Molecular Ecology (2007) **16**, 977–992 doi: 10.1111/j.1365-294X.2006.03216.x

Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus*)

AMY G. VANDERGAST,* ANDREW J. BOHONAK,† DAVID B. WEISSMAN‡ and ROBERT N. FISHER*
*US Geological Survey, Western Ecological Research Center, San Diego Field Station, 4165 Spruance Road Suite 200, San Diego, CA 92101, USA, †San Diego State University, Department of Biology, 5500 Campanile Drive, San Diego, CA 92182-4614, USA, ‡California Academy of Sciences, Department of Entomology, 875 Howard Street, San Francisco, CA 94103, USA

Abstract

Habitat loss and fragmentation due to urbanization are the most pervasive threats to biodiversity in southern California. Loss of habitat and fragmentation can lower migration rates and genetic connectivity among remaining populations of native species, reducing genetic variability and increasing extinction risk. However, it may be difficult to separate the effects of recent anthropogenic fragmentation from the genetic signature of prehistoric fragmentation due to previous natural geological and climatic changes. To address these challenges, we examined the phylogenetic and population genetic structure of a flightless insect endemic to cismontane southern California, *Stenopelmatus* '*mahogani*' (Orthoptera: Stenopelmatidae). Analyses of mitochondrial DNA sequence data suggest that diversification across southern California began during the Pleistocene, with most haplotypes currently restricted to a single population. Patterns of genetic divergence correlate with contemporary urbanization, even after correcting for (geographical information system) GIS-based reconstructions of fragmentation during the Pleistocene. Theoretical simulations confirm that contemporary patterns of genetic structure could be produced by recent urban fragmentation using biologically reasonable assumptions about model parameters. Diversity within populations was positively correlated with current fragment size, but not prehistoric fragment size, suggesting that the effects of increased drift following anthropogenic fragmentation are already being seen. Loss of genetic connectivity and diversity can hinder a population's ability to adapt to ecological perturbations commonly associated with urbanization, such as habitat degradation, climatic changes and introduced species. Consequently, our results underscore the importance of preserving and restoring landscape connectivity for long-term persistence of low vagility native species.

Keywords: anthropogenic habitat fragmentation, gene flow, genetic population structure, insect, Quaternary inundation, *Stenopelmatus*, urbanization

Received 14 July 2006; revision received 9 October 2006; accepted 30 October 2006

Introduction

Coastal southern California has been widely recognized as a hotspot for biodiversity and endangerment (e.g. Cincotta *et al*. 2000; Myers *et al*. 2000). Roughly one-third of California's native flora and 487 native vertebrates are known to occur in this region (California Department of Fish and Game 2005). Although the invertebrate fauna has been less well-characterized, levels of endemism and diversity are undoubtedly high (Prentice *et al*. 1998, 2001; Ward 2005; Bond *et al*. 2006). Coastal southern California is also one of

Correspondence: A. G. Vandergast, Fax: 6192256436; E-mail: avandergast@usgs.gov

the most highly fragmented and urbanized regions in North America (Atwood 1993). Approximately 20 million people (60% of California's population) reside in the six counties of coastal southern California (2000 US Census data; Hunter *et al*. 2003). Urban development and human population pressures have already led to loss of habitat and increased habitat fragmentation. For example, over 90% of southern California's coastal sage scrub habitat, 99% of its coastal prairies, and 95% of its vernal pools have been lost to urbanization (McCaull 1994; Mattoni & Longcore 1997; Bauder & McMillan 1998). Consequently, approximately 400 plant and animal species are considered endangered or threatened in this region (Hunter *et al*. 2003).

The ecological effects of urban fragmentation on native populations and communities in southern California are largely negative, and include competition with exotics, modification of behaviour, changes in species composition and overall loss of native species diversity (Suarez *et al*. 1998; Bolger *et al*. 2000; Tigas *et al*. 2002; Crooks *et al*. 2004). Even species that appear to persist in fragments may be negatively impacted. For example, fragmentation may reduce dispersal, and consequently, genetic connectivity among populations (Cunningham & Moritz 1998; Dayanandan *et al*. 1999; Gerlach & Musolf 2000). Decreased levels of gene flow among small populations can lead to decreased genetic variability and increased inbreeding due to drift, and reductions in survival and reproductive success (Frankham *et al*. 2002; Reed & Frankham 2003). Lowered immigration rates can also increase the likelihood that local populations will go extinct, and lower the probability of recolonization (Andren 1994; Crooks *et al*. 2001; Templeton *et al*. 2001).

Previous studies addressing genetic connectivity in southern California have mainly focused on large, wide-ranging mammals (e.g. Ernest *et al*. 2003; Epps *et al*. 2005; Riley *et al*. 2006) or endangered species with highly restricted ranges (e.g. Metcalf *et al*. 2001; Swei *et al*. 2003). Nonetheless, fragmentation has had demonstrable effects on population genetic structure even in highly mobile species. In the Santa Monica Mountains northwest of Los Angeles, bobcats and coyotes were found to have reduced migration and genetic connectivity across a major highway, when compared to secondary roads and continuous habitat (Riley *et al*. 2006). A recent study of desert bighorn sheep in southern California linked reductions in both genetic connectivity and genetic diversity to as few as 40 years of anthropogenic isolation (Epps *et al*. 2005). Ernest *et al*. (2003) found that southern populations of mountain lion (*Puma concolor*) were genetically distinct from other regions in California, and gene flow was lower among southern populations than elsewhere. Similarly, genetic structure among populations of the Pacific pocket mouse, *Perognathus longimembris pacificus* in coastal southern California was greater than genetic structure in *P. l. longimembris* throughout the Mojave Desert, despite similar geographical scales in each case (Swei *et al*. 2003). Swei *et al*. (2003) also concluded that high genetic structure within *P. l. pacificus* predated recent habitat fragmentation, because genetic distances among the three remaining extant populations were of the same magnitude as those among three extinct populations (sampled from museum specimens that predate widespread urbanization).

As for any large region, estimating the effects of anthropogenic fragmentation on population genetic structure in southern California is difficult. Southern California has undergone complex geological and climatic changes over the past several million years characterized by mountain uplift, marine incursion and land movements along fault zones (Yanev 1980; Jacobs *et al*. 2004). Because all of these processes have contributed to current patterns of genetic diversity (e.g. Tan & Wake 1995; Rodriguez-Robles *et al*. 1999; Lovich 2001; Maldonado *et al*. 2001; reviewed in Calsbeek *et al*. 2003), the genetic signatures of these events could theoretically overwhelm population genetic changes due to very recent anthropogenic fragmentation. This is particularly true in areas where recent urbanization is geographically concordant with fragmentation on geological timescales. For example, the urbanized Los Angeles Basin has undergone several major marine incursions, with the most recent occurring roughly 100 000 years ago (Jacobs *et al*. 2004). In addition, transportation corridors often follow valleys (e.g. Highway 101 through the Santa Monica Mountain range), river basins (e.g. Interstate 91 along the Santa Ana River) and fault zones (e.g. Interstate 15 along the Elsinore and San Andreas Fault Zones), all of which may have historically constituted natural barriers to movement for some species.

Several methods can be used to separate the effects of historical and contemporary processes on population genetic structure. First, genealogically informative markers such as DNA sequences can be used to separate historical processes (represented in deeper nodes and more inclusive clades) from more recent events (represented at the tips of gene trees; Templeton 1998). Second, comparative studies can help to resolve the relative importance of factors hypothesized to influence population genetic structure (e.g. Bohonak 1999). For example, comparisons of genetic differentiation in fragmented and nonfragmented conditions can determine the genetic impacts of recent habitat fragmentation, even in cases where drift/gene flow equilibria are unlikely to have been reached (e.g. Williams *et al*. 2003; Vandergast *et al*. 2004). Finally, parametric bootstrapping can be used to test the validity of nonequilibrium scenarios (including recent fragmentation) under various parameter combinations (e.g. Bohonak *et al*. 2001; Mardulyn & Milinkovitch 2005). We used all three of these approaches in this study.

We focused on population genetic structure and phylogeographic history in a large flightless insect, the mahogany Jerusalem cricket, *Stenopelmatus 'mahogani'*. The mahogany Jerusalem cricket is an ideal indicator species for monitoring the genetic effects of anthropogenic habitat fragmentation throughout southern California. It is endemic to cismontane southern California and is widespread throughout this region. Mating song cohesion throughout its range (see Weissman 2001a) suggests that mahogany Jerusalem cricket gene pools have maintained genetic connectivity historically. However, many populations may now be isolated due to urbanization and heavily trafficked highways. *S. 'mahogani'* has a relatively short generation time of 2 years (Weissman, unpublished data), which may allow for the accumulation of detectable genetic changes on the timescale of widespread urbanization in southern California (60–100 BP; Atwood 1993).

To understand current genetic population structure in the context of the long-term species history, we analysed mitochondrial DNA (mtDNA) sequence variation in *S. 'mahogani'*. We combined phylogenetic and population genetic approaches, simulation models, and (geographical information system) GIS-based reconstructions of current and prehistoric habitat fragmentation to test the degree to which genetic differentiation reflects current and prehistoric levels of habitat fragmentation. We specifically addressed the following questions: (i) To what extent can current patterns of genetic differentiation be attributed to prehistoric fragmentation? (ii) Has recent urban fragmentation further increased genetic differentiation among populations? (iii) Is it theoretically possible that genetic differentiation could have significantly increased in the short time since urban development? (iv) Is genetic diversity correlated with fragment size? Our results demonstrate that the signatures of ancient and contemporary fragmentation events can be successfully decoupled using multiple analytical approaches.

Methods

Study organism

Stenopelmatus Jerusalem crickets (Orthoptera: Stenopelmatidae) are widespread throughout western North America. They are nocturnally active, retreating underground during the day; and omnivorous, feeding on roots, small invertebrates and detritus. Recent work has revealed greater species diversity within the group than had been previously described, with 40–50 putative species occurring in California alone. A taxonomic revision of the genus is currently underway (Weissman 2001a, b). *Stenopelmatus 'mahogani'* is a morphologically distinctive species. (Per Article 8.3 of the International Code of Zoological Nomenclature, we use the manuscript name of *S. 'mahogani'* while disclaiming this name as 'not available' at the present time). On average, *S. 'mahogani'* is the heaviest insect in California with live females weighing as much as 12.96 g (DBW, personal observation). Its range is restricted to cismontane southern California, bordered by the Transverse and Peninsular mountain ranges to the north and east and the San Diego River to the south. Within this range, it is found primarily on sandy soils in woodland, coastal sage scrub, chaparral and riparian habitats, with a preference for moister microclimates than other co-occurring stenopelmatids (D.B.W., personal observation).

Sampling sites and collections

Individuals were collected from 33 sampling locations throughout the species' range in pitfall traps or by hand (Fig. 1; Table S1, Supplementary material). A sampling location was defined as a group of individuals collected within approximately a 5-km radius and not separated by potential geographical barriers to movement (e.g. rivers, mountains, highways, urban development). Initial examination of genetic isolation-by-distance plots showed little or no genetic differentiation among individuals at geographical distances of 10 km or less (results not shown). The average distance among individuals grouped within a sampling location was 2.3 km (range 0–10.9 km). In contrast, the average distance among sampling locations was 82 km (range 2.4–220 km). Each sampling location was geographically referenced at the centre of individuals within it.

Most individuals were collected in pitfall traps as part of a large-scale study conducted by R.N.F. and colleagues. Over 700 pitfall traps located throughout southern California have been sampled for herpetofauna, small mammals and invertebrates between 1998 and present (see Case & Fisher 2001; Fisher *et al*. 2002; for methods). Jerusalem crickets from these sites were initially preserved in 70% and later transferred to 95% ethanol. Additional locations within the known species' range of *S. mahogani* were sampled by hand by A.G.V. and D.B.W. Hand-caught specimens were obtained at oatmeal trails, and by searching under rocks and other debris. Specimens were brought back to the laboratory live, and tissue was frozen at –80 °C. Individuals were identified to species level using morphological characters (body colouration, abdominal stripping patterns and rear tibial spines), combined with mating song and karyotype information.

Amplification and sequencing of mtDNA

Sequences were collected from a total of 260 *S. 'mahogani'* individuals. Genomic DNA was isolated from the femur of one leg of each specimen using DNeasy Tissue Kits (QIAGEN). A 708-bp region of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the universal primer pair LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG, and

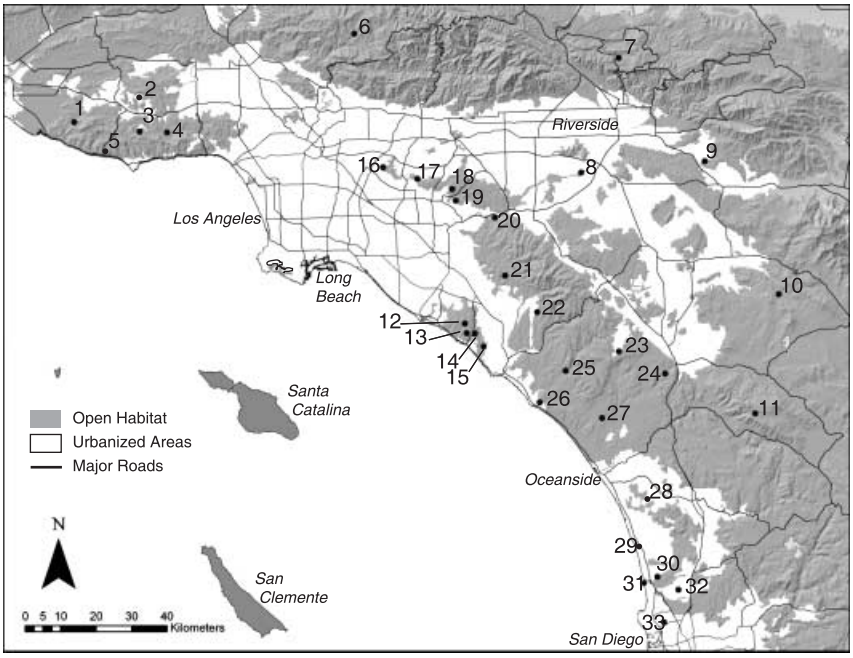

Fig. 1 Map of collection localities throughout southern California, created in ARCGIS 9.1 from the 1995 California Gap Analysis Land-Cover/Vegetation layer, the California Major Roads layer, and a shaded relief for California. Land cover polygons categorized as urban WHR (Wildlife Habitat Relationships) habitat types are shown in white. Remaining habitat types are shown in grey, and include (in descending order of total area covered), chaparral, coastal sage scrub, forest, orchards, grasslands, croplands, oak woodland and desert scrub. Desert scrub habitat is unsuitable for *Stenopelmatus 'mahogani'* and is shown in light grey.

HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA (Folmer *et al*. 1994). Polymerase chain reaction (PCR) amplifications were as follows: 95 °C for 2 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s; 72 °C for 7 min. Amplification reactions consisted of 2 μL of DNA, 0.5 U *Taq* Polymerase (Invitrogen), 1.8 mM $MgCl_2$, 0.2 mM each dNTP, and 0.4 mM each primer in 25 μL total volume. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN). PCR products were cycle sequenced in the forward direction using Big Dye Terminator III (Applied Biosystems) and run on an ABI 377 automated sequencer. Resulting sequences were aligned manually in SEQUENCHER (version 3.1.1; Gene Codes). No insertions or deletions were found, and ambiguous end regions were clipped so that all individuals were analysed over the same sequence length of 623 bases. Unique haplotypes were identified using the program COLLAPSE version 1.1 (Posada 1999).

Phylogenetic analyses and molecular dating

Phylogenetic trees. To reconstruct the phylogenetic relationships among haplotypes, we utilized a Bayesian search method. Nine individuals from four of the closest known relatives of *S. 'mahogani'* were included as outgroups (based on song characteristics and a preliminary molecular phylogeny of the genus; Weissman and Vandergast unpublished data). Bayesian searches were performed in MRBAYES 3.0 (Huelsenbeck & Ronquist 2001) using a general time reversible model with invariable sites and gamma distribution (GTR+I+Γ, Rodríguez *et al*. 1990; determined to be the most appropriate model using a hierarchical log ratio test in MODELTEST, Posada & Crandall 1998). Searches were run for 10 million generations, sampling every 1000 generations, with branch lengths recorded. The first 1 million generations (10%) were discarded as burn in, and the remaining 9000 trees retained. Two searches were performed to confirm stationarity. From the resulting 18 000 trees in the stationary distribution, a 50% majority rule consensus tree was built and average branch lengths calculated, rooted on outgroups. Following Wilcox *et al*. (2002), branches containing posterior probabilities ≥ 95% were considered highly supported.

Stenopelmatus n.sp. (Catalina), endemic to Santa Catalina Island, is presumed to be a close relative of *S. 'mahogani'* based on similarities in mating song structure and a preliminary molecular phylogeny of the genus *Stenopelmatus* (Weissman & Vandergast, unpublished data). Geologic and fossil evidence suggests that Santa Catalina Island subsided to a depth of at least 1000 m below sea level during the late Miocene and did not gain sub-aerial exposure until at least the Pliocene (Vedder & Howell 1980; Rowland 1984). Therefore, we dated the maximal divergence of the Santa Catalina Island species to the boundaries of the Pliocene (1.8–5.0 million years ago), and used this as a calibration point to determine the per-site mutation rate for our sequences under the assumption of a molecular clock. To determine whether sequence data follow clock-like behaviour, Bayesian searches were repeated under the assumption of a molecular clock. The average log likelihood from the stationary distribution of this constrained analysis was then compared to the unconstrained stationary distribution using a standard log likelihood ratio test (LRT) for nested models given by −2 ln(likelihood ratio of the

unconstrained model/model with clock). The LRT was evaluated using the χ^2 distribution for a two-tailed test with 1 d.f. (Casella & Berger 2002).

Networks. Traditional tree-building techniques tend to poorly resolve intraspecific gene genealogies when the number of mutations between haplotypes is small, ancestral haplotypes are retained, and recent multifurcations are common (Crandall 1994; Crandall & Templeton 1996). Therefore, we also examined relationships among haplotypes with a network reconstructed in the program TCS version 1.21 using a 95% maximum parsimony connection criterion (Clement *et al*. 2000). Ambiguous connections among sets of haplotypes (i.e. loops) were resolved when possible using the criteria outlined in Crandall & Templeton (1993) and Crandall *et al*. (1994).

Population genetic analyses

Population differentiation and molecular diversity. Nucleotide diversity (π, Tajima 1983), the number of segregating sites (S), and Tajima's test of selective neutrality (Tajima's D: Tajima 1989) were calculated for each collection location and across all samples in the program ARLEQUIN 3.0 (Excoffier *et al*. 2005). The null hypothesis of $D = 0$ was assessed in ARLEQUIN using a coalescent simulation algorithm under the hypothesis of selective neutrality and drift-mutation equilibrium.

Global and pairwise estimates of genetic differentiation were examined using Φ_{ST}, an analogue of F_{ST} that incorporates haplotype frequency and relatedness (Excoffier *et al*. 1992). Significance was assessed with 10 000 randomizations of the AMOVA test statistic. Singleton collection localities were removed from these analyses. Euclidean geographical distances among all population pairs were measured in ARCGIS 9.1 (ESRI). Pairwise matrices of geographical distance and Φ_{ST} were compared using a Mantel Test for matrix correlation (Mantel 1967), with significance assessed by 10 000 randomizations of the genetic distance matrix. These isolation-by-distance (IBD) analyses were performed using IBDWS 2.5 (Jensen *et al*. 2005).

Effects of prehistoric fragmentation. Because we could not find spatial reconstructions for all of southern California during specific periods of marine inundation during the Pleistocene or Holocene, we approximated prehistoric fragmentation from the Digital Geologic Map of California (Saucedo *et al*. 2000), in ARCGIS 9.1 (ESRI). From the Geologic Map layer, we selected all areas characterized by Quaternary sedimentary rock deposits. These are comprised of alluvium (primarily Holocene, some Pleistocene; 3000 years to 1.5 million BP), Quaternary marine and Quaternary nonmarine sediments. Because sedimentary rocks are formed and deposited in rivers, lakes or sea-beds, these areas are assumed to have been underwater at some point during the Quaternary period. Both internally and externally calibrated molecular divergence times suggest that diversification within the *S. 'mahogani'* lineage is roughly contemporaneous with this period (see Results). Although this method does not accurately reflect the southern Californian landscape at any exact point in time, it should represent the maximum potential for fragmentation due to inundation and flooding during this period. Collection location points were overlaid on the selected geology layer, and a categorical (binary) fragmentation matrix was created with values of 1 for population pairs separated by Quaternary inundation and 0 for pairs assumed to be continuously connected. We assessed the correlation between the pairwise genetic differentiation matrix and fragmentation index after controlling for geographical distance using a partial Mantel test (Legendre & Legendre 1983) with IBDWS 2.5 (Jensen *et al*. 2005).

Effects of recent urban fragmentation. A similar method was used to estimate the effects of recent anthropogenic fragmentation. Recent fragmentation was estimated in a GIS by mapping urban wildlife habitat relationships (WHR) habitat types from the 1995 California Gap Analysis Land-Cover/Vegetation layer (http://www.biogeog.ucsb.edu/projects/gap/gap_data.html), and mapping state and interstate highways from the California Major Roads layer (California Spatial Information Library; http://gis.ca.gov/data.epi). As above, collection location points were overlaid onto the urban WHR and road layers, and a binary fragmentation index was created. (Population pairs separated by urbanized areas and/or highways were categorized as fragmented.) For this analysis, we did not consider the Ortega Highway (State Route 74 between Interstates 5 and 15) as a barrier to movement because it is a small, relatively low-traffic route (two lanes, 10 000 cars per day at the Riverside/Orange County line; Caltrans 2005 Traffic Volumes; http://www.dot.ca.gov/hq/traffops/saferesr/trafdata), with almost no flanking development along most of its length, and no medians or berms. Transportation corridors that were considered barriers were travelled by approximately 20 000 cars per day or more, with most exceeding 100 000 cars per day over some segments (Caltrans 2005 Traffic Volumes), and did not contain wildlife underpasses in the vicinity of sampling. As above, a partial Mantel test was employed to determine the correlation between genetic similarity and fragmentation, after controlling for geographical distance.

We noted that no populations separated in the Quarternar model are currently in the same fragment (i.e. fragmentation has increased or remained the same in all cases). We determined the effects of recent urban fragmentation above and beyond prehistoric fragmentation with a two-step analysis.

First, we assumed that Quaternary population subdivision would have been higher among fragments than within them. Therefore, we calculated the residuals from reduced major axes (RMA) regressions of genetic differentiation vs. geographical distance separately for population pairs that were and were not separated by water under the Quaternary fragmentation model. RMA regression is considered more appropriate than ordinary least squares regression for data sets in which the independent variable is measured with error (Sokal & Rohlf 1995), and has been found to be a more appropriate estimator of slope in genetic IBD analyses (Hellberg 1994). These RMA residuals represent excess genetic divergence or similarity that is not explained by prehistoric conditions. We then used a Mantel test to determine whether these residuals were significantly correlated with the urban fragmentation matrix. Conceptually, this analysis determines whether contemporary fragmentation can account for differentiation that is not explained by prehistoric patterns of IBD, which are permitted to differ in continuous and fragmented conditions. RMA regressions were calculated using the program RMA for Java (Bohonak & van der Linde 2004).

Theoretical evaluation of potential urban fragmentation impacts. We tested whether observed increases in genetic divergence associated with urban fragmentation could theoretically reflect very recent decreases in gene flow. For current populations of *S. 'mahogani'*, highways frequently subdivide optimal habitat. For example, the San Joaquin Hills in coastal Orange County are dominated by coastal sage scrub habitat and contain mahogany Jerusalem crickets (collection locations 12–15). While this area was not fragmented by inundation according to our Quaternary model, suitable habitat is currently divided by the Laguna Highway (Route 133). The Laguna Highway was built in the early 1900s and currently carries approximately 30 000 vehicles per day (www. cahighways.org). We constructed a simulation model for this region to determine whether the genetic effects of fairly recent highway construction may already be evident. Simulations were conducted using the program ESP (Bohonak *et al.* 2001) which follows the evolution of nonrecombining DNA sequences across multiple populations, incorporating random genetic drift, mutation and gene flow. This program incorporates empirical sampling error as well as stochasticity in drift, mutation and gene flow. Our approach was as follows:

1 Evolutionary divergence was simulated under historic conditions using the following parameters, judged to be biologically reasonable for our data set, given our collective field expertise of over 30 years with this species: a regional set of 100 populations corresponding to the coastal southern California region prior to urbanization, each of size $(0.5)N_e = 500$ mtDNA genomes. A minimum mutation rate of $\mu = 1.1 \times 10^{-8}$ per bp per generation (for 623 bases) was derived from our data (see Results below), and is very close to the average rate of $\mu = 1.0 \times 10^{-8}$ for mitochondrial and nuclear synonymous substitutions (Li 1997). Gene flow was set to $m = 1\%$. We simulated 60 000 generations under these conditions to allow the system to reach equilibrium.

2 We validated the historic model based on intra- and interpopulation levels of diversity by comparing Φ_{ST} and the number of alleles (unique haplotypes) per population K in the simulations and our empirical data. For this region, mean $\Phi_{ST} = 0.02$ between pairs of populations not separated by highways (range 0.00–0.03). In the simulated data, we sampled 1000 replicates of two populations (24 gene copies each) to incorporate sampling error and bias comparable to the empirical data. The median and mean values from the model fell well within the empirical bounds (median $\Phi_{ST} = 0.005$, mean $\Phi_{ST} = 0.03$). Diversity within populations in the model (median $K = 3.5$, mean $K = 2.0$ for 1000 replicate samples of two populations, 24 gene copies each) and the empirical data (mean $K = 3.5$, range 2–5) were also concordant.

3 The model was extended an additional 100 generations under no gene flow (representing division by the Laguna Highway). Each generation, Φ_{ST} was calculated for 1000 replicate samples of 2 populations (24 gene copies each).

4 We compared the theoretical results to actual levels of genetic divergence between population pairs that are currently separated by the Laguna Highway (mean $\Phi_{ST} = 0.14$, range 0.12–0.15) over timescales that represent the age of the highway (30–50 generations before present, assuming a generation time of 2 years).

Loss of genetic diversity. We examined the relationship between fragment size and genetic diversity in current patches. Fragment areas were calculated in ARCGIS 9.1 by summing polygon areas of nonurbanized habitats within a 'fragment' defined by urbanization and/or roads. Diversity was estimated as (i) θ_π, which estimates the population parameter $\theta = 4N_e\mu$ from the average sequence divergence π, and (ii) θ_K, which estimates $\theta = 4N_e\mu$ from the number of haplotypes (K). Because bottlenecks purge populations of rare alleles more quickly than common alleles, θ_K is expected to reflect recent fragmentation more than θ_π. Calculations were performed in ARLEQUIN, with fragments containing only one individual excluded from the analysis. For both θ_K and θ_π, we performed a multiple regression on current fragment size and Pleistocene fragment size in DATADESK, version 6.2.1 (Velleman 1997). Because the results were identical whether or not any of the four variables were log-transformed, we only present here the analyses without transformation.

Results

Phylogenetic analyses

The 260 mtDNA COI sequences of *Stenopelmatus 'mahogani'* contained 65 unique haplotypes and 71 polymorphic sites, of which 51 sites were parsimony informative (GenBank Accession nos EF030116–EF030189, EF057493–EF057687). The mean proportion of pairwise differences was relatively low among haplotypes, with overall $\pi = 0.01557$ (range: 0.0016–0.0321, mean = 0.0111 including all individuals). The Bayesian phylogenetic analysis highly supported monophyly of *S. 'mahogani'* (Fig. 2, posterior probability = 1.00). Within this lineage, haplotypes from the Chino and Puente Hills are basal, with monophyly for the remainder of the lineage supported by a posterior probability of 0.96. Haplotypes from higher altitude regions that surround the Los Angeles Basin (San Bernardino Mountains, Santa Monica Mountains, San Gabriel Mountains, San Jacinto Mountains, and Palomar Mountain) form a well-supported subclade (posterior probability = 1.00). Few other clades with specific geographical affinities were strongly supported (posterior probability < 0.95).

Based on the log-likelihood ratio test, we could not reject a molecular clock (LRT = 0.01309, 1 d.f., $P > 0.90$). Because the Santa Catalina Island clade, the Winchester clade and the mahogany clade form a basal polytomy, we calculated the rate of sequence divergence from the average pairwise distances among these three clades. Applying the dates bounding the Pliocene (1.8–5.0 million years ago) translates into a mutation rate of 1.1×10^{-8} to 3.15×10^{-8} per base pair per generation corresponding to an average rate of pairwise sequence divergence of 2.2–6.3% per million years. The lower bound of this estimate is concordant with previously

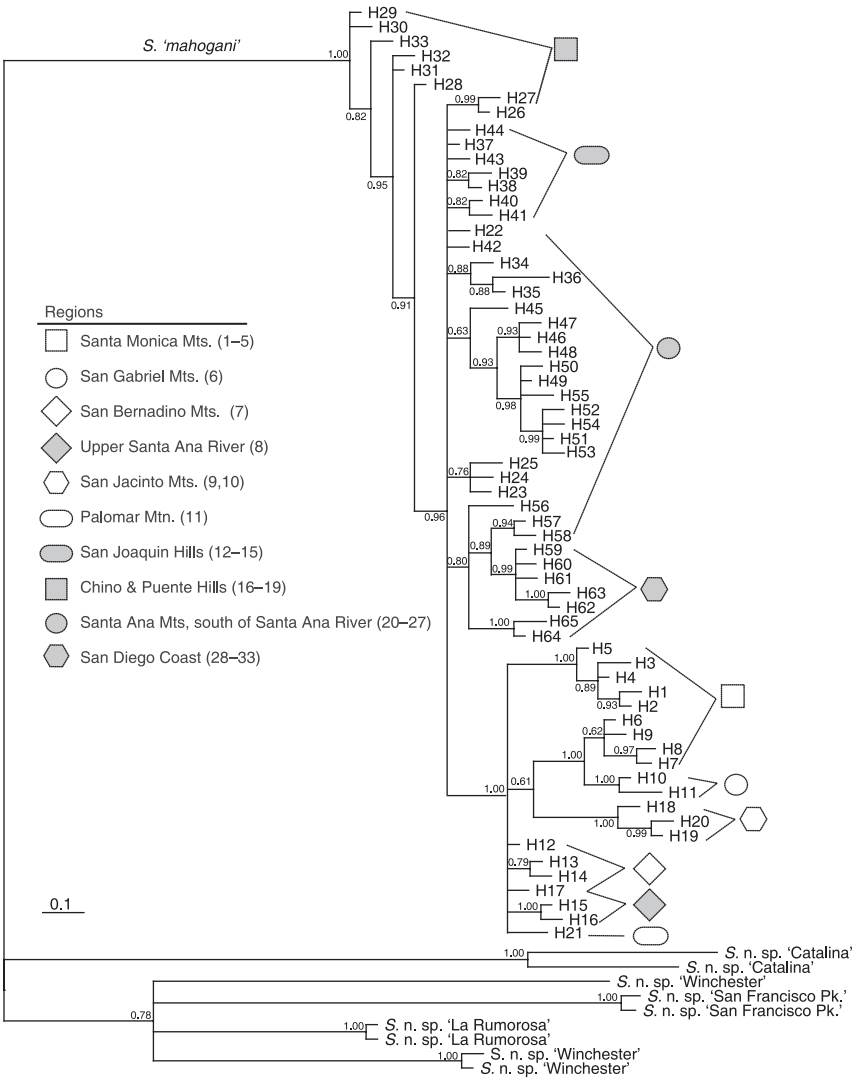

Fig. 2 Majority rule consensus tree derived using Bayesian inference (GTR + I + Γ; average ln L = –2721.002, r(A↔C) = 0.005, r(A↔G) = 0.374, r(A↔T) = 0.006, r(C↔G) = 0.001, r(C↔T) = 0.474, r(G↔T) = 0.004, p(A) = 0.283, p(C) = 0.202, p(G) = 0.147, p(T) = 0.368, α = 0.103, I = 0.482). Branch lengths were averaged from the posterior stationary distribution, and posterior clade probabilities > 50% are listed at each node. Haplotypes are grouped by major geographical regions, denoted by shapes. Open shapes represent higher elevation mountain populations, and shaded shapes represent lower elevation sites within the Santa Ana Mountains and coastal regions.

reported and widely used rates of pairwise sequence divergence in invertebrate mtDNA of 2.0–2.3% per million years (Desalle *et al*. 1987; Brower 1994). Bond *et al*. (2001) reported 4% sequence divergence per million years in trapdoor spiders endemic to coastal dunes, calibrated using inundation of the Los Angeles basin.

The parsimony network elucidated finer scale relationships among haplotypes, emphasizing that haplotypes tended to cluster by geographical area (Fig. 3). (For example, 77% of haplotypes were restricted to a single population, and 98% were restricted to a single region.) A cursory examination of haplotype relatedness by region suggests either a stepping-stone model of colonization, or that low gene flow in an IBD pattern prevails over the majority of the species' range. From the root supported by the Bayesian analysis, the network suggests that *S. 'mahogani'* may have spread from the Chino and Puente Hills across the Santa Ana River into the southern Santa Ana Mountains and then colonized the coastal regions and higher altitude mountains.

Population genetic analyses

Nucleotide diversity within each population was low, ranging from 0 to 0.00985, and Tajima's *D* was not significantly different from zero in any population (Table 1). The majority of haplotypes (49 of 65) were restricted to a single sampling location, and most haplotypes observed in more than one location were restricted to geographically proximate sampling locations (Table S2, Supplementary material). In general, more haplotypes were shared among populations in the largely nonfragmented areas of southeastern Orange, western Riverside and northern San Diego Counties (sampling locations 20–27) than elsewhere in the species' range. In only one case was a haplotype shared between two distant collection locations. Haplotype 17 was found in both the San Bernardino and Tequesquite collection locations (roughly 34 km away). Because the San Bernardino site is near the headwaters of the Santa Ana River, and Tequesquite is located downstream, the shared haplotype could imply a single long-distance dispersal event (e.g. during a flooding event), or a functional dispersal corridor along the Santa Ana River.

Genetic structure was high, with overall $\Phi_{ST} = 0.756$ ($P \leq 0.0001$) and 352 of 406 pairwise population comparisons significantly divergent ($P \leq 0.05$; 115 significant with Bonferroni corrected $P \leq 0.000123$; Table S3, Supplementary material). Genetic differentiation and geographical distance were significantly correlated with strong IBD (Mantel Test; Z score = 26361, $r = 0.455$, $P \leq 0.0001$).

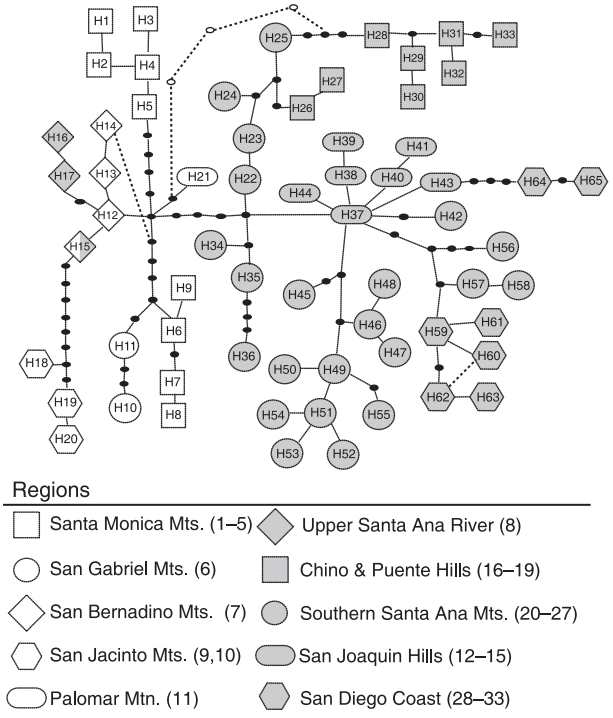

Fig. 3 Parsimony haplotype network. Haplotypes are numbered, and labelled by geographical region. Open shapes represent higher elevation mountain populations, and shaded shapes represent lower elevation sites within the Santa Ana Mountains and coastal regions. Mutational steps connecting haplotypes are depicted as small circles. Alternative connections among haplotypes are shown with dashed lines.

Effects of habitat fragmentation

Pairwise genetic differentiation estimates were significantly correlated with both the prehistoric and the current urban fragmentation matrices (Table 2, Fig. 4). Furthermore, residuals from the RMA regressions accounting for geographical distance under prehistoric fragmentation were significantly higher for population pairs that are *currently* separated by urban fragmentation (partial Mantel test: $P = 0.0375$; Table 2; Figure S1, Supplementary material). This strongly suggests that recent anthropogenic changes to the landscape have reduced gene flow beyond historical levels. Contemporary fragmentation may have also depleted populations of genetic diversity, as both θ_π and θ_K are significantly greater in larger fragments, but uncorrelated with prehistoric fragment size (multiple regressions; $P = 0.0006$ for θ_π on current fragment size and $P = 0.03$ for θ_K on current fragment size; $P > 0.8$ for prehistoric fragment size in both models). Detailed inspection of the genetic diversity analyses revealed that the decline was most pronounced in contemporary fragments ≤ 22 km^2 (see Table 1).

Theoretical evaluation of potential urban fragmentation impacts

As described above, intrapopulation genetic diversity and interpopulation divergence in the 'historical conditions'

Table 1 Collection locations, number of individuals collected, molecular indices and fragment sizes

	Collection location	*N*	No. of haplotypes (*K*)	Nucleotide diversity (π)	Tajima's *D*	θ_K	θ_π	Current fragment size (km²)	Prehistoric fragment size (km²)
1	Santa Monica Mountains 1	10	6	0.0083	0.1914	5.4075	5.1556	299.1	5251.8
2	Santa Monica Mountains 2*	1	1	NA	NA	NA	NA	6.4	5251.8
3	Santa Monica Mountains 3	7	5	0.0028	1.8112	1.4226	1.7143	331.3	5251.8
4	Santa Monica Mountains 4	5	2	0.0010	1.2247	0.6911	0.6000	331.3	5251.8
5	Santa Monica Mountains 5*	1	1	NA	NA	NA	NA	331.3	5251.8
6	San Gabriel	2	2	0.0048	0.0000	NA†	3.0000	1117.5	5251.8
7	San Bernardino	14	5	0.0016	0.1838	1.4944	1.0000	167.5	5251.8
8	Tequesquite	5	3	0.0035	0.9571	2.2254	2.2000	44.7	8221.7
9	Riverside	2	1	0.0000	0.0000	0.0000	0.0000	1.9	16.0
10	San Jacinto	5	2	0.0006	−0.8165	0.6911	0.4000	757.3	8221.7
11	Palomar Mountain	2	1	0.0000	0.0000	0.0000	0.0000	444.2	8221.7
12	San Joaquin Hills 1	28	5	0.0021	0.7101	1.5019	1.3069	81.5	8221.7
13	San Joaquin Hills 2	23	4	0.0018	0.0991	1.1276	1.1225	81.5	8221.7
14	Aliso Woods Canyon 1	32	3	0.0019	1.2856	0.5980	1.1593	22.4	8221.7
15	Aliso Woods Canyon 2	14	2	0.0008	−0.4376	0.3669	0.5275	22.4	8221.7
16	Puente Hills 1	9	1	0.0000	0.0000	0.0000	0.0000	16.3	311.9
17	Puente Hills 2	2	1	0.0000	0.0000	0.0000	0.0000	21.1	311.9
18	Chino Hills 1	3	3	0.0032	0.0000	NA†	2.0000	45.8	311.9
19	Chino Hills 2	15	5	0.0053	−1.3426	2.2028	2.1333	94.5	311.9
20	Chino Hills 3	5	3	0.0071	−0.5963	2.2254	4.4000	1701.3	8221.7
21	Limestone Canyon	11	3	0.0033	0.0468	0.9864	2.0727	1701.3	8221.7
22	Starr Ranch	6	5	0.0034	−0.1443	11.4423	2.1333	1701.3	8221.7
23	Tenaja	8	5	0.0098	−0.0530	4.6935	6.1071	1701.3	8221.7
24	Santa Margarita	5	1	0.0000	0.0000	0.0000	0.0000	1701.3	8221.7
25	Camp Pendleton 1	2	2	0.0064	0.0000	NA†	4.0000	1701.3	8221.7
26	Camp Pendleton 2	3	3	0.0096	0.0000	NA†	6.0000	1701.3	8221.7
27	Camp Pendleton 3	6	6	0.0098	−0.7713	NA†	6.1333	1701.3	8221.7
28	Carlsbad*	1	1	NA	NA	NA	NA	7.8	8221.7
29	San Elijo Lagoon*	1	1	NA	NA	NA	NA	0.4	8221.7
30	Carmel Mountain	2	1	0.0000	0.0000	0.0000	0.0000	0.6	8221.7
31	Torrey Pines	20	3	0.0028	−0.7272	0.7185	1.7684	5.8	8221.7
32	Mira Mesa	5	1	0.0000	0.0000	0.0000	0.0000	0.7	8221.7
33	Tecolote Canyon	5	2	0.0006	−0.8165	0.6911	0.4000	4.5	8221.7
	Total	260	65	0.0139	−1.2010				

*removed from population-level analyses of F_{ST} due to sample size of 1.
†θ_K cannot be computed when all gene copies are unique.

Table 2 Results from the Mantel tests and partial Mantel tests

Mantel test	*r*	*P* value
Standard Mantel test		
Genetic distance vs. geographical distance	0.455	0.0001
Partial Mantel tests with Quaternary inundation model		
Genetic distance vs. geographical distance (controlled for Quaternary fragmentation)	0.329	0.0008
Genetic distance vs. Quaternary fragmentation (controlled for geographical distance)	0.259	0.0045
Partial Mantel tests with urban fragmentation model		
Genetic distance vs. geographical distance (controlled for urban fragmentation)	0.316	0.0002
Genetic distance vs. urban fragmentation (controlled for geographical distance)	0.523	0.0001
Mantel test of Quaternary model residuals and urban fragmentation		
Residuals from separate Quaternary regressions within and among fragments vs. urban fragmentation	0.126	0.0375

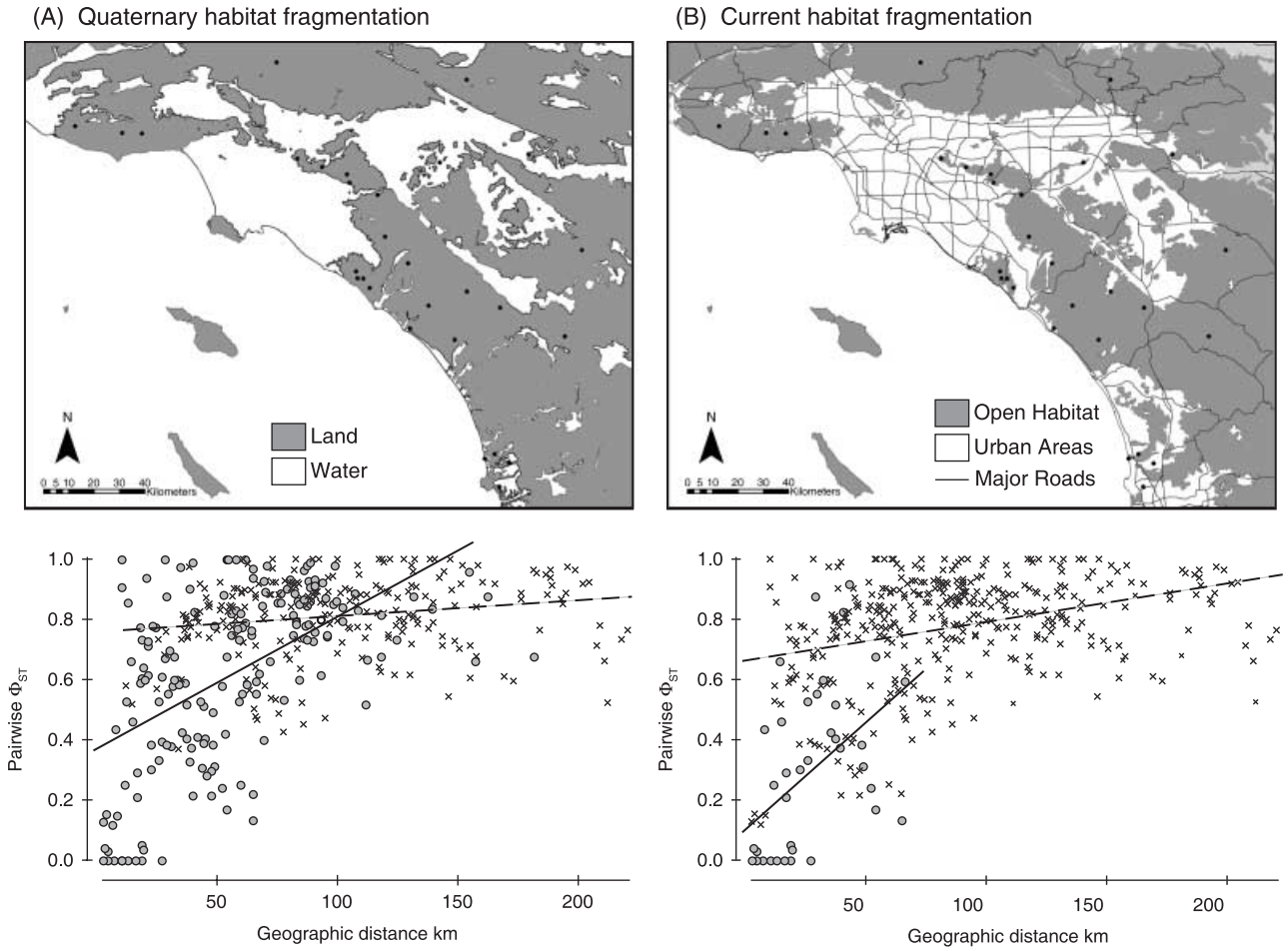

Fig. 4 Fragmentation scenarios and corresponding isolation-by-distance genetic plots for (A) Quaternary inundation and (B) recent urban fragmentation. Isolation by distance plots depict pairs of populations separated by inundated or urban areas as crosses, and shaded circles denote pairs of populations within fragments. In both scenarios, genetic differentiation is significantly correlated with fragmentation (Table 2).

simulation model matched empirical data for populations in the San Joaquin Hills that are not separated by urbanization or major highways. Mean and median values for 100 additional generations continuing under historical conditions remained constant (data not shown). In contrast, genetic divergence among population pairs increased approximately linearly for 100 generations when fragmentation was simulated in a 'no gene flow' scenario (Fig. 5). The mean value from the model fell very close to the 95% CI for Φ_{ST} that is empirically observed in the focal area, and the 95% CI from the model was so broad that it completely contained the empirical 95% CI. It is clear that habitat fragmentation by major highways is theoretically compatible with observed patterns of genetic structure, if this large flightless invertebrate has experienced no gene flow across a major highway during the last 30–50 generations.

Discussion and conclusions

In general, genetic analyses revealed that mahogany Jerusalem cricket populations are genetically divergent, with largely fixed differences among regions. These crickets are large, relatively slow moving, and most likely have small home ranges that would contribute to high genetic divergence among regions. This is most evident in the high mountains, where the terrain and patchiness of preferred habitats likely limits dispersal across canyons and mountain tops. In contrast, the coastal and foothill regions of southern California have less topographic relief and historically larger continuous expanses of appropriate habitats; thus, these populations may have been more continuously distributed and larger spatially and numerically, resulting in higher historical levels of genetic connectivity.

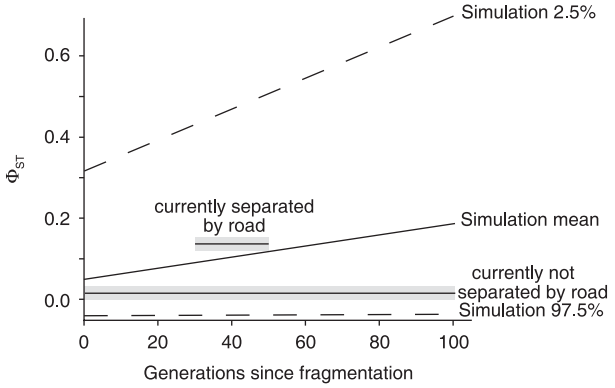

Fig. 5 Simulation of urban fragmentation effects in the San Joaquin Hills. Gray boxes depict the empirical mean and range for population pairs separated by a major road for 30–50 generations (Φ_{ST} mean = 0.14, range = 0.12–0.15), and population pairs in the same fragment (Φ_{ST} mean = 0.02, range = 0.00–0.03). Mean and 95% confidence intervals for 1000 replicate simulations are plotted for Φ_{ST}, based on two populations sampled (24 gene copies each) from a set of 100 regional populations. Time 0 represents the generation in which major roads prevent gene flow among historically connected populations (see Methods). After cessation of gene flow, Φ_{ST} increases rapidly towards empirically observed levels.

Phylogeographic history of S. 'mahogani'

Low levels of sequence divergence suggest that the observed diversification within *S. 'mahogani'* occurred fairly recently. Applying our minimal divergence rate of 2.2% per million years to the maximum pairwise divergence among haplotypes (3.2%), diversification within the lineage may have begun in the early to mid-Pleistocene (roughly sometime after 1.6 million years ago). Our estimated divergence rate is based on a polytomous branch, which may introduce additional error in rate estimation, but it is probably much less substantial than the uncertainty in the dating of the geological event used as a calibration point. Given these and other uncertainties in molecular clock estimation (reviewed in Arbogast *et al*. 2002), we feel that our results (as with all molecular clock estimates) should be interpreted with some caution. However, our internally calibrated clock represents the best means of estimating divergence times in this group based on the available data. From floral fossil evidence, Pleistocene conditions are thought to have been generally cooler and wetter (Axelrod 1966; Searcy 1969; Axelrod 1986), with climatic conditions in coastal southern California approximately similar to those found today near Monterey, California (Axelrod & Govean 1996). A cooler and wetter climate may have promoted the expansion of mesic-adapted species throughout southern California, including the mahogany Jerusalem cricket (Yanev 1980; Axelrod 1986). At glacial maxima, the exposure of additional near-shore habitats would have also provided temporary migration corridors among coastal populations. Pleistocene climates were also quite variable, ranging from wet and cool during glacial maxima interspersed with warm interglacials, with shifts in climate occurring on timescales of hundreds to thousands of years (Roy *et al*. 1996). It is during these interglacial time periods that sea levels were at their highest and extensive fragmentation due to inundation along the southern California coast probably occurred.

Effects of fragmentation on current population structure

IBD analyses suggest that both past and present habitat fragmentation has decreased genetic connectivity among populations of *S. 'mahogani'*. After accounting for the residual effects of prehistoric fragmentation, recent fragmentation still has a measurable impact on genetic differentiation. Analysis of residuals from the prehistoric fragmentation model suggests that urban fragmentation has led to an average increase in Φ_{ST} of 0.087. Our use of the total coverage of Quaternary sedimentary rock (the best available data for the study area) represents the maximum amount of inundation possible at any given time during this period. Because actual inundation at any single point in time was probably much less than the maximum, historical connectivity may have been somewhat higher than we estimate. Thus, contemporary urban fragmentation is likely to have had an even larger effect on the genetic structure of Jerusalem cricket populations than we were able to estimate with this method. We would also expect to gain additional inferential power through the analysis of spatial variation for molecular markers that have higher mutation rates than mtDNA, such as microsatellites.

Theoretical simulations

It is generally difficult to separate the effects of recent changes in genetic connectivity from historical processes when interpreting population genetic structure. Any particular data set can be interpreted through numerous combinations of specific mutation, gene flow and drift parameters, even without considering whether some of these parameters have recently changed. We were able to overcome these challenges by constraining parameter values in our simulation models to biologically realistic values for *S. 'mahogani'*, and focusing on a dominant landscape feature (highways) that is likely to have recently affected population structure. Although we did not conduct a detailed sensitivity analysis of model parameters (e.g. Bohonak *et al*. 2001), our results demonstrate that the construction of highways may already be increasing genetic isolation between populations of terrestrial arthropods. As one would expect, our conclusions do depend to some extent on assumptions about effective population size and rates of gene flow (supplementary analyses not presented).

Our validation procedure for the specific ancestral parameters that we chose included comparisons with empirical data for populations that are not separated by highways, under realistic parameter values. For example, if population sizes are decreased by an order of magnitude, only one allele is present in the sampled individuals. Our choice of $N_e = 1000$ likely represents an upper bound based on extensive field surveys (D.B.W. and A.G.V., unpublished data), and drift over the last 30–50 generations of fragmentation occurs even more quickly if N_e is lower.

We also note that our qualitative conclusions are dependent on the number of populations that are modelled. For example, neither Epps *et al*. (2005) nor Riley *et al*. (2006) were able to replicate observed increases in genetic differentiation due to highways, using simulation models for desert bighorn sheep and midsized carnivores, respectively. However, their models followed the evolution of two populations in isolation, rather than an empirical sample of two populations from a larger regional pool (as in this study). The errors associated with sampling two populations from a larger regional gene pool are considerably greater than those associated with sampling all populations in a set of two. For *S. 'mahogani'*, we conducted additional simulations with only two interacting populations and could find no combination of gene flow, mutation and population size parameters that could be empirically validated using nonfragmented populations. That is, we could not create a two-population model for which sample sizes of 24 gene copies yield both $K \approx 3.5$ alleles and $\Phi_{ST} \approx 0.02$ at equilibrium. More quantitative conclusions and smaller confidence intervals from simulation models may be possible if additional molecular markers and populations are incorporated.

Consequences of loss of genetic connectivity

Loss of genetic connectivity among fragmented populations is hypothesized to be detrimental to long-term species persistence. Small, isolated populations are more vulnerable to stochastic extinction events where there is no available rescue effect from immigration or recolonization (Gilpin & Soulé 1986; Templeton *et al*. 1990). Loss of gene flow also increases the effects of genetic drift, leading to loss of genetic diversity, particularly in small populations where drift is strong. Because we observed a strong IBD pattern and correlations between genetic diversity and fragment size, mahogany Jerusalem crickets may be particularly vulnerable to these effects. Reduced genetic diversity can limit the ability of populations to evolve in response to ecological perturbations such as climate change, habitat degradation, and introduced predators, competitors, disease and parasites (Frankham *et al*. 2002; Spielman *et al*. 2004). If patterns of genetic diversity detected in mtDNA are representative of the entire genome, then we can infer that small isolated populations of *S. 'mahogani'* and other native species with similar sensitivity to fragmentation will have reduced adaptive potential. These effects are most detrimental in small fragments that abut urban edges, where ecological perturbations are most pervasive (McKinney 2002; Radeloff *et al*. 2005).

Implications for natural area conservation in southern California

The mahogany Jerusalem cricket has a wide distribution and persists in many habitat fragments, sometimes reaching moderate to high local abundances. Yet, even this species has experienced demonstrable impacts to its genetic structure from fragmentation and isolation throughout its range. If taken as an indicator species representing small, low vagility taxa, then our results underscore the importance of maintaining and restoring connectivity among remnant wildlands for long-term population viability (Beier *et al*. 2006).

Our study lends particular support for focused land conservation in the Santa Ana Mountains, including the Chino and Puente Hills. High levels of genetic connectivity were measured throughout the Santa Ana Mountains (sites 20–27, south of the Santa Ana River and Highway 91) emphasizing that this region has long contained a relatively intact swath of open and natural habitat, which is now threatened by proposed new transportation corridors that will connect inland Riverside County to coastal Orange County (http://www.rcocconnection.info/map.html). Previous studies have also identified this area as an important core habitat for wildlife, with a characteristic representation of the southern California coastal ecoregion (Beier 1995; Spencer *et al*. 2001; Hunter *et al*. 2003; Dickson *et al*. 2005). Our phylogenetic analysis suggests that the Chino and Puente Hills are the centre of ancestral genetic diversity in *S. mahogani*. The most basal haplotypes were found in the Chino and Puente Hills, north of the Santa Ana River (sites 16–19). Whether this pattern is unique to *S. 'mahogani'*, or extends to a centre of endemism for other taxa remains to be seen. Many phylogenetic studies have found that lineages endemic to the southern California coastal ecoregion have expanded from Baja California in the south (Rodriguez-Robles *et al*. 1999; Bond *et al*. 2001), or across mountain barriers in the north (Jockusch & Wake 2002; Sgariglia & Burns 2003). However, the most basal lineage in the southern California endemic trapdoor spider genus, *Apomastus*, may be near Cajalco Canyon, on the eastern edge of the Santa Ana Mountains (Bond *et al*. 2006), which is somewhat coincident with this study. If other species or lineages endemic to cismontane southern California show concordant root placement, then the area could be considered an important component of evolutionary diversity within the ecoregion worthy of conservation (*sensu* Moritz & Faith 1998; Moritz 2002).

There is already considerable conservation and restoration focus in the Santa Ana Mountains by land management agencies (e.g. California Department of Parks and Recreation; Noss *et al*. 2002; Royte 2002; Koelle 2003). However, development pressures continue to threaten the region. The Chino and Puente Hills currently form a 'peninsula' of open space surrounded by urban development by Los Angeles, Riverside and Orange Counties. Although a large portion of this area is protected in state or local wildlife preserves (e.g. Chino Hills State Park, Schabarum Park, Whittier Narrows Recreation Area), segmentation by roads and development hinder wildlife movement within the area to some extent, despite the creation of underpasses and culverts (Haas & Crooks 1999; Cooper 2000; Haas 2000; Haas *et al*. 2002). Additional proposed residential development in the centre of this region threatens to effectively isolate the entire peninsula west of Chino Hills State Park (Spencer 2005; west of collection site 20 in Fig. 1). In general, development pressures in unprotected areas throughout the Santa Ana Mountains are likely to increase, as the human population in southern California continues to grow and urban development is driven farther inland from the coast and farther west from Riverside. We advocate that land conservation efforts throughout this region continue to focus on preserving large areas of intact habitat with maximum levels of connectivity (see also Beier *et al*. 2006).

Conclusions

Although the footprints of Quaternary inundation and urban fragmentation are highly coincidental, Fig. 4 visually demonstrates that in some areas, particularly near the coast, urbanization has already reduced and fragmented the amount of available habitat for wildlife beyond that which has occurred naturally in the last 1.5 million years. This major landscape alteration is likely to change the relative importance of gene flow and genetic drift as evolutionary processes for a wide variety of organisms, as observed in *Stenopelmatus 'mahogani'*.

Acknowledgements

This work was supported by NSF grant DBI-0204447 to AGV and by the USGS Western Ecological Research Center. We thank Carrie Charlton, Lars Bell, Eric Lewallen, Joe Deas Jr., and Amanda Scidmore for laboratory assistance, and Dr Tod Reeder for use of his laboratory at San Diego State University. We also thank Michael Caterino for sharing preliminary data. Collections were permitted by California Department of Fish and Game, California State Parks, National Park Service, Audubon Society, Camp Pendleton Marine Base, TNC, NROC, MRCA, OC Regional Parks, City of San Diego, and SDSU Field Stations. The manuscript was improved based on comments from three anonymous reviewers. Use of trade names does not imply USGS endorsement.

Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3216/MEC3216sm.htm

Fig. S1 Bar chart showing the mean – or + 1 s.e. of Quaternary model residuals for populations pairs that are (a) currently within the same fragment, or (b) currently in different fragments. The difference is statistically significant ($P = 0.0375$; Table 2).

Table S1 Geographical coordinates (Datum: North American 1983) and site description for each collection location.

Table S2 mtDNA COI haplotypes by collection location.

Table S3 Pairwise estimates of Φ_{ST} (below diagonal) and associated *P* values (above diagonal) between collection locations. Collection locations 1, 5, 28 and 29 were removed from the analysis because they each contained only one individual.

References

Andren H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos*, **71**, 355–366.

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB (2002) Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics*, **33**, 707–740.

Atwood JL (1993) California gnatcatchers and coastal sage scrub: the biological basis for endangered species listing. In: *Interface Between Ecology and Land Development in California* (ed. Keeley JE), pp. 149–169. Southern California Academy of Science, Los Angeles, California.

Axelrod DI (1966) The Pleistocene Soboba flora of southern California. *University of California Publications, Geological Science*, **60**, 1–109.

Axelrod DI (1986) Cenozoic history of some western American pines. *Annual Review of the Missouri Botanical Gardens*, **73**, 565–641.

Axelrod DI, Govean F (1996) An Early Pleistocene closed-cone pine forest at Costa Mesa, southern California. *International Journal of Plant Sciences*, **157**, 323–329.

Bauder ET, McMillan S (1998) Current distribution and historical extent of vernal pools in southern California and northern Baja California, Mexico. In: *Ecology, Conservation and Management of Vernal Pool Ecosystems* (eds Witham CW, Bauder ET, Belk D, Ferren WR Jr, Ornduff R), pp. 56–70. California Native Plant Society, Sacramento, California.

Beier P (1995) Dispersal of juvenile cougars in fragmented habitat. *Journal of Wildlife Management*, **59**, 228–237.

Beier P, Penrod KL, Luke C, Spencer WD, Cabanero C (2006) South coast missing linkages: restoring connectivity to wildlands in the largest metropolitan area in the United States. In: *Connectivity Conservation* (eds Crooks KR, Sanjayan MA). Cambridge University Press, Cambridge, Massachusetts.

Bohonak AJ (1999) Dispersal, gene flow, and population structure. *Quarterly Review of Biology*, **74**, 21–45.

Bohonak AJ, Davies N, Villablanca FX, Roderick GK (2001) Invasion genetics of New World medflies: testing alternative colonization scenarios. *Biological Invasions*, **3**, 103–111.

Bohonak AJ, van der Linde K (2004) RMA: software for reduced major axis regression, version 1.17 for Java.

Bolger DT, Suarez AV, Crooks KR, Morrison SA, Case TJ (2000) Arthropods in urban habitat fragments in southern California: Area, age, and edge effects. *Ecological Applications*, **10**, 1230–1248.

Bond JE, Hedin MC, Ramirez MG, Opell BD (2001) Deep molecular divergence in the absence of morphological and ecological change in the Californian coastal dune endemic trapdoor spider *Aptostichus simus*. *Molecular Ecology*, **10**, 899–910.

Bond JE, Beamer DA, Lamb T, Hedin M (2006) Combining genetic and geospatial analyses to infer population extinction in mygalamorph spiders endemic to the Los Angeles region. *Animal Conservation*, **9**, 145–158.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences, USA*, **91**, 6491–6495.

California Department of Fish and Game (2005) California wildlife habitat relationships database system version 8.1. California Department of Fish and Game, Sacramento, California. http://www.dfg.ca.gov/whdab/html/cwhr.html.

Calsbeek R, Thompson JN, Richardson JE (2003) Patterns of molecular evolution and diversification in a biodiversity hotspot: The California Floristic Province. *Molecular Ecology*, **12**, 1021–1029.

Caltrans Traffic and Vehicle Data Systems Unit (2005) *All Traffic Volumes on CSHS*. California Department of Transportation, Sacramento, California. http://www.dot.ca.gov/hq/traffops/saferesr/trafdata/2005all.htm.

Case TJ, Fisher RN (2001) Measuring and predicting species presence: coastal sage scrub case study. In: *Spatial Uncertainty in Ecology: Implications for Remote Sensing and GIS Applications* (eds Hunsaker CT, Goodchild MF, Friedl MA, Case TJ), pp. 47–71. Springer, New York.

Casella G, Berger RL (2002) *Statistical Inference*, 2nd edn. Duxbury.

Cincotta RP, Wisnewski J, Engelman R (2000) Human population in the biodiversity hotspots. *Nature*, **404**, 990–992.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Cooper DS (2000) Breeding landbirds of a highly threatened open space: the Puente-Chino Hills, California. *Western Birds*, **31**, 213–234.

Crandall KA (1994) Intraspecific cladogram estimation: accuracy at higher levels of divergence. *Systematic Biology*, **43**, 222–235.

Crandall KA, Templeton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics*, **134**, 959–969.

Crandall KA, Templeton AR (1996) Applications of intraspecific phylogenetics. In: *New Uses for New Phylogenies* (eds Harvey PH, Brown AJL, Smith JM, Nee S), pp. 81–99. Oxford University Press, Oxford.

Crandall KA, Templeton AR, Sing CF (1994) Intraspecific phylogenetics: Problems and solutions. In: *Models in Phylogeny Reconstruction* (eds Scotland RW, Siebert DJ, Williams DM), pp. 273–297. Clerendon, Oxford.

Crooks KR, Suarez AV, Bolger DT, Soulé ME (2001) Extinction and colonization of birds on habitat islands. *Conservation Biology*, **15**, 159–172.

Crooks KR, Suarez AV, Bolger DT (2004) Avian assemblages along a gradient of urbanization in a highly fragmented landscape. *Biological Conservation*, **115**, 451–462.

Cunningham M, Moritz C (1998) Genetic effects of forest fragmentation on a rainforest restricted lizard (Scincidae: *Gnypetoscincus queenslandiae*). *Biological Conservation*, **83**, 19–30.

Dayanandan S, Dole J, Bawa K, Kesseli R (1999) Population structure delineated with microsatellite markers in fragmented populations of a tropical tree, *Carapa guianensis* (Meliaceae). *Molecular Ecology*, **8**, 1585–1592.

Desalle R, Freedman T, Prager EM, Wilson AC (1987) Tempo and mode of sequence evolution in mitochondrial DNA of Hawaiian *Drosophila*. *Journal of Molecular Evolution*, **26**, 157–164.

Dickson BG, Jenness JS, Beier P (2005) Influence of vegetation, topography, and roads on cougar movement in southern California *Journal of Wildlife Management*, **69**, 264–276.

Epps CW, Palsbøll PJ, Wehausen JD *et al.* (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters*, **8**, 1029–1038.

Ernest HB, Boyce WM, Bleich VC *et al.* (2003) Genetic structure of mountain lion (*Puma concolor*) populations in California. *Conservation Genetics*, **4**, 353–366.

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.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fisher RN, Suarez AV, Case TJ (2002) Spatial patterns in the abundance of the coastal horned lizard. *Conservation Biology*, **16**, 205–215.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. *Conservation Biology*, **14**, 1066–1074.

Gilpin ME, Soulé ME (1986) Minimum viable populations: processes of species extinction. In: *Conservation Biology* (ed. Soulé ME), pp. 19–34. Sinauer Associates., Massachusetts.

Haas C (2000) *Distribution, relative abundance, and roadway underpass responses of carnivores throughout the Puente-Chino Hill*s. Masters Thesis, California State Pollytechnic University, Pomona, California.

Haas C, Crooks K (1999) Carnivore abundance and distribution throughout the Puente/Chino Hills. Final report. Prepared for Mountains Recreation and. Conservation Authority and State of California Department of Transportation. US Geological Survey, Western Ecological Research Center.

Haas C, Backlin AR, Rochester C, Fisher RN (2002) Monitoring reptiles and amphibians at long-term biodiversity monitoring stations: the Puente-Chino Hills. Final Report. Prepared for Mountains Recreation and Conservation Authority, Puente Hills Landfill Native Habitat. Preservation Authority, and California State Parks. US Geological Survey, Western Ecological Research Center.

Hellberg ME (1994) Relationships between inferred levels of gene flow and geographic distance in a philopatric coral, *Balanophyllia elegans*. *Evolution*, **48**, 1829–1854.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754–755.

Hunter RD, Fisher RN, Crooks KR (2003) Landscape-level connectivity in coastal southern California, USA, as assessed through carnivore habitat suitability. *Natural Areas Journal*, **23**, 302–314.

Jacobs DK, Haney TA, Louie KD (2004) Genes, diversity, and geologic process on the Pacific coast. *Annual Review of Earth Planetary Sciences*, **32**, 601–652.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics*, **6**, 13.

Jockusch EL, Wake DB (2002) Falling apart and merging: Diversification of slender salamanders (Plethodontidae: *Batrachoseps*) in the American West. *Biological Journal of the Linnean Society*, **76**, 361–391.

Koelle A (2003) Cougar corridors: restoring the missing link in California's Chino Hills. *Road-RIPorter*, **8**, 3–5.

Legendre L, Legendre P (1983) *Numerical Ecology*. Elsevier Scientific Publishing, Amsterdam.

Li W-H (1997) *Molecular Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Lovich R (2001) Phylogeography of the night lizard, *Xantusia henshawi*, in southern California: evolution across fault zones. *Herpetologica*, **57**, 470–487.

Maldonado JE, Vila C, Wayne RK (2001) Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). *Molecular Ecology*, **10**, 127–147.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Mardulyn P, Milinkovitch MC (2005) Inferring contemporary levels of gene flow and demographic history in a local population of the leaf beetle *Gonioctena olivacea* from mitochondrial DNA sequence variation. *Molecular Ecology*, **14**, 1641–1653.

Mattoni R, Longcore T (1997) The Los Angeles coastal prairie, a vanished community. *Crossosoma*, **23**, 71–102.

McCaull J (1994) The natural community conservation planning program and the coastal sage scrub ecosystem of southern California. In: *Environmental Policy and Biodiversity* (ed. Grumbine RE). Island Press, Washington, D.C.

McKinney ML (2002) Urbanization, biodiversity, and conservation. *Bioscience*, **52**, 883–890.

Metcalf AE, Nunney L, Hyman BC (2001) Geographic patterns of genetic differentiation within the restricted range of the endangered Stephens' Kangaroo Rat *Dipodomys stephensi*. *Evolution*, **55**, 1233–1244.

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology*, **51**, 238–254.

Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. *Molecular Ecology*, **7**, 419–429.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Noss RF, Beier P, Shaw W (2002) Evaluation of the Coal Canyon biological corridor. Unpublished report. Prepared for Hills for Everyone, Brea, California, p. 19. Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.

Posada D (1999) *Collapse*. Department of Zoology, Brigham Young University, Provo, Utah.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. In: *Bioinformatics*, pp. 817–818.

Prentice TR, Burger JC, Icenogle WR, Redak RA (1998) Spiders from Diegan coastal sage scrub (Arachnida: Araneae). *Pan-Pacific Entomologist*, **74**, 181–202.

Prentice TR, Burger JC, Icenogle WR, Redak RA (2001) Spiders from Riversidian coastal sage scrub with comparisons to Diegan scrub fauna (Arachnida: Araneae). *Pan-Pacific Entomologist*, **77**, 90–122.

Radeloff VC, Hammer RB, Stewart SI *et al.* (2005) The wildland–urban interface in the United States. *Ecological Applications*, **15**, 799–805.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology*, **17**, 230–237.

Riley SPD, Pollinger JP, Sauvajot RM *et al.* (2006) A southern California freeway is a physical and social barrier to gene flow in carnivores. *Molecular Ecology*, **15**, 1733–1741.

Rodríguez F, Oliver JF, Marín A, Medina JR (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology*, **142**, 485–501.

Rodriguez-Robles JA, Denardo DF, Staub RE (1999) Phylogeography of the California mountain kingsnake, *Lampropeltis zonata* (Colubridae). *Molecular Ecology*, **8**, 1923–1934.

Rowland SM (1984) Geology of Santa Catalina Island: A review. In: *Geology of Santa Catalina Island and Nearby Basins* (ed. Pipkin BW), pp. 1–27. University of Southern California, Los Angeles, California.

Roy K, Valentine JW, Jablonski D, Kidwell SM (1996) Scales of climatic variability and time averaging in Pleistocene biotas: implications for ecology and evolution. *Trends in Ecology &Evolution*, **11**, 458–463.

Royte E (2002) Wilding America. *Discover*, **23** issue 9.

Saucedo GJ, Bedford DR, Raines GL, Miller RJ, Wentworth CM (2000) *GIS Data for the Digital Map of California*, Version 2.0. California Department of Conservation, Division of Mines and Geology, CDROM 2000-007, Sacramento, California.

Searcy KB (1969) Variation in *Cercocarpus* in Southern California. *New Phytologist*, **68**, 829–839.

Sgariglia EA, Burns KJ (2003) Phylogeography of the California thrasher (*Toxostoma redivivum*) based on nested-clade analysis of mitochondrial-DNA variation. *Auk*, **120**, 346–361.

Sokal RR, Rohlf FJ (1995) *Biometry: the Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman, New York.

Spencer WD (2005) Maintaining ecological connectivity across the 'missing middle' of the Puente-Chino Hills wildlife corridor. Final report. Prepared for Resources Legacy Fund Foundation, p. 52. Conservation Biology Institute, San Diego, California.

Spencer WD, White MD, Stallcup JA (2001) On the global and regional ecological significance of southern Orange County: conservation priorities for a biodiveristy hotspot. Unpublished report prepared for Endangered Habitat League, p. 44. Conservation Biology Institute, San Diego, California.

Spielman D, Brook BW, Briscoe DA, Frankham R (2004) Does inbreeding and loss of genetic diversity decrease disease resistance? *Conservation Genetics*, **5**, 439–448.

Suarez AV, Bogler DT, Case TJ (1998) Effects of fragmentation and invasion on native ant communities on coastal southern California. *Ecology*, **79**, 2041–2056.

Swei A, Brylski PV, Spencer WD, Dodd SC, Patton JL (2003) Hierarchical genetic structure in fragmented populations of the Little Pocket Mouse (*Perognathus longimembris*) in Southern California. *Conservation Genetics*, **4**, 501–514.

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**, 585–596.

Tan A-M, Wake DB (1995) mtDNA phylogeography of the California newt, *Taricha torosa* (Caudata, Salamandridae). *Molecular Phylogenetics and Evolution*, **4**, 383–394.

Templeton AR (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology*, **7**, 381–397.

Templeton AR, Robertson RJ, Brisson J, Strasburg J (2001) Disrupting evolutionary processes: the effect of habitat fragmentation on collared lizards in the Missouri Ozarks. *Proceedings of the National Academy of Sciences, USA*, **98**, 5426–5432.

Templeton AR, Shaw K, Routman E, Davis SK (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden*, **77**, 13–27.

Tigas LA, Van Vuren DH, Savajot RM (2002) Behavioral responses of bobcats and coyotes to habitat fragmentation and corridors in an urban environment. *Biological Conservation*, **108**, 299–306.

Vandergast AG, Gillespie RG, Roderick GK (2004) Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology*, **13**, 1729–1743.

Vedder JG, Howell DG (1980) Topographic evolution of the southern California borderland during Late Cenozoic time. In: *The California Islands: Proceedings of a Multidisciplinary Symposium* (ed. Power DM), pp. 7–31. Santa Barbara Museum of Natural History, Santa Barbara, California.

Velleman PF (1997) *DataDesk*. Data Description, Ithaca, New York.

Ward PS (2005) A synoptic review of the ants of California (Hymenoptera: Formicidae). *Zootaxa*, **936**, 1–68.

Weissman DB (2001a) Communication and reproductive behaviour in North American Jerusalem crickets (*Stenopelmatus*) (Orthoptera: Stenopelmatidae). In: *The Biology of Wetas, King Crickets and Their Allies* (ed. Field LH), pp. 351–375. CABI Publishing, Wallingford, Oxfordshire, UK.

Weissman DB (2001b) North and Central America Jerusalem crickets (Orthoptera: Stenopelmatidae): taxonomy, distribution, life cycle, ecology and related biology of the American species. In: *The Biology of Wetas, King Crickets and Their Allies* (ed. Field LH), pp. 57–72. CABI Publishing, Wallingford, Oxfordshire, UK.

Wilcox TP, Zwickl DJ, Heath TA, Hillis DM (2002) Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution*, **25**, 361–371.

Williams BL, Brawn JD, Paige KN (2003) Landscape scale genetic effects of habitat fragmentation on a high gene flow species: *Speyeria idalia* (Nymphalidae). *Molecular Ecology*, **12**, 11–20.

Yanev KP (1980) Biogeography and distribution of three parapatric salamander species in coastal and borderland California. In: *The California Islands: Proceedings of a Multidisciplinary Symposium* (ed. Power DM), pp. 531–550. Santa Barbara Museum of Natural History, Santa Barbara, California.

Amy Vandergast is a Geneticist with the USGS, Western Ecological Research Center with interests in landscape genetics and conservation of terrestrial invertebrates. Andrew Bohonak (http://www.bio.sdsu.edu/pub/andy) is an Associate Professor at San Diego State University with interests in conservation genetics, invertebrate population biology and freshwater biology. David Weissman is a Research Associate at California Academy of Sciences with interests in revisionary studies on *Stenopelmatus* Jerusalem crickets, *Gryllus* field crickets, western US decticine katydids, oedipod grasshoppers, and Baja California, Mexico, Orthoptera. Robert Fisher is a Biologist with the USGS, Western Ecological Research Center. His research focuses on the effects of urbanization on biodiversity using evolution, ecology, and natural history.
