Ecological coassociations influence species' responses to past climatic change: an example from a Sonoran Desert bark beetle

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Abstract

Ecologically interacting species may have phylogeographical histories that are shaped both by features of their abiotic landscape and by biotic constraints imposed by their coassociation. The Baja California peninsula provides an excellent opportunity to examine the influence of abiotic vs. biotic factors on patterns of diversity in plant-insect species. This is because past climatic and geological changes impacted the genetic structure of plants quite differently to that of codistributed free-living animals (e.g. herpetofauna and small mammals). Thus, 'plant-like' patterns should be discernible in host-specific insect herbivores. Here, we investigate the population history of a monophagous bark beetle, *Araptus attenuatus*, and consider drivers of phylogeographical patterns in the light of previous work on its host plant, *Euphorbia lomelii*. Using a combination of phylogenetic, coalescent-simulation-based and exploratory analyses of mitochondrial DNA sequences and nuclear genotypic data, we found that the evolutionary history of *A. attenuatus* exhibits similarities to its host plant that are attributable to both biotic and abiotic processes. Southward range expansion and recent colonization of continental Sonora from the Baja peninsula appear to be unique to this taxon pair and probably reflect influences of the host plant. On the other hand, abiotic factors with landscape-level influences on a diverse suite of codistributed arid-adapted taxa, such as Plio- and Pleistocene-aged marine incursions in the region, also left genetic signatures in beetle and host plant populations. Superimposed on these similarities, bark beetle-specific patterns and processes were also evident: our data revealed two secondarily sympatric, reproductively isolated genetic lineages, as well as a previously unrecognized mid-peninsular warm desert refuge. Taken together, this work illustrates that the evolutionary history of species-specific insect herbivores may represent a mosaic of influences, including—but not limited to—those imposed by the host plant.

Keywords: Baja California, Coleoptera, landscape history, population genetics, range expansion, vicariance

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Introduction

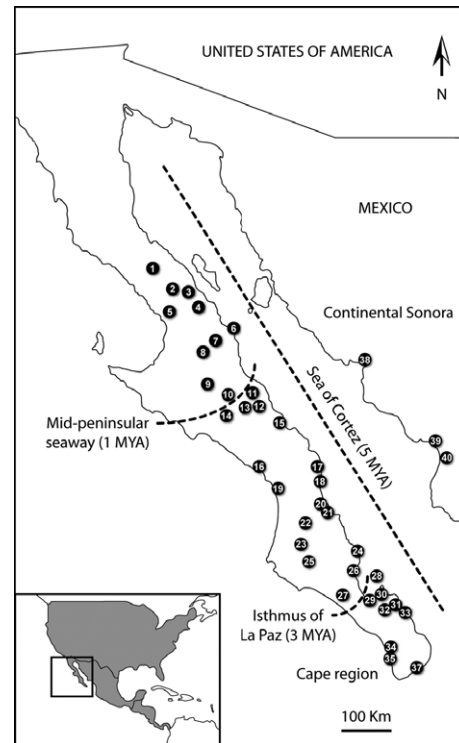Phylogeographical studies are of broad practical and theoretical importance because characterizations of species' responses to past climate change provide critical baseline data for predicting future impacts (McLachlan *et al.* 2005; Cordellier & Pfenninger 2009; Norgate *et al.* 2009). Similarly, determining the causes and consequences of evolutionary changes occurring at different historical stages along the continuum of population divergence [e.g. allele frequency shifts during

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range expansion (Excoffier *et al.* 2009) vs. partial- or complete reproductive incompatibility at secondary contact zones of long-isolated lineages (Moritz *et al.* 2009)] provides new insights into speciation processes. Empirical studies have shown that the interaction between physiogeographical landscape context and certain life history characteristics—particularly dispersal ability—can generate predictable outcomes for how species responded to Pleistocene climatic oscillations (e.g. Hugall *et al.* 2002; Garrick *et al.* 2008). Less well-known, however, is the extent to which evolutionary constraints imposed by biotic interrelationships among taxa have shaped present-day patterns of diversity within and among species. Indeed, ecologically interacting taxa may be particularly likely to show nonindependent histories (Nason *et al.* 2002; Smith *et al.* 2008, 2011; Aoki *et al.* 2011; Yu & Nason 2013).

Plant-insect species pairs from the Baja California peninsula, together with an adjacent section of the Sonoran Desert on mainland Mexico ('Baja peninsula' and 'continental Sonora' herein), provide useful models for examining the degree to which constraints imposed by biotic interactions may influence a species' capacity to track the changing distribution of habitats. This is because the dynamic geological and environmental history of this landscape seems to have impacted the genetic structure of plants and animals in different ways (e.g. Riddle *et al.* 2000; Zink *et al.* 2000; Nason *et al.* 2002; Clark-Tapia & Molina-Freaner 2003; Crews & Hedin 2006; Pfeiler *et al.* 2007, 2009; Garrick *et al.* 2009a), such that the influence of host plant phylogeographical history upon its obligate insect parasite or pollinator should be discernible.

Plio- or Pleistocene-aged vicariance events most likely underpin geographically clustered genetic breaks in numerous animal species along the Baja peninsula (Riddle & Hafner 2006). Based on molecular, geological and paleo-ecological data, Riddle *et al.* (2000) synthesized a spatially and temporally explicit general hypothesis of vicariance-driven diversification in the region. Three temporally discrete events are thought to have been particularly important in promoting allopatric divergence in arid-adapted species: (i) formation of the Sea of Cortez separating the Baja peninsula from continental Sonora [5 million years ago (Ma); Roberts 1989]; (ii) marine incursion across the Isthmus of La Paz that temporarily isolated most of the southern Cape Region from the rest of the peninsula (3 Ma; Riddle *et al.* 2000); and (iii) a 'cryptic' mid-peninsular seaway bisecting northern and central Baja peninsula (1 Ma; Upton & Murphy 1997; Fig. 1). Subsequent studies suggest that the mid-peninsular seaway was the most taxonomically pervasive vicariance event (e.g. Rodríguez-Robles & De Jesús-Escobar 2000; Whorley *et al.* 2004; Lindell *et al.* 2005, 2008; Crews &

Fig. 1 Map of the Baja peninsula and continental Sonora showing three major vicariance-driven phylogeographical breaks (dashed lines; Riddle *et al.* 2000 and references therein). Solid black circles represent *Araptus attenuatus* sampling localities (i.e. local populations, labelled 1–40 and correspond with those in Garrick *et al.* 2009a; site 36 had no beetle samples and is omitted). *Inset:* map of Central and North America showing the location of the study area (boxed).

Hedin 2006; Devitt 2006; Douglas *et al.* 2006; Recuero *et al.* 2006). However, geological evidence for a transient seaway at this location has remained elusive, and divergence times seem to vary considerably (Lindell *et al.* 2006 and references therein). Thus, an alternative explanation for spatial clustering of phylogeographical breaks is that this has consistently been a region of low-quality habitat (Grismer 2000, 2002). While these ideas are amenable to testing using paleo-ecological niche modelling, to date, they have not been investigated in this way.

In contrast to most animals, patterns of genetic diversity within and among Sonoran Desert plant populations seem to have been most profoundly influenced by post-Pleistocene range expansion. Plants may track changing local environmental conditions more closely than animals do because their phenologies and physiological tolerances can be strongly tied to precipitation or the frequency and severity of winter frosts (Van Devender 2002). Macrofossils preserved in packrat middens indicate early- to mid-Holocene northward range shifts of floristic communities in the region (Van Devender *et al.*

1994). Furthermore, molecular data from two similarly distributed cacti (*Lophocereus schottii* and *Stenocereus gummosus*) also show evidence of strictly northward range expansions (Nason *et al.* 2002; Clark-Tapia & Molina-Freaner 2003; also see Swenson & Howard 2005). Overall, patterns of genetic diversity in cacti indicate that the southern Cape Region served as an important refuge for frost intolerant taxa during Pleistocene glacial cycles, perhaps owing to its low latitude and deeply dissected, heterogeneous topography (Wells & Hunziker 1976). Interestingly, this *a priori* expectation of northward range expansion contrasts sharply with observed patterns of genetic variation in a long-lived Sonoran Desert euphorb (*Euphorbia lomelii* V.W. Steinm., synon. *Pedilanthus macrocarpus*), for which there is strong evidence for southward range expansion (Garrick *et al.* 2009a; Dyer *et al.* 2010).

While the dynamic geological and environmental history of the Sonoran Desert region may differentially affect the phylogeography of animals and plants, a diversity of plant-feeding insects are specialists and through a long history of ecological coassociation may be expected to exhibit geographical congruence in genetic structure with their host plant. The extent to which codistributed species share a common biogeographical and, consequently, phylogeographical history has been argued to vary depending on the strength and specificity of ecological relationships between them (Whiteman *et al.* 2007; Mikheyev *et al.* 2008). Although the influence of host plant phylogeographical history on that of associated insects has rarely been tested (Smith *et al.* 2008, 2011; Aoki *et al.* 2011), we expect the congruence of their genetic structures to increase with host specificity. It should be especially strong in obligate pollination mutualisms because migration in the pollinator may be associated with pollen-mediated gene flow in the plant. However, it should also be strong in monophagous insect herbivores and seed predators despite antagonistic interactions with host plants, given that the sizes and geographical distributions of their populations are expected to track that of their hosts through time.

In the present study, we extend our previous work on the comparative population structure of arid-adapted plant-insect communities in the Sonoran Desert (Nason *et al.* 2002; Dyer & Nason 2004; Garrick *et al.* 2009a; Dyer *et al.* 2010). Specifically, we evaluate the impact of the phylogeographical history of a host plant, *E. lomelii,* on that of its bark beetle herbivore, *Araptus attenuatus* Wood (Curculionidae). Our primary expectation is that ecologically interacting species pairs will show strong congruence in spatial-genetic patterns and underlying demographic processes.

Materials and methods

Focal species and ecological niche models

Araptus attenuatus is known only from senescing stems of its host plant, *E. lomelii.* Adults are small (1.4–1.6 mm long), and as with many bark beetles that exhibit sexual dimorphism in the frons, *A. attenuatus* has an out-breeding mating system (L. R. Kirkendall, pers. comm.; Garrick *et al.* 2009b). To explore evidence for spatial co-occurrence of regions of sustained low-quality habitat along the Baja peninsula with locations of hypothesized historical marine inundations, we constructed Ecological Niche Models (ENMs) for *A. attenuatus* based on its host plant's distribution. Host plant locations ($n = 380$) were determined via physical census by the authors and a survey of all available herbarium records with spatial coordinates of sufficient accuracy. Two models were estimated: a present-day ENM and a Last Glacial Maximum (LGM) projection ('paleo-ENM' herein). Climate layers used for the estimation of ENMs were based on 18 BioClimatic layers for current conditions at a resolution of 30-arc seconds (Hijmans *et al.* 2005). These layers represent mean and variance in temperature and precipitation that have previously been shown to influence the spatial distribution of genetic variance in the host plant (Dyer *et al.* 2010). Corresponding layers describing conditions during the LGM were retrieved from the Model for Interdisciplinary Research on Climate (MIROC v3.2, K-1 Model Developers 2004). ENMs for present-day occurrence were created using presence-only data, with the algorithm implemented in MAXENT v3.3.3k (Phillips *et al.* 2006). This was performed using the default convergence threshold (10–5), random seed, 5000 maxiterations, 20 replicate runs and 25% of the sites for training of the model. We allowed MAXENT to select the variable regularization and functions automatically. Once specified, the ENM was projected onto LGM climate layers. The output was a continuous probability of host plant occurrence for current and LGM landscapes.

Population sampling and molecular data sets

Araptus attenuatus individuals were sampled from up to 10 plants at each of 39 sites ('local populations' herein) along the Baja peninsula and in continental Sonora, encompassing the known range of the species (Fig. 1; Appendix I). In most cases, only one beetle per plant was assayed for genetic variation to avoid sampling kin groups. Genomic DNA was extracted using a DNeasy® animal tissue kit (Qiagen). A ~500-bp fragment of mitochondrial *cytochrome oxidase I* (mt*COI*) was amplified via polymerase chain reaction using primers C1-J-1718 (5′-GGAGGATTTGGAAATTGATTAGTTCC-3′, Simon *et al.*

1994) and ArCOI-R (5′-TAGAATGTATAMTTCKGGGTGT
CC-3′, this study). Amplifications were performed with a
PTC-200 thermocycler (MJ Research) in 10 μL volumes
containing 2 μL 5 × *GoTaq®* Flexi buffer (Promega), 2 mM
MgCl$_2$, 200 μM each dNTP, 5% bovine serum albumin
(New England Biolabs), 0.5 μM each primer, 0.5U *GoTaq®*
DNA polymerase (Promega) and 1 μL template DNA. The
following profile was used: 95 °C 2 min (one cycle), 95 °C
30 s, 46 °C 30 s, 72 °C 1 min (35 cycles), 72 °C 2 min (one
cycle). Amplicons were purified using ExoSAP-IT® (USB
Corporation) and then sequenced on a MegaBACE 1000
(Amersham Biosciences) following the manufacturer's
recommendations. Sequences were edited and aligned in
MEGA v4.0 (Tamura *et al.* 2007), generating a final unambig-
uous alignment of 403-bp from 292 individuals. Individuals
(n = 365) were also genotyped at eight codominant nuclear
loci (Appendix I). This marker set comprised intron-span-
ning DNA regions from the following genes: *enolase, elonga-
tion factor-1α, wingless, muscle protein 20, kuzbanian, ATP
synthetase* subunit *α, lysidyl aminoacyl transfer RNA synthetase*
and an anonymous microsatellite locus. Molecular assays
included restriction-fragment-length polymorphism for five
loci, and insertion–deletion mutation screening for three
length-variable loci (full details in Garrick *et al.* 2009b).

Deep-time phylogeographical analyses

Phylogenetic tree estimation. To assess evidence for ancient
lineage splitting events potentially associated with Plio-
or Pleistocene-aged vicariance events, Bayesian phyloge-
netic analysis of *A. attenuatus* mt*COI* sequences (n = 117
nonredundant haplotypes) was performed with BEAST
v1.4.8 (Drummond & Rambaut 2007). Searches used the
best-fit substitution model (GTR+I+G) identified via AIC
in MODELTEST v3.7 (Posada & Crandall 1998), empirical
base frequencies, a Yule process tree prior, user-optimized
tuning, with other priors set as default. Final searches
used 2×10^6 MCMC generations burn-in followed by
2×10^7 generations, sampling every 2000th step. Chain
convergence was assessed on the basis of effective sample
size values (all > 300) using TRACER v1.4, as well as compari-
son of two replicate runs. These were pooled in LOGCOMBIN-
ER v1.4.8, and then the maximum clade credibility tree and
posterior probability node support values were calculated
using TREEANNOTATOR v1.4.8 (Drummond & Rambaut 2007).
Tree rooting used the best available out-group, *Araptus* sp.
SCH05 (GenBank accession AF187123). For comparison,
relationships among haplotypes were also estimated
using a statistical parsimony network approach, imple-
mented in TCS v. 1.21 (Clement *et al.* 2000).

Approximate Bayesian computation. To further assess
higher-level phylogenetic relationships among three
well-supported major mt*COI* clades identified using the

above phylogenetic analysis (see Results), and to estimate
splitting times among populations in which these mt*COI*
sequences had evolved, we used Approximate Bayesian
Computation implemented in DIY-ABC v1.0.4.46b (Cornuet
et al. 2010). This approach employs summary statistics
to characterize an empirical data set and then calculates
the same summary statistics from numerous data sets
simulated under alternative historical scenarios. The
posterior probability of each scenario is then used to
identify the best-fit model (Bertorelle *et al.* 2010).

In the present study, alternative scenarios included
all three possible topologies of a bifurcating three-taxon
tree. Scenarios had the same parameters and uniform
priors as follows: five effective population size (N_e)
parameters (one for each of the three contemporary
major mt*COI* clades, plus two for ancestral populations;
$N_e = 1.5 \times 10^3$–1.5×10^7) and two splitting time (t)
parameters (one for each bifurcation in a three-taxon tree;
$t = 6 \times 10^4$–1.8×10^7 generations before present, and
with conditional prior $t_2 > t_1$). Assuming three genera-
tions per year (based on related bark beetles from similar
climates; Dreistadt *et al.* 2004), the lower bound on split-
ting times corresponds with the height of the LGM, and
the upper bound pre-dates formation of the Sea of Cortez
by 1 Ma. We used the best available mutation model
(HKY+I+G) with the proportion of invariable sites and
gamma shape distribution parameters taken from MODEL-
TEST estimates. Following Ruiz *et al.* (2009), we used a
broad uniform prior for Coleoptera mtDNA mutation
rate (8.3×10^{-10}–5.8×10^{-8} substitutions per lineage
per generation), which encompasses the vast majority
of insect mtDNA rates reported in the literature. We
used summary statistics that measure aspects of within-
population genetic variation, and so redundant mt*COI*
haplotypes were included. The following were calcu-
lated: number of distinct haplotypes and segregating
sites, number of private segregating sites, mean and
variance of pairwise nucleotide differences and mean and
variance of the rarest nucleotide at segregating sites.
DIY-ABC runs consisted of 4.5×10^6 simulations (1.5×10^6
per scenario). Following Cornuet *et al.* (2010), model
checking was performed via principal components analy-
sis, and then posterior probabilities of scenarios were
determined via logistic regression performed on 1% of
simulated data sets closest to the empirical data set.
Finally, posterior distributions of the parameters of the
best-fit model were then re-estimated using the closest
1% of simulated data sets, with a 'logit' transformation.

Shallow-time phylogeographical analyses

Regional populations. Codominant nuclear marker data
from individuals sampled along the Baja peninsula,
where the distribution of *A. attenuatus* is relatively

continuous, were analysed using STRUCTURE v2.2.3 (Pritchard *et al.* 2000). This method can identify the number and spatial distributions of distinct genotypic clusters ('regional populations' herein). Furthermore, because all sampled individuals were geo-referenced, this clustering analysis has the potential to pinpoint locations of abrupt genetic discontinuities on the landscape. Searches were performed for $K = 1–7$ (four replicates each) using 'correlated allele frequency' and 'admixture ancestry' models, 10^5 MCMC generations burn-in and a run length of 10^6 generations. The smallest K-value that captured the major structure in the data was determined following the study by Pritchard *et al.* (2000), and the estimated membership coefficient (Q-value) for each individual ($n = 327$) determined the composition of clusters. Relationships among regional populations were estimated via neighbour-joining using Cavalli-Sforza & Edwards' (1967) chord distance, with node support assessed via 1000 bootstrap replicates, calculated in PHYLIP v3.5c (Felsenstein 1993).
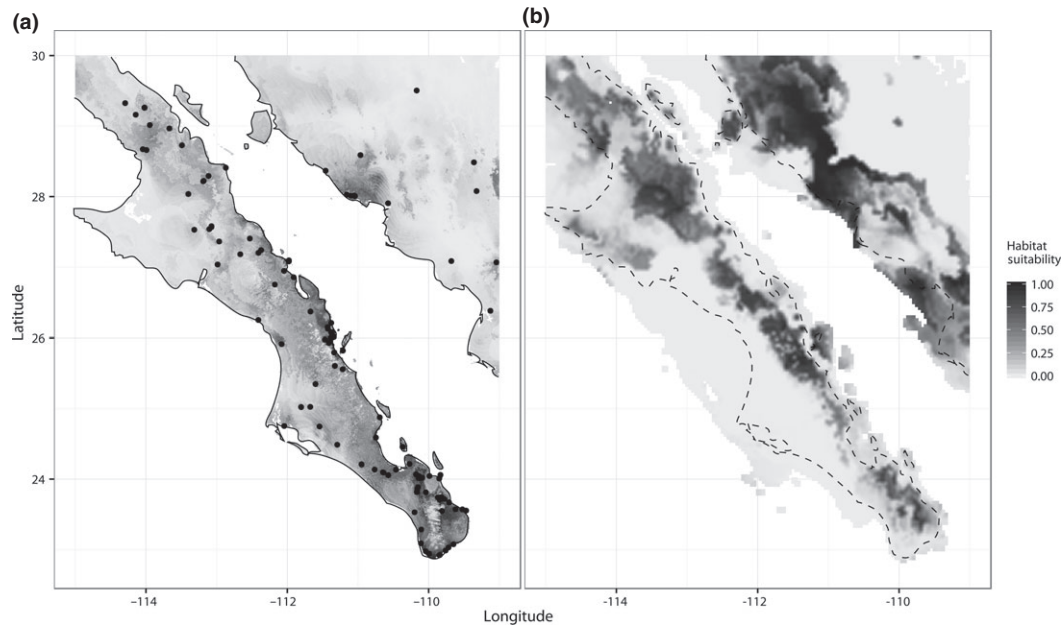
Phylogeographical structure among regional populations. To relate observed patterns of genetic variation to underlying processes, we wanted to determine whether differentiation among regional populations had arisen on short timescales where drift is the primary driver of allele frequency change vs. longer timescales where both drift and mutation play a role. One approach that follows the rationale of Pons & Petit (1996) and Hardy *et al.* (2003) is to test whether evolutionary relationships among DNA sequences contain signal beyond that in haplotype frequencies alone (Garrick *et al.* 2010). To do this, we used an analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) framework to analyse mt*COI* sequences from pairs of regional populations using two alternative measures of between-group variance. The first measure (Φ_{ST}), an analogue of Weir & Cockerham's (1984) estimate of F_{ST}, treats all mt*COI* haplotypes equally, whereas the second measure ($\Phi_{ST\ corrected}$) incorporates maximum-likelihood corrected genetic distances among haplotypes (GTR+I+G model as determined by MODELTEST). In the absence of phylogeographical structure, the ratio of the two measures is expected to be approximately equal. Conversely, when phylogeographical structure is present, $\Phi_{ST\ corrected} > \Phi_{ST}$. Maximum-likelihood corrected distances were calculated in PAUP* v4.0b10 (Swofford 2002), and AMOVAs were performed using ARLEQUIN v.3.11 (Excoffier *et al.* 2005), with significance of Φ_{ST} determined via 10 000 permutations.

Range expansion

Spatial diversity gradients. Given the existing evidence for southward range expansion of the host plant,

E. lomelii, along the Baja peninsula (Garrick *et al.* 2009a), here we focussed on identifying the geographical origin and directionality of range expansion in *A. attenuatus*. Latitudinal trends in levels of within-population genetic diversity can be informative about the directionality of past expansion, with lower diversity expected in more recently established populations (Hewitt 1996). Accordingly, we assessed spatial trends in diversity among local populations within each *A. attenuatus* regional population from the Baja peninsula. We estimated nuclear allelic richness (A_R) using rarefaction as implemented in HP-RARE v1.0 (Kalinowski 2005), subsampling three diploid individuals per local population to correct for unequal sample sizes (local populations with insufficient data were omitted). A_R-values were summed across loci to yield a multilocus diversity measure. The same general approach was used for mt*COI* sequence data, except that we used Nei's (1987) haplotypic diversity (H) estimated in DNASP v4.10.3 (Rozas *et al.* 2003). Significance of systematic changes in diversity for both genetic data types was determined by nonparametric regression on latitude using the least squares method.

Demographic growth. Spatial expansion is often associated with rapid growth, and so we used Bayesian skyline analyses (Drummond *et al.* 2005) of mt*COI* sequences to examine the nature and magnitude of size changes over time in Baja peninsula regional populations. Searches were implemented in BEAST and used a coalescent (Bayesian skyline) tree prior, linear skyline model, the best-fit mt*COI* substitution model from MODELTEST, user-optimized tuning and other priors as default. For the purpose of exploring the directionality, magnitude and relative timing of past population size changes (and for generating coarse estimates of absolute timing), we used Brower's (1994) standard arthropod mtDNA rate (assuming three generations/year). Final searches were performed using $1–1.5 \times 10^7$ MCMC generations and sampling parameters every 2000th step (10% discarded as burn-in), with two replicate runs combined. The frequency distribution of haplotypes can also carry signal of recent growth or decline, and so a series of complementary analyses were performed. For each Baja peninsula regional population, we estimated Fu's (1997) F_S from mt*COI* sequences using DNASP. The null hypothesis of constant population size was tested by comparing the observed value with a null distribution simulated via neutral coalescence (10^4 replicates). Significance at the lower tail was assessed at the 0.02 level, following Fu (1997). Ramos-Onsins & Rozas' (2002) R_2, a statistic based on the difference between the number of singleton sites and the average number of nucleotide differences, was used to re-evaluate the same null hypothesis in regional populations with < 50 sequences.

Fig. 2 Ecological Niche Models (ENMs) for *Araptus attenuatus* based on the distribution of its host plant, *Euphorbia lomelii*. (A) Distribution of habitat suitability given current climatic conditions, with all ENM sample locations shown as solid dots. (B) Projection of current ENM onto MIROC Last Glacial Maximum climate reconstructions.

Results

Ecological niche models

Historical habitat suitability. The present-day ENM estimated for *A. attenuatus*, based on its host plant, is shown in Fig. 2A. The best-fit ENM has variables representing mean temperature range and precipitation seasonality (Table S1, Supporting information). Model validation, as determined by 20 replicate random partitions, resulted in the following mean (±standard deviation where appropriate) parameters: $AUC_{Training} = 0.949$, $AUC_{Test} = 0.873$ ($\pm$ 0.042), Area = 0.313 ($\pm$0.025), $Omission_{Test} = 0.232$ ($\pm$0.066) and Binomial probability = 1.24×10^{-8}. Estimated habitat suitability during the LGM (Fig. 2B) revealed a discontinuous distribution along the Baja peninsula. Of particular note in the re-projection is the division of suitable habitat into three discrete regions (i.e. north, central and south) and that poor-quality habitats roughly coincide with locations of hypothesized historical sources of vicariance (Fig. 1).

Deep-time phylogeographical analyses

Phylogenetic tree estimation. We detected 117 nonredundant mt*COI* haplotypes from 292 *A. attenuatus* individuals. All sequences had open reading frames when translated and were consistent with Lunt *et al.*'s (1996) model of insect *COI*. Three well-supported and geographically cohesive major clades were recovered on the maximum

clade credibility phylogenetic tree (Fig. 3; these same major groupings were also reflected by the statistical parsimony network shown in Fig. S1, Supporting information). On the peninsula, Clade B (Baja) is widespread and diverse (*n* = 57 haplotypes, 199 individuals), whereas the Clade C (Cape Region) is predominantly in the south and has fewer haplotypes (*n* = 40 haplotypes, 60 individuals)—interestingly, its geographical distribution is completely nested within the range of Clade B. Conversely, Clade S (Sonora) is allopatric and found only on continental Sonora (*n* = 20 haplotypes, 33 individuals). Among-clade divergences were large (mean uncorrected *p* = 8.4–10.1%; net *p*-distances = 7.0–8.9%), as reflected by disconnected haplotype networks being formed (i.e. the 95% confidence eight-step connection limit was exceeded; Fig. S1, Supporting information). The tree indicates a sister relationship between Clades C and S (albeit with only modest node support, posterior probability = 0.88, Fig. 3), whereas Clade B is early-branching with respect to sister Clades C and S. Topologically, these relationships are not consistent with the hypothesized temporal sequence of vicariance events in the region (Riddle *et al.* 2000; Introduction).

Approximate Bayesian computation. DIY-ABC analysis of the complete mt*COI* sequence data set strongly supported the same relationships as in the maximum clade credibility phylogenetic tree (posterior probability = 0.90; type I error rate = 0.12). Point estimates and confidence inter-
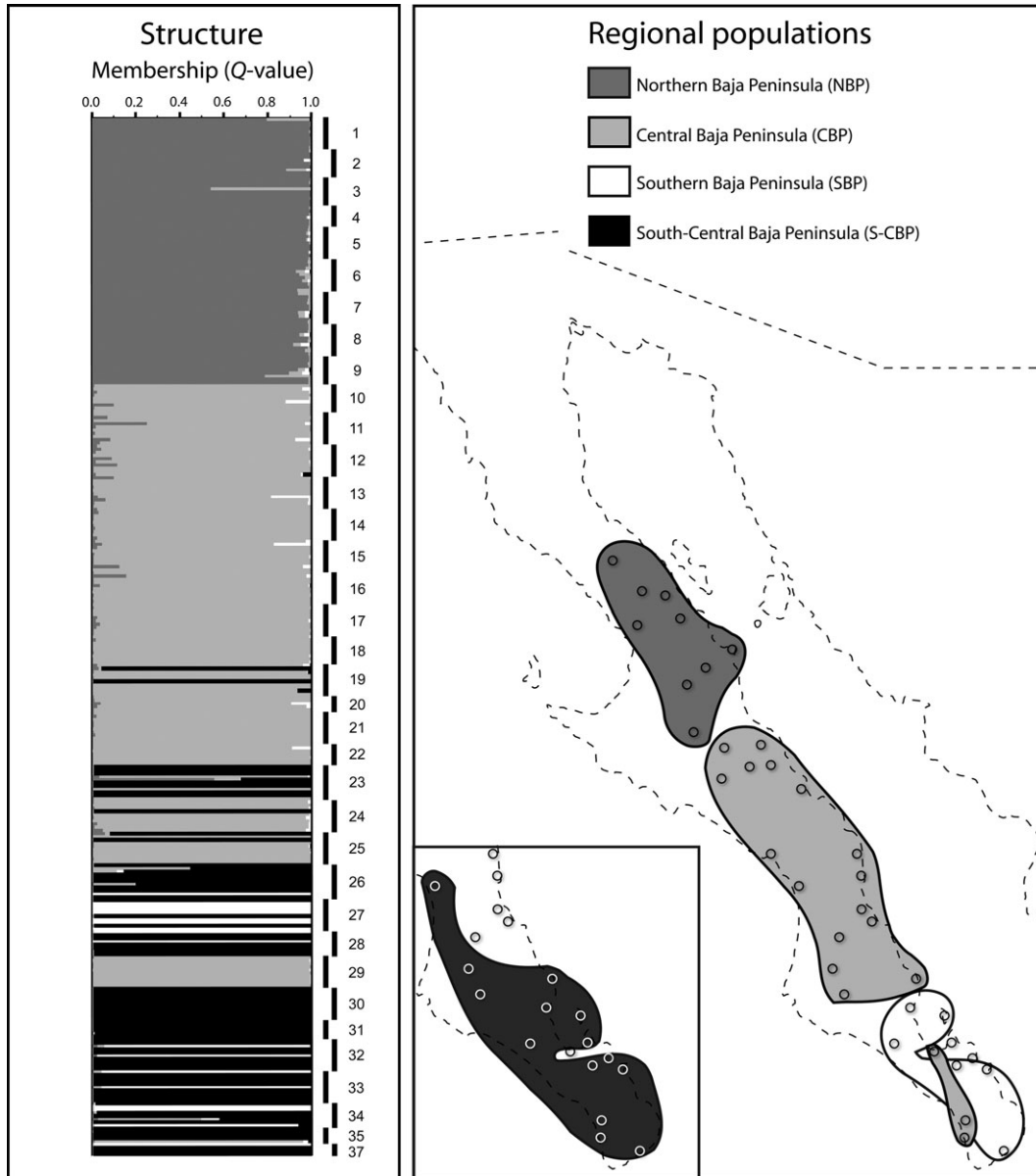
Mitochondrial DNA (*COI*)

Fig. 3 Maximum clade credibility phylogenetic tree estimated from 117 *Araptus attenuatus* mt*COI* haplotypes. Gray numbers above nodes are posterior probability support values. The in-group is divided into three reciprocally monophyletic major clades; Clade B (Baja) and Clade C (Cape Region) are sympatric and restricted to the Baja peninsula, whereas Clade S (Sonora) is allopatric and restricted to continental Sonora.

vals (CIs) for all parameters of the best-fit DIY-ABC model are given in Fig. S2 (Supporting information). The estimated mean splitting time of populations in which Clade C and S mt*COI* lineages evolved ($t_1 = 0.68$ Ma, 90% CI = 0.13–2.04) post-dates all three hypothesized major vicariance events in the region (i.e. mid-peninsular seaway, 1 Ma; Isthmus of La Paz, 3 Ma, Sea of Cortez, 5 Ma; see Introduction). Conversely, the earlier splitting event between the two populations that presently co-occur in parts of the Baja peninsula and carry Clade B or C haplotypes ($t_2 = 4.30$ Ma, 90% CI = 0.44–13.60) pre-dates mid-peninsular and Isthmus of La Paz events.

As the mt*COI* tree topology and estimated splitting times were not consistent with Riddle *et al.*'s (2000) general hypothesis of vicariance-driven diversification in the region, we conducted a *post hoc* analysis to investigate an alternative dispersal-based divergence history.

Considering the inferred topological relationship [i.e. (B, (C, S))], two competing scenarios were plausible: (i) a single colonization of continental Sonora from a peninsular Cape Region ancestor, (ii) and two independent and temporally spaced colonizations of the Baja peninsula from a continental ancestor. The key difference between these scenarios relates to bottleneck events that occur during long-distance overwater dispersal and subsequent founding of a new population. Thus, in our *post hoc* DIY-ABC analysis, we attempted to distinguish among these two competing scenarios on the basis of these putative bottleneck events (see Appendix S1, Supporting information). This showed that the single-event colonization scenario in which a peninsular Cape Region ancestor was the original source of the present-day continental Sonoran lineage was most strongly supported (posterior probability = 0.68; type I error = 0.22). Point estimates

Fig. 4 Left panel: Bayesian inference of the number of distinct genotypic clusters ('regional populations') of *Araptus attenuatus* along the Baja peninsula, and individual membership in each. Sampled bark beetles are represented by a horizontal column divided into $K = 4$ segments (white, pale gray, dark gray and black), where area is proportional to membership (Q-value) in each cluster. Individuals are arranged by local population (numbered 1–37), sequentially ordered from north (top) to south (bottom). Right panel: Spatial distributions of the four Baja peninsula clusters. Local populations are represented as open circles and correspond to those in Fig. 1.

and CIs for all parameters of the best-fit model that includes a bottleneck event in the population in which mt*COI* Clade S evolved are given in Fig. S3 (Supporting information).

Shallow-time phylogeographical analyses

Regional populations. The minimum number of clusters capturing the major structure in the nuclear genotypic data from individuals along the Baja peninsula was
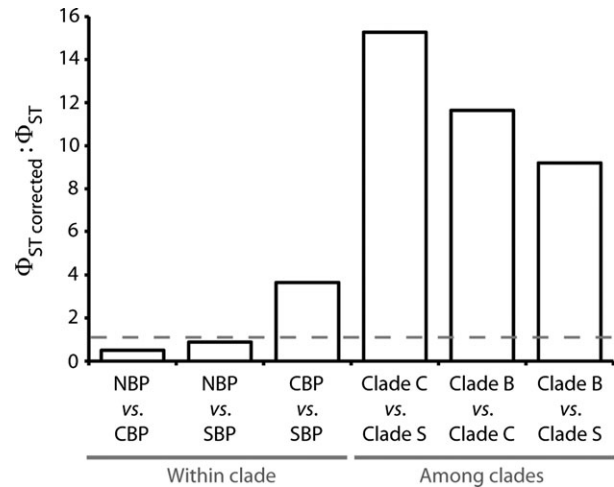
$K = 4$ (Fig. S4, Supporting information). Three of these (i.e. northern, central and southern Baja peninsula; NBP, CBP and SBP, respectively) show little or no geographical overlap (Fig. 4), and their members carry only mt*COI* haplotypes that belong to Clade B (Fig. 3). Furthermore, two zones of abrupt spatial-genetic discontinuity that mark the boundaries of these regional populations NBP vs. CBP and CBP vs. SBP (Fig. 4) roughly coincide with the locations of hypothesized sources of historical vicariance (i.e. mid-peninsular seaway and Isthmus of La Paz

inundation; Fig. 1), as well as the locations of poor habitat suitability during the LGM (Fig. 2B). Neighbour-joining clustering of nuclear allele frequency data indicates that the NBP/CBP split is the most recent (Fig. S5, Supporting information). Members of the fourth regional population identified by STRUCTURE, south-central Baja peninsula (S-CBP), carry only mt*COI* haplotypes that belong to Clade C (Fig. 3). The geographical range of S-CBP overlaps with that of CBP and SBP, yet despite this sympatry, 93.6% of S-CBP individuals were strongly assigned ($Q \geq 0.90$) to their own cluster. Overall, weakly assigned individuals were few (i.e. only 1.8% of the total sample had $Q = 0.70–0.79$), and in this area of co-occurrence, there was no indication of any meaningful admixture [only two individuals had approximately equal ancestry in both S-CBP and another co-occurring cluster ($Q = 0.46–0.55$), but both also had substantial missing data, that is, 37–62% of loci not scored]. Thus, our data strongly suggest reproductive isolation of the S-CBP regional population.

Phylogeographical structure among regional populations. AM-OVAs revealed significant mt*COI* differentiation for all pairwise combinations of regional populations NBP, CBP and SBP (all with $P < 0.01$). However, drift alone can account for differentiation of regional populations flanking the location of putative mid-peninsular vicariance (NBP vs. CBP $\Phi_{ST\ corrected}$: $\Phi_{ST} < 1$), and there was only very modest phylogeographical structure between those flanking the Isthmus of La Paz (CBP vs. SBP $\Phi_{ST\ corrected}$: $\Phi_{ST} = 3.7$; (Fig. 5, left columns). Conversely, all among-clade comparisons showed strong phylogeographical structure, with $\Phi_{ST\ corrected}$: Φ_{ST} ratios > 9 (Fig. 5, right columns).

The lack of strong mt*COI* phylogeographical structure among regional populations with spatial-genetic boundaries that nonetheless roughly correspond with Riddle *et al.*'s (2000) predictions based on ancient vicariance was surprising, and so we investigated this further. One often-invoked cause of gene tree-species tree discordance is incomplete lineage sorting. To test the plausibility of this explanation, a series of coalescent simulations were performed using MESQUITE v2.5 (Maddison & Maddison 2008; Fig. S6, Supporting information). We focussed on the proposed timescales of hypothesized Pleistocene-/Pliocene-aged marine inundations (i.e. mid-peninsular seaway, 1 Ma; Isthmus of La Paz, 3 Ma; plus a composite 'two-tiered vicariance' scenario including both events). In addition, given the close similarly between the paleo-ENM habitat stability surface (Fig. 2B) and geographical ranges of the three Baja peninsula regional populations (Fig. 4), we also ran simulations under a model of simultaneous divergence at the height of the LGM (0.18 Ma). Deep Coalescences (Maddison 1997) was used to mea-

Fig. 5 Assessment of mt*COI* phylogeographical structure based on the ratio of $\Phi_{ST\ corrected}$: Φ_{ST} (*y*-axis), calculated within an AMOVA framework. Dashed gray line indicates 1:1 ratio, above which phylogeographical structure exists. Within-clade comparisons include regional populations NBP, CBP and SBP, all of which are comprised of individuals that carry only mt*COI* Clade B haplotypes (left columns); among-clade comparisons are included as benchmarks for strong phylogeographical structure (right columns).

sure the expected level of gene tree vs. population tree discord that is attributable to incomplete lineage sorting. Significance of deviation from the null hypothesis of incomplete lineage sorting was assessed with $\alpha = 0.05$ (two-tailed test). Additionally, as *A. attenuatus* is flight-capable, we also explored the impacts on phylogeographical inference of incorporating low levels of postdivergence migration into the coalescent simulations (Appendix S1, Supporting information).
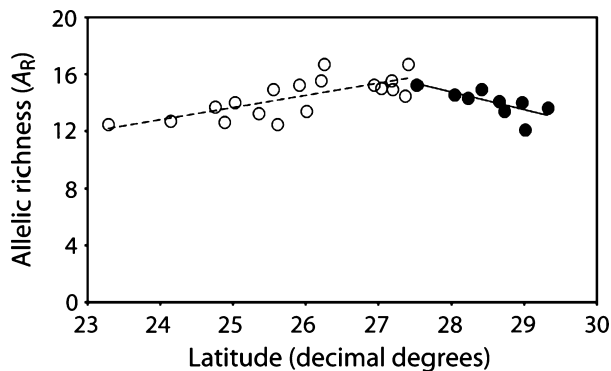
Coalescent simulations showed that the LGM scenario of simultaneous divergence among regional populations NBP, CBP and SBP was easily rejected, irrespective of assumptions about migration. Conversely, provided that at least moderate postdivergence migration occurred among regional populations (i.e. probability of migration/individual/generation ranging from 10^{-4} to 10^{-5}), incomplete lineage sorting could not be rejected either for the Isthmus of La Paz vicariance scenario or for the 'two-tiered' model that includes both the mid-peninsular and Isthmus of La Paz vicariance events (Table 1). More generally, our simulations showed that postdivergence migration has a strong impact on null distributions generated—and ultimately, historical inferences made—under this commonly used analytical framework (Figs S6 and S7, Supporting information).

Range expansion

Spatial diversity gradients. Consistent with spatial-genetic patterns in the host plant (Garrick *et al.* 2009a), latitudinal

Table 1 Coalescence-with-migration simulations performed under four alternative Baja peninsula vicariance scenarios, focussing on regional populations NBP, CBP and SBP. For each scenario/migration probability combination, we list the probability of the empirical Deep Coalescences (*DC*) value under the null distribution of *DC*-values obtained under of model neutral coalescence (two-tailed test with significance assessed at the 0.05 level; ns = not significant, [Y] = younger population splitting time than predicted by incomplete lineage sorting, [O] = older; see Figs S6 and S7, Supporting information)

		Migration probability			
Scenario	Timing (Ma)	Zero	1.0×10^{-6}	1.0×10^{-5}	1.0×10^{-4}
Last Glacial Maximum	0.018	<0.002[O]	<0.002[O]	<0.002[O]	<0.002[O]
Mid-peninsular seaway	1	<0.002[Y]	<0.002[Y]	ns	<0.002[O]
Isthmus of La Paz	3	<0.002[Y]	<0.002[Y]	ns	ns
Two-tiered vicariance	1–3	<0.002[Y]	0.006[Y]	ns	ns

Fig. 6 Regression of genetic diversity (*y*-axis) on latitude (*x*-axis). Genetic diversity was measured via nuclear allelic richness within local populations along the Baja peninsula, which are plotted from south (left) to north (right). Relationships were analysed separately for regional populations NBP (solid circles and trend line) and CBP (open circles and dashed trend line). Regional population SBP had too few data points and is not shown.

changes in nuclear allelic richness indicated that *A. attenuatus* regional population CBP had also expanded southward (slope = 0.854, R^2 = 0.544, d.f. = 1,16, F = 19.057, P < 0.001; Fig. 6). In contrast, regional population NBP showed a signature of northward expansion (slope = −1.239, R^2 = 0.534, d.f. = 1,7, F = 8.001, P = 0.025). Regional population SBP was omitted from the analysis owing to insufficient sample size (Appendix I). Taken together, spatial patterns of allelic richness are consistent with two expansion events sourced from a single geographical origin (~27.5 degrees latitude, between local populations 9 and 10). No significant latitudinal correlation in mt*COI* haplotypic diversity was detected for any regional population.

Demographic growth. The Bayesian skyline plot for regional population NBP indicated a signature of abrupt and rapid growth. Assuming Brower's (1994) standard arthropod mtDNA rate (and three generations/year),

this growth originated perhaps only ~0.05 Ma (i.e. 150 000 generations ago, Fig. S8, top, Supporting information). A more gradual and smaller-magnitude increase in N_e over time can be seen on the skyline plot for regional population CBP (Fig. S8, middle, Supporting information), and this growth may have begun earlier than in NBP. In contrast to regional populations NBP and CBP, the skyline plot for SBP is indicative of constant size. Additional support for growth, based on the frequency distribution of mt*COI* haplotypes, was evident only for regional population CBP (Table S2, Supporting information).

Discussion

Direct interactions between organisms and abiotic landscape features (e.g. mountains, streams, local climate) have been a focus of studies that aim to infer past events that generated present-day spatial patterns of intraspecific biodiversity, and the processes that maintain them. Few studies, however, have considered how additional constraints imposed by biotic interactions may influence a species' capacity to track the changing distribution of preferred habitats through periods of climate change. Species-specific associations between insect herbivores (or parasites) and their host plant may promote lock-step responses to landscape-level changes (Nason *et al.* 2002). In such cases, demographic histories may be tightly linked and thus show congruent spatial and phylogenetic patterns of genetic structure (e.g. Smith *et al.* 2008, 2011).

Generally speaking, codifferentiation of plant-insect herbivore species pairs should be largely driven by factors affecting the host distribution, given that an asymmetry of interdependence exists (i.e. the plant can survive without its insect herbivore, but not *vice versa*). In the case of frost-sensitive Sonoran Desert flora, range contraction and re-expansion out of southern refugia in response to Pleistocene glacial–interglacial cycles is expected to have most strongly impacted genetic structure (Nason

et al. 2002; Garrick *et al.* 2009a). Thus, coassociated insect herbivores are likely to exhibit spatial patterns of genetic variation that evolved under the constraints of frost-sensitivity, even if the insect itself is relatively frost tolerant. In addition, there may be abiotic factors that impose restrictions to gene flow that affect only the insect herbivore, such that multiple divergent lineages arise within the geographical range of a single host plant lineage (Nason *et al.* 2002). Thus, while the phylogeographical history of an insect herbivore should more closely resemble that of its host plant compared with other codistributed but free-living animals, some species-specific differences may be superimposed on its 'plant-like' evolutionary history.

In the present study, phylogeographical inferences for *A. attenuatus* are consistent with the idea that this bark beetle has tracked the changing distribution of its host plant, *E. lomelii*, with high fidelity. However, several differences also exist. Below, we highlight aspects of the phylogeography of *A. attenuatus* that are likely a consequence of this bark beetle's evolutionary trajectory being closely tied to that of its host plant (i.e. driven by biotic constraints) and then note additional similarities that may simply be due to shared landscape experience (i.e. common abiotic influences). Next, we discuss unique aspects of the phylogeography of *A. attenuatus* and conclude by pointing to areas of future research.

Phylogeographical history influenced by the host plant

Southward range expansion. A noticeable similarity between the phylogeographical histories of *A. attenuatus* and its host plant, which appears to be unique to this taxon pair, is the directionality of past range expansion along the Baja peninsula. The unexpected southward expansion previously reported for *E. lomelii* (Garrick *et al.* 2009a; Dyer *et al.* 2010) was generally mirrored by its insect herbivore. For *A. attenuatus*, southward expansion in regional population CBP was inferred from latitudinal patterns of genetic diversity at nuclear loci (Fig. 6), coupled with evidence for demographic growth based on mt*COI* sequences (Table S2, Fig. S8, Supporting information). This event was characterized by a relatively gradual increase in N_e, perhaps during a time frame coincident with increasing aridity following the penultimate (or an earlier) glaciation (Fig. S8, Supporting information).

In contrast to *A. attenuatus* and *E. lomelii*, genetic signatures of strictly northward range expansions are seen in codistributed cacti (*Lophocereus schottii* and *Stenocereus gummosus*; Nason *et al.* 2002; Clark-Tapia & Molina-Freaner 2003) and Sonoran Desert cactus beetles (*Moneilema armatum* and *M. gigas*; Smith & Farrell 2005). In the case of cacti, the timing of expansion is considered to be

relatively recent (i.e. Holocene-aged), as is demographic growth in Sonoran Desert brittlebush (*Encelia farinosa*) inferred from patterns of variation in chloroplast DNA (cpDNA) sequences (Fehlberg & Ranker 2009). For cactus beetles, however, this timing was inferred to be considerably older (~1.5 Ma), and molecular evidence for demographic growth of considerable antiquity also exists for several other Sonoran Desert insects (Pfeiler *et al.* 2007, 2009). In considering possible explanations for these temporal differences, we speculate that invertebrates may have a greater propensity to capture and retain signatures of past population size changes owing to generally faster molecular evolutionary rates and shorter generation times.

Colonization of continental Sonora from the Baja peninsula. The second prominent similarity between *A. attenuatus* and *E. lomelii* is that long-distance overwater dispersal seems to have been the main driver of differentiation between mainland and peninsular populations (Garrick *et al.* 2009a; Figs S2 and S3, this study, Supporting information). The Sea of Cortez represents a long-standing, permanent physiogeographical barrier, with an absence of land bridges estimated at 5 Ma (Roberts 1989). Genetically isolated peninsular and continental lineages have been reported for diverse organisms and at a variety of levels of biological organization [e.g. congeneric species: deer mice, pocket mice and antelope squirrels (Riddle *et al.* 2000), spiders (Crews & Hedin 2006) and cactus beetles (Pfeiler *et al.* 2010); subspecies: lyresnakes (Devitt 2006); populations: toads (Riddle *et al.* 2000), pseudoscorpions (Pfeiler *et al.* 2009) and fruit flies (Smith *et al.* 2012)]. These taxa exhibit deep molecular divergences that seem to be consistent with ancient vicariance, rather than more recent dispersal. Thus, successful colonization following long-distance overwater dispersal, as seen in *A. attenuatus* and *E. lomelii*, is not typical. Furthermore, for both members of this plant-insect species pair, DNA sequence data (cpDNA, Garrick *et al.* 2009a; mt*COI*, this study) identified the Baja peninsula as hosting the source population, whereas continental Sonora was the recipient of colonists. With the exception of *Drosophila mojavensis* (Smith *et al.* 2012), the directionality of this source vs. sink relationship is also unusual.

Mitochondrial DNA sequence data from *A. attenuatus* suggest a single colonization of continental Sonora from the Baja peninsula, tentatively estimated at ~0.60–0.68 Ma (Figs S2 and S3, Supporting information). However, temporal concordance with long-distance colonization by its host plant is difficult to assess with the present data. Anecdotally, however, continental *E. lomelii* populations do harbour some unique cpDNA haplotypes. Thus, given these DNA sequence mutations probably arose

after the initial dispersal event, colonization by Baja peninsular founders is not likely to have been very recent (Garrick *et al.* 2009a). Taken together, inferences for this plant-insect species pair confirm that even rare events such as occasional long-distance dispersal may nonetheless have large impacts on spatial-genetic structure (de Queiroz 2005). Given the close ecological association between *A. attenuatus* and *E. lomelii*, we hypothesize that their arrival in continental Sonora was contemporaneous; however, the mode of long-distance overwater dispersal (e.g. rafting, phoresy) remains unclear.

Phylogeographical history influenced by abiotic factors

Two-tiered vicariance on the Baja peninsula. Several features of the phylogeographical histories of *A. attenuatus* and *E. lomelii* are similar not only to one another, but also to those inferred for a broader suite of codistributed arid-adapted taxa. Past landscape-level events that elicited shared community-wide responses are indicative of major changes in abiotic environment (e.g. geological or climatic; Avise 2000). On this basis, Riddle *et al.* (2000) hypothesized that spatially clustered, multi-taxon phylogeographical breaks along the Baja peninsula were attributable to past marine inundations at or near these present-day suture zones. Although still controversial—particularly the proposed mid-peninsular seaway for which corroborating geological evidence remains elusive (Lindell *et al.* 2006 and references therein)—subsequent studies have provided some support for this general model.

Molecular data from *E. lomelii* showed strong signal of mid-peninsular vicariance consistent with the proposed 1 Ma time frame, and there was also some indication of an Isthmus of La Paz nuclear genetic discontinuity (Garrick *et al.* 2009a). In *A. attenuatus*, the spatial locations of nuclear genetic discontinuities between three regional populations (NBP, CBP and SBP) closely correspond with those of hypothesized mid-peninsular and Isthmus of La Paz vicariance (Figs 1 and 4). Furthermore, topological relationships among these regional populations are consistent with the hypothesized temporal sequence of vicariance events (Fig. S3, Supporting information). Although no strong mt*COI* phylogeographical structure was associated with nuclear genetic breaks, simulations (Fig. S6, Supporting information) showed that this can be explained by incomplete lineage sorting (assuming low-to-modest postdivergence migration rates; Table 1, Fig. S7, Supporting information). Indeed, even when population sizes are extremely large, as may be common for euphorb-associated bark beetles (Jordal & Hewitt 2004), nuclear genotypic data are expected to reveal spatial substructure more rapidly than DNA sequence genealogies, given that diploid combinations of alleles are reshuffled every generation, whereas new mutations are usually slow to accumulate and spread throughout a population (Crandall *et al.* 2000; Sunnucks 2000; Garrick *et al.* 2010; Bohonak & Vandergast 2011). Female-biased dispersal represents an alternative explanation for the observed discrepancy between nuclear marker and mtDNA-based genetic structuring that warrants investigation. However, based on the available data and outcomes from coalescent simulations, a two-tiered vicariance model seems plausible for both *A. attenuatus* and *E. lomelii*, as it does for several codistributed animals. Thus, some aspects of the phylogeographical histories of this plant-insect species pair follow Riddle *et al.*'s (2000) geologically driven predictions for the whole region.

Interestingly, the paleo-ENM for *A. attenuatus* (Fig. 2B) supports an alternative hypothesis of ecologically driven gene flow limitation (Grismer 2000, 2002). During the LGM, potentially suitable habitats were partitioned into three discrete regions, with intervening poor-quality habitats spatially coincident with historical sources of vicariance (i.e. mid-peninsular seaway, and Isthmus of La Paz; Fig. 2B). Given that secondary contact zones may gravitate towards poor-quality habitats owing to concomitant population 'density troughs' (Moritz *et al.* 2009), present-day spatial clustering of multi-taxon phylogeographical breaks does not necessarily pinpoint a single geographical or temporal origin of past vicariance. Indeed, reanalysis of genetic data sets from several vertebrate species from the Baja peninsula has indicated multiple, temporally distinct, mid-peninsular vicariance events (Leaché *et al.* 2007). It remains unclear, however, whether persistently inhospitable Pleistocene habitat alone is sufficient to generate the observed abrupt spatial-genetic discontinuities, or instead, has simply reinforced or amplified genetic bifurcations generated by geologically driven vicariance.

Bark beetle-specific patterns and processes

Sympatric Baja peninsula lineages. We found evidence for two reproductively isolated genetic lineages of *A. attenuatus* that are presently sympatric in the southern part of the Baja peninsula. Average net sequence divergences between members of these major mt*COI* clades were large, with initial divergence of populations estimated at ~4.6 Ma (Figs S2 and S3, Supporting information). Furthermore, membership coefficients estimated from nuclear genotypic data indicated that no apparent F_1 hybrids exist (i.e. STRUCTURE *Q*-values were well above thresholds established in both simulation and empirical studies; Vähä & Primmer 2006 and Garrick *et al.* 2012, respectively). Based on preliminary examination of representative specimens, the members of these two lineages are morphologically indistinguishable (A. Cognato,

pers. comm.). To our knowledge, this is the first report of sympatric 'cryptic' species from the Baja peninsula.

In the case of *A. attenuatus* from southern Baja, sympatric speciation seems improbable, given that co-occurring lineages do not show a sister relationship (Fig. 3), and the peninsula's complex tectonic history—characterized by repeated uplift, submergence and fragmentation (Grismer 2000, 2002)—makes past allopatric phases likely. Similarly, co-speciation of bark beetles and their host plant (e.g. Farrell & Sequeira 2004; Jordal & Hewitt 2004) is not supported by data from *E. lomelii*, which shows no evidence of cryptic species (Garrick *et al.* 2009a). That said, hydrocarbon profiles and growth form morphotypes of the host plant may show some broad-scale spatial structuring along the Baja peninsula (Sternburg & Rodriguez 1982), and microgeographical separation at the level of individual plants remains to be determined. Thus, an ecological driver of lineage splitting (and maintenance of lineage integrity) remains plausible. Nonetheless, at present, we favour a scenario of allopatric divergence followed by secondary sympatry, as inferred for euphorb-associated bark beetles in the Canary Islands (Jordal *et al.* 2006). Given the current geographical range and divergence time of the lineage represented by mt*COI* Clade C, a Pliocene-aged phylogeographical break in the vicinity of Loreto, which has been seen in two lizard species (Lindell *et al.* 2005, 2008), may have also played a role here.

A mid-peninsular refuge. Contrary to our expectation that *A. attenuatus* would show evidence of the same strictly southward spatial expansion along the Baja peninsula as inferred for its host plant, nuclear marker data indicated northward expansion of regional population NBP (Fig. 6). This event also carried the mt*COI* signature of recent and rapid demographic growth, perhaps initiated as recently as 0.05 Ma (Fig. S8, Supporting information). Consistent with a scenario of very recent northward expansion, a single mt*COI* haplotype (H029) occurred at relatively high-frequency in every NBP local population (this DNA sequence also occupies an internal position in a haplotype network and has many recent descendants radiating directly from it; Fig. S1, Supporting information). Most notably, however, this northward expansion of regional population NBP originated at or near the same mid-peninsular location as did southward expansion of regional population CBP (Fig. 6). Bidirectional range expansion from a single source likely pinpoints the location of an important habitat refuge (Garrick *et al.* 2008). Accordingly, we hypothesize that a mid-peninsular desert refuge for *A. attenuatus* occurred at ~27.5 degrees latitude. Given the indications of different temporal origins of NBP and CBP expansion events (Fig. S8, Supporting information),

this mid-peninsular refuge may have been relatively stable, repeatedly insulating local populations from regional climatic changes.

The fact that the same bidirectional range expansion was not seen in *E. lomelii* (Garrick *et al.* 2009a) may be attributable to contrasting levels of spatial and temporal resolution inherent in plant vs. insect molecular data sets, or to the genetic stochasticity associated with spatial expansions (Excoffier *et al.* 2009). Given this possibility of pseudo-incongruence, and assuming our molecular data are indicative of a genuine refuge, the sorts of landscape features that might facilitate retention of small, warm semi-deserts warrant consideration. Axelrod (1979) suggested that during the cooler and wetter periods of the Pleistocene, semi-desert habitats would have contracted into drier valleys and protected slopes, or survived in the driest sites bordering woodland, grassland or shrubby vegetation communities. Similarly, Wells & Hunziker (1976) noted that topographical features producing rain shadow effects were likely a key characteristic of Pleistocene refuges for the xerophytic creosote bush (*Larrea* spp.). The approximate location of the inferred *A. attenuatus* mid-peninsular refuge lies near the intersection of the Vizcaíno and Magdalena phytogeographical provinces (Roberts 1989). Interestingly, this region of considerable topographical complexity, with a series of high-elevation (>2000 m) volcanic mountains with north–south oriented ridgelines (i.e. Sierra San Francisco), was identified as having retained high-suitability habitat for *A. attenuatus* during the LGM (~28 degrees latitude, Fig. 2B). Taken together, a mid-peninsular desert refuge seems plausible.

Future research

The impact of postdivergence gene flow on phylogeographical reconstructions of past population splitting events has only recently begun to be examined (e.g. Muster *et al.* 2009). We found that inferences drawn from coalescent-simulation-based tests of alternative vicariance scenarios (Fig. S6, Supporting information) can be critically dependent on assumptions about levels migration—even when rates are very low (Table 1, Fig. S7, Supporting information). If this outcome applies broadly, long-term averages of postdivergence migration will need to be treated as a key component of any divergence model, much as N_e is currently recognized as a critical parameter. Thus, the generality of this conclusion warrants further investigation. The main finding of the present work, however, was that ecological co-associations may strongly influence species' responses to past climatic change. The present study provides an empirical example of how the demographic history of a flight-capable bark beetle can be constrained by its dispersal-limited, frost-

sensitive host plant. Again, given that the generality of this discovery remains unknown, further study of additional plant-insect species pairs is required. For this reason, we are following up the present work by investigating the comparative population structure of *L. schottii* (senita cactus) and *Upiga virescens* (senita moth) mutualism in the Sonoran Desert.

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Data accessibility

Appendix I

Araptus attenuatus collection sites (local populations are labelled 1–40 as in Garrick *et al.* 2009a; location 36 had no beetles and is omitted). Sample sizes refer to the number of individuals per local population screened for variation at eight nuclear markers (N_{nDNA}) and for mt*COI* sequence variation (N_{mtCOI})

Local population	Latitude	Longitude	Sample sizes	
			N_{nDNA}	N_{mtCOI}
Baja peninsula				
1	29.32541	−114.29353	10	10
2	29.01457	−113.94486	9	9
3	28.96651	−113.66787	9	8
4	28.72796	−113.48973	7	3
5	28.66056	−113.99141	10	3
6	28.40846	−112.86985	10	7
7	28.22308	−113.18263	10	7
8	28.03661	−113.39991	10	9
9	27.52944	−113.31609	9	7
10	27.36320	−112.96400	9	9
11	27.40498	−112.52959	10	7
12	27.20280	−112.40800	10	7
13	27.18232	−112.66552	10	9
14	27.03670	−112.98600	10	7
15	26.94589	−112.04613	10	10
16	26.24905	−112.40948	10	7
17	26.20876	−111.37833	10	8
18	26.01550	−111.35475	9	9
19	25.91409	−112.08062	10	7
20	25.60521	−111.32638	5	5
21	25.55757	−111.21563	10	8
22	25.34819	−111.60056	7	7
23	25.02470	−111.67500	11	7
24	24.87611	−110.69175	10	10
25	24.74642	−111.54410	10	7
26	24.58843	−110.74599	11	10
27	24.21150	−110.95100	10	7
28	24.45879	−110.36857	8	5
29	24.13389	−110.46236	10	10
30	24.21441	−110.27252	10	10
31	24.04380	−109.98900	6	5
32	24.01950	−110.09600	10	9
33	24.00789	−109.85071	10	9
34	23.28550	−110.10429	8	2
35	23.08984	−110.10910	5	1
37	23.07570	−109.64869	4	4
Continental Sonora				
38	27.90509	−110.57436	9	7
39	26.63783	−109.32700	19	18
40	26.38014	−109.12633	8	8

Supporting information

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

Appendix S1 Supplementary methods.

Table S1 Bioclimatic layers contributing ($\geq 5\%$) to the estimated Ecological Niche Model in terms of the percent to the estimated model and permutation importance.

Table S2 Tests of population growth within Clade B (Baja) based on the frequency distribution of mt*COI* haplotypes.

Table S3 Estimates of Θ for *Araptus attenuatus* regional populations used in coalescence-with-migration simulations (abbreviations follow Fig. 4 in the main text).

Fig. S1 Relationships among mitochondrial DNA haplotypes estimated using Statistical Parsimony in TCS v. 1.21 (Clement *et al.* 2000).

Fig. S2 Best-fit model of evolutionary relationships among populations in which major mt*COI* clades evolved (Clade B, Baja; Clade C, Cape Region; Clade S, Sonora), estimated using DIY-ABC.

Fig. S3 Best-fit model of colonization history of populations in which major mt*COI* clades evolved (Clade B, Baja; Clade C, Cape Region; Clade S, Sonora), estimated using DIY-ABC.

Fig. S4 Plot of the number of clusters (K; x-axis) vs. negative log likelihood ($-\ln L$) of the data (y-axis), based on four replicate runs per K using STRUCTURE.

Fig. S5 Neighbor-joining tree showing relationships among regional populations based on nuclear allele frequencies (chord distance, Dc).

Fig. S6 Model parameters of alternative vicariance scenarios tested using mt*COI* sequence data from *Araptus attenuatus*.

Fig. S7 Coalescence-with-migration tests of four alternative Baja peninsula vicariance scenarios: (A) Last Glacial Maximum, LGM, (B) mid-peninsular seaway, MPS, (C) Isthmus of La Paz, IoL, and (D) two-tiered vicariance, TTV.

Fig. S8 Bayesian skyline plots showing population sizes changes over time, estimated using BEAST.