

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

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Introduction

In urbanized landscapes, preserving small tracts of natural lands (microreserves) may help retain some aspects of terrestrial arthropod diversity (Shafiq 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 combination of other environmental perturbations associating populations in small habitat fragments may ated with urban development (e.g., increased predation experience a loss of genetic connectivity. The isolation of risks due to lack of cover, interactions with non-native local populations generally leads to an increase in genetic species, chemical and light pollutants). We specifically divergence and a loss of genetic variability within frag- focused on (1) whether the presence of major highways and ments (Keller and Largiadè 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 Sould 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 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. 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 through telemetry, mark/recapture efforts). Understanding both males and females (at 20 C), the presence of a male contemporary population genetic structure in the context of sex-clari cation drum, a male chromosome count of 25, historical genetic integrity can help inform reserve managers of potential population declines in key species of levels of mtDNA sequence divergence when compared to other closely related species (Epperson 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: Stenopelmatae) in fragments throughout the Simi Hills of southern California and in the adjacent and more contiguous Santa Monica Mountains. Although 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

Study organism 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 1999; Gerlach and Musol 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 both males and females (at 20 C), the presence of a male contemporary population genetic structure in the context of sex-clari cation drum, a male chromosome count of 25, historical genetic integrity can help inform reserve managers of potential population declines in key species of 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.).

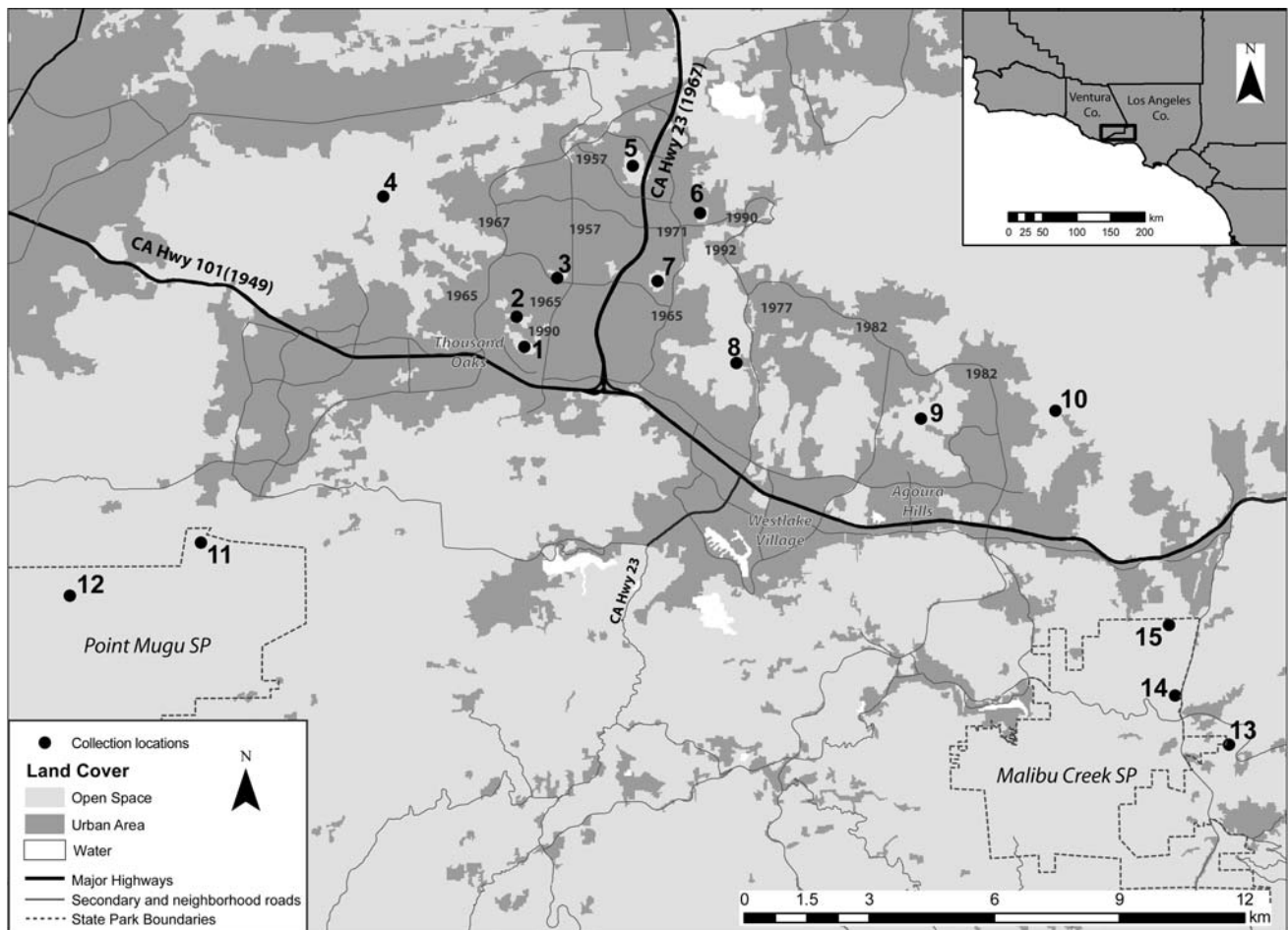


Fig. 1 Map of the study area within the Simi Hills (collection chaparral, coastal scrub, annual grasslands, coastal oak woodlands, locations 1–10) and the more contiguous open space south and riparian habitat types. Highways and local roads further subdivide Highway 101 (collection locations 11–15) in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

Thousand Oaks, Westlake Village and Agoura Hills. Urban (see Case and Fisher 2001; Fisher et al. 2002 for pitfall development in this region has grown substantially over the sampling methods). We defined a sampling location as a last several decades, beginning in the late 1930s through a group of pitfall trap arrays (1–7 arrays) within approximately the present. The patchwork of remaining open spaces are rarely 2 km of one another and not separated by potential largely protected and managed by several agencies geographic barriers to movement (e.g., rivers, dry river including National Park Service (NPS), California beds, mountains, roads, urban development, cultivated Department of Parks and Recreation, Santa Monica cañons). Sampling locations included 6 microreserves (Mountains Conservancy, Conejo Open Space Conservation km², locations 1, 2, 3, 5, 6, 7), 2 mid-sized fragments (Agency, Conejo Recreation and Park District, Mountains km², locations 8, 9) and 2 large fragments (20 km², Recreation and Conservation Authority, City of Thousand locations 4, 10) in the Simi Hills, north of CA State Highway 101 (Fig. 1). Vegetation types in these fragments

Individuals were collected from 15 sampling locations in consist primarily of coastal sage scrub and grasslands with pitfall trap arrays between 2000–2002 and 2005, and presume mixed chaparral. These fragments are separated from served in 70% or 95% ethanol. Most samples were collected each other by residential and commercial development, by NPS personnel during herpetofaunal and ground-dwelling several roads and a major highway (CA State Highway 23). ing invertebrate monitoring activities (Bustera 2003), and The ve remaining sampling locations were located south were supplemented by additional pitfall collections by EAL of Highway 101, in more contiguous chaparral, scrub and

grasslands of the Santa Monica Mountains. However, more comparable to AFLPs (Wolfe 2005). Because these 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 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'-GTCAACAATCATAAAGATATTGG, and HCO2198: 5'-TAACCTTCAGGGTGACCAAAAATCA (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 MgCl₂, 0.2 µM each dNTP, and 0.4 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 Colapase 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

(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 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, genetic 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 G_{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 F_{ST} (Weir and Cockerham 1984), an estimator of F_{ST} . Overall ISSR divergence among all sites was quantified 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 "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 TFGA (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 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.

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 (krf)	No. captured/ array/trap year	N	No. of mtDNA 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	4.0000	-0.8241	4	2	0.0122	0.0686
2	Botanical Gardens	0.0315	4.00	6	4	0.8000	4.0627	7.9330.8601	5	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	6.40090.8124	5	8	8	0.0440	0.0786
6	Erbes	0.1071	4.50	9	5	0.7222	3.8288	1.3333-1.7278	7	10	10	0.0733	0.0903
7	Old Meadows	0.1206	3.50	9	5	0.7222	3.8288	5.2500-1.8811	9	6	6	0.0271	0.0646
8	Hillcrest Patch	4.6643	4.83	24	10	0.7065	5.9124	3.09782.1388	23	19	19	0.0545	0.0772
9	Rancho Simi Patch	6.1840	1.5	8	4	0.7500	2.5006	6.17861.7468	8	7	7	0.0371	0.0709
10	Pala Camado	162.0962	2.75	8	6	0.9286	9.2308	2.32140.6668	7	6	6	0.0393	0.0727
11	Pt. Mugu SP A	213.5114	5.00	5	5	1.0000	NA	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	2.25640.4831	11	14	14	0.0608	0.0828
14	Malibu Crk SP B	355.1453	3.00	12	9	0.9545	14.6866	4.90910.0501	10	18	18	0.1084	0.1239
15	Malibur Crk SP C	355.1453	4.67	12	10	0.9697	25.5629	7.46970.4353	11	9	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 signi cantly different from zero $P \leq 0.02$

- A categorical (binary) fragmentation matrix was created from mtDNA sequences as $(1)_{\pi}$, which estimates the 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 the 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 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.

divergence $d = 4N_e\mu$ from the average sequence $\theta = 4N_e\mu$ from the number of haplotypes s . Calculations were performed in Arlequin. For ISSRs we estimated diversity with both Nei’s (1973) gene diversity (calculated in PopGene) and the average panmictic heterozygosity within each population, calculated in Hickory 1.0 under the 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 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).

The 164 sequences of *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

Assessing loss of genetic diversity

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).

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 FragStat (McGarigal and Marks 1995). The isolation age of each fragment was calculated as the time since the year 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

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 of these haplotype groups may reflect large 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 shared across two regions (Simi Hills and Point Mugu: haplotype, found in Point Mugu (H21 to H58, Fig. 2). The genetic distances among these haplotype groups may reflect large geographic distances between the three regions that were sampled. Of the 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 connected to the major network at the 94% but not 95% connection limit. Another haplotype group contained

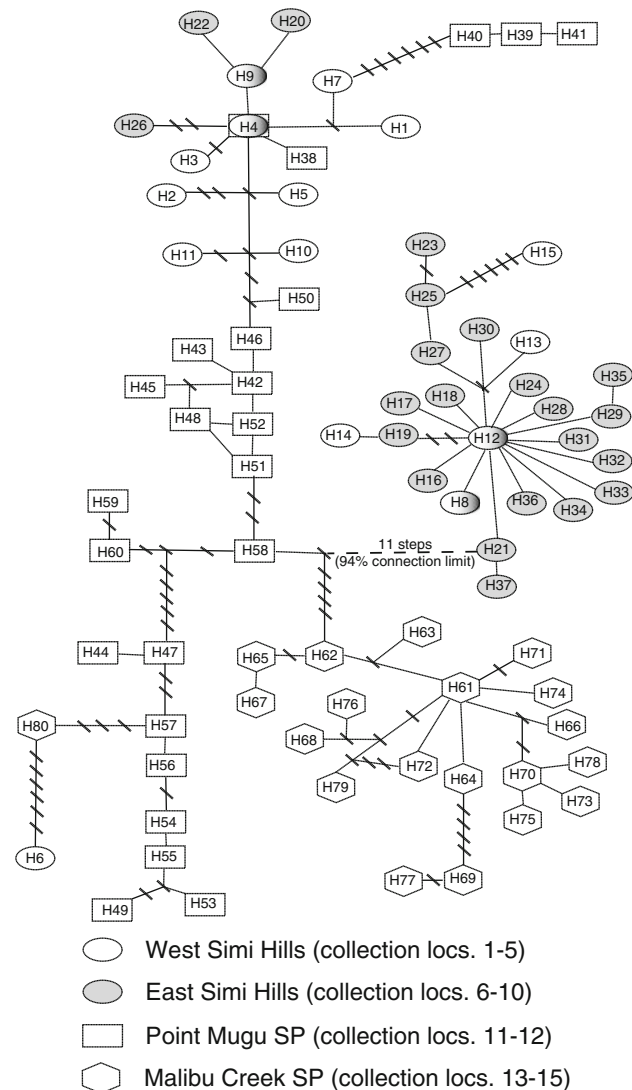


Fig. 2 Ninety-five percent parsimony network depicting the relationship among mtDNA haplotypes. Base changes between haplotypes are represented as lines. Haplotype shapes represent populations (open ovals represent those found in the Simi Hills, east of Highway 23, open ovals represent those found in the Simi Hills west of Highway 23, open 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 H11) constitute the largest age class. A locally weighted regression smoother (Cleveland 1979) is plotted to visualize trends

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 ($F_{ST} = 0.63, P \leq 0.0001$). Pairwise genetic and geographic distances were positively correlated (Mantel test $r = 0.45, P \leq 0.0001$, Fig. 3a).

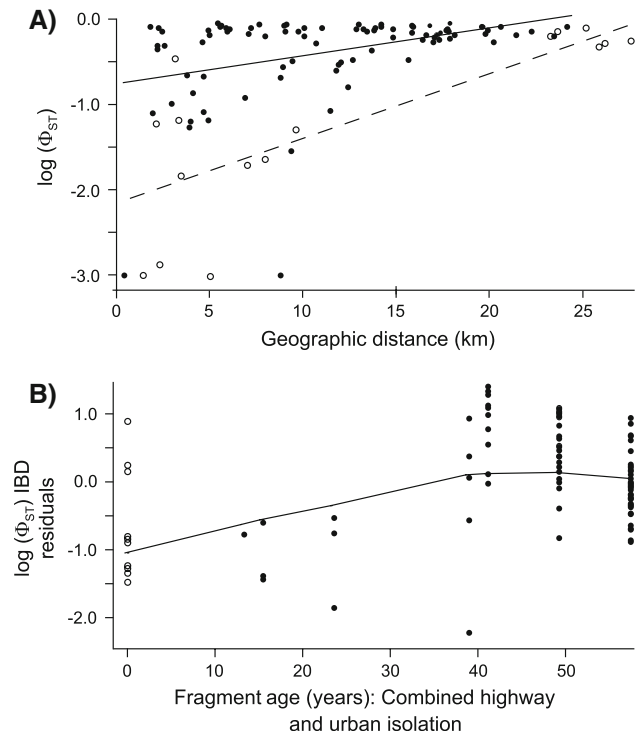


Fig. 3 Results of isolation by distance analyses for mtDNA sequence data. a) Scatterplot of log genetic distance by geographic distance. Open circles represent population pairs in contiguous habitat and filled circles represent populations fragmented by urban areas and highways. Regression lines are drawn for population pairs separated by urban areas and highways (solid line) and for contiguous comparisons (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 are shown as hexagons. 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 free model, $\theta_{\beta} = 0.07$ (95% credible intervals: 0.05–0.10); and assuming H–W genotype frequencies, $\theta_{\beta} = 0.06$ (95% CI: 0.03–0.09). These two estimates did not differ substantially from one another (i.e., 95% credible intervals overlapped). Similar to mtDNA data, ISSR pairwise genetic distances were also positively correlated with the same 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, 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

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. 3b).

Both fragment size and fragment age were significant predictors of mtDNA genetic diversity as measured by H_e , but neither factors explained variation in mtDNA, ISSR gene diversity, or 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 (-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 among the sampled sites. Finally, mtDNA genetic diversity 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

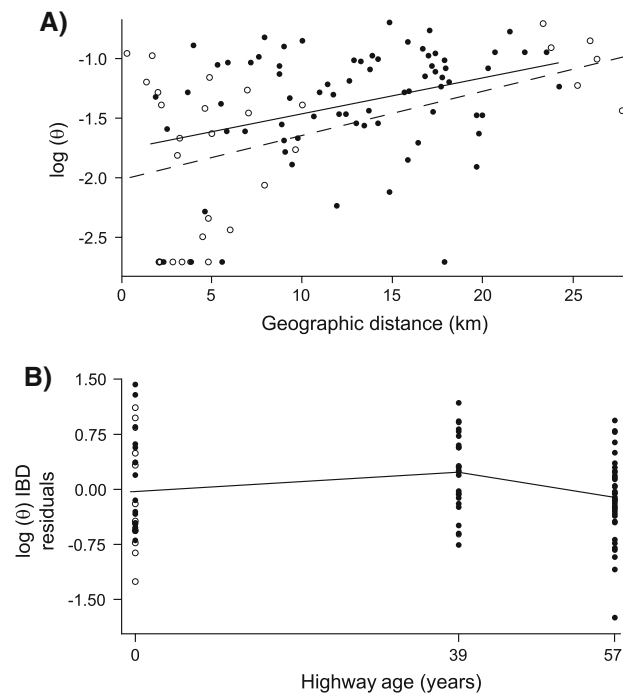


Fig. 4 Results of isolation by distance analyses for ISSRs. Filled circles represent population pairs separated by major highways and open circles represent population pairs not separated by highways. Regression lines are drawn for population pairs separated by major highways (solid line) and for other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

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

Test	mtDNA (θ_{ST})		ISSR (θ)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
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			d.f.	Fragment size			Fragment age		
	R^2	<i>t</i> -ratio	<i>P</i>	R^2	<i>t</i> -Ratio	<i>P</i>		R^2	<i>t</i> -Ratio	<i>P</i>	<i>t</i> -Ratio	<i>P</i>	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 lizards *Sceloporus occidentalis* and *Uta stansburiana* (K. Semple-Delaney pers. comm.). However, these studies do 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, these underpasses may be offset to some extent by 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 ancestral polymorphism in these markers or greater mixing among populations in the nuclear genome.

Avoidance of unsuitable substrates may also limit 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 traps separated by major highways. Yards and other vegetated arrays, captures tended to occur in distinct pulses with 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, by 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. Previous genetic work on *Stenoplematus n. sp.* (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 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_s . (based on average sequence divergence). Lack of pattern in ISSR gene diversity and heterozygosity is also consistent with either retention of

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 some species have been restricted to only a few micro-small isolated reserves may slow the loss of biodiversity in reserves (e.g., Delhi Sands flower-loving fly *Rhaphiomidas terminatus abdominalis*, Grandberry and Nagant 1998). El Segundo blue butterfly *Euphilotes bernardino allyni*, *Glaucopsyche lygdamus palosverdesensis*, Mattoni and Powers 2000). In the absence of efforts to increase con-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). MSCP are usually designed to protect a subset of species that include few if any invertebrates (Redal 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 Noon 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*

edita quino, Mattoni et al. 1997; trapdoor spiders *omastus* sp., Bond et al. 2006). In the most severe cases, some species have been restricted to only a few micro-small isolated reserves (e.g., Delhi Sands flower-loving fly *Rhaphiomidas terminatus abdominalis*, Grandberry and Nagant 1998). El Segundo blue butterfly *Euphilotes bernardino allyni*, *Glaucopsyche lygdamus palosverdesensis*, Mattoni and Powers 2000). In the absence of efforts to increase con-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). MSCP are usually designed to protect a subset of species that include few if any invertebrates (Redal 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 Noon 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*

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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

Appendix 1 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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