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ORIGINAL PAPER

Loss of genetic connectivity and diversity in urban microreserves in a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus* n. sp. "santa monica")

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Abstract Microreserves may be useful in protecting native arthropod diversity in urbanized landscapes. However, species that do not disperse through the urban matrix may eventually be lost from these fragments. Population extinctions may be precipitated by an increase in genetic differentiation among fragments and loss of genetic diversity within fragments, and these effects should become stronger with time. We analyzed population genetic structure in the dispersal limited Jerusalem cricket *Stenopelmatus* n. sp. "santa monica" in the Santa Monica Mountains and Simi Hills north of Los Angeles, California (CA), to determine the impacts of fragmentation over the past 70 years. MtDNA divergence was greater among urban fragments than within contiguous habitat and was positively correlated with fragment age. MtDNA genetic diversity within fragments increased with fragment size and decreased with fragment age. Genetic divergence across 38 anonymous nuclear Inter-Simple Sequence Repeat (ISSR) loci was influenced by the presence of major highways and highway age, but there was no effect of additional urban fragmentation. ISSR diversity was not correlated with fragment size or age. Differing results between markers may be due to male-biased dispersal, or different effective population sizes, sorting rates, or mutation rates among sampled genes. Results suggest that genetic connectivity among populations has been disrupted by highways and urban development, prior to declines in local population sizes. We emphasize that genetic connectivity can rapidly erode in fragmented landscapes and that flightless arthropods can serve as sensitive indicators for these effects.

Keywords Habitat fragmentation · Insects · ISSR loci · mtDNA sequences · Population genetic structure

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Introduction

In urbanized landscapes, preserving small tracts of natural lands (microreserves) may help retain some aspects of terrestrial arthropod diversity (Shafer 1995; Connor et al. 2002; Watts and Lariviere 2004; Bond et al. 2006; Cook and Faeth 2006). However, the components of biodiversity that remain in these fragments will almost certainly be a subset of the original faunal assemblage. Specialized, rare and poorly dispersing species disappear more quickly in small reserves than generalists with larger population sizes and greater abilities to disperse through the urban matrix (Shochat et al. 2004; Watts and Lariviere 2004; Cook and

Faeth 2006). Even species that initially appear to maintain functioning populations in small habitat fragments may experience a loss of genetic connectivity. The isolation of local populations generally leads to an increase in genetic divergence and a loss of genetic variability within fragments (Keller and Largiader 2003; Keyghobadi et al. 2005; Vandergast et al. 2007). Loss of genetic variability coupled with increased inbreeding in small, isolated populations may lead to significant reductions in survival and reproductive success (Reed and Frankham 2003) and loss of adaptive potential (Stockwell et al. 2003; Spielman et al. 2004). These effects may be most detrimental in small fragments that abut urban edges where ecological perturbations are most pervasive (McKinney 2002; Radeloff et al. 2005). Small, isolated populations are also more susceptible to stochastic extinction, with little chance of recolonization from remaining populations. Over time, the accumulated loss of local populations and the genetic diversity they harbor can potentially lead to the decline of the entire meta-population (Gilpin and Soulé 1986; Templeton et al. 1990).

Standard field monitoring techniques focused on qualitative estimates of abundance may not detect declining populations until they reach the point of large-scale demographic decline or local extinctions (Taylor and Gerrodette 1993; Leon-Cortes et al. 1999; Maxwell and Jennings 2005; but see Pollock 2006). However, for apparently widespread species, genetic monitoring may detect declines in genetic connectivity and variability that precede local extinctions, and identify the landscape features that are most influential in this loss (Keyghobadi et al. 1999; Gerlach and Musolf 2000; Vos et al. 2001). Genetic monitoring is particularly useful in species and habitats where it is difficult to monitor movement directly (e.g., through telemetry, mark/recapture efforts). Understanding contemporary population genetic structure in the context of historical genetic integrity can help inform reserve managers of potential population declines in key species or groups of species that share similar life history traits (Epps et al. 2005; Riley et al. 2006).

Using mitochondrial DNA sequences and anonymous nuclear Inter-Simple Sequence Repeat (ISSR) markers, we examined the genetic structure of the Jerusalem cricket, *Stenopelmatus* n. sp. "santa monica" (Orthoptera: Stenopelmatidae) in fragments throughout the Simi Hills of southern California and in the adjacent and more contiguous Santa Monica Mountains. Although *S.* n. sp. "santa monica" persists in relatively high abundances in fragments, it is a wingless, large-bodied, and slow-moving insect. These characteristics suggest that this species' ability to move through the urban landscape may be impeded by roads, additional modified or unsuitable urban habitat (sensu Vandergast et al. 2007), and/or a combination of other environmental perturbations associated with urban development (e.g., increased predation risks due to lack of cover, interactions with non-native species, chemical and light pollutants). We specifically focused on (1) whether the presence of major highways and additional urban development were associated with higher levels of genetic divergence, (2) whether genetic divergence was correlated with temporal patterns of urban development, and (3) whether genetic diversity within fragments was associated with fragment size or age.

Methods

Study organism

Stenopelmatus Jerusalem crickets are distributed throughout western North America (Weissman 2001a). They are nocturnally active, retreating underground during the day; and omnivorous, feeding on roots, small invertebrates and detritus. Jerusalem crickets are large-bodied and heavy (adult females of *S.* n. sp. "santa monica" can reach weights up to 11 g), with low vagility. They lack wings and have short, stocky hind legs that are modified for digging, rather than jumping. Recent work on mating calls (produced by abdominal drumming), morphology, cytology and genetics of the group has revealed much greater species diversity than had been previously described, with 100 putative species occurring in the U.S. alone. A taxonomic revision of the genus is currently underway (Weissman 2001a). *Stenopelmatus* n. sp. "santa monica" is distinguished from other co-occurring species based on morphological characters (rear tibia spination and abdominal coloration), a mating call of 7 drums per second in both males and females (at 20 C), the presence of a male sex-clarification drum, a male chromosome count of 25, lack of an adult reproductive dormancy period, and high levels of mtDNA sequence divergence when compared to other closely related species (>8% average pairwise divergence). Per Article 8.3 of the International Code of Zoological Nomenclature, we use the manuscript name of *Stenopelmatus* n. sp. "santa monica" herein, while disclaiming this name as "not available" at the present time. The species' range is restricted to southern California, where it is found primarily in scrubland, oak woodland, and grassland habitats (DBW pers. obs.).

Study site and sample collection

This study was conducted in the Santa Monica Mountains and the Simi Hills of Los Angeles, California (Fig. 1). Urban areas found mainly north of CA State Highway 101 (which we denote as the Simi Hills) comprise the cities of

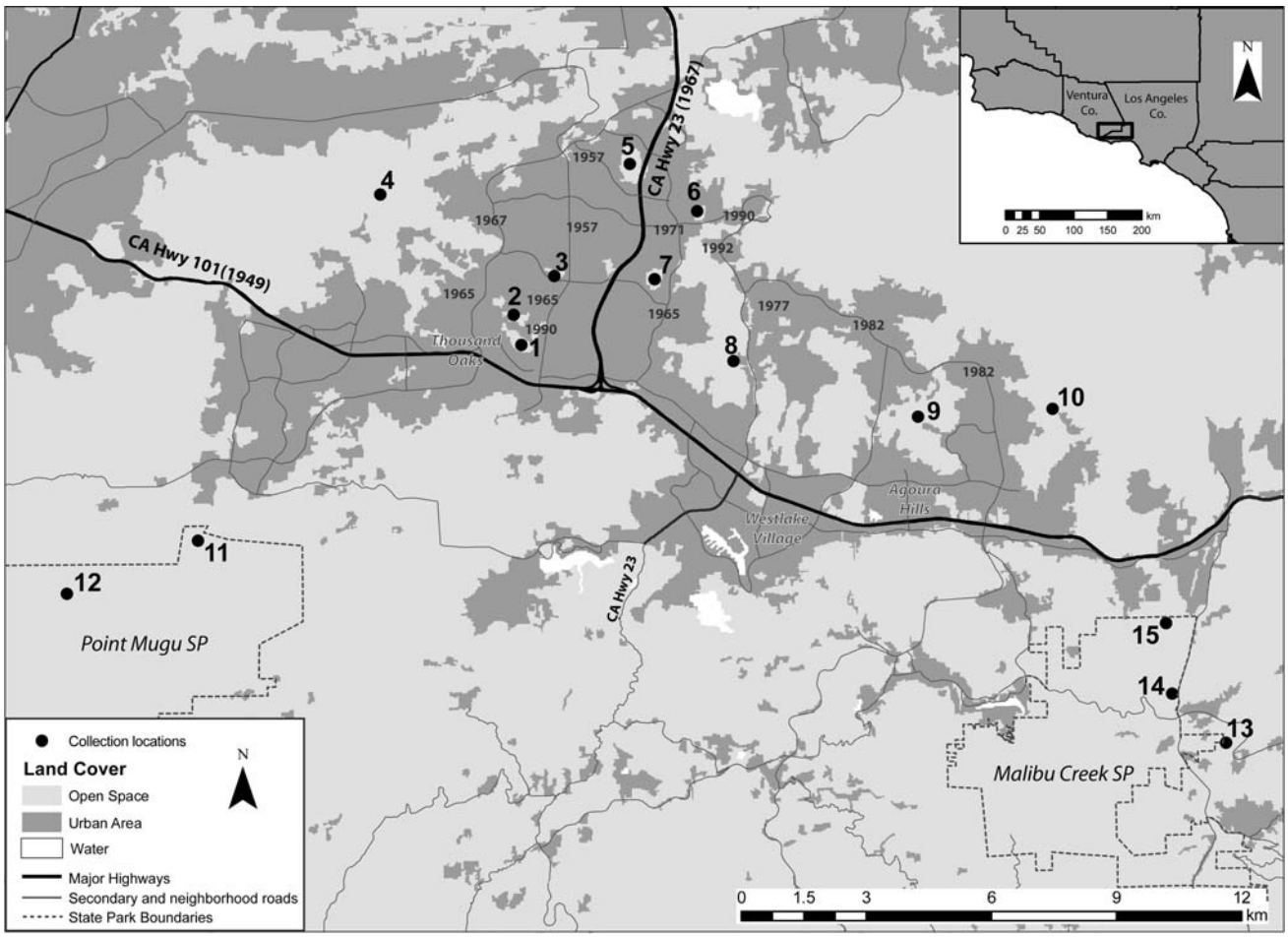

Fig. 1 Map of the study area within the Simi Hills (collection locations 1–10) and the more contiguous open space south of Highway 101 (collection locations 11–15) in Los Angeles and Ventura Counties, California. Shaded areas are urbanized and light grey represents open space which is mainly comprised of mixed chaparral, coastal scrub, annual grasslands, coastal oak woodlands, and riparian habitat types. Highways and local roads further subdivide the study area. The earliest subdivision or road build years are marked between study fragments. Cities are labeled in grey italic text

Thousand Oaks, Westlake Village and Agoura Hills. Urban development in this region has grown substantially over the last several decades, beginning in the late 1930s through the present. The patchwork of remaining open spaces are largely protected and managed by several agencies, including National Park Service (NPS), California Department of Parks and Recreation, Santa Monica Mountains Conservancy, Conejo Open Space Conservation Agency, Conejo Recreation and Park District, Mountains Recreation and Conservation Authority, City of Thousand Oaks, and Rancho Simi Recreation and Park District.

Individuals were collected from 15 sampling locations in pitfall trap arrays between 2000–2002 and 2005, and preserved in 70% or 95% ethanol. Most samples were collected by NPS personnel during herpetofaunal and ground-dwelling invertebrate monitoring activities (Busteed 2003), and were supplemented by additional pitfall collections by EAL (see Case and Fisher 2001; Fisher et al. 2002 for pitfall sampling methods). We defined a sampling location as a group of pitfall trap arrays (1–7 arrays) within approximately 2 km of one another and not separated by potential geographic barriers to movement (e.g., rivers, dry river beds, mountains, roads, urban development, cultivated fields). Sampling locations included 6 microreserves (<1 km^2, locations 1, 2, 3, 5, 6, 7), 2 mid-sized fragments ($\sim$4 km^2, locations 8, 9) and 2 large fragments (>20 km^2, locations 4, 10) in the Simi Hills, north of CA State Highway 101 (Fig. 1). Vegetation types in these fragments consist primarily of coastal sage scrub and grasslands with some mixed chaparral. These fragments are separated from each other by residential and commercial development, several roads and a major highway (CA State Highway 23). The five remaining sampling locations were located south of Highway 101, in more contiguous chaparral, scrub and

grasslands of the Santa Monica Mountains. However, western sampling locations (11, 12) and eastern locations (13–15) within the Santa Monica Mountains are separated by several smaller, single lane roads including the southern extension of CA State Highway 23 (Fig. 1).

Mitochondrial DNA genotyping

Sequences were collected from a total of 164 *S.* n. sp. "santa monica" individuals. Genomic DNA was isolated from the femur of one leg of each specimen using DNeasy Tissue Kits (Qiagen, Valencia, CA). A 708 base pair region of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the universal primer pair LCO1490: 5′GGT CAACAAATCATAAAGATATTGG, and HCO2198: 5′TA AACTTCAGGGTGACCAAAAAATCA (Folmer et al. 1994). Polymerase chain amplifications were as follows: 95 C for 2 min; 35 cycles of 95 C for 30 s, 54 C for 30 s, and 72 C for 40 s; 72 C for 7 min. Amplification reactions consisted of 5 µl of DNA, 1.5U Platinum Taq Polymerase (Invitrogen), 2.3 mM $MgCl_2$, 0.2 µM each dNTP, and 0.48 µM each primer in 25 µl total volume. PCR products were purified using the Ultraclean PCR Purification Kit (Qiagen, Valencia, CA), cycle sequenced in the forward direction using Big Dye Terminator III (Applied Biosystems), and run on an ABI 3100 automated sequencer at the San Diego State University Microchemical Core Facility. Resulting sequences were aligned manually in Sequencher (v. 3.1.1; Gene Codes Corporation). No insertions or deletions were found, and ambiguous end regions were clipped so that all individuals were analyzed over the same sequence length of 639 bases. Unique haplotypes were identified using the program Collapse v. 1.1 (Posada 1999).

ISSR genotyping

Inter-Simple Sequence Repeats (ISSRs) are anonymous, presumably nuclear fragments amplified between simple sequence repeats with a single, anchored primer that binds to the tandem repeat motif. While simple repeat motifs are common throughout the nuclear genome, they rarely occur in animal mitochondrial genomes (Nardi et al. 2001; Snäll et al. 2002; Mayer and Kerth 2005). ISSRs have been used extensively in population genetic studies of invertebrates (Chatterjee et al. 2004, 2005; Kar et al. 2005; Maltagliati et al. 2005) and typically yield large numbers of polymorphic loci (Wolfe 2005). Although the amplification protocols are similar to those used for random amplified polymorphic DNA (RAPDs), the annealing temperature for ISSR amplification is much higher, resulting in higher stringency (Wolfe and Liston 1998; Wolfe 2005). Therefore, ISSRs tend to be more reproducible than RAPDs and more comparable to AFLPs (Wolfe 2005). Because these markers require little prior genetic information on the species of interest, they provide a time efficient and a cost effective method for sampling multiple nuclear markers (Wolfe 2005).

One hundred di-repeat ISSR primers with a single nucleotide anchor at the 3′ end (University of British Columbia Nucleic Acid-Protein Service Unit, Primer Set #9) were tested at an annealing temperature of 55 C, and 64 of these successfully amplified *S.* n. sp. "santa monica" DNA. From these, we selected a primer that reliably produced many polymorphic bands visible with agarose gel electrophoresis. Primer 827 (5′-(AC)8G -3′) was optimized for annealing temperature to the nearest 0.5 C using unlabeled primer, and subsequently amplified in separate reactions with a 6FAM (blue) fluorescent dye-labeled primer. All ISSR PCR amplifications were performed with a final volume of 25 µl using 18.5 µl Platinum Taq Supermix (Invitrogen), 1.5 µl of primer (15 µM), and 5 µl of DNA. Amplifications were performed under the following conditions: 94 C for 2 min; 40 cycles of 94 C for 30 s, 64.5 C for 30 s, 72 C for 2 min; 72 C for 7 min. Positive and negative controls were performed for each set of PCR amplifications. ISSR genotyping was performed on an ABI 3100 capillary machine. The presence or absence of a locus (defined as a segment of DNA that may or may not amplify) was determined visually using GeneMapper 3.7 (ABI). In order to ensure repeatability, we ran one positive control sample six times (once with each batch of genotyping) and 17 samples were repeated twice. We limited our genotype scoring to bands that were present in repeated samples, and loci with discrepancies among positive controls for the presence or absence of amplified DNA were removed. Based on comparisons of repeated samples, we determined that fragments could be sized unambiguously to the nearest 2 bases. We converted genotype data for all loci into binary code with "0" representing absence and "1" representing the presence of an amplified DNA fragment.

Population genetic analyses

Many tree-building techniques tend to poorly resolve intraspecific gene genealogies when ancestral haplotypes are retained, multifurcations exist and the number of mutations between haplotypes is small (Crandall 1994). Therefore, we estimated haplotypic relationships using a parsimony network reconstructed in the program TCS (Clement et al. 2000). A 95% maximum parsimony connection criterion was used and ambiguous connections among sets of haplotypes were resolved when possible using previously established criteria (Crandall and Templeton 1993; Crandall 1994).

For mtDNA sequences, the number of haplotypes, gene diversity (Nei 1973), nucleotide diversity (Tajima 1983), the number of segregating sites (S), and Tajima's test of selective neutrality (Tajima 1989) were calculated for each collection location and across all samples in the program Arlequin 3.1 (Excoffier et al. 2005). The null hypothesis of $D = 0$ was tested in Arlequin using a coalescent simulation algorithm under the hypothesis of selective neutrality and drift-mutation equilibrium. To estimate ISSR variation within sampling sites, we used Popgene 1.31 (Yeh et al. 1999) to calculate the number of polymorphic loci and gene diversity.

Population subdivision was quantified using F-statistics. For mtDNA sequences, 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). Uncorrected pairwise differences were used to calculate relatedness. Significance was assessed with 10,000 randomizations of the AMOVA test statistic. ISSRs are dominant markers for which allele frequencies, observed heterozygosity (H_o) and inbreeding coefficients ($f = F_{IS}$) cannot be estimated directly. For these markers, we used two methods to calculate θ (Weir and Cockerham 1984), an estimator of F_{ST}. Overall ISSR divergence among all sites was quantified as θ_β using Hickory 1.0 (Holsinger et al. 2002). This program uses a Bayesian algorithm to estimate heterozygosity within each sampling site (H_e, Table 1), which is subsequently used to estimate θ_β. We performed our analyses using the "f-free model" in Hickory 1.0, which chooses the inbreeding coefficient, f, randomly from a non-informative prior during sampling. Ninety-five percent credible intervals around θ_β were used to test whether population subdivision was greater than zero. We also used the program TFPGA (Miller 1993) to estimate Weir and Cockerham's (1984) θ under the more strict assumption of Hardy–Weinberg genotype frequencies within each population. The frequency of recessive alleles was estimated using a Taylor expansion, a less biased estimator than the square root of the count of absent bands (Lynch and Milligan 1994). Ninety-five percent confidence intervals around θ were generated with 10,000 bootstrap replications across loci.

Each sampling location was estimated at the geographic coordinates of the pitfall trap array (estimated with a GPS), or the geographic center of arrays if multiple arrays were combined. Euclidean geographic distances among all sampling locations were measured in ArcGIS 9.1 (ESRI). Pairwise matrices of geographic distance and Φ_{ST} or θ were compared using Mantel tests for matrix correlation (Mantel 1967), with significance assessed by 10,000 randomizations of the genetic distance matrix. Genetic distances were log-transformed. These "isolation by distance" (IBD) analyses were performed using IBDWS 2.5 (Jensen et al. 2005).

Assessing loss of genetic connectivity

Following our earlier work (Vandergast et al. 2007), we first examined whether natural barriers to gene flow may have existed prior to widespread urbanization throughout the sampled area since these would have influenced genetic structure prior to urban fragmentation. Examination of geologic maps revealed patchy deposits of Pleistocene and early Holocene aged sedimentary rocks in the valley between the Simi Hills and the Santa Monica Mountains (along Highway 101), and along present day Malibu creek (separating localities 11–12 from 13–15). This suggests some flooding of low lying areas during this period. However, preliminary analyses using partial Mantel tests revealed no relationship between genetic differentiation and these potential barriers (data not shown). This is likely due to the fact that sampling locations were never surrounded by these deposits, and so sampling locations were unlikely to have been completely isolated (at least by these water barriers) prior to urbanization. Riley et al. (2006) also suggested that natural habitat in this region was contiguous prior to urbanization.

We estimated the effect of fragmentation between pairs of sampling locations by (1) major highway presence, (2) highway age, (3) combined urban development and highway presence, and (4) combined urban and highway age. These were defined as follows.

(1) *Major highway presence*: Major highways in the study area include CA State Highway 101 and CA State Highway 23 north of 101, which dissect the study area into three sections. These routes were considered to be potential barriers to dispersal due to the high traffic volumes, multiple lanes, berms, medians and/or fences along the majority of these routes. Highway 101 had an annual average daily traffic volume of over 400,000 cars per day in 2005, and Highway 23 north of 101 had an annual average daily traffic volume of 80,000–190,000 cars per day (Caltrans 2005). South of Highway 101, CA State Highway 23 was traveled by approximately 80,000 cars per day in the northern portion of the route through Westlake Village, although traffic volume dropped substantially south of Westlake Village (1000 or fewer cars per day, Caltrans 2005). Given low traffic volume and the absence of berms, curbs, multiple lanes, fencing and medians along this stretch of road, we did not consider Highway 23 south of Highway 101 to be a major highway in our analyses.

Table 1 Collection locations, fragment size estimates, number of crickets per unit sampling effort, and mtDNA and ISSR summary statistics

Sampling location	Site name	Fragment size (km^2)	No. captured/ array/trap year	N mtDNA	No. of haplotypes	mtDNA gene diversity	θ_K	θ_π	Tajima's D	*N* ISSRs	Polymorphic loci	ISSR gene diversity	H_e
1	Firework Hill	0.2151	3.00	4	4	1.0000	NA[a]	4.0000	−0.8241	4	2	0.0122	0.0686
2	Botanical Gardens	0.0315	4.00	6	4	0.8000	4.0627	7.9333	−0.8601	5	5	0.0321	0.0730
3	Labisco Hill	0.0945	4.00	10	3	0.5111	1.0522	10.3556	1.2973	9	14	0.0554	0.0817
4	Wildwood	53.4061	1.50	8	4	0.8214	2.5006	3.7500	1.0417	8	15	0.0535	0.0823
5	Pederson	0.3861	3.00	5	4	0.9000	7.1062	6.4000	−0.8124	5	8	0.0440	0.0786
6	Erbes	0.1071	4.50	9	5	0.7222	3.8288	1.3333	−1.7278[b]	7	10	0.0733	0.0903
7	Old Meadows	0.1206	3.50	9	5	0.7222	3.8288	5.2500	−1.8811[b]	9	6	0.0271	0.0646
8	Hillcrest Patch	4.6643	4.83	24	10	0.7065	5.9124	3.0978	−2.1386[b]	23	19	0.0545	0.0772
9	Rancho Simi Patch	6.1840	1.5	8	4	0.7500	2.5006	6.1786	−1.7466[b]	8	7	0.0371	0.0709
10	Pala Camado	162.0962	2.75	8	6	0.9286	9.2308	2.3214	−0.6668	7	6	0.0393	0.0727
11	Pt. Mugu SP A	213.5114	5.00	5	5	1.0000	NA[a]	6.8000	1.3058	5	6	0.0383	0.0771
12	Pt. Mugu SP B	213.5114	4.13	31	19	0.9591	19.9092	9.4258	1.5918	33	22	0.0489	0.0648
13	Malibu Crk SP A	355.1453	13.00	13	7	0.7949	5.4253	2.2564	−0.4831	11	14	0.0608	0.0828
14	Malibu Crk SP B	355.1453	3.00	12	9	0.9545	14.6866	4.9091	−0.0501	10	18	0.1084	0.1239
15	Malibur Crk SP C	355.1453	4.67	12	10	0.9697	25.5629	7.4697	−0.4353	11	9	0.0585	0.0806
Total				164	79	0.9655	58.4260	14.6342	0.1659	155	37	0.0592	0.0793

N refers to the number of individuals sequenced (mtDNA) or genotyped (ISSRs)

[a] θ_K cannot be computed when all gene copies are unique

[b] Tajima's D significantly different from zero, $P \leq 0.02$

A categorical (binary) fragmentation matrix was created with values of 1 for population pairs separated by major highways and 0 for pairs not separated.

(2) *Highway age*: Genetic isolation effects are expected to increase with time since isolation. Highway ages were estimated from route inception dates (www.cahighways.org) for both major highways (see Fig. 1). To create a "highway age" matrix, pairs of sampling locations separated by highways were assigned that highway's age and pairs not separated by a highway were assigned an age of 0.

(3) *Combined Urban development and highway presence*: Urban development further fragments areas between major highways, particularly to the north of Highway 101. A "combined urban and highway presence" matrix was created by combining major highways and the presence of urban development (estimated from the California Department of Forestry and Fire Protection's (2005) LCMMP Vegetation data (http://frap.cdf.ca.gov/projects/land_cover/mapping/index.html) and aerial photographs). Collection points separated by urban areas and/or major highways were categorized as 1, and those within contiguous open habitat were categorized as 0.

(4) *Combined urban and highway (fragment) age*: We examined maps of subdivision and road development years (created by U. S. National Park Service (NPS), available upon request) to create a combined urban and highway (fragment) age matrix consisting of the time (in years) since fragment pairs were isolated from one another by intervening roads or urban development. Pairs of collection locations connected by continuous habitat were assigned an age of 0.

In all cases, we assessed the correlation between the pairwise genetic differentiation matrix and fragmentation indices *after* controlling for geographic distance using partial Mantel tests (Legendre and Legendre 1983) with IBDWS 2.5 (Jensen et al. 2005). We also visually examined IBD scatterplots with different symbols for fragmented and unfragmented pairs of sites.

Assessing loss of genetic diversity

We examined the relationship between genetic diversity and fragmentation measures including fragment size and fragment isolation age. Fragment size was calculated from habitat and road coverages in the program FragStats (McGarigal and Marks 1995). The isolation age of each fragment was calculated as the time since the year a fragment was at least 95% surrounded by road and urban development, again using development year maps created by NPS. Genetic diversity within fragments was estimated from mtDNA sequences as (1) θ_π, which estimates the population parameter $\theta = 4N_e\mu$ from the average sequence divergence π, and (2) θ_K, which estimates $\theta = 4N_e\mu$ from the number of haplotypes (K). Calculations were performed in Arlequin. For ISSRs we estimated diversity with both Nei's (1973) gene diversity (calculated in Popgene) and H_e, (the average panmictic heterozygosity within each population, calculated in Hickory 1.0 under the f-free model). For each genetic diversity estimate, we used linear regressions to determine whether patterns of genetic diversity were explained by fragment size or fragment age. All variables were log-transformed to remove skew, mute the effect of outliers, and to help achieve linearity. We also used two-sample t-tests to determine whether diversity estimates from samples within microreserves (<1 km^2) were less than those from larger fragments and contiguous areas. Statistical analyses were performed in DataDesk, v. 6.2.1 (Vellman 1997).

Results

MtDNA COI sequence variation

The 164 sequences of *S.* n. sp. "santa monica" yielded 80 unique haplotypes (Genbank accession nos: EU833485–EU833648). Over a total sequence length of 639 bases, there were 79 polymorphic sites (68 transitions, 19 transversions) and 59 parsimony informative characters. All mutations were silent (i.e., did not result in amino acid substitutions). Gene diversity within sampling locations (equivalent to expected heterozygosity with a sample size correction) ranged from 0.5111 to 1 (Table 1). Four sampling locations had significantly negative values of Tajima's D (sampling locations 6–9, Table 1), suggesting purifying selection, a selective sweep, recovery from a temporary bottleneck, or recent population growth (Tajima 1989). For the total set of sequences pooled across all locations, Tajima's D did not deviate significantly from neutrality.

ISSR variability

From a total of 155 individuals, 38 bands could be reliably scored, of which 37 were variable. The number of polymorphic loci varied from 2 to 22 within collection sites and gene diversity ranged from 0.0122 to 0.1084 (Table 1).

Population genetic structure

The majority of mtDNA haplotypes (70 of 80) were restricted to a single sampling location, while the remaining ten haplotypes were found in multiple locations (Appendix 1). Our haplotype network did not support

exclusive haplotype groups among regions (East and West Simi Hills, Point Mugu State Park, Malibu Creek State Park), suggesting incomplete lineage sorting or limited gene flow (past or present) at this scale. However, some geographically isolated haplotype groups exist. For example, a group containing haplotypes restricted to the Simi Hills was twelve steps away from the most closely related haplotype, found in Point Mugu (H21 to H58, Fig. 2), and connected to the major network at the 94% but not 95% connection limit. Another haplotype group contained haplotypes restricted to Malibu Creek sampling locations and was five steps away from the closest related haplotype in Point Mugu (H62 to H58, Fig. 2). The genetic distances among these haplotype groups may reflect large geographic distances between the three regions that were sampled. Of haplotypes shared across collecting locations, H4 was shared across two regions (Simi Hills and Point Mugu: sampling locations 1–3, 7 and 11). Haplotype H1 was shared among four Simi Hills fragments west of State Route 23 (sampling locations 1–4). Three haplotypes (H8, H9, H12) were found in the Simi Hills fragments on both sides of State Highway 23 (sampling locations 1–10). The remaining five haplotypes (H61, H64, H69, H70, and H71) were shared among Malibu Creek sampling locations (13–15). Accordingly, population genetic structure measured with mtDNA was high ($\Phi_{ST} = 0.63$, $P \leq 0.0001$). Pairwise genetic and geographic distances were positively correlated (Mantel test $r = 0.45$, $P \leq 0.0001$, Fig. 3a).

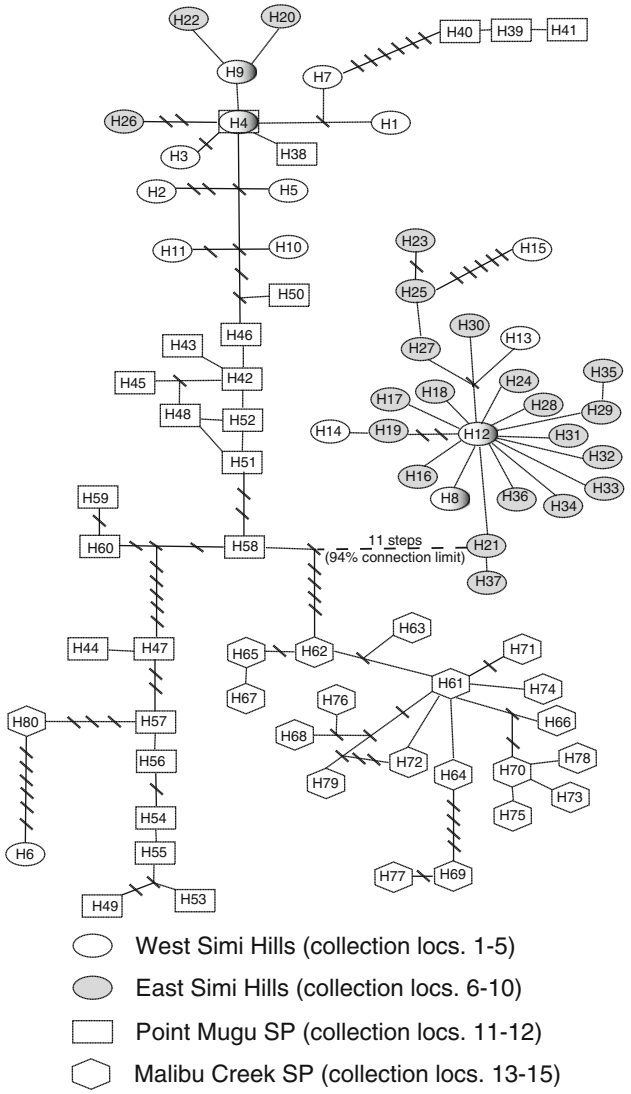

Fig. 2 Ninety-five percent connection criterion parsimony network depicting the relationship among mtDNA haplotypes. Base changes between haplotypes are represented as lines. Haplotype shapes reflect region of origin. Grey ovals represent haplotypes found in the Simi Hills, east of Highway 23, open ovals represent those found in the Simi Hills west of Highway 23. Rectangles depict haplotypes found in Pt. Mugu State Park, and haplotypes found in Malibu Creek State Park are shown as hexagons. Four haplotypes (H4, H8, H9 and H12) are shared among regions

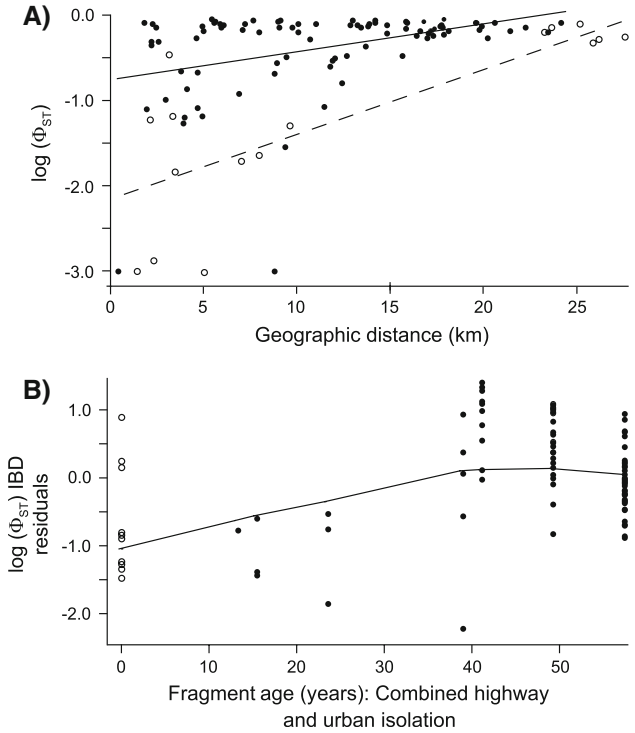

Fig. 3 Results of isolation by distance analyses for mtDNA sequence data. (**a**) Scatterplot of log genetic distance by geographic distance. Open circles represent population pairs in contiguous habitat and filled circles represent populations fragmented by urban areas and major highways. Regression lines are drawn for population pairs separated by urban areas and highways (solid line) and for contiguous populations (dashed line). (**b**) Scatterplot of residuals from the IBD regression against combined highway and urban age. Open circles represent comparisons between contiguous collection locations south of Highway 101, and filled circles represent comparisons between collection locations north of Highway 101. Contrasts across Highway 101 constitute the largest age class. A locally weighted regression smoother (Cleveland 1979) is plotted to visualize trends

In comparison to mtDNA data, ISSR data showed markedly lower, but still statistically significant levels of population differentiation. Estimated under the *f*-free model, $\theta_\beta = 0.07$ (95% credible intervals: 0.05–0.10); and assuming H–W genotype frequencies, $\theta = 0.06$ (95% CI: 0.03–0.09). These two estimates of θ did not differ substantially from one another (i.e., 95% credible and confidence intervals overlapped). Similar to mtDNA results, ISSR pairwise genetic and geographic distances were also positively correlated with the same correlation coefficient (Mantel test $r = 0.45$, $P \leq 0.0003$, Fig. 4a).

Genetic effects of habitat fragmentation

The effects of fragmentation on genetic connectivity were more pronounced in mtDNA sequence data than in ISSRs. For mtDNA sequences, highway presence, highway age, combined highway and urban presence, and fragment age were all significantly correlated with genetic differentiation when controlling for geographic distance (Table 2, Fig. 3). Measures that accounted for urban development combined with highways were more strongly correlated with genetic distances than the measures of highway presence or highway age alone. Interestingly, the effects of fragment age increased only up to 40 years, remaining roughly constant thereafter (Fig. 3b). Conversely, highway presence and highway age showed only weak positive correlations with ISSR genetic distance after controlling for geographic distance, and the indices describing additional urban fragmentation, and fragment age were not significant in the partial Mantel tests (Table 2). The significant partial correlation for highway age was due primarily to the isolating effects of CA Highway 23 after 39 years, since the older Highway 101 (age of 57 years) showed no increase in population structure over sites not separated by a major highway (plotted at 0 years; Fig. 4b).

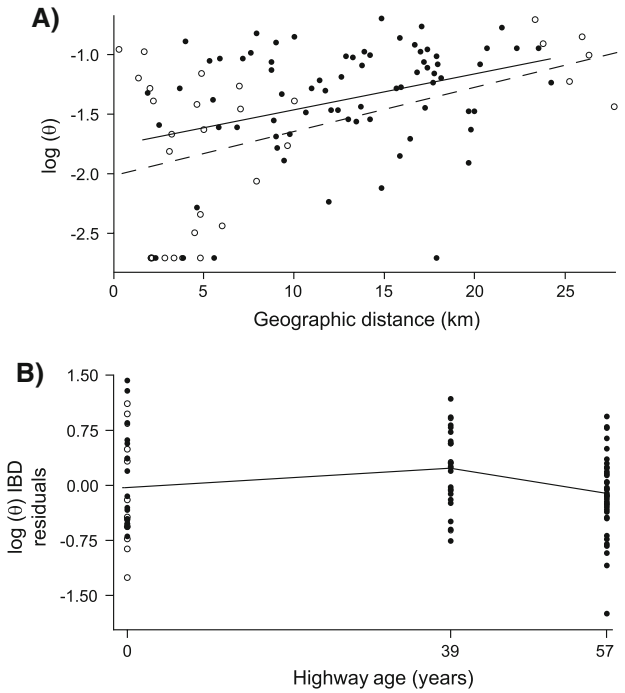

Fig. 4 Results of isolation by distance analyses for ISSRs. Filled circles represent population pairs separated by major highways and open circles represent population pairs not separated by highways. Regression lines are drawn for population pairs separated by major highways (solid line) and for other populations (dashed line). (**a**) Scatterplot of log genetic distance by geographic distance. (**b**) Scatterplot of residuals from the IBD regression against highway age. Symbols are the same as in Fig. 3

Both fragment size and fragment age were significant predictors of mtDNA genetic diversity as measured by θ_K, but neither factors explained variation in mtDNA θ_π, ISSR gene diversity, or ISSR H_e (Table 3). When fragment size and fragment age were combined in a multiple regression on θ_K, only fragment age approached statistical significance ($P \leq 0.07$). This result may, in part, be due to a high correlation between the two explanatory variables ($r = -0.77$), and low power due to relatively few sampling locations ($N = 13$). Therefore, it is difficult to determine which of these two variables better explains differences in θ_K among the sampled sites. Finally, mtDNA genetic diversity (θ_K) was significantly lower in microreserves than in larger fragments and contiguous sites (difference between means = 6.60, $t_8 = 2.06$, 1-sided $P = 0.036$). No other genetic diversity measure differed significantly between microreserves and other sites.

Discussion

Effects of fragmentation

Our results indicate that fragmentation by highways and urbanization is associated with increased genetic differentiation, when compared to levels of differentiation across contiguous habitat. Furthermore, the temporal scale of fragmentation is positively associated with genetic divergence when controlling for geographical distance, suggesting that older fragments are more divergent from one another than those separated more recently. Finally, smaller fragments isolated for longer periods of time contained less mtDNA genetic diversity than larger areas. Together these results suggest that gene movement and gene flow among fragments have been reduced as a consequence of habitat fragmentation.

Genetic studies of other animals in this study region have shown similar responses to fragmentation. Using microsatellite markers, Riley et al. (2006) found that bobcats were genetically delineated into three populations by

Table 2 Results for Mantel tests for correlations between genetic distance (measured as Φ_{ST} and θ) and geographic distance among pairs of sampling locations

Test	mtDNA (Φ_{ST})		ISSR (θ)	
	r	*P*	*r*	*P*
Mantel test for correlation between geographic and genetic distance	0.4485	0.0001	0.4469	0.0003
Partial test for hwy presence, controlling geo. dist.	0.2949	0.0075	0.1546	0.0536
Partial test for geo. dist., controlling hwy presence	0.3861	0.0001	0.4044	0.0013
Partial test for hwy age, controlling for geo. dist.	0.3002	0.0085	0.1537	0.0451
Partial test for geo. dist., controlling for hwy age	0.3359	0.0006	0.3725	0.0011
Partial test for hwy & urban presence, controlling geo. dist.	0.5146	0.0004	−0.0153	0.5211
Partial test for geo. dist., controlling hwy & urban presence	0.5226	0.0001	0.4460	0.0004
Partial test for hwy & urban age, controlling geo. dist.	0.4725	0.0010	−0.0096	0.4905
Partial test for geo. dist., controlling hwy & urban age	0.4490	0.0001	0.4452	0.0002

Partial Mantel test results for partial correlation of genetic distance and measures of fragmentation

Table 3 Single and multiple regression analyses of genetic diversity measures (dependent variables) and fragment size and/or fragment age (independent variables)

	Single regressions							Multiple regressions					
	Fragment size			Fragment age				Fragment size			Fragment age		
	R^2	*t*-ratio	*P*	R^2	*t*-Ratio	*P*	d.f.	R^2	*t*-Ratio	*P*	*t*-Ratio	*P*	d.f.
mtDNA θ_K	0.413	2.780	0.018	0.570	−3.820	0.003	11	0.585	0.605	0.559	−2.04	0.069	10
mtDNA θ_π	0.030	−0.185	0.856	0.019	−0.497	0.628	13	0.077	−0.871	0.401	−0.983	0.345	12
ISSR gene diversity	0.183	1.17	0.112	0.152	−1.53	0.151	13	0.193	0.774	0.454	−0.376	0.713	12
ISSR H_e	0.071	0.997	0.337	0.970	−1.180	0.259	13	0.097	0.162	0.874	−0.607	0.555	12

All variables were log-transformed

Highway 101 and northern Highway 23, and that coyote populations were divided into a northern and southern group along Highway 101. Significant genetic differentiation across these highways has also been found in the lizards *Sceloporus occidentalis* and *Uta stansburiana* (K. Semple-Delaney pers. comm.). However, these studies did not report genetic differentiation among fragments separated by urban development in addition to major highways. This may indicate that Jerusalem crickets are more sensitive to urban fragmentation than these larger, more mobile animals.

Large, busy highways are likely to represent formidable barriers to dispersal for Jerusalem crickets for several reasons. Raised, steep banks, berms, curbs, medians and fences common along these routes may be difficult for Jerusalem crickets to scale. Even if individuals make it past these barriers, high traffic flow leads to a high probability of mortality, particularly across multi-lane roads. Finally, Jerusalem crickets may avoid unsuitable or non-natural substrates, and simply choose to turn around at paved edges, as documented in ground beetles (Mader 1984), and many other small animals (Merriam et al. 1989; Baur and Baur 1990; Trombulak and Frissell 2000; Andrews and Gibbons 2005).

Highway undercrossings are often built or modified in order to facilitate wildlife movement across major highways (Noss 1987; Clevenger 2005). However, the utility of these undercrossings has rarely been studied for animals other than large mammals (Clevenger and Waltho 2000; Ascensao and Mira 2007; Gagnon et al. 2007; Kleist et al. 2007 but see Ng et al. 2003). There are several culverts and two spanning bridges along Highway 101 within the study area. Interestingly, genetic divergence across Highway 101 for both mtDNA and ISSRs was slightly lower than across other urban and highway barriers established for at least 39 years (Figs. 3b, 4b). This may indicate that Jerusalem crickets are able to cross this highway more easily than other barriers, perhaps utilizing undercrossings. However, movement through these underpasses may be offset to some extent by an increased risk of predation. A recent study documenting small mammal use of undercrossings showed that crossings under this section of Highway 101 were mostly used by raccoons, opossums, skunks and house cats, all of

which are nocturnally active and potential predators of Jerusalem crickets (Ng et al. 2003).

Avoidance of unsuitable substrates may also limit Jerusalem crickets from moving among fragments in urbanized landscapes even when these fragments are not separated by major highways. Yards and other vegetated areas present throughout urban areas are usually irrigated and contain exotic plants and animals, which can adversely affect some arthropod species and alter community structure (McIntyre and Hostetler 2001; Shochat et al. 2004). Microclimatic changes associated with urbanization (Kim 1992) may also impact ectotherms, such as invertebrates, altering abundance and diversity patterns (McIntyre et al. 2001). Environmental pollutants have been linked to declines in arthropod diversity in urban settings (Tyler et al. 1989; Pouyat et al. 1994) as well as changes in selective pressures on populations (Kettlewell 1973). Finally, the presence of introduced predators and competitors may also reduce survivorship of native species in urban areas (Suarez et al. 1998; Bolger et al. 2000).

Contrasts between genetic markers

Discrepancies in measurable fragmentation effects between the two genetic markers may reflect stochastic lineage sorting, differences in effective population sizes between nuclear and mitochondrial DNA, differences in mutation rates among genes, and/or sex-biased dispersal. Single copy, maternally inherited mtDNA has roughly one quarter the effective population size of nuclear genes (assuming an equal sex ratio among breeding adults). This difference may allow mtDNA to sort more quickly, revealing a stronger signal of fragmentation than nuclear markers. Given the relatively short time since urbanization began in this region (within the last 70 years), it is unlikely that an equilibrium between drift and gene flow has been reached (Bohonak and Roderick 2001). Therefore, we would expect to see a stronger signal in mtDNA until a new gene flow/drift equilibrium is reached for both sets of markers. Because fragmentation effects strengthen with time, the significant relationship between genetic differentiation and road/fragment age may reflect non-equilibrium conditions. Finally, it may take considerably longer to detect changes in genetic diversity than in genetic divergence in response to fragmentation (Latter 1973; Varvio et al. 1986; Keyghobadi et al. 2005). In our analyses, only θ_K showed significant associations with fragment size and fragment age. Because bottlenecks purge populations of rare alleles more quickly than common alleles, θ_K (based on the number of haplotypes) is expected to reflect recent fragmentation more than θ_π. (based on average sequence divergence). Lack of pattern in ISSR gene diversity and heterozygosity is also consistent with either retention of ancestral polymorphism in these markers or greater mixing among populations in the nuclear genome.

Greater nuclear gene flow may be due to male-biased dispersal in Jerusalem crickets. Although males and females were captured at roughly equal numbers in pitfall trap arrays, captures tended to occur in distinct pulses with mature males captured at higher rates earlier in the season, and females later (AGV, data not presented). This may be related to mate-searching behavior, in which males wander earlier in the mating season in search of mates, and mated females wander later in search of oviposition sites or food. Jerusalem cricket mating drums can differ substantially by sex, and may provide insight into their dispersal patterns. In other *Stenoplematus* species, females may drum for minutes at a time while males may drum in short bursts of only a few seconds each (Weissman 2001b). In the laboratory, *S.* n. sp. "santa monica" females drum 2–3 times longer than males (Weissman, unpublished data). In other insects that display similar sexual differences in vibrational communication patterns, these differences tend to reflect different mating strategies; females remain stationary while calling and males search for hidden females between short calling bouts (Field and Bailey 1997; Stewart 1997). Thus, average dispersal distances for male *S.* n. sp. "santa monica" are likely to be larger than those for females, if females remain stationary during mating and are philopatric in selecting oviposition sites.

Conservation through microreserves

Jerusalem crickets may be particularly susceptible to loss of population connectivity due to their poor movement abilities. Previous genetic work on *Stenoplematus* n. sp. "mahogani", another Jerusalem cricket endemic to southern California, also found an effect of urban fragmentation on genetic structure (Vandergast et al. 2007). Within our current study area, *S.* n. sp. "mahogani" is much less abundant than *S.* n. sp. "santa monica", and is restricted primarily to the more contiguous areas south of Highway 101. Only one *S.* n. sp. "mahogani" was captured in a Simi Hills fragment over a 2-year sampling period. In contrast, the number of *S.* n. sp. "santa monica" individuals captured per unit of sampling effort was not significantly correlated with fragment size ($r_{14} = 0.26$, $0.25 > P > 0.10$). Similarly, the mean capture rates of sampling locations in the microreserves were not significantly lower than those in the larger fragments and contiguous regions (mean difference $= -0.82$, $t_8 = -0.69$, 1-sided $P = 0.253$). From these capture data alone, it would appear that small urban reserves throughout the Simi Hills are adequately maintaining populations of *S.* n. sp. "santa monica". However, long-term declines in genetic connectivity among and genetic diversity within microreserves may

increase the likelihood of local population declines and eventual extinction (Templeton et al. 1990; Saccheri et al. 1998). Our results suggest that while the protection of small isolated reserves may slow the loss of biodiversity in urban landscapes, this approach may be insufficient to protect all components of arthropod diversity in perpetuity.

These results may have implications for the long-term success of multi-species habitat conservation plans (MSCPs) in southern California, as these often include microreserves along with larger (but disjunct) fragments of open space (e.g., San Diego MSCP 1998; Western Riverside County MSHCP 2003; Orange County Southern Subregion NCCP 2006). MSCPs are usually designed to protect a subset of species that include few if any invertebrates (Redak 2000), under the assumption that these larger "umbrella species" satisfactorily represent other aspects of biodiversity. Unfortunately, the use of umbrella species does not always lead to the adequate protection of arthropod diversity (Rubinoff 2001). Furthermore, wildlife corridors in large-scale conservation plans are most often designed and implemented to facilitate movement of vertebrates, rather than arthropods (Beier and Noss 1998; Hunter et al. 2003; Beier et al. 2006; reviewed in Ockinger and Smith 2008). The hypothesis that arthropods present in microreserves may eventually become rare is supported by severe range reductions in several species in southern California (e.g., Quino checkerspot butterfly *Euphydryas editha quino*, Mattoni et al. 1997; trapdoor spiders *Apomastus* sp., Bond et al. 2006). In the most severe cases, some species have been restricted to only a few microreserves (e.g., Delhi Sands flower-loving fly *Rhaphiomidas terminatus abdominalis*, Grandberry and Nagano 1998; El Segundo blue butterfly *Euphilotes bernardino allyni*, Longcore et al. 2000; the Palos Verdes blue butterfly *Glaucopsyche lygdamus palosverdesensis*, Mattoni and Powers 2000). In the absence of efforts to increase connectivity among isolated fragments, additional arthropods could display the loss of genetic diversity that we are observing in the Santa Monica Jerusalem cricket, eventually requiring further active management such as captive breeding, translocation or supplementation from larger source populations (e.g., Mattoni et al. 2003; Hannon and Hafernik 2006; Hochkirch et al. 2007).

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Disclaimers Use of trade names does not imply the endorsement of the U.S. Geological Survey.

Appendix 1

MtDNA haplotypes by sampling location

Haplotype	Sampling locations															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
H1	1	3	1	2												7
H2	1															1
H3	1															1
H4	1		2				1				1					5
H5		1														1
H6		1														1
H7		1														1
H8			7							2						9
H9				2			5		1							8
H10				1												1
H11				3												3
H12					2	5		13	4							24
H13					1											1
H14					1											1
H15					1											1
H16						1										1
H17						1										1

Appendix1 continued

Haplotype	Sampling locations															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
H18						1										1
H19						1										1
H20							1									1
H21							1									1
H22							1									1
H23								1								1
H24								3								3
H25								1								1
H26								1								1
H27								1								1
H28								1								1
H29								2								2
H30								1								1
H31									2							2
H32									1							1
H33										2						2
H34										1						1
H35										1						1
H36										1						1
H37										1						1
H38											1					1
H39											1					1
H40											1					1
H41											1					1
H42												5				5
H43												1				1
H44												1				1
H45												1				1
H46												1				1
H47												2				2
H48												1				1
H49												2				2
H50												1				1
H51												2				2
H52												1				1
H53												1				1
H54												2				2
H55												1				1
H56												3				3
H57												2				2
H58												1				1
H59												1				1
H60												2				2
H61													6	1	2	9
H62													1			1
H63													1			1
H64													2	1		3

Appendix1 continued

Haplotype	Sampling locations															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
H65													1			1
H66													1			1
H67													1			1
H68														2		2
H69														2	1	3
H70														2	1	3
H71														1	2	3
H72														1		1
H73														1		1
H74														1		1
H75															1	1
H76															1	1
H77															1	1
H78															1	1
H79															1	1
H80															1	1
	4	6	10	8	5	9	9	24	8	8	5	31	13	12	12	164

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