) *Stenosis smyrnensis*, (c) *Dailognatha hellenica*, (d) *Dailognatha quadricollis*, (e) *Pimelia sericella*, (f) *Graecopachys quadricollis*, (g) *Zophosis punctata*, (h) *Tentyria rotundata*, (i) *Ammobius rufus*, (j) *Eutagenia smyrnensis*, (k) *Micrositus orbicularis*, (l) *Dichomma dardanum* and (m) *Erodius orientalis*. See Table S1 (Supporting information) for further details regarding the length and width of each species and their taxonomy. A simplified representation of a Cycladic island shows a typical distribution of the two main soil types: sand-obligate taxa (i–m) are confined to restricted sandy areas by the coastline (in yellow), while ‘soil’ (geophilous) taxa (a–f) are associated with shrublands (‘phrygana’ and maquis) and meadows, which are the most dominant habitat types throughout the island (in green). Generalist species (g–h) are abundant in both sandy and ‘soil’ habitats.

data set and (ii) combined analyses of both loci using the ‘locus-by-locus’ option, which produces a weighted average of the fixation indices across loci. For the latter, we reduced the data set to the individuals that were sequenced successfully for both loci and the two gene fragments were combined using a phased Mp20 haplotype chosen at random for each individual.

To approximate the expected degree of population connectivity over time, we further used bathymetric data and the local sea level change curve at Paros (Lambeck 1996) as predicted by models taking into account the eustatic global sea level changes and the regional effects of glacio-hydro isostasy (note that local tectonics are considered negligible in this part of the archipelago; see also Lambeck 1995; Lambeck & Chappell 2001; Lambeck & Purcell 2005). Specifically, we divided the currently submerged shelf of the PAIC into 10-m-depth intervals, and inferred the relative time of exposure of each interval during the last glacial cycle. We then used the relative times (%) of exposure as conductance values on a 0–100 scale (with all currently exposed shelf receiving a score of 100) to rescale geographic distances among sampling localities under a circuit theory approach (McRae & Beier 2007; McRae *et al.* 2008). Pair-

wise resistance distances among sampling localities of each species were calculated in CIRCUITSCAPE 4.0.5 (McRae *et al.* 2013), and the resulting rescaled distance matrices were used to assess for the effect of sea level change on population isolation, while taking into account the spatial configuration of corridors formed among islands over time. To test for isolation by distance and isolation by bathymetry among populations of each species, we calculated genetic distances (average number of pairwise differences, π_{xy}) among sampling localities in ARLEQUIN v3.5.1.3 (Excoffier & Lischer 2010) and conducted Mantel tests (Mantel 1967) between the pairwise genetic distance matrix and either the Euclidean geographic distance matrix, respectively, using functions of the *vegan* package (Oksanen *et al.* 2013). Additionally, partial Mantel tests (Smouse *et al.* 1986) were used to test the significance of the association between resistance distances and genetic differentiation, while controlling for geographic distance. The above tests were repeated after removing the populations of the four small islets, as long-term population persistence over successive connectivity cycles is less probable on these islands, and thus, they may not fit an isolation-by-bathymetry model.

For these correlational analyses that were conducted at the level of individual sampling localities (instead of the island level used for AMOVAS), we calculated genetic distances based only on the *Cox1* sequences to maximize the number of localities used in the tests (i.e. to minimize the effects of missing data).

Lineage sorting and the duration of island connections

To assess the degree of lineage sorting on each island, *Cox1* gene trees for each species were estimated using Bayesian inference in BEAST v1.8.0 (Drummond *et al.* 2012), under a lognormal uncorrelated relaxed clock (Drummond *et al.* 2006), with a mean rate of 0.0168 substitutions/site/My, as estimated previously for the same gene fragment in tenebrionid taxa based on biogeographic calibration (for details, see Papadopoulou *et al.* 2010), a coalescent tree prior and a best-fit nucleotide substitution model selected for each data set based on the Bayesian information criterion in jMODELTEST (Darriba *et al.* 2012). Four independent runs of 20 million generations (sampling every 2000th generation) were conducted for each data set, and the convergence and mixing of the MCMC chains was assessed by inspection of the trace plots and the effective sample sizes using TRACER 1.6 (Rambaut *et al.* 2014). Sampled trees from all independent runs were then pooled after removing a 10% burn-in using LOGCOMBINER v1.8.0, and the means of node heights were summarized on a 'maximum clade credibility' tree using TREEANNOTATOR 1.8.0 (Drummond & Rambaut 2007).

The degree of lineage sorting on each island was quantified using the genealogical sorting index (*gsi*; Cummings *et al.* 2008), which provides a standardized way to quantify the exclusive ancestry of a given group on a rooted gene tree, along the continuum from polyphyly to paraphyly and monophyly. To account for uncertainty in gene tree topology, we integrated across 100 trees sampled from the posterior distribution (i.e. the credible set of 100 gene tree topologies with highest posterior density) and calculated an ensemble statistic (*gsiT*) weighting each topology relatively to its posterior probability. All calculations were performed using the GENEALOGICAL SORTING INDEX web server (www.genealogicalsorting.org). Statistical significance was evaluated with 1000 permutations, and only significant *gsiT* index values ($P < 0.05$) were used for further analyses. In subsequent analyses, we assessed whether the relative duration of island connections during the last glacial cycle (T_c ; calculated from bathymetric data and the local sea level curve as explained above) was a significant predictor for the degree of genealogical sorting on each island, and whether including additional factors as predictors (soil-type preference, body size, island size

and elevation) would improve model fitting. Model comparisons were conducted based on the corrected Akaike information criterion (AICc) using the R package *AICcmodavg* (Mazerolle 2014).

Testing for simultaneous divergence

Focusing on the major bathymetric break between the northern and southern plateau (Fig. 1; see Table S3, Supporting information for sample sizes), we tested for simultaneous divergence either among all 13 taxa or among the six 'soil' taxa. We used a hierarchical approximate Bayesian computation (hABC) approach that allows for across-species demographic variation, intergene variability in coalescent times and heterogeneity in mutation rate and captures the congruence in divergence times through three hyperparameters: Ψ , the number of different divergence times across Y taxon pairs, $E(\tau)$ the mean divergence time and Ω , the dispersion index of τ ($\text{Var}(\tau)/E(\tau)$) (Hickerson *et al.* 2007; Huang *et al.* 2011). DNA sequence data were simulated taking into account different sample sizes and fragment lengths for each species and locus, with base frequencies and a transition/transversion ratio estimated from the real data using BEAST under an HKY model, and a mutation scalar for the two gene fragments based on mutation rate estimates from biogeographic calibration (Papadopoulou *et al.* 2010).

Because of problems with the prior distributions used in some implementations of the hABC method (i.e. *msbayes*; Hickerson *et al.* 2007; Huang *et al.* 2011) that cause a bias towards supporting clustered divergences (Oaks *et al.* 2013, 2014), we used the recent modification of the hABC method (*dpp-msbayes*; Oaks 2014), which has an improved performance using more flexible prior distributions. More specifically, we used (i) exponential prior distributions on demographic and divergence-time parameters, as the uniform distributions result in prohibitively small marginal likelihoods for models with high number of divergence events (Oaks 2014), and (ii) a dirichlet process prior on the divergence models, because a uniform prior on the number of divergence events favours models with either very few or very many divergence events (i.e. a uniform prior on the number of divergence events actually produces a U-shaped prior on the divergence models, as there are many more ways to assign taxa in models with intermediate numbers of divergence events; see Oaks 2014; Oaks *et al.* 2014). Broad exponential distributions were selected for the priors on divergence times [$\tau \sim \text{Exp}(\text{mean} = 10)$], and on the population mutation parameter of ancestral (θ_A) and of descendant populations [$\theta_A \sim \text{Exp}(\text{mean} = 0.005)$, $\theta_D \sim \text{Exp}(\text{mean} = 0.005)$], on the basis of biologically meaningful expectations, as well as

on the recommendations of Oaks *et al.* (2014). Given the limited number of available loci, we kept the model simple to avoid problems of overparameterization and did not include bottleneck or migration parameters. Note that a model without migration appears reasonable for the 'soil' taxa (see Results section) but not necessarily for the sand-obligate or generalist taxa. Therefore, as explained above, the analyses were conducted either on the full set of taxa or exclusively on the six 'soil' species.

To test for codivergence among taxa using hABC, we compared patterns of sequence diversity between observed and simulated data using four summary statistics, which have been shown to capture efficiently information about codivergence (Hickerson *et al.* 2006a). Specifically, for each population pair, we calculated the average number of pairwise differences among all sequences (π ; Tajima 1983), net average pairwise differences between two descendant populations (π_{net} ; Tachibana & Nei 1985), Watterson's θ (θ_w ; Watterson 1975) and SD ($\pi - \theta_w$) (Tajima 1989). Given the unequal sample sizes among taxa (Table S3, Supporting information), the vector of the simulated summary statistics was retained in the taxon order of the observed data as suggested by Oaks (2014) instead of re-ordering the vector by descending values of average pairwise differences between the descendant populations (π_b) as in *ms-bayes*, which reduces the number of required simulations but also discards important information in the data and inhibits estimation of marginalized divergence times for each taxon. Absolute divergence times were estimated assuming $\mu_{\text{Cox1}} = 0.0168$ mutations/site/generation, $\mu_{\text{M20}} = 0.0033$ mutations/site/generation (Papadopoulou *et al.* 2010) and a generation time of 1 year. Convergence was assessed by plotting the results for 50–100 million simulations, where incremental increases in the number of simulations were applied to reach convergence.

Rejection sampling was performed using Euclidean distances, after standardizing observed and simulated summary statistics using the mean and standard deviation of the statistics from the prior sample, as implemented in *eureject* (Oaks 2014). In each analysis, the set of 1000 samples with the smallest Euclidean distance from the observed summary statistics was retained as an approximate posterior. Postsampling adjustment was conducted with GLM (general linear model) regression (in *ABCtoolbox*; Wegmann *et al.* 2010).

To validate the power and accuracy of our analyses, we generated pseudo-observed data sets (100 for each possible Ψ value; i.e. 1300 data sets in total for the analyses with all taxa included) by sampling parameters from the same prior distributions used for the above analyses and then performing ABC for each of

these pseudo-observed data sets. True and estimated hyperparameter values were compared to assess the power of our ABC procedure to distinguish among alternative divergence models and estimate mean divergence times. Additional analyses were performed to assess sensitivity to prior distributions, as well as the effect of re-ordering the summary statistics vector, and the impact of the postsampling adjustment method (see Appendix S1, Supporting information).

Results

Linking bathymetry with population connectivity and genetic differentiation

Two major bathymetric barriers were identified within the PAIC (Fig. 1): (i) between the northern and the southern Cycladic plateau (minimum depth 95 m) and (ii) between the small island of Donoussa and the southern Cycladic plateau (minimum depth 105 m). Hierarchical AMOVAS across the first bathymetric barrier between the northern and southern Cycladic plateau attributed the largest proportion of the genetic variance to (i) the bathymetric barrier in four 'soil' and one sand-obligate species, (ii) among island variation within each region in two 'soil' and four sand-obligate species and (iii) within island variation in the two generalist species (Table 1). In the case of the deepest bathymetric barrier between the small Donoussa Island and the southern Cycladic plateau, AMOVAS attributed most of the genetic variance to the bathymetric barrier in the case of the small-sized 'soil' species *Eutagenia* sp. (Fig. 2a), and to a lesser extent in its congeneric and very similar morphologically sand-obligate species *E. smyrnensis* (Fig. 2j), but not in other taxa (Table 1). The results were qualitatively robust to the inclusion or exclusion of the nuclear locus and a more or less inclusive set of individuals (Table S4, Supporting information).

In all 'soil' taxa, there was a strong association between genetic differentiation and resistance distances based on bathymetric data (Table 2). In all cases apart from *D. hellenica*, the fit for the isolation-by-bathymetry model was better than for a simple isolation-by-distance model. Moreover, the correlation between genetic differentiation and bathymetric resistance remained highly significant when controlling for geographic distance (partial Mantel tests; Table 2), either with all the sampled islands included in the model (*E. sp.*, *P. sericella*, *S. syrensis*), or after excluding the small islets (*Dailognatha quadricollis*, *Graecopachys quadricollis*). This strong correlation observed in the 'soil' taxa contrasted with the sand-obligate and generalist species in which only weak and/or marginally significant correlation

Table 1 Results of hierarchical AMOVAS assessing the proportion of the genetic variance attributable to the two major bathymetric splits shown in Fig. 1: (a) the bathymetric split separating the northern from the southern Cycladic plateau (b) the one separating the southern plateau from Donoussa Island. The results are presented as percentages of variance corresponding to each of three hierarchical levels: between groups of islands on either side of the inferred bathymetric split (Φ_{CT}), among islands within such island groups (Φ_{SC}) and within islands (Φ_{ST}). These analyses are based on the *Cox1* gene and the most inclusive set of individuals (see Table S4, Supporting information for the AMOVA results based on both genes and a reduced set of individuals)

Species	Southern vs. Northern plateau	Φ_{CT}	Among islands within groups	Φ_{SC}	Within islands	Φ_{ST}
(a)						
<i>A. Eutagenia</i> sp.	64.84	0.65*	20.32	0.58***	14.84	0.85***
<i>B. Stenosis syrensis</i>	-6.43	0 ns	85.44	0.80***	20.99	0.79***
<i>C. Dailognatha hellenica</i>	87.6	0.88*	3.22	0.26**	9.18	0.91***
<i>D. Dailognatha quadricollis</i>	59.73	0.60*	24	0.60***	16.27	0.84***
<i>E. Pimelia sericella</i>	89.8	0.9*	5.38	0.53*	4.82	0.95***
<i>F. Graecopachys quadricollis</i>	13.74	0.14 ns	65.56	0.76***	20.7	0.79**
<i>G. Zophosis punctata</i>	25.33	0.25**	13.86	0.18***	60.8	0.39***
<i>H. Tentyria rotundata</i>	9.72	0.1 ns	43.28	0.48***	47	0.53***
<i>I. Ammobius rufus</i>	3.44	0.03 ns	87.82	0.91***	8.75	0.91***
<i>J. Eutagenia smyrnensis</i>	16.35	0.16 ns	62.76	0.75***	20.89	0.79***
<i>K. Micrositus orbicularis</i>	87.89	0.87*	7.87	0.65***	4.24	0.96***
<i>L. Dichomma dardanum</i>	21.43	0.21**	55.73	0.71***	22.84	0.77***
<i>M. Erodium orientalis</i>	5.47	0.05 ns	76.13	0.80***	18.4	0.82***
Species	Donoussa vs. Southern plateau	Φ_{CT}	Among islands within groups	Φ_{SC}	Within islands	Φ_{ST}
(b)						
<i>A. Eutagenia</i> sp.	97.26	0.97*	0.92	0.33*	1.82	0.98***
<i>D. Dailognatha quadricollis</i>	-22.52	0 ns	52.24	0.43***	70.28	0.30***
<i>G. Zophosis punctata</i>	32.12	0.32 ns	47.07	0.69*	20.81	0.79***
<i>J. Eutagenia smyrnensis</i>	59.03	0.59 ns	39.72	0.97*	1.26	0.99***
<i>L. Dichomma dardanum</i>	-29.76	0 ns	77.03	0.59***	52.72	0.47***
<i>M. Erodium orientalis</i>	-37.37	0 ns	113.04	0.82***	24.32	0.76***

Asterisks indicate the significance of the variance components (*** $P \leq 0.001$, ** $P \leq 0.005$, * $P \leq 0.05$, ns $P > 0.05$). (a-f): soil, (g,h): generalists, (i-m): sand-obligate taxa.

(*T. rotundata*, *A. rufus*, *E. smyrnensis*, *E. orientalis*) or a complete lack of correlation (*Z. punctata*, *M. orbicularis*, *D. dardanum*) was observed between genetic differentiation and bathymetric resistance when controlling for geographic distance (Table 2).

Lineage sorting and the duration of island connections

The mtDNA gene trees showed different degrees of geographical structure and lineage sorting across taxa in a manner that reflected differences in the habitats of the taxa (Fig. 3). Specifically, all 'soil' species (Fig. 3a-f) showed a deep subdivision between the northern and the southern Cycladic plateau, and a general pattern of stronger geographical structure among the northern islands than among the southern ones, whereas there was less clear geographic structure in most sand-obligate and generalist taxa (Fig. 3g-m).

The degree of mtDNA lineage sorting on each island, as quantified using the genealogical sorting index, also

differed greatly among taxa and among islands (Fig. S1, Supporting information). Model comparisons based on AICc showed that the duration of island connections during the last glacial cycle (Tc; calculated from bathymetric data and the local sea level curve) in combination with habitat type (soil, sand or both) were the best predictors for the degree of genealogical sorting on each island (Table S5, Supporting information). Including body size or island size as additional predictors in the model yielded similar AIC scores ($\Delta AICc \sim 0.9$ or $\Delta AICc \sim 2$, respectively; Table S5, Supporting information). Island elevation was highly correlated with island size ($r = 0.95$, $P \ll 0.001$) and thus not included as a separate predictor in the models. In 'soil' taxa, there was a significant positive linear relationship between the genealogical sorting index and the relative duration of island connections during the last glacial cycle ($R_{adj}^2 = 0.48$, $P \ll 0.001$; Fig. 4a), but this correlation was not significant in the case of the sand-obligate species ($R_{adj}^2 = 0.01$, $P = 0.54$; Fig. 4b). These results were not altered when

Table 2 Results of Mantel tests and partial Mantel tests to assess the relative effect of isolation by distance (IBD, i.e. the correlation between genetic distances and geographic distances) and ‘isolation by bathymetry’ (IBB, i.e. the correlation between genetic distances and resistance values, as calculated based on bathymetric data and the local sea level curve, see Methods for more details). Partial Mantel tests assess the correlation between genetic distances and resistance values, after controlling for geographic distances. The analyses were performed either considering all islands, or after excluding the four islets

Species	Both larger islands and islets			Only larger islands		
	IBB	IBD	Partial	IBB	IBD	Partial
<i>A. Eutagenia</i> sp.	0.90***	0.61***	0.84***	0.85***	0.72***	0.67***
<i>B. Stenosis</i> <i>syrensis</i>	NA	NA	NA	0.52***	0.40***	0.53***
<i>C. Dailognatha</i> <i>hellenica</i>	0.68***	0.82***	−0.05 ns	0.56***	0.83***	−0.25 ns
<i>D. Dailognatha</i> <i>quadricollis</i>	0.45***	0.82***	−0.09 ns	0.94***	0.82***	0.82***
<i>E. Pimelia</i> <i>sericella</i>	0.95*	0.91*	0.7*	0.95*	0.94 ns	0.40 ns
<i>F. Graecopachys</i> <i>quadricollis</i>	0.38*	0.36**	0.21 ns	0.60***	0.28*	0.66***
<i>G. Zophosis</i> <i>punctata</i>	0.23 ns	0.22*	0.13 ns	0.16 ns	0.12 ns	0.07 ns
<i>H. Tentyria</i> <i>rotundata</i>	0.34**	0.27*	0.21*	0.32**	0.22*	0.25*
<i>I. Ammobius</i> <i>rufus</i>	NA	NA	NA	0.73*	0.66*	0.43*
<i>J. Eutagenia</i> <i>smyrniensis</i>	0.22*	0.14 ns	0.18*	−0.11 ns	−0.02 ns	−0.16 ns
<i>K. Micrositus</i> <i>orbicularis</i>	0.33***	0.68***	−0.12 ns	0.48***	0.67***	−0.19 ns
<i>L. Dichomma</i> <i>dardanum</i>	0.28 ns	0.68***	−0.19 ns	0.61***	0.71***	−0.03 ns
<i>M. Erodius</i> <i>orientalis</i>	0.05 ns	0.23*	−0.09 ns	0.46***	0.19 ns	0.58*

The Mantel test statistic is reported and the significance level is indicated (*** $P \leq 0.001$, ** $P \leq 0.005$, * $P \leq 0.05$, ns $P > 0.05$). (a–f): soil, (g,h): generalists, (i–m): sand-obligate taxa.

we controlled for number of localities or number of sequences sampled per island (‘soil’ taxa: $R^2_{adj} = 0.49$, $P \ll 0.001$; sand-obligate taxa: $R^2_{adj} = 0.04$, $P = 0.24$). In ‘soil’ taxa, the degree of lineage sorting ranged along a continuum from monophyly on islands that have been connected for 10% or less of the glacial cycle such as Donoussa, Syros or Mykonos, to complete lack of lineage sorting on islands that have been connected almost 90% of the time, such as Paros and Antiparos (Fig S1).

Testing for simultaneous divergence

The results of the hABC analyses for the full 13-taxon data set supported two divergence events, but with considerable posterior uncertainty (Fig. 5a). Retaining the order of the summary statistics allowed us to approximate the marginalized estimates of divergence times for each taxon (Fig. 6), which showed considerably older divergence times for all ‘soil’ taxa and the smallest of the sand-obligate taxa (*A. rufus*), than for the generalist and the larger sand-obligate taxa. Specifically, for the ‘soil’ taxa, divergence times ranged between 3.6 and $13N_C$ (corresponding to ~1.3–4.9 My), whereas for the generalist and the larger sand-obligate taxa $\tau \ll N_C$, apart from the case of *M. orbicularis* where $\tau \sim N_C$ (corresponding to ~372 ky), where $4N_C = \theta_C/\mu$ and $\theta_C = 0.005$ is the mean of the prior distribution on descendant population size. When the six ‘soil’ taxa were analysed separately, the results supported clearly a model with a single divergence event (Fig. 5b), with a mean diver-

gence time of $\sim 6.2N_C$ or ~ 2.3 My (95% HPD intervals 1.2–12.4 N_C and 0.5–4.7 My, respectively).

Validation analyses using pseudo-observed data sets to assess the power and precision of our ABC procedure showed that there were no important biases in the estimation of the mean divergence time ($E(\tau)$; Fig. S2b, Supporting information) and the dispersion index (Ω ; Fig. S2c, Supporting information). Both the precision and accuracy of the estimated number of divergence events (Ψ) were reduced for high values of true Ψ (Fig. S2a, Supporting information; see also Oaks 2014), but not for models involving from one to three divergence events (Fig. S2a, Supporting information). Sensitivity to prior distributions also did not appear to be an important concern (i.e. the results were generally robust under the alternative prior sets used; Fig. S3, Supporting information). The approximated posterior distribution of the hyperparameter Ψ (the inferred number of divergence events) was not significantly altered either when using unadjusted estimates of model parameters (Fig. S4a, Supporting information) or when the vector of the summary statistics was re-ordered by π_b to reduce the sample space of possible divergence models and thus the number of required simulations (Fig. S5, Supporting information). In contrast, using local multinomial logistic regression for postsampling adjustment (the default option in the original *msbayes* implementation) produced a posterior distribution with the same mode (=2), but practically no posterior uncertainty (Fig. S4c, Supporting information).

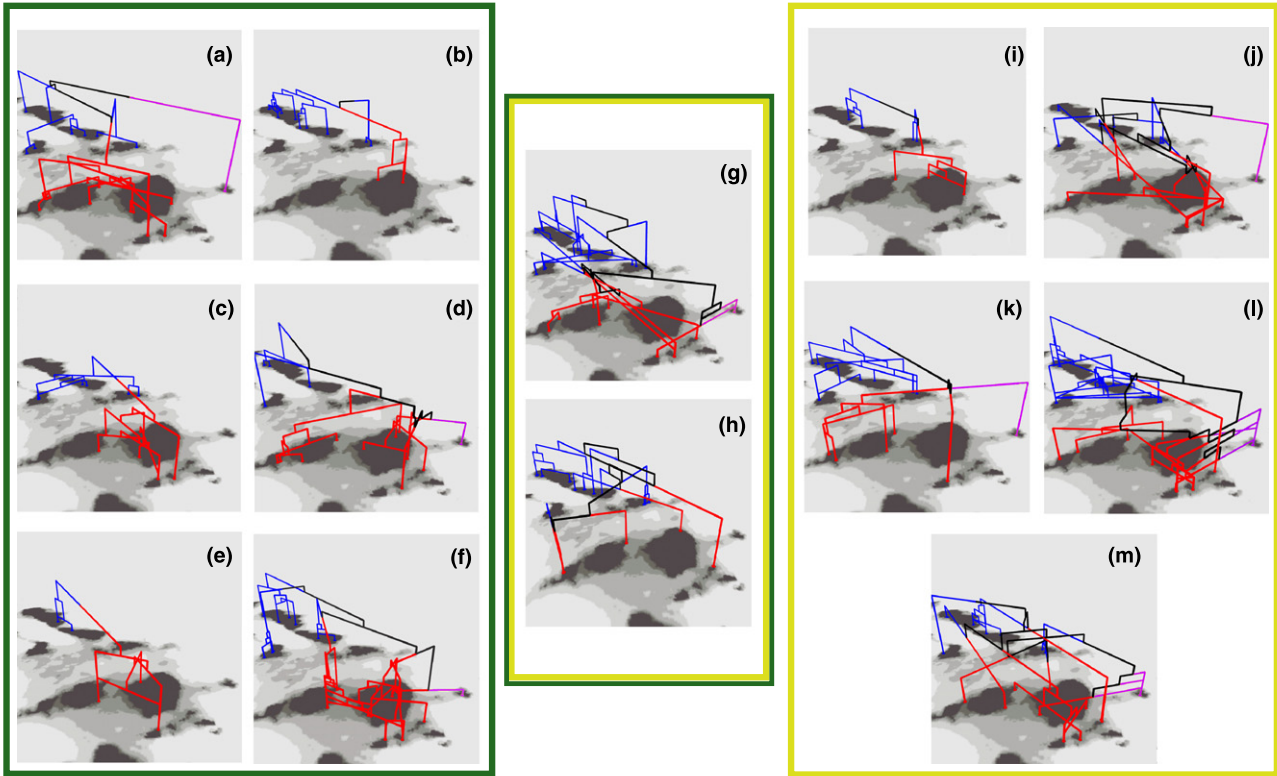


Fig. 3 Mitochondrial gene trees for each of the 13 taxa as estimated by BEAST, plotted on the map of the Cycladic plateau. The branches of the trees are coloured to represent the main three groupings of islands as defined by the two deepest bathymetric splits shown in Fig. 1. Blue, north plateau; Red, southern plateau; Purple, Donoussa islet. The 13 taxa are numbered as (a–m) following Fig. 2. (a–f): soil, (g,h): generalists, (i–m): sand-obligate taxa.

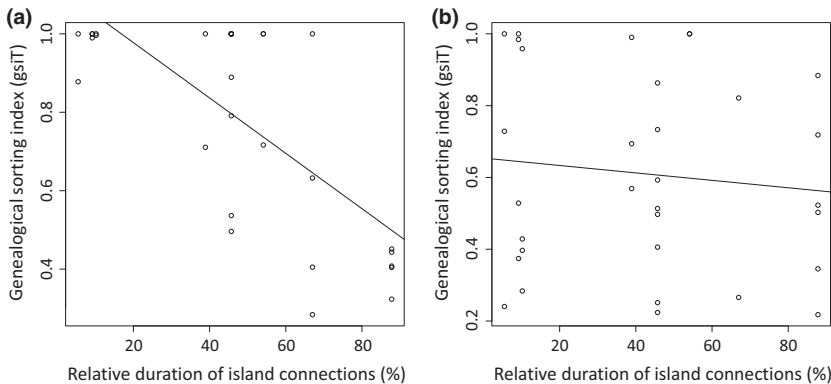


Fig. 4 Correlation between the degree of lineage sorting (as quantified using the genealogical sorting index) and the relative duration of island connections (as approximated based on bathymetric data and the local sea level change curve by Lambeck 1996) for (a) stable-habitat ('soil') taxa ($R^2_{adj} = 0.48$, $P \ll 0.001$), (b) disturbed-habitat (sand-obligate) taxa ($R^2_{adj} = 0.01$, $P = 0.54$). See Fig. S1 (Supporting information) for the actual range of values of the genealogical sorting index per taxon and per island.

Discussion

The observed phylogeographic concordance among species associated with stable habitats, despite strong discordance across the whole assembly of darkling beetle species considered here, supports the role of island connectivity cycles as drivers of divergence across the Cycladic plateau, while also demonstrating that a global model of phylogeographic concordance is unrealistically

too conservative for evaluating dynamic models of diversification. Moreover, given that the taxa targeted here are closely related, with similar inherent dispersal capabilities, our study highlights the need for similarly refined models of concordance across disparate taxa and/or across sets of islands with different levels of connectivity. Below, we discuss what these findings imply about the expectations for concordance under the PAIC model of diversification, and more generally

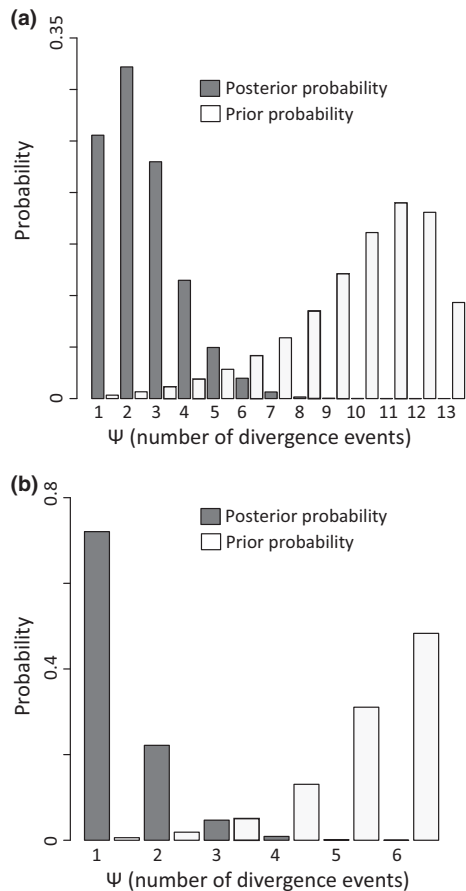


Fig. 5 Results of hierarchical approximate Bayesian computation analyses using *dpp-msbayes*. Prior and posterior distributions of the number of divergence events (Ψ) under (a) a null model of phylogeographic concordance across all 13 taxa and (b) a refined model of phylogeographic concordance across six stable-habitat ('soil') taxa.

about the use of refined versus generic null models of concordance in comparative phylogeography.

Properties of PAIC systems that affect population divergence

Our results highlight how certain properties of PAIC systems, such as topography, bathymetry or surface area, can play a major role in shaping patterns of population divergence across such island complexes. Specifically, for stable-habitat taxa, the levels of population divergence across the Cycladic plateau can be largely predicted by bathymetry. The degree of mitochondrial lineage sorting on each island is positively correlated with the relative duration of island connections during the last glacial cycle (Fig. 4a), while a model of 'isolation by bathymetry', taking into account the changes in population connectivity via landbridges through time,

fits the data significantly better than a simple model of isolation by distance, in most stable-habitat lineages (Table 2).

These results in combination suggest an important role of the periodically exposed shelf in maintaining gene flow among islands: the longer the duration of the island connections, the higher the amount of migrants exchanged between islands, either purely as a function of time, or potentially due to increased habitat suitability on landbridges that are exposed for longer periods. Additionally, the width of the corridor itself may play a role too, similarly to the width of habitat corridors in continental landscapes (Tischendorf & Wissel 1997; Haddad 1999). For example, Andros Island, which is connected to Tinos by relatively shallow waters but through a narrow corridor (Fig. 1), shows high degrees of lineage sorting (Fig. S1, Supporting information), while such an effect would be difficult to disentangle in the case of Donoussa where the narrow corridor coincides with the deepest bathymetric split (Fig. 1).

These observations highlight further the need to refine the traditional PAIC paradigm, which targets groups of islands connected at the last glacial maximum (by tracing the 120-m bathymetric contour; e.g. Heaney 1986; Brown & Diesmos 2009; Siler *et al.* 2010) without distinguishing further among them based on detailed bathymetric data. While we here followed a fairly simplified approach, based on the local sea level curve for the last glacial cycle and present-day bathymetric data to approximate at a coarse scale the exposure of the currently submerged shelf over time, more detailed geological models are being developed to simulate paleo-configurations of island archipelagos (Ali & Aitchison 2014; Rijdsdijk *et al.* 2014) and can be employed for informing phylogeographic models in PAIC systems.

Bathymetric data alone do not appear to be the only factor structuring patterns of divergence. For example, despite the predominant influence of bathymetry, it is not by itself a good predictor for the levels of population divergence on the small island Donoussa on the Cycladic plateau. While Donoussa is separated by the deepest bathymetric split (105 m) and a very narrow corridor (Fig. 1) from Naxos, this split only accounts for a high proportion of the genetic variance in the case of the two small-sized *Eutagenia* species (hierarchical AMOVA results, Table 1b), but not in the rest of the sampled taxa. The only stable-habitat lineage showing deep divergence across this split is *Eutagenia* sp. (Fig. 3a; an approximate estimate of divergence time based on the BEAST analysis of *Cox1* is 1.7–11.6 My 95% HPD), while *D. quadricollis* (Fig. 3d; 0.05–0.3 My) and *G. quadricollis* (Fig. 3f; 0.2–0.7 My) appear to have colonized the island

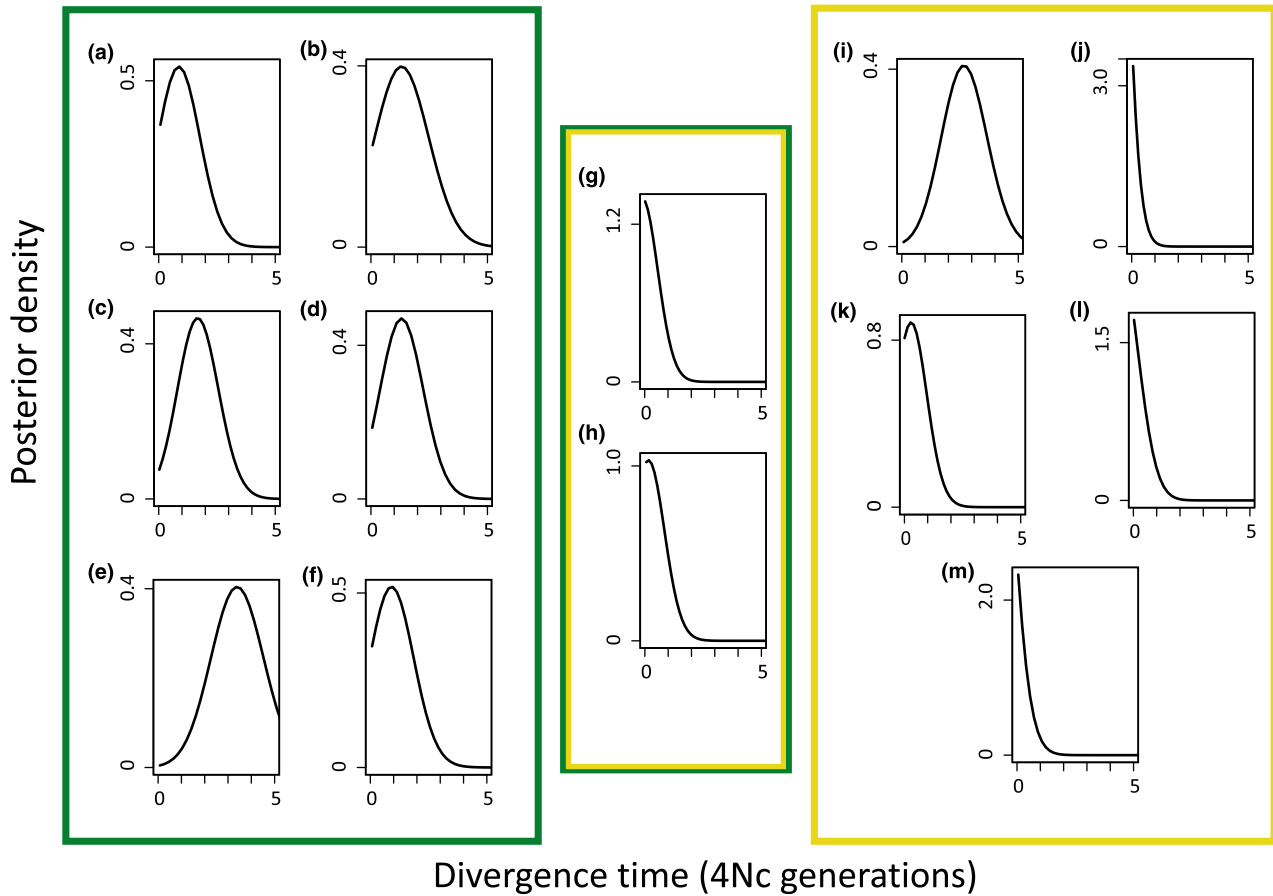


Fig. 6 Posterior density distributions of the marginalized estimates of divergence times for each taxon as approximated based on the 13-taxon hABC analyses (see Fig. 5a). Estimates of divergence times are given in units of $4N_C$ generations ($4N_C = \theta_C \mu$ where θ_C is the expectation of the prior on descendant population size). Given the mutation rates of the two loci (as estimated by Papadopoulou *et al.* 2010) and our prior distribution on descendant population size, and assuming a generation time of 1 year, one unit of $4N_C$ corresponds to approximately 1 490 000 years. The 13 taxa are numbered as (a–m) following Fig. 2. (a–f): soil, (g,h): generalists, (i–m): sand-obligate taxa.

more recently. This is also reflected in the partial Mantel test results for the latter two taxa, which only show a significant effect of bathymetry when the small islands are removed from the analysis (Table 2). This deviation from the general pattern predicted by the bathymetric data could reflect higher local extinction rates on these islands (Simberloff 1976; Heaney 1986), due to demographic stochasticity, reduced genetic variability and other related factors causing high vulnerability of small populations (Harrison 1991; Gaggiotti & Hanski 2004). Island elevation may also be an important factor affecting local extinction rates (Heaney 1984), although it is often tightly correlated with island size as found here, and thus, the effect of the two factors cannot be easily distinguished.

These results demonstrate that islands within a PAIC system cannot be treated as equivalent in phylogeographic models, especially if they differ greatly in size

and elevation during high sea level periods, which can affect long-term population persistence on each island and thus the propensity for population divergence and speciation (Dynesius & Jansson 2014). As such, using expectations of concordance of divergence times as a general test of the species-pump hypothesis when the taxa actually inhabit different islands (see Oaks *et al.* 2013) is not a reasonable test (i.e. we do not learn much about the effect of island connectivity cycles on the diversification process, neither about the taxa themselves, by rejecting a null hypothesis of concordance because of the inherent heterogeneity in a data set comprising islands with very different properties).

Trait-mediated responses to connectivity cycles

The effect of the aforementioned geographic properties of islands on levels of population divergence is not

uniform across species, but instead is clearly mediated by species-specific ecological traits. Importantly, the effect of bathymetry as described above is only apparent in the case of the stable-habitat taxa, while in the disturbed-habitat and generalist taxa there is no correlation between the degree of lineage sorting and the duration of island connections (Fig. 4b), with a very weak or non-significant fit to an 'isolation-by-bathymetry' model in most cases (Table 2) and very shallow divergence times across the major bathymetric split between the northern and southern plateau (Fig. 6g,h,j-m).

It is not that sand-obligate species (disturbed-habitat specialists) do not show evidence of geographic structure. Indeed, generally high genetic variance among islands is evident from AMOVA, but this structure is not associated with the major bathymetric splits (Table 1). The high partitioning of genetic variance among islands is consistent with frequent local extinction and recolonization due to habitat disturbance causing repeated population bottlenecks (Wade & McCauley 1988; Lande 1992; see also Papadopoulou *et al.* 2009), a process not strictly linked to the island connectivity cycles per se (hence, the lack of strong partitioning of genetic divergence across the bathymetric barriers discussed in the previous section among the sand dwelling taxa).

The two generalist species in contrast show low differentiation even among islands (Table 1), indicating higher levels of gene flow among island populations than both the stable- and disturbed-habitat specialists. This lack of geographic structure across the PAIC found in *Zophosis punctata* (Fig. 3g) and *Tentyria rotundata* (Fig. 3h) is somewhat surprising given their high levels of genetic divergence across the major biogeographic barriers of the Aegean archipelago (see Papadopoulou *et al.* 2010); however, this concordant response to the vicariant events between the generalist and stable-habitat taxa occurs at larger geographic scales than the one studied here. This difference in patterns found within the PAIC vs. across the permanent geological barriers indicates a role of the connectivity cycles and could be related to a high ability of the generalist taxa to migrate readily through the exposed landbridges during low sea level periods, irrespective of the actual duration of the island connections (given the lack of an isolation-by-bathymetry effect; Table 2; Fig. S1, Supporting information). That is, in these generalist flightless tenebrionids, the island connectivity cycles appear to inhibit, rather than promote diversification. A crucial role of habitat association in accounting for population connectivity across a PAIC system has also been proposed for Philippine mammals (Heaney *et al.* 2005), where tolerance of disturbed habitat appears to be as important as the mode of dispersal itself (flighted vs. flightless taxa) in structuring levels of gene flow among island populations.

Apart from habitat association, other species-specific traits such as body size may also affect population connectivity and local extinction rates across a PAIC system, as in many animal groups body size is correlated with dispersal propensity (Peters 1983; Gaston & Blackburn 1996) or area requirements (Biedermann 2003). Even though in the group of beetles studied here body size did not appear to be the most important predictor for the degree of lineage sorting (Table S5, Supporting information), there are some indications that population divergence in the smallest-sized species (<0.4 cm; *Ammobius rufus* and *Eutagenia* spp.) of both habitat types reflects more closely the effects of bathymetry than their larger-bodied counterparts (e.g. Table 1b; Figs S1 and 6i). These observations are consistent with reduced local extinction risk and/or reduced propensity to cross the exposed shelf in small-bodied taxa, although a more extensive sampling would be required to provide statistical evidence for this effect. Additional ecological traits not considered here may also be very relevant, such as trophic level, or reliance on mutualist species, which have been shown to affect species responses to fragmentation (for a review see Ewers & Didham 2006). Instead of selecting taxa that differ along these trait axes, we chose to focus on a uniform group of taxa in terms of trophic ecology and environmental niche (all taxa are detritivores and ground dwelling and adapted to warm and dry conditions), as well as inherent dispersal capabilities (all apterous), such that variation in patterns of genetic variation that might arise because of habitat associations, and secondarily body size, can be detected. Moreover, by demonstrating that even though the taxa are closely related and share many characteristics, a null model of phylogeographic concordance across this assemblage is nonetheless unrealistic, we underscore the limitations of approaches that rely on concordance across entire island communities (e.g. Oaks *et al.* 2013) as an evaluation of the role of the island connectivity cycles in driving diversification. In other words, the work highlights how crucial an understanding of ecological traits is for refining models of concordance across a PAIC system and providing insights into whether the cycles of population connectivity structure species divergence.

Refined vs. null models of concordance in comparative phylogeography

Our study clearly demonstrates how the support (or lack thereof) for the role of PAIC systems as drivers of divergence is very much a function of the null model used in the hABC analyses. Specifically, a prediction of concordance in divergence times is supported for a model with a single divergence event for the set of six

stable-habitat taxa (Fig. 5b). In contrast, when the same analysis was applied on the full set of thirteen taxa, there was moderate support for a model with two divergence events, with considerable posterior uncertainty (Fig. 5a) and great variation in divergence times (Fig. 6), as expected due to habitat-mediated differences in local extinction rate and dispersal propensity. Moreover, using the most recent implementation of the hABC pipeline (*dpp-msbayes*; Oaks 2014) with exponential/dirichlet process prior distributions and GLM regression postsampling adjustment, we detected a great amount of posterior uncertainty (Fig. 5a), which could have gone undetected because of the biases related to the uniform/U-shaped prior distributions implemented in *msbayes* (for details see Oaks *et al.* 2013, 2014; also see Fig. S4c (Supporting information) for the effect of the postsampling adjustment method on estimating posterior uncertainty). Additionally, by retaining the order of the summary statistics vector, we were able to obtain marginalized divergence time estimates for each taxon (Fig. 6), which helped considerably with interpreting our results, and this too would not have been possible with the conventional default option of re-sorting the summary statistics vector (see also Oaks 2014). In that sense, the new hABC implemented in *dpp-msbayes* (Oaks 2014) facilitates the detection and interpretation of discordance in divergence times, instead of seeking and emphasizing phylogeographic concordance, which has been a major trend in comparative phylogeography since its infancy (Avice 2000; Soltis *et al.* 2006; Hickerson *et al.* 2010) and has been indirectly encouraged by tests conducted with *msbayes* (see Oaks *et al.* 2013, 2014).

This is not to disavow the important insights provided by the commonalities in patterns of genetic variation among codistributed taxa, which have improved our understanding of the biogeographic and ecological processes that drive the evolution of entire communities (Arbogast & Kenagy 2001; Hewitt 2004; Hickerson *et al.* 2010). However, this emphasis has also promoted the tendency to attribute the lack of concordance to the vagaries of history (e.g. Kropf *et al.* 2003; Marske *et al.* 2012) and thus potentially disregard important deterministic processes associated with species-specific responses, such as microhabitat affinity (Massatti & Knowles 2014) or climatic niche (Moussalli *et al.* 2009). Within the context of climate change research, phylogeographic concordance has been widely interpreted as evidence for community-level responses and used for assigning conservation priorities (Carnaval *et al.* 2009; Fouquet *et al.* 2012), while individualistic responses to climate change have brought into question the refugia concept and the view of ecological communities as historical entities (Stewart *et al.* 2010; Marske *et al.*

2012). Recognizing and interpreting phylogeographic discordance under an appropriate study design can also be very meaningful as shown here (see also Dawson 2014; Massatti & Knowles 2014). Moreover, recognition that discordant patterns can have deterministic underpinnings can also help to develop refined models of concordance for sets of ecologically equivalent taxa with the potential to offer greater insights into the dynamics of species diversification compared with adhering to null models of global concordance that are unsurprisingly predisposed to be rejected (unless performed at large geographic scales where historical geologic events will override any taxon-specific responses to temporal and spatial differences in population connectivity).

Apart from the choice of analytical tools that can be critical for evaluating the prevalence of concordance and avoiding potential biases, the temporal scale and the resolution provided by the genetic markers are also critical for identifying discordance in divergence times among taxa. Relying on a limited number of loci unavoidably yields a great amount of uncertainty in divergence time estimation (Fig. 6). Only with sufficient resolution for rejecting models of simultaneous divergence can hABC models be applied effectively. Given that increasing the number of independent loci will reduce the confidence limits on divergence time estimates (Edwards & Beerli 2000; Zhu *et al.* 2015), and thereby will enhance the resolution required for rejecting models of simultaneous divergence, it remains to be seen how the shift towards genomic data in comparative phylogeographic studies will change the perceived support for phylogeographic concordance, not to mention open new opportunities for tests of more refined models in phylogeography (see He *et al.* 2013; Nadechowska-Brzyska *et al.* 2013; Reitzel *et al.* 2013; Massatti & Knowles 2014).

Concordance as a criterion for the 'species-pump' model

Even with refined expectations for concordance, is congruence in divergence times a good criterion for evaluating dynamic models of diversification like the 'species-pump' model? Our hABC results support a model of simultaneous divergence among all stable-habitat taxa, which could be taken as evidence in favour of the PAIC model (Oaks *et al.* 2013). However, the estimated mean divergence time is older than what expected under a dynamic 'species-pump' model, as it dates back to the early Pleistocene (~2.3 My, with 95% HPD intervals 0.5–4.7 My). Therefore, population divergence between the northern and southern plateau either could be associated with glacial cycles that predate the last glacial maximum, or might even coincide with the

original break-up of the Cycladic plateau into the present-day islands in the Late Pliocene (Dermitzakis 1990). This would suggest that the initial fragmentations of the Cycladic plateau promoted population divergence in stable-habitat taxa, which has not been erased by the repeated reconnections of the islands. This is not an unreasonable scenario given the relatively short periods of the connections between the northern and the southern plateau (i.e. the connections only makeup ~8% of the temporal period associated with the last glacial cycle). That is, the hABC results support the role of island fragmentation in promoting diversification in stable-habitat taxa, but not the role of island reconnections in providing opportunities for colonization, in order for the system to act as a 'species pump' (see also Gorog *et al.* 2004; Esselstyn & Brown 2009). As such, the results support a relatively static rather than a dynamic model of diversification. However, this could be partly due to the current focus on the deepest bathymetric splits separating islands. It remains to be determined whether for other islands, where the duration of connections might predominate the recent geologic history because of shallower waters among islands, the 'species pump' might better apply (e.g. Papadopoulou & Knowles 2015).

With the unprecedented resolution provided by genomic data, it would certainly be interesting to evaluate how the support for the 'species-pump' hypothesis may vary depending on the tempo of the island connections. For example, analysis of genomewide SNP data provided more direct evidence for the role of island connectivity cycles in driving divergence of cricket populations across a Caribbean PAIC system separated by shallow waters (Papadopoulou & Knowles 2015). In that study, estimated divergence times based on the site frequency spectrum coincided with a recent period of repeated connection and fragmentation of the islands at 75–130 ka (Papadopoulou & Knowles 2015). Unfortunately, the limited number of loci available here prohibits similar analyses to be performed across shallower splits, where there is a predominance of incomplete lineage sorting (Fig. S1, Supporting information). Moreover, given the limited number of loci, the use of the hABC approach within a comparative phylogeographic framework (as opposed to estimating divergence times individually in single species analyses) was necessary to account for coalescent stochasticity and gain analytical power from the simultaneous analysis of multiple population pairs (see Hickerson *et al.* 2006b; Hickerson & Meyer 2008). Nevertheless, the potential insights offered by refined models of concordance in comparative phylogeographic studies (as demonstrated here), when coupled with the increasingly accessible genomic data on a

range of taxa and PAIC systems, promises to elucidate some of the most interesting (albeit challenging) dynamics postulated about the contribution of island connectivity cycles to diversification patterns in archipelagos around the world, as well as the often debated merit of the 'species-pump' hypothesis.

Conclusions

The role of island connectivity cycles in driving population divergence and diversification has been surprisingly understudied, partly due to a long-standing emphasis on seeking phylogeographic concordance, even among ecologically dissimilar taxa, as a criterion for evaluating climate-driven diversification models. Here, instead of relying upon generic expectations of concordance, we focused on identifying and interpreting phylogeographic discordance under an appropriate study design that highlighted some of the geographic factors and ecological traits that affect population connectivity and persistence across a PAIC system, thus offering insights into the dynamic diversification process hypothesized by the 'species pump'. Our results (in particular, the demonstration of how support for the 'species-pump' hypothesis differs depending on how we refine the model of concordance in the hABC analyses) also highlight that phylogeographic studies on PAIC systems should incorporate detailed geological models and take advantage of genomic data to provide the required resolution for exploring trait-mediated responses and refining the contribution of island connectivity cycles across taxa and regions. Lastly, our study emphasizes the greater insights comparative phylogeographic work can provide when we move away from the concordance vs. discordance dichotomy.

Acknowledgements

This work was funded by NSF (DEB 1118815 to LLK). This study was made possible by the availability of specimens and sequence data collected as part of A.P.'s PhD project supervised by Alfried P. Vogler at Imperial College London and funded by the Greek Scholarships Foundation and the National Environmental Research Council (NERC grant NE/C510908/1 to APV). Collection of specimens was assisted by Ioannis Anastasiou, Malda Stalimerou, Sofia Terzopoulou and Fotini Spagopoulou and supported by a Collections Enhancement Grant from The Natural History Museum in London and funds from the University of Athens. We are grateful to Jamie Oaks for tips on the hABC analyses, to Jeet Sukumaran, Lawrence Heaney, Kurt Lambeck, Kenneth Rijdsdijk and Johannes Foufopoulos for discussions, to Christos Georgiadis for help with photographs, as well as to the Subject Editor and two anonymous referees for their helpful comments during the review process.

References

- Ali JR, Aitchison JC (2014) Exploring the combined role of eustasy and oceanic island thermal subsidence in shaping biodiversity on the Galápagos. *Journal of Biogeography*, **41**, 1227–1241.
- Arbogast BS, Kenagy GJ (2001) Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, **28**, 819–825.
- Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.
- Bell RC, MacKenzie JB, Hickerson MJ *et al.* (2012) Comparative multi-locus phylogeography confirms multiple vicariance events in co-distributed rainforest frogs. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 991–999.
- Biedermann R (2003) Body size and area-incidence relationships: is there a general pattern? *Global Ecology and Biogeography*, **12**, 381–387.
- Brown R, Diesmos A (2009) *Philippines, Biology. Encyclopedia of Islands*, pp. 723–732. University of California Press, Berkeley.
- Brown RM, Siler CD, Oliveros CH *et al.* (2013) Evolutionary processes of diversification in a model island Archipelago. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 411–435.
- Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C (2009) Stability predicts genetic diversity in the Brazilian Atlantic Forest hotspot. *Science*, **323**, 785–789.
- Carnaval AC, Waltari E, Rodrigues MT *et al.* (2014) Prediction of phylogeographic endemism in an environmentally complex biome. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20141461.
- Cummings MP, Neel MC, Shaw KL (2008) A genealogical approach to quantifying lineage divergence. *Evolution*, **62**, 2411–2422.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 772.
- Dawson MN (2014) Natural experiments and meta-analyses in comparative phylogeography. *Journal of Biogeography*, **41**, 52–65.
- Dermitzakis DM (1990) Palaeogeography, geodynamic processes and event stratigraphy during the Late Cenozoic of the Aegean area. *International Symposium on: Biogeographical Aspects of Insularity*, pp. 263–288. Accad. Naz. Lincei, Roma.
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, 699–710.
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.
- Dynesius M, Jansson R (2014) Persistence of within-species lineages: a neglected control of speciation rates. *Evolution*, **68**, 923–934.
- Edwards SV, Beerli P (2000) Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*, **54**, 1839–1854.
- Esselstyn JA, Brown RM (2009) The role of repeated sea-level fluctuations in the generation of shrew (Soricidae: Crocidura) diversity in the Philippine Archipelago. *Molecular Phylogenetics and Evolution*, **53**, 171–181.
- Ewers RM, Didham RK (2006) Confounding factors in the detection of species responses to habitat fragmentation. *Biological Reviews*, **81**, 117–142.
- Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.
- Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.
- Fouquet A, Noonan BP, Rodrigues MT *et al.* (2012) Multiple quaternary refugia in the eastern Guiana shield revealed by comparative phylogeography of 12 frog species. *Systematic Biology*, **61**, 461–489.
- Futuyma DJ (1987) On the role of species in anagenesis. *American Naturalist*, **130**, 465–473.
- Gaggiotti OE, Hanski IA (2004) Mechanisms of population extinction. In: *Ecology, Genetics and Evolution of Metapopulations* (eds Hanski IA, Gaggiotti OE), pp. 337–366. Elsevier Academic Press, San Diego.
- Gaston KJ, Blackburn TM (1996) Conservation implications of geographic range size-body size relationships. *Conservation Biology*, **10**, 638–646.
- Gorog AJ, Sinaga MH, Engstrom MD (2004) Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). *Biological Journal of the Linnean Society*, **81**, 91–109.
- Gutierrez D, Menéndez R (1997) Patterns in the distribution, abundance and body size of carabid beetles (Coleoptera: Caraboidea) in relation to dispersal ability. *Journal of Biogeography*, **24**, 903–914.
- Haddad NM (1999) Corridor use predicted from behaviors at habitat boundaries. *The American Naturalist*, **153**, 215–227.
- Harrison S (1991) Local extinction in a metapopulation context: an empirical evaluation. *Biological Journal of the Linnean Society*, **42**, 73–88.
- He Q, Edwards DL, Knowles LL (2013) Integrative testing of how environments from the past to the present shape genetic structure across landscapes. *Evolution*, **67**, 3386–3402.
- Heaney LR (1984) Mammalian species richness on islands on the Sunda Shelf, Southeast Asia. *Oecologia*, **61**, 11–17.
- Heaney LR (1986) Biogeography of mammals in SE Asia: estimates of rates of colonization, extinction and speciation. *Biological Journal of the Linnean Society*, **28**, 127–165.
- Heaney LR, Walsh JS, Peterson AT (2005) The roles of geological history and colonization abilities in genetic differentiation between mammalian populations in the Philippine archipelago. *Journal of Biogeography*, **32**, 229–247.
- Hewitt GM (2004) The structure of biodiversity – insights from molecular phylogeography. *Frontiers in Zoology*, **1**, 4.
- Hickerson MJ, Meyer CP (2008) Testing comparative phylogeographic models of marine vicariance and dispersal using a hierarchical Bayesian approach. *BMC Evolutionary Biology*, **8**, 322.
- Hickerson M, Dolman G, Moritz C (2006a) Comparative phylogeographic summary statistics for testing simultaneous vicariance. *Molecular Ecology*, **15**, 209–223.
- Hickerson MJ, Stahl EA, Lessios HA (2006b) Test for simultaneous divergence using approximate Bayesian computation. *Evolution*, **60**, 2435–2453.

- Hickerson MJ, Stahl E, Takebayashi N (2007) msBayes: pipeline for testing comparative phylogeographic histories using hierarchical approximate Bayesian computation. *BMC Bioinformatics*, **8**, 268.
- Hickerson M, Carstens B, Cavender-Bares J *et al.* (2010) Phylogeography's past, present, and future: 10 years after. *Molecular Phylogenetics and Evolution*, **54**, 291–301.
- Hickerson MJ, Stone GN, Lohse K *et al.* (2014) Recommendations for using msBayes to incorporate uncertainty in selecting an ABC model prior: a response to Oaks *et al.* *Evolution*, **68**, 284–294.
- Hope AG, Takebayashi N, Galbreath KE *et al.* (2013) Temporal, spatial and ecological dynamics of speciation among amphiberingian small mammals. *Journal of Biogeography*, **40**, 415–429.
- Hosner PA, Sanchez-Gonzalez LA, Peterson AT, Moyle RG (2014) Climate-driven diversification and Pleistocene refugia in Philippine birds: evidence from phylogeographic structure and paleoenvironmental niche modeling. *Evolution*, **68**, 2658–2674.
- Huang W, Takebayashi N, Qi Y, Hickerson MJ (2011) MTML-msBayes: approximate Bayesian comparative phylogeographic inference from multiple taxa and multiple loci with rate heterogeneity. *BMC Bioinformatics*, **12**, 1.
- Jordan S, Simon C, Foote D, Englund RA (2005) Phylogeographic patterns of Hawaiian Megalagrion damselflies (Odonata: Coenagrionidae) correlate with Pleistocene island boundaries. *Molecular Ecology*, **14**, 3457–3470.
- Kropf M, Kadereit JW, Comes HP (2003) Differential cycles of range contraction and expansion in European high mountain plants during the Late Quaternary: insights from *Pritzelago alpina* (L.) O. Kuntze (Brassicaceae). *Molecular Ecology*, **12**, 931–949.
- Lambeck K (1995) Late Pleistocene and Holocene sea-level change in Greece and south-western Turkey: a separation of eustatic, isostatic and tectonic contributions. *Geophysical Journal International*, **122**, 1022–1044.
- Lambeck K (1996) Sea-level change and shore-line evolution in Aegean Greece since upper palaeolithic time. *Antiquity*, **70**, 588–611.
- Lambeck K, Chappell J (2001) Sea level change through the last glacial cycle. *Science*, **292**, 679–686.
- Lambeck K, Purcell A (2005) Sea-level change in the Mediterranean Sea since the LGM: model predictions for tectonically stable areas. *Quaternary Science Reviews*, **24**, 1969–1988.
- Lande R (1992) Neutral theory of quantitative genetic variance in an island model with local extinction and colonization. *Evolution*, **46**, 381–389.
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.
- Marske KA, Leschen RA, Buckley TR (2012) Concerted versus independent evolution and the search for multiple refugia: comparative phylogeography of four forest beetles. *Evolution*, **66**, 1862–1877.
- Massatti R, Knowles LL (2014) Microhabitat differences impact phylogeographic concordance of codistributed species: genomic evidence in montane sedges (*Carex* L.) from the Rocky Mountains. *Evolution*, **68**, 2833–2846.
- Mazerolle M (2014) AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R package version 2.00. <http://CRAN.R-project.org/package=AICcmodavg>.
- McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences*, **104**, 19885–19890.
- McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology*, **89**, 2712–2724.
- McRae B, Shah V, Mohapatra T (2013) *Circuitscape 4 User Guide*. The Nature Conservancy. <http://www.circuitscape.org>.
- Morgan K, O'Loughlin SM, Chen B *et al.* (2011) Comparative phylogeography reveals a shared impact of pleistocene environmental change in shaping genetic diversity within nine *Anopheles* mosquito species across the Indo-Burma biodiversity hotspot. *Molecular Ecology*, **20**, 4533–4549.
- Moussalli A, Moritz C, Williams SE, Carnaval AC (2009) Variable responses of skinks to a common history of rainforest fluctuation: concordance between phylogeography and palaeo-distribution models. *Molecular Ecology*, **18**, 483–499.
- Nadachowska-Brzyska K, Burri R, Olason PI *et al.* (2013) Demographic divergence history of pied flycatcher and collared flycatcher inferred from whole-genome re-sequencing data. *PLoS Genetics*, **9**, e1003942.
- Oaks JR (2014) An improved approximate-Bayesian model-choice method for estimating shared evolutionary history. *BMC Evolutionary Biology*, **14**, 150.
- Oaks JR, Sukumaran J, Esselstyn JA *et al.* (2013) Evidence for climate-driven diversification? A caution for interpreting ABC inferences of simultaneous historical events. *Evolution*, **67**, 991–1010.
- Oaks JR, Linkem CW, Sukumaran J (2014) Implications of uniformly distributed, empirically informed priors for phylogeographical model selection: a reply to Hickerson *et al.* *Evolution*, **68**, 3607–3617.
- Oksanen J, Blanchet FG, Kindt R *et al.* (2013) *vegan: Community Ecology Package*. R package version 2.0-9.
- Papadopoulou A, Knowles LL (2015) Genomic tests of the species-pump hypothesis: recent island connectivity cycles drive population divergence but not speciation in Caribbean crickets across the Virgin Islands. *Evolution*, **69**, 1501–1517.
- Papadopoulou A, Bergsten J, Fujisawa T *et al.* (2008) Speciation and DNA barcodes: testing the effects of dispersal on the formation of discrete sequence clusters. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 2987–2996.
- Papadopoulou A, Anastasiou I, Keskin B, Vogler AP (2009) Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: the effect of dispersal ability and habitat preference. *Molecular Ecology*, **18**, 2503–2517.
- Papadopoulou A, Anastasiou I, Vogler AP (2010) Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. *Molecular Biology and Evolution*, **27**, 1659–1672.
- Papadopoulou A, Anastasiou I, Spagopoulou F *et al.* (2011) Testing the species-genetic diversity correlation in the Aegean archipelago: toward a haplotype-based macroecology? *The American Naturalist*, **178**, 241–255.
- Peters RH (1983) *The Ecological Implications of Body Size*. Cambridge University Press, Cambridge.
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) *Tracer* version 1.6. Available from <http://beast.bio.ed.ac.uk/Tracer>.
- Reitzel A, Herrera S, Layden M, Martindale M, Shank T (2013) Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine inverte-

- brate phylogeography and population genomics. *Molecular Ecology*, **22**, 2953–2970.
- Rijsdijk KF, Hengl T, Norder SJ *et al.* (2014) Quantifying surface-area changes of volcanic islands driven by Pleistocene sea-level cycles: biogeographical implications for the Macaronesian archipelagos. *Journal of Biogeography*, **41**, 1242–1254.
- Roberts TE (2006) Multiple levels of allopatric divergence in the endemic Philippine fruit bat *Haplonycteris fischeri* (Pteropodidae). *Biological Journal of the Linnean Society*, **88**, 329–349.
- Salvi D, Schembri PJ, Sciberras A, Harris DJ (2014) Evolutionary history of the Maltese wall lizard *Podarcis filfolensis*: insights on the ‘Expansion-Contraction’ model of Pleistocene biogeography. *Molecular Ecology*, **23**, 1167–1187.
- Siler CD, Oaks JR, Esselstyn JA, Diesmos AC, Brown RM (2010) Phylogeny and biogeography of Philippine bent-toed geckos (Gekkonidae: Cyrtodactylus) contradict a prevailing model of Pleistocene diversification. *Molecular Phylogenetics and Evolution*, **55**, 699–710.
- Simberloff D (1976) Experimental zoogeography of islands: effects of island size. *Ecology*, **57**, 629–648.
- Smith BT, McCormack JE, Cuervo AM *et al.* (2014) The drivers of tropical speciation. *Nature*, **515**, 406–409.
- Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, **35**, 627–632.
- Soltis DE, Morris AB, McLachlan JS, Manos PS, Soltis PS (2006) Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**, 4261–4293.
- Stewart JR, Lister AM, Barnes I, Dalen L (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 661–671.
- Stone GN, Lohse K, Nicholls JA *et al.* (2012) Reconstructing community assembly in time and space reveals enemy escape in a Western Palearctic insect community. *Current Biology*, **22**, 532–537.
- Tajima F (1983) Evolutionary relationship of DNA-Sequences in finite populations. *Genetics*, **105**, 437–460.
- Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.
- Takahata N, Nei M (1985) Gene genealogy and variance of interpopulational nucleotide differences. *Genetics*, **110**, 325–344.
- Tischendorf L, Wissel C (1997) Corridors as conduits for small animals: attainable distances depending on movement pattern, boundary reaction and corridor width. *Oikos*, **79**, 603–611.
- Wade MJ, McCauley DE (1988) Extinction and recolonization – their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.
- Watterson G (1975) On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology*, **7**, 256–276.
- Wegmann D, Leuenberger C, Neuenschwander S, Excoffier L (2010) ABCtoolbox: a versatile toolkit for approximate Bayesian computations. *BMC Bioinformatics*, **11**, 116.
- Zhu T, dos Reis M, Yang Z (2015) Characterization of the uncertainty of divergence time estimation under relaxed molecular clock models using multiple loci. *Systematic Biology*, **64**, 267–280.

A.P. and L.L.K. designed the study, A.P. generated and analysed the data, and A.P. and L.L.K. wrote the manuscript.

Data accessibility

All sequence data have been submitted to the European Nucleotide Archive and are available through GenBank, and all voucher specimens have been deposited to The Natural History Museum in London (see Table S7, Supporting information for Accession nos and voucher numbers, as well as for geographic coordinates of sampling localities). Alignments, tree files and input files for hABC and Mantel test analyses are available on Dryad (doi:10.5061/dryad.mk3dc).

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Additional hABC analyses.

Table S1 Taxonomic classification and average size of the 13 darkling beetle species.

Table S2 Topographic characteristics of the studied islands.

Table S3 Sampling sizes per taxon, per island and per locus.

Table S4 Results of locus-by-locus hierarchical AMOVA analyses.

Table S5 Model comparisons to assess the relative importance of four factors that may affect the degree of lineage sorting on each island.

Table S6 Four alternative prior sets for hABC analyses.

Table S7 Coordinates, voucher numbers and sequence accession numbers of all specimens included in this study.

Fig. S1 Heatmap showing the degree of lineage sorting per taxon and per island.

Fig. S2 Validation analyses based on 1300 pseudo-observed datasets.

Fig. S3 Assessing sensitivity to alternative prior distributions.

Fig. S4 Assessing different post-sampling adjustment regimes.

Fig. S5 Comparing the two alternative regimes of ordering the summary statistics vector.