

Combining genetic and geospatial analyses to infer population extinction in mygalomorph spiders endemic to the Los Angeles region

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Abstract

Although hyperdiverse groups like terrestrial arthropods are almost certainly severely impacted by habitat fragmentation and destruction, few studies have formally documented such effects. In this paper, we summarize the results of a multifaceted research approach to assess the magnitude and importance of anthropogenic population extinction on the narrowly endemic trapdoor spider genus *Apomastus*. We used geographical information systems modeling to reconstruct the likely historical distribution of *Apomastus*, and used molecular phylogeographic data to discern population genetic structure and detect genetic signatures of population extinction. In combination, these complementary lines of inference support direct observations of population extinction, and lead us to conclude that population extinction via urbanization has played an important role in defining the modern-day distribution of *Apomastus* species. This population loss implies coincident loss of genetic and adaptive diversity within this genus, and more generally, suggests a loss of ground-dwelling arthropod population diversity throughout the Los Angeles Basin. Strategies for minimizing this loss are proposed.

Introduction

The 'biodiversity crisis' of recent times includes not only the loss of species diversity but also, fundamentally, the extinction of populations that comprise species. Hughes, Daily & Ehrlich (1997) estimated the rate of loss of distinct populations at *c.* 16 million per year for tropical systems, whereas Hobbs & Mooney (1998) summarized similar patterns of rampant population extinction in temperate ecosystems. Many other well-documented examples exist. Population extinction is a microcosm of species extinction, with species extinction typically representing the endpoint of an ongoing process of degradation and loss (see Ehrlich & Daily, 1993; Hobbs & Mooney, 1998). This loss of local diversity is exceedingly important from an ecological and evolutionary perspective; population extinction disrupts fundamental evolutionary and ecological processes, and greatly impacts future potential for evolutionary response and change (see Myers & Knoll, 2001; Templeton *et al.*, 2001; Frankham, Ballou & Briscoe, 2002).

Southern California is home to a large number of endemic plant, vertebrate and invertebrate species (Cincotta, Wisniewski & Engelman, 2000; Myers *et al.*, 2000; Brooks *et al.*, 2002), vying for space with two of the largest urban centers in North America (Los Angeles and San Diego).

Conversion and fragmentation of this landscape have taken their toll on native biodiversity, as evidenced by a disproportionately large number of US federally listed endangered species in the region (Dobson *et al.*, 1997). Direct evidence for population decline and extinction has been documented in plants (Soulé *et al.*, 1988; Skinner & Pavlik, 1994) and vertebrate species (e.g. Soulé *et al.*, 1988; Soulé, Alberts & Bolger, 1992; Hobbs & Mooney, 1998; Crooks *et al.*, 2001; Fisher, Suarez & Case, 2002). Although a few studies have documented arthropod population extinctions (Suarez, Bolger & Case, 1998; Rubinoff, 2001), the ratio of diversity to endangerment or documented extinction is extremely high.

The Mediterranean scrub habitats of southern California are rich in spider species diversity (Prentice *et al.*, 1998, 2001), including many species in the infraorder Mygalomorphae (tarantulas, trapdoor spiders and kin). Because these spiders are in a basal clade well diverged from all other spiders, the presence of mygalomorphs in any arthropod community increases the phylogenetic diversity (*sensu* Faith, 1992) of that community. Mygalomorphs (trapdoor spider lineages in particular) possess life-history traits that differ markedly from other spiders, and from arthropods in general; for example some species live for 15–30 years and require 5–6 years to reach reproductive maturity (e.g. Main, 1978; Vincent, 1993). Most species are habitat specialists,

and are extraordinarily sedentary (e.g. Main, 1987; Vincent, 1993; Coyle & Icenogle, 1994). Site fidelity leads to considerable spatial clumping in appropriate microhabitats and extreme population genetic structuring (Bond *et al.*, 2001; Ramirez & Chi, 2004). These life-history traits promote geographic fragmentation over space and time, resulting in a large number of taxa that have small geographic distributions. Overall, this combination of life-history characteristics (long-lived, habitat specialists with poor dispersal abilities and small geographic ranges) parallels general characteristics of well-studied taxa that are 'extinction prone', either at the population or species level (see McKinney, 1997; Purvis, Jones & Mace, 2000). We believe that mygalomorph species are probably extinction prone as well, although this has never been formally documented (but see Main, 1999).

We report on direct and indirect evidence for population extirpation in the narrowly endemic mygalomorph genus *Apomastus* (Bond, 2004). *Apomastus* includes two allopatric species confined in the present day to habitats in and around the Los Angeles (LA) Basin (Bond, 2004; Fig. 1a). These spiders are habitat specialists, constructing subterranean burrows on shaded banks and slopes of wooded or chaparral-dominated ravines. With the exception of a few outlying lowland populations (PTH, PVD, CJE; Fig. 1a), the majority of extant populations appear confined to relatively undisturbed ravines peripheral to urban development of the LA Basin.

Multiple lines of direct evidence suggest that *Apomastus* has suffered both population decline and extinction in the LA Basin. During field surveys conducted over the past 10 years, we were unable to find *Apomastus* at sites for which we have historical records of presence, meaning either that the populations have become extinct or that we were unable to locate extant populations (unlikely, as the burrows of these spiders are conspicuous). In addition, we failed to find *Apomastus* at a large number of sites (> 70) appropriate for the species – we believe that some of these sites must have held *Apomastus* populations that have become extinct. Finally, in addition to this extinction evidence, several extant populations are imperiled, restricted to small patches of remnant habitat in an otherwise urbanized habitat matrix.

The direct observations of decline and extinction suggest that *Apomastus* has had a much larger distribution in pre-urbanization times. We used geographical information systems (GIS) modeling to reconstruct the historical, pre-urban, distribution of *Apomastus* and use phylogeographic data to understand population genetic structure and detect genetic signatures of population extinction. In combination, these complementary lines of inference support our direct observations, leading us to postulate that population extinction via urbanization has played an important role in defining the modern-day distribution of *Apomastus*.

Methods

GIS spatial analyses

We generated a dataset of 28 presence and 72 absence observations for *Apomastus*. Our absence data come from

field observations over the last 10 years of collecting in the LA Basin area. *Apomastus* specimens are easily located by experienced arachnologists; they construct open burrows whose entrance is lined with white silk (Bond & Opell, 2002). Latitude and longitude coordinates were recorded with a global positioning system (GPS) receiver for each searched locality. Additional museum material from the American Museum of Natural History and California Academy of Sciences [see material examined sections of Bond (2004) for detailed collection information] was georeferenced on USGS 1:25 000 topographic maps; only specimens with sufficiently detailed locality data were georeferenced (see Stockman, Beamer & Bond, 2006). All georeferenced points were eventually confirmed in the field (Table 2). As mentioned in the Introduction, two populations of *Apomastus* are now extinct. These data were treated as presence observations in all spatial analyses because the habitat and climate at these locations were once suitable. Coordinates representing presence and absence were imported into Arcview 3.3 (ESRI, Redlands, CA) and converted into shapefiles.

Datasets for land cover, gap vegetation and elevation were obtained from the US Environmental Protection Agency. The land cover data were derived from 30-m land remote sensing satellite (Landsat) thematic mapper data, which were classified into 21 different land cover types. This coverage was clipped from the National Landcover dataset. The gap vegetation data were derived from the California Gap Analysis Project (Davis *et al.*, 1995). Elevation data were derived from the National Elevation Dataset.

Coverage data for precipitation were obtained from the California Spatial Information Library. These data represent lines of equal rainfall based on long-term mean annual precipitation data compiled from USGS, California Department of Water Resources, and California Division of Mines map and information sources collected over a 60-year period (1900–1960). The minimum mapping unit was *c.* 1000 acres. Average temperature coverage data for the LA Basin area were obtained from WORLDCLIM global climate layers (Hijmans *et al.*, 2004). Coverage for recent vegetation in vector format was obtained from the California Department of Forestry and Fire Protection for Los Angeles, Orange, San Bernardino, Riverside and Ventura counties. These data were created from 1977 Landsat imagery and then digitized from 1:1 000 000 scale maps with a minimum mapping unit of 400 acres. Vegetation was divided into 78 classes for the entire state of California. STATSGO soil data were obtained from the United States Department of Agriculture in raster format. Soil maps for STATSGO were compiled by generalizing more detailed (SSURGO) soil survey maps. Where more detailed soil survey maps were not available, data on geology, topography, vegetation and climate were assembled, together with Landsat images.

Two coverages, slope and aspect, were derived from the National Elevation Dataset by using the slope and aspect functions in the image interpreter topographic analysis of ERDAS Imagine 8.7 (Leica Geosystems GIS & Mapping,

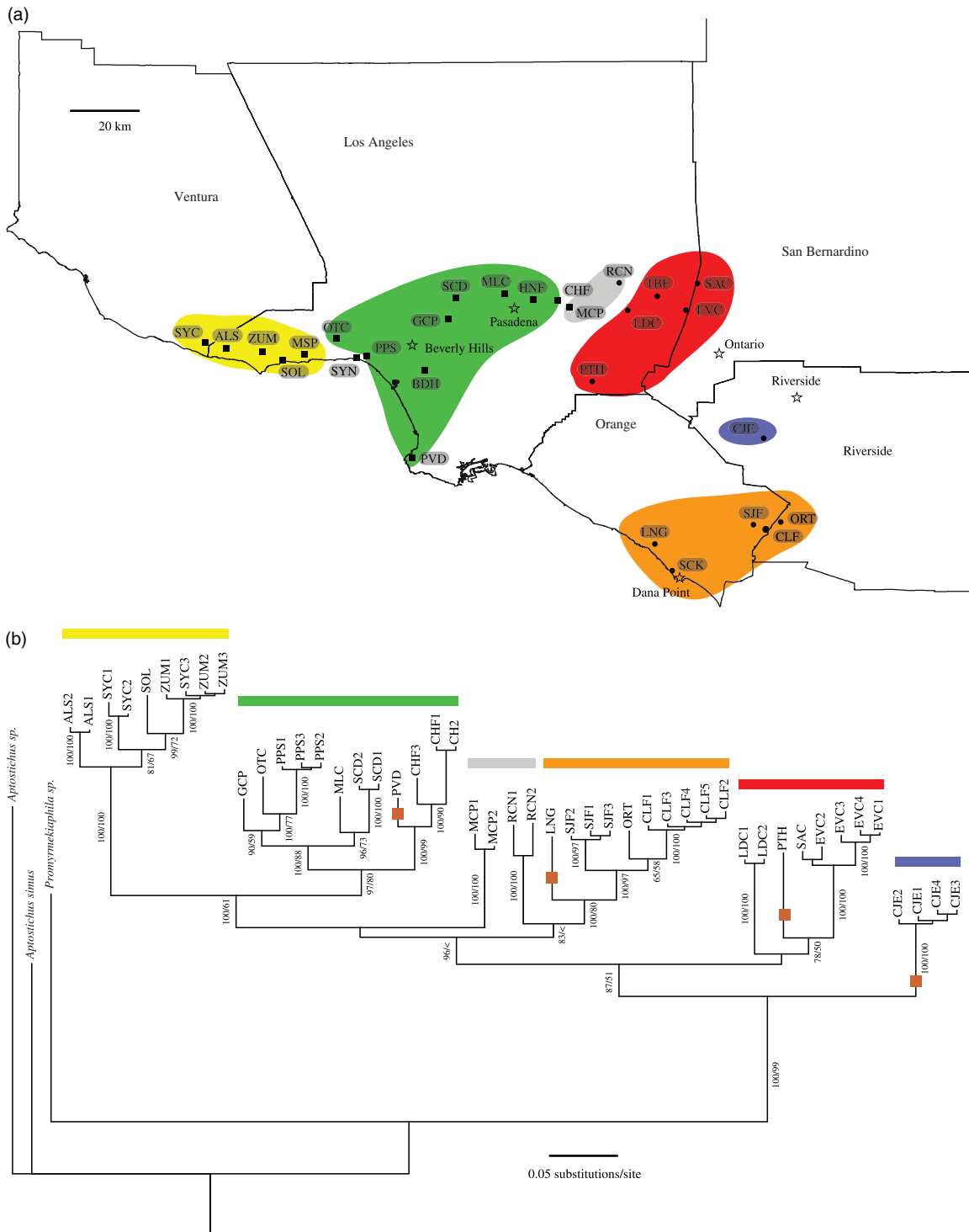


Figure 1 (a) Known distribution of *Apomastus*. ■ represent *Apomastus schlingerii* localities, ● represent *Apomastus kristenae* localities and ☆ represent cities. (b) *COI* gene tree. Relationships were established using Bayesian inference (illustrated model used = F81 + Γ , $\ln = -5967.42$, $\alpha = 0.262546$) and parsimony (964 steps, $CI = 0.46$, $RI = 0.76$). Branch lengths depicted are averaged from the posterior distribution (after burn-in). Posterior clade probabilities and non-parametric bootstrap values > 50% are listed at each node (posterior clade probability/bootstrap). Boxes on branches indicate nodes at which coastal scrub habitat is derived. Single individuals of two *Aptostichus* species [*A. simus* Chamberlin 1917 (from Zuma Beach, LA County) and *Aptostichus* new species (from San Diego County, Anza Borrego Desert State Park, CA)] and a *Promyrmekiaphila* species (from Glenn County, CA) were used to root trees. The choice of outgroup taxa was based on the phylogeny proposed by Bond & Opell (2002).

Table 1 Summary of environmental layer coverage data (30 m resolution) used in binary logistic regression and GARP analyses

Environmental coverage	Logistic regression	GARP
Elevation	X	X
Aspect	X ^a	X
Slope	X	X
Recent vegetation	X ^a	X
Land cover	X ^a	X
Potential vegetation		X
Gap vegetation	X ^a	X
Precipitation	X	X
Average temperature		X
Soil	X ^a	

^aInitially considered in logistic regression model but removed based on significance in forward and reverse stepwise removal of parameters.

GARP, genetic algorithm for rule-set prediction.

LLC, Atlanta, GA). Slope and aspect were each output as degrees. A coverage representing the potential vegetation of California (see Saunders *et al.*, 1987), divided into 54 classes, was obtained from the United States Bureau of Reclamation in vector format.

All data coverages (summarized in Table 1) and presence/absence points were projected to the Teale Albers projection. Vector coverages were rasterized using the vector to raster function in the image interpreter utilities of ERDAS Imagine. The value from the environmental coverage at each collection point was then extracted from the raster coverages by creating a model in Model Maker in ERDAS Imagine. This model used the 'zonal min' and 'focal min' functions to extract the center value out of a 3 × 3 pixel matrix centered on each collection point. All values were then appended to the attribute table of the presence/absence shapefile.

A binary logistic regression (BLR; Stockwell & Peters, 1999; Fertig & William, 2002) was used to predict the probability that *Apomastus* would occur at a given site. In total, eight environmental variables (Table 1) were initially included in the regression model, performed in SPSS 11.0. A logistic regression model utilizing only statistically significant variables was constructed in Model Maker ERDAS Imagine. The EXP function in Model Maker was used to transform the data and to produce a probability map of *Apomastus* occurrence.

Additional spatial analyses using genetic algorithm for rule-set prediction (GARP; Stockwell & Peters, 1999) were implemented using the Desktop GARP software version 1.1.3 (Scachetti-Pereira, 2002). First, seven coverage variables corresponding directly to those used in the binary logistic regression analysis (land cover, gap vegetation, recent vegetation, potential vegetation, elevation, slope and aspect) were used in a conservative model. The analysis was performed with optimization parameters set for 500 runs, 0.01 convergence limit and 100 Max iterations. The four available rule types (atomic, range, negated range and logistic regression) were included. Fifty per cent of the

points were used for training and data were output as ARC/INFO grids. A second analysis, using the same parameters as described above, was conducted with the optimization parameters set for 500 runs. The best-subset procedure in desktop GARP with default settings was utilized. This procedure should select the best models in cases where the species have moderate to large potential distributions in the study area, which is consistent with our distributional dataset (Anderson, Lew & Peterson, 2003).

A second GARP model, using the same procedures described above, was produced using a subset of variables considered to have remained constant throughout the urbanization of the Los Angeles basin. This dataset included the following parameters (Table 1): elevation, slope, aspect, pre-urban vegetation, average temperature and average rainfall. These variables were chosen because they should either be identical to (elevation, slope, aspect and hypothesized vegetation) or are climatic features that approximate (average rainfall and average temperature) the pre-urban LA environment.

Genetic and phylogenetic analyses

Three to five individuals were sampled per collecting locality from sites throughout the known distribution of each *Apomastus* species (Fig. 1a, Table 2). In total, we obtained sequences of the mitochondrial cytochrome *c* oxidase I (COI) gene for 24 in-group populations (Table 2). DNA was extracted using the DNAeasy tissue kit (Qiagen, Valencia, CA), and amplified using standard polymerase chain reaction (PCR) protocols. PCR primers C1J–1751SPID and C1N–2776 (Hedin & Maddison, 2001) were used to amplify a *c.* 1000 base pair region. PCR products were column purified and sequenced directly with both PCR primers. Sequences were edited and aligned using the computer program Sequencher (Genecodes Inc., Madison, WI). We detected no length variation in the data.

Parsimony analysis of these data was conducted using the branch and bound algorithm implemented in PAUP* version 4.0b10 (Swofford, 2002). Relative branch support was evaluated by nonparametric bootstrap analysis based on 10 000 pseudoreplicates using the heuristic search algorithm with TBR branch swapping. Modeltest version 3.1 (Posada & Crandall, 1998) was used to determine the appropriate model of DNA substitution (by likelihood ratio test – lrt). The computer program MrBayes version 3.0b4 (Huelsenbeck & Ronquist, 2001) was used to infer tree topology based on the best-fit DNA substitution model. Four simultaneous Markov chain Monte Carlo (MCMC) chains were run for one million generations, saving the current tree to file every 100 generations. Trees before –ln likelihood stabilization (burn-in) were discarded, and clade posterior probabilities were computed from the trimmed set of trees by computing a 50% majority rule consensus tree in PAUP*. Average branch lengths and average likelihood scores based on the post burn-in tree set were computed using the *sumt* and *sump* commands in MrBayes. Bayesian analyses were repeated three times to ensure topological

Table 2 List of all known *Apomastus* collecting localities

Place name and GenBank Accession #	Acronym	Latitude/longitude	<i>n</i>
Ventura County, Sycamore Canyon DQ388577–DQ388579	SYC	N34.08848° W118.94862°	5
LA County, Los Alisos Canyon DQ388580, DQ388581	ALS	N34.06314° W118.89697°	5
LA County, Point Dume DQ388582–DQ388584	ZUM	N34.05922° W118.79942°	5
LA County, Solstice Canyon DQ388585	SOL	N34.03771° W118.74751°	5
LA County, Malibu Creek State Park	MSP	N34.05130° W118.69060°	0
LA County, Old Topanga Canyon ^a AY621484	OTC	N34.09860° W118.61630°	5
LA County, Pacific Palisades AY621482, AY621483	PPS	N34.06220° W118.53040°	5
LA County, Santa Ynez Canyon	SYN	N34.04470° W118.55210°	0
LA County, Palos Verdes DQ388586	PVD	N33.77734 W118.40751	5
LA County, Baldwin Hills	BDH	N34.00660° W118.37370°	0
LA County, Griffith City Park AY621504	GCP	N34.14543° W118.30818°	4
LA County, Sunset Canyon AY621487, AY621488	SCD	N34.20100° W118.28980°	5
LA County, Millard Canyon AY621486	MLC	N34.21030° W118.16240°	3
LA County, Henninger Flats	HNF	N34.19250° W118.08670°	0
LA County, Chantry Flats DQ388587–DQ388589	CHF	N34.19606° W118.02300°	5
LA County, Rincon Fire Station DQ388590, DQ388591	RCN	N34.23798° W117.86337°	5
LA County, Monrovia Canyon AY621489, AY621490	MCP	N34.17440° W117.98890°	3
LA County, Puente Hills DQ388592	PTH	N33.98162° W117.93351°	5
LA County, Tan Bark Flats	TBF	[N34.20390° W117.75970°]	0
LA County, Evey Canyon AY621503	EVC	N34.16730° W117.68380°	5
LA County, Little Dalton Canyon DQ388593, DQ388594	LDC	N34.16372° W117.83891°	5
San Bernardino County, Santa Antonio Canyon DQ388595	SAC	N34.1880° W117.6772°	5
Riverside County, Cajalco Canyon ^b AY621494–AY621497	CJE	N33.82560° W117.49570°	4
Orange County, Cleveland Ntl. Forest AY621499–AY621501	CLF	N33.59194° W117.47694°	5
Orange County, Ortega Highway DQ389886	ORT	N33.61276° W117.43363°	5
Orange County, San Juan Fire Station AY621491–AY621493	SJF	N33.59070° W117.47500°	5
Orange County, Salt Creek	SCK	N33.48190° W117.72060°	0
Orange County, Laguna Beach AY621502	LNG	N33.1770° W117.76783°	1

Note: Acronyms correspond to those used in Fig. 1; latitude/longitude estimated from topographic maps given in square brackets; *n*, number of specimens sampled for DNA studies.

^aType locality for *Apomastus schlingeri*.

^bType locality for *Apomastus kristenae*.

convergence and homogeneity of posterior clade probabilities (Huelsenbeck *et al.*, 2002).

For particular genetic clades of in-group populations, we examined genetic divergence as a function of geographic distance using reduced major axis regression. Pairwise geographic distances were estimated in the computer program ArcGIS version 8 (ESRI, Redlands, CA). Average pairwise genetic distances between populations were estimated using PAUP* (using the best-fit model). Correlations between genetic and geographic distance were based on a Mantel test matrix correlation with 10 000 randomizations, implemented in the computer program IBD version 1.52 (Bohonak, 2002).

Lineage-through-time (LTT; Nee *et al.*, 1994) plots were used to visualize the accumulation of mitochondrial lineages over time. The COI data were significantly non-clocklike ($P < 0.001$; lrt). Thus, we used non-parametric rate smoothing (NPRS; Sanderson, 1997), implemented in the computer program r8s version 1.50 (Sanderson, 2002), to minimize localized changes in substitution rate across the tree. Branch lengths for the NPRS tree were estimated using the Powell algorithm in r8s, with 10 random initializations, each followed by 10 repeated perturbations. An LTT plot was calculated from the NPRS tree using the computer program GENIE version 3.0 (Pybus & Rambaut, 2002).

Results

Spatial analyses

The variables of slope, elevation and precipitation contributed significantly ($P < 0.05$) to the binary logistic model. This model predicts a high probability of *Apomastus* occurrence in high-relief habitats surrounding the LA Basin, but a very low probability of occurrence within the LA Basin proper (Fig. 2). However, *contra* predictions of the model, we know that several populations do or have occurred within the LA Basin proper [e.g. Puente Hills (PTH), Baldwin Hills (BDH, now extinct), Palos Verdes (PVD) and Cajalco Canyon (CJE)], occupying low-probability sites. Perhaps these are indeed low-quality habitats, with the isolated lowland populations resulting from chance and infrequent colonization events? Alternatively, the model itself may be biased by the inclusion of absence data, particularly if the absence data are more a reflection of recent population extinction than habitat quality *per se*.

As an alternative to logistic regression, GARP analyses were used to search for non-random correlations between environmental parameters and *Apomastus* distribution. GARP analysis differs fundamentally from binary logistic regression by optimizing presence data. Although areas of high topographic relief were again recovered with a high probability of occurrence (Fig. 3a), the GARP model also predicts that *Apomastus* should be more extensively distributed in the LA Basin proper (Fig. 3b and c). This prediction is especially apparent in the less conservative model using only climatic, physical and pre-urban parameters (Fig. 3c). Predicted distributions within the Basin depict close ties to

riverine corridors (highly modified for flood control) and uplands of moderate topographic relief (Fig. 3b and c).

The GARP analysis suggests an alternative explanation for the presence of extant lowland populations in the LA Basin proper. This alternative hypothesis posits that LA Basin proper populations are *remnants* of a once more-widespread lowland population distribution. Under this hypothesis, the general absence of populations in the LA Basin proper is due to population extinction associated with recent urbanization, rather than naturally poor habitat quality. The GARP results also suggest potential geographic and genetic ties between now-isolated internal populations and populations that ring the Basin. For example, the spatial distribution of favorable habitat suggested by the GARP analyses would predict that PVD should have genetic connections to northern populations, rather than populations to the east (Fig. 3). Similarly, we expect the PTH population to be connected to populations to the northeast. These phylogeographic predictions, and population extinction predictions in general, are further explored below.

Genetic and phylogenetic analyses

The 100 *Apomastus* individuals sampled carried 47 unique COI haplotypes (GenBank accession nos. AY621482–AY612508 and DQ388577–DQ388595, DQ389886). Sampled populations of *Apomastus* are genetically unique at the mtDNA level. All haplotypes are restricted to a single sampling site, and multiple haplotypes sampled from the same site form exclusive genetic clades in all but two cases. These results indicate that female-based gene flow in *Apomastus* is extremely limited, as has been found in other mygalomorph species. This result also implies that population extinction necessarily involves the loss of unique genetic variation.

Most genetic clades corresponding to sample sites are genealogically arranged into four larger geographic clades, although three sites [Monrovia County Park (MCP), Rincon Fire Station (RCN) and CJE] are genealogically isolated (Fig. 1b). There is evidence for isolation-by-distance within the larger geographic clades (see below), but across geographic clades and isolated populations there is marked population divergence that cannot be predicted by geography. For example, *A. schlingeri* haplotypes from MCP and RCN appear closely related to haplotypes from more distant localities (Fig. 1b). As predicted by GARP analyses, PVD haplotypes are related to a northern genetic clade, and PTH haplotypes are related to those from adjacent montane populations to the northeast.

We evaluated the relationship between geographic and genetic distance for several different genetic groupings (see Fig. 4). Although low sample sizes prevent statistical significance in some cases (Table 3, Fig. 4), all analyses show a general relationship between geographic and genetic distance, indicative of an isolation-by-distance model of gene exchange. Although female-based gene flow seems limited in these spiders (evidenced by genealogical exclusivity of sampled sites), the genetic exchange that is occurring (or has occurred) is predicted by geographic proximity.

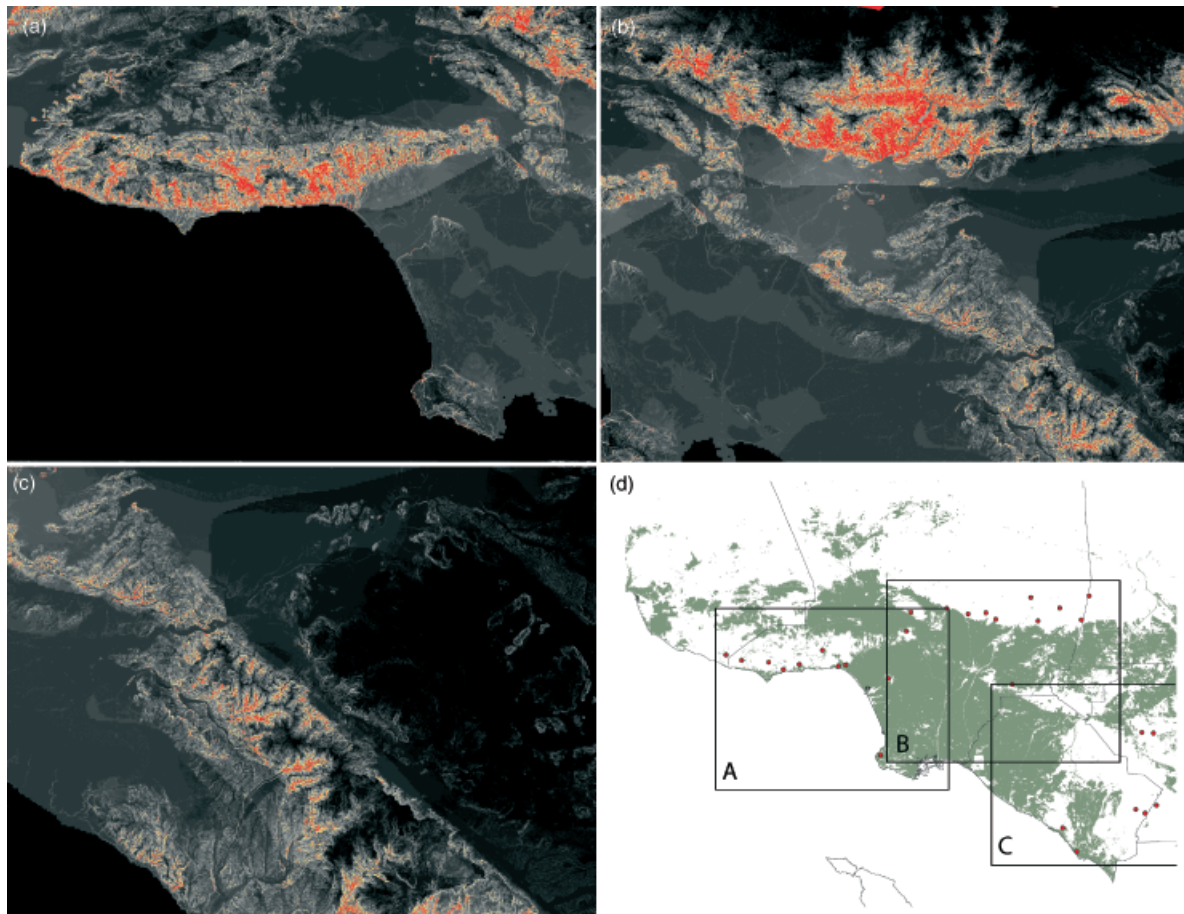


Figure 2 (a–c) Occurrence probabilities based on presence–absence data using a binary logistic regression model. Red coloration represents probability of *Apomastus* occurrence between 82 and 99%, yellow 70 and 81.99%; $P = \exp [3.667 - 0.179(\text{precipitation}) + 0.005(\text{elevation}) - 0.117(\text{slope})] / 1 + \exp [3.667 - 0.179(\text{precipitation}) + 0.005(\text{elevation}) - 0.117(\text{slope})]$, model success = 79.4%, Nagelkerker $R^2 = 0.382$. (a) Santa Monica Mountains. (b) San Gabriel Mountains. (c) Santa Ana Mountains. (d) Distribution of *Apomastus* with respect to urbanization. Urbanized regions across the Los Angeles Basin are shaded in green, the red dots correspond to known populations and boxes demarcate insets for (a–c).

This pattern is most consistent with an isolation-by-distance model of gene flow, where outlying populations were until recently connected by contiguous habitat (see Hutchison & Templeton, 1999). We would not expect this relationship under alternative scenarios that might explain the distribution of these remnant populations (see fig. 1 of Hutchison & Templeton, 1999). For example, if these populations were the result of long-distance dispersal across poor-quality habitats (e.g. via ballooning), we would expect geographic distance to be a poor predictor of genetic divergence. Conversely, these lowland populations might have been naturally fragmented from upland peripheral populations long before urban-induced fragmentation. But under this scenario, we would expect genetic divergence under drift to dominate, again eroding the relationship between genetic and geographic distance.

The LTT plot including all sampled haplotypes reveals a sharp upturn in lineage number in the recent past (Fig. 5a). We view this recent upturn as an artefact of including

multiple tip haplotypes per sampled site. Because we are interested in genetic evidence for population extinction (rather than haplotype extinction), we also conducted an LTT analysis using only a single representative haplotype per sampled site. Here, we assume that distinct mitochondrial lineage diversity is a reasonable surrogate for population diversity, which seems a fair assumption given that most sampled *Apomastus* populations carry genealogically exclusive mtDNA variation. An LTT plot using only representative mitochondrial diversity results in a generally convex curve, with a noticeable plateau at the tail (Fig. 5b). If we assume that a standard birth–death process explains these data (see Nee *et al.*, 1994), this curve is consistent with an increase in lineage extinction rate (or a decrease in lineage birth rate) in recent times. We should caution that we present the LTT plots only as a corroboration of our hypothesis of population extinction. Because these plots lack an absolute temporal context, we posit only that they are consistent with the loss of *Apomastus* populations in the recent past.

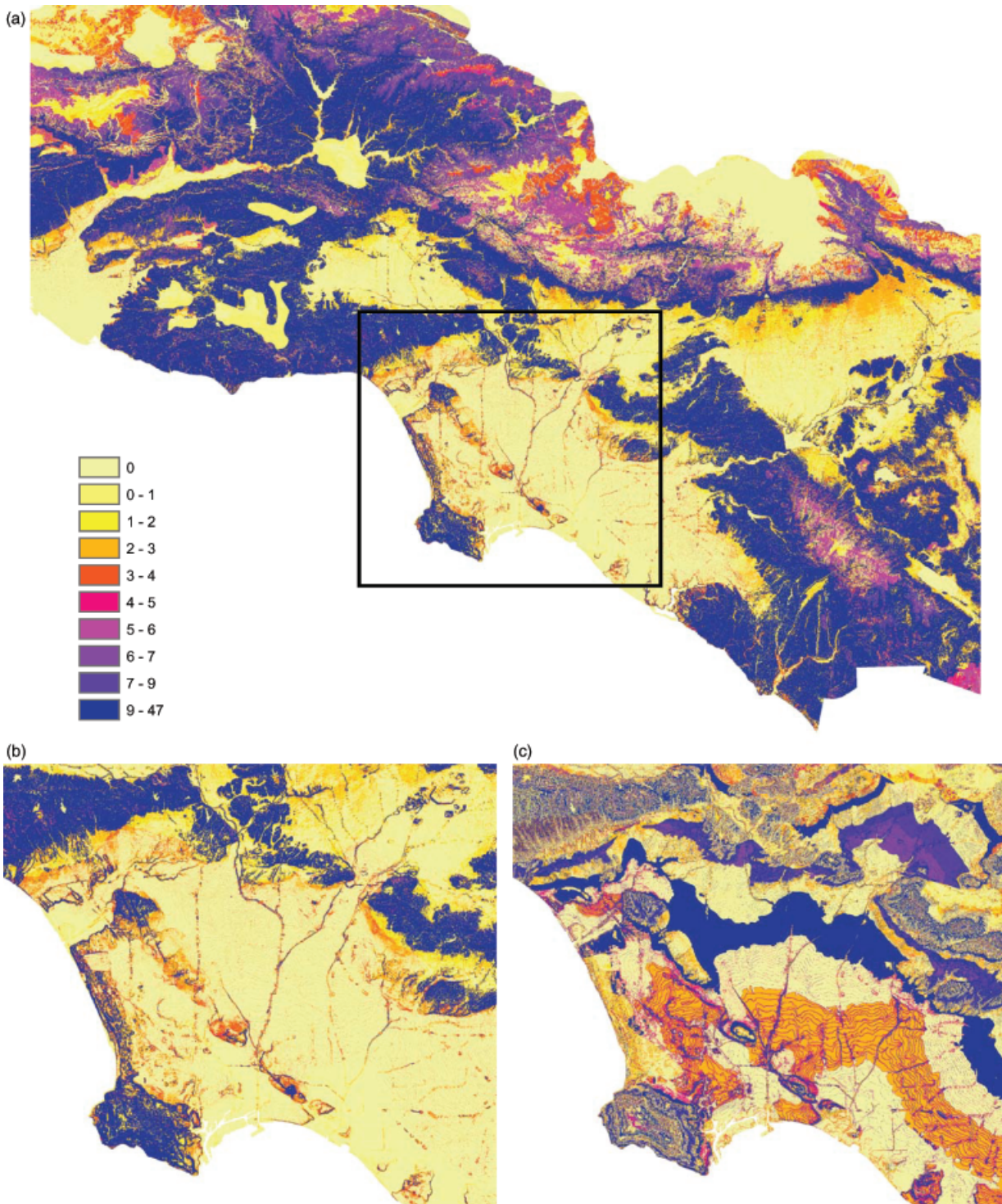


Figure 3 *Apomastus* occurrence probabilities based on genetic algorithm for rule-set prediction (GARP) analyses (best-subset procedure) (a) GARP analysis for the entire region using pre- and post-urbanization model parameters [inset box indicates the area depicted in (b) and (c)]. (b) Inset of the Los Angeles Basin based on model parameters used in (a). (c) GARP model based on analysis using only physical, climatic and pre-urban parameters. Higher numbers in the legend correspond to shaded regions with a high probability of occurrence; areas whose shading corresponds to lower numbers are regions with very low occurrence probabilities.

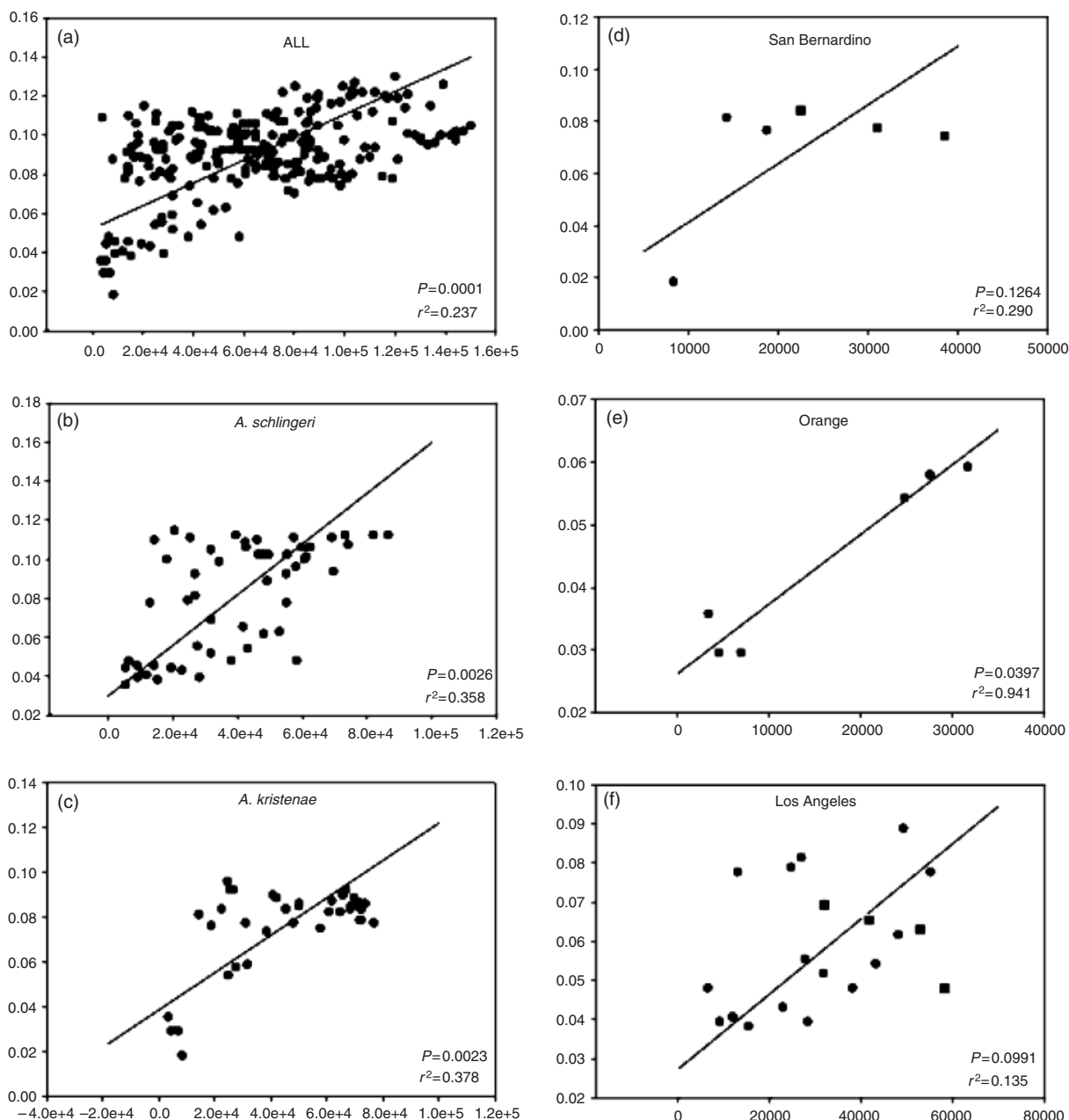


Figure 4 Isolation-by-distance plot for various genetic groupings of *Apomastus* populations. (a) All populations from both species combined, (b) *A. schlingeri* populations, excluding haplotypes from the divergent MCP site, (c) *A. kristenae* populations, excluding haplotypes from the divergent RCN site, (d) San Bernardino clade, (e) Orange County clade, and (f) Los Angeles clade (see Fig. 1b). Pairwise comparisons involving Palos Verdes and Puente Hills (points plotted as ■ on the Los Angeles and San Bernardino plots, respectively) populations are highlighted.

Discussion

On the basis of a combination of field surveys, compilation of historical records providing direct evidence that extinction has occurred and GIS-based modeling, we confirmed that the current distribution of *Apomastus* is largely exclusive of urban development. A GIS model incorporating both presence and absence data identifies high-relief topographic settings as optimal habitat, and indicates a low

probability of occurrence in the LA Basin proper. However, different GARP models, optimizing presence data, predict that *Apomastus* populations should be more prevalent in lowlands of the LA Basin. We hypothesize that their absence from such sites is due to population extinction, and tested predictions of this hypothesis using phylogeographic data. Although inferences from these genetic data are mostly indirect, general patterns are consistent with an extinction hypothesis. Taken together, our results suggest a perhaps

Table 3 Relationship between geographic distance and genetic distance

	Z	P<	r ²
<i>Apomastus</i>			
No transformation	1432357.41	0.0001	0.237
Log transformation	99.00	0.0001	0.303
<i>Apomastus schlingeri</i>			
No transformation	193513.21	0.0026	0.358
Log transformation	20.29	0.0008	0.377999
<i>Apomastus kristenae</i>			
No transformation	131838.85	0.0023	0.378
Log transformation	12.63	0.0007	0.590
San Bernardino			
No transformation	9889.86	0.1264	0.290
Log transformation	1.79	0.1246	0.501
Orange			
No transformation	5271.49	0.0397	0.941
Log transformation	1.11	0.2136	0.865
Los Angeles			
No transformation	42293.73	0.0991	0.135
Log transformation	5.52	0.089	0.150

Notes: Analyses based on reduced major axis regression for all *Apomastus* populations combined, *Apomastus schlingeri* populations only and *Apomastus kristenae* populations only. Analyses were performed on non-transformed and log-transformed geographic distances. Genetic distances were corrected using a single substitution rate model with a Γ shape distribution of nucleotide substitution (F81+ Γ).

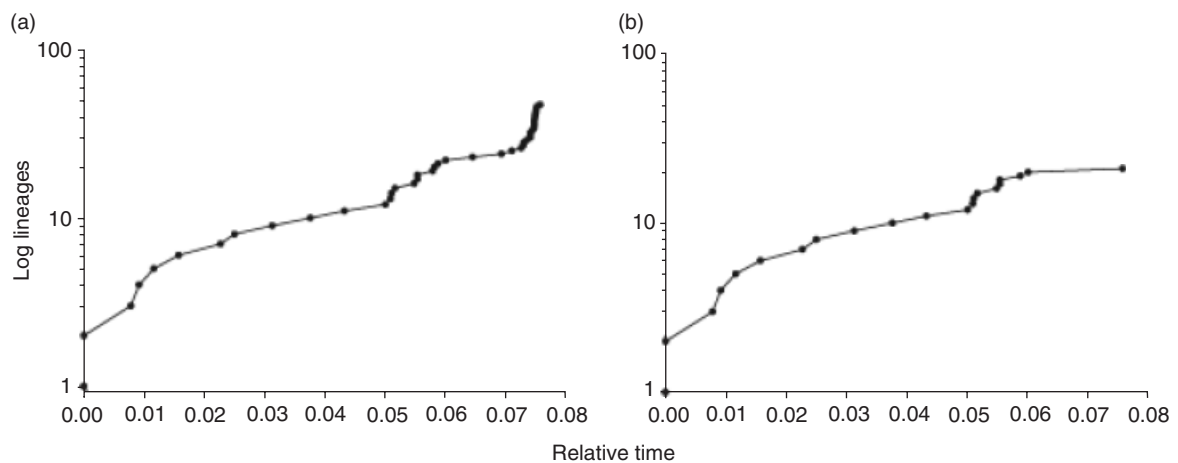


Figure 5 Lineages-through-time plots for (a) all in-group haplotypes included, and (b) single representative haplotype per sampled site. Relative time and numbers of lineages are derived from the Bayesian phylogeny, with branch lengths estimated using non-parametric rate smoothing.

considerable loss of population diversity in *Apomastus*. This population loss implies coincident loss of genetic and adaptive diversity within this genus, and more generally suggests a loss of ground-dwelling arthropod population diversity throughout the LA Basin.

Conservation

Genetic diversity is fundamental to the evolutionary or adaptive potential of both populations and species, particularly in the face of natural or artificially induced environmental change (e.g. response to climate change). In species characterized by high gene flow, any single local population is expected to carry most of the genetic variation found in

the entire species, perhaps minimizing the genetic impact of population loss (but see Leonard, Vilà & Wayne, 2005). This is not the case in naturally structured species, where genetic drift and limited gene flow combine to promote local genetic differentiation over space and through time. In such species, the loss of local populations carries a coincident loss of unique genetic diversity (e.g. Bouzat *et al.*, 1998; Wisely *et al.*, 2002), eroding the adaptive and evolutionary potential of the species (Templeton *et al.*, 2001; Frankham *et al.*, 2002).

In *Apomastus*, local genetic differentiation occurs over very fine spatial scales. All *Apomastus* populations, even those separated by as little as 3 km, appear to house some unique mtDNA genetic variation. Most carry an entire

exclusive array of such variation, which is revealed even with relatively small samples sizes. If we consider the expected greater differentiation in other portions of the genome (e.g. nuclear microsatellite DNA), it becomes apparent that population loss in *Apomastus* has carried with it the loss of significant amounts of novel genetic variation. If we conservatively estimate that populations separated by at least 5 km are genetically diverged, a single drainage system consisting of suitable habitat, once spanning the LA Basin, may have contained as many as eight unique population groups. The phylogenetic isolation of lowland populations such as CJE further suggests the possibility that entire genetic clades have been lost (e.g. see Leonard *et al.*, 2005).

Population extinction may have also eroded adaptive diversity in *Apomastus*. All lowland remnant populations occur in coastal sage scrub (CSS; see Fig. 1b), and reconstructions of pre-urbanization habitat in the LA Basin proper have classified the region as coastal sage habitat (Küchler, 1967). CSS is structurally different from the chaparral and oak woodland habitats typical of most *Apomastus* populations, and is generally more xeric. Studies on other trapdoor spider taxa have shown that populations inhabiting xeric habitats often show a suite of behavioral, life-history and phenotypic differences when compared with more mesic-habitat relatives (e.g. burrow structure and depth, entrance plugging, microsite selection, etc.; see Main, 1978, 1982, 1996; Coyle & Icenogle, 1994). Whether such differences exist in *Apomastus* is an open question, but if such differences do exist, then the extinction of lowland CSS populations is expected to have eroded both genetic and adaptive diversity.

Although the future of *Apomastus* populations that ring the LA Basin seems reasonably secure, saving the extant lowland CSS populations from extinction will require special conservation effort. The direction of these efforts is severely constrained by both the nature of the urban landscape and the biology of these spiders. The habitats are largely discontinuous, and will remain that way. Dispersal corridors are both politically and financially infeasible, and are not expected to work in these small, dispersal-limited taxa. Reintroductions would rely upon some knowledge of historical genetic configurations, which is lacking. Despite these constraints, we see several possible avenues for future research and conservation activity. Our focus is not only directed at *Apomastus*, but is more generally aimed at preserving at least some vestige of a rich and unique CSS ground-dwelling arthropod fauna in the LA Basin.

Focused ecological studies of extant lowland populations (e.g. PVD and PTH) are needed. These populations are restricted to small patches of habitat completely surrounded by urbanization, and appear very limited in numbers of individuals. Although the impact of invasive Argentine ants, non-native vegetation and peripheral development is unknown, we expect this impact to be great. For example, non-native vegetation, by blanketing favorable microsites, will affect the foraging abilities of these spiders. And even if the 'internal' habitat is not directly influenced, development in adjacent habitats will have negative demographic impacts

via roads, sidewalks and swimming pools, which represent deathtraps for wandering adult males. These considerations are general for mygalomorph spiders of the region (both trapdoor spiders and tarantulas), where long-lived sedentary females wait patiently for males that never materialize, while the habitats in which they are embedded continue to degrade.

More generally, we suggest that more consideration be given to the development and/or further inventory of urban 'microreserves' in the LA Basin region. Currently, reserve selection and design in southern California CSS habitats is based, in large part, on the presence of vertebrate umbrella species. However, studies on CSS habitats have shown that reserves based on the presence or absence of vertebrates may not capture invertebrate diversity (Rubinoff, 2001). In particular, we suggest the development of small reserves that retain viable populations of unique arthropods and other invertebrates, even if these sites are otherwise viewed as 'suboptimal' because they lack certain vertebrate taxa. Other studies have shown that such urban microreserves can harbor important elements of a remnant arthropod fauna, and act as reservoirs of diversity, even in a highly modified landscape (e.g. Connor *et al.*, 2002; Watts & Larivière, 2004). The CSS ground-dwelling arthropod fauna of the region is very distinctive, with many endemics. By targeting lowland sites that retain *Apomastus* or other mygalomorph spider taxa (e.g. *Aptostichus*, *Aliatypus*, *Bothriocyrtum*, *Aphonopelma*), we might be able to retain some of this special diversity.

Although Rubinoff (2001) suggests that 'the future is bleak for CA coastal sage scrub invertebrate biodiversity', we suggest that this future is *not entirely* inevitable. The native diversity that urbanization continues to claim can be tempered through modern approaches to biodiversity study. In the light of our work, we endorse multifaceted assessment – combining fieldwork, museum science, phylogeography and GIS spatial analyses – to best identify elements of diversity that remain and how they might be best conserved. We also emphasize the need for additional studies of terrestrial arthropods. Despite their fundamental ecological and economic importance (e.g. Wilson, 1987; Kremen *et al.*, 1993), and global dominance in species diversity (e.g., Clark & May, 2002), terrestrial arthropods have received limited consideration in biological inventory and conservation efforts (see Wilson, 1987; Skerl, 1999). Our research on *Apomastus* reminds us that the loss of biodiversity not only affects vertebrate taxa. We hope that this work provokes interest, concern and further study.

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