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Population genetic structure reveals terrestrial affinities for a headwater stream insect

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SUMMARY

1. The spatial distribution of stream-dwelling organisms is often considered to be limited primarily according to the hierarchical structure of the hydrologic network, and previous conceptual models of population genetic structure have reflected this generality. Headwater specialists, however, are confined to short upstream sections of the network, and therefore are unlikely to respond in the same way as species with a broader range of habitat tolerance.
2. Here, we propose a model to describe spatial patterns of genetic diversity in headwater specialists with a limited ability for among-stream dispersal. The headwater model predicts a partitioning of genetic variance according to higher-elevation 'islands' of terrestrial habitat that provide required headwater stream conditions. The model therefore expects a geographic pattern of genetic variance similar to that expected for low-dispersal terrestrial species occupying the adjacent habitat.
3. Using a 1032-bp mitochondrial DNA fragment encompassing parts of the COI and COII genes, we demonstrate that Madrean Sky Islands populations of the giant water bug *Abedus herberti* conform to the proposed headwater model. Furthermore, they exhibit phylogeographic patterns broadly concordant with those shown for several terrestrial species in the region, including a major zone of discontinuity in the Chiricahua mountain range.
4. Overall, populations are highly isolated from one another, and a nested clade analysis suggested that *A. herberti* population structure, similarly to terrestrial Sky Islands species studied previously, has been influenced by Pleistocene climatic cycles causing expansion and contraction of temperate woodland habitat.
5. Because they have no ability to disperse among present-day mountaintop habitat islands, *A. herberti* and other headwater species with limited dispersal ability are vulnerable to the projected increasing rate of climatic warming in this region.

Keywords: Belostomatidae, headwater streams, nested clade analysis, population genetics, stream hierarchy model

Introduction

The hierarchical structure of stream networks and drainage basins provides a natural spatial framework for considering the effects of dispersal and resulting gene flow in resident species (Meffe & Vrijenhoek, 1988; see also Grant, Lowe & Fagan, 2007). For aquatic organisms with limited terrestrial (trans-basin) dispersal propensity, gene flow typically is thought to occur primarily within versus among drainage basins (e.g. Bilton, Freeland & Okamura, 2001). Under this scenario, population genetic variance is expected to partition significantly according to drainage basin hierarchical structure. This pattern has been demonstrated in several low-dispersal stream insects, particularly for species in mountainous systems, where the intervening steep topography is expected to render

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terrestrial among-drainage dispersal even less likely (Hughes *et al.*, 1999, 2003; Wishart & Hughes, 2001, 2003; Monaghan *et al.*, 2002; Schultheis, Weight & Hendricks, 2002).

Recent studies (e.g. McGlashan, Hughes & Bunn, 2001; Wishart & Hughes, 2003; Huey, Hughes & Baker, 2006) have invoked Meffe & Vrijenhoek's (1988) stream hierarchy model (SHM) as an overarching conceptual framework describing the hierarchical distribution of genetic diversity according to drainage structure. Meffe & Vrijenhoek (1988), who were writing specifically in regard to desert fishes, developed the SHM for cases in which there are more or less continuous hydrologic connections along dendritic networks. An alternative model proposed by these authors, the 'Death Valley model' (DVM), was developed for isolated populations unconnected hydrologically and predicts that in such cases populations will be highly differentiated genetically without regard for landscape structure (e.g. drainage divides) and with no isolation by distance (see Fig. 1). As such, the DVM is expected to hold under conditions of zero gene flow.

Interestingly, some recent evidence suggests that neither the DVM nor the SHM is a sufficient framework for explaining patterns of genetic diversity among populations of low-dispersal headwater stream specialists (i.e. species restricted to higher-elevation primary tributaries; Finn *et al.*, 2006). Although overall strong among-stream genetic structure is predicted for these species, populations are likely to be more connected by limited overland dispersal across drainage divides than by longitudinal movement within the stream network through suboptimal or lethal lower-elevation reaches. In the absence of overland movement (as in most fish), populations of headwater specialists would be completely isolated and probably well-described by the DVM. However, for species that have some potential for terrestrial dispersal (as in many insects), the result should be a pattern of genetic diversity not unlike what would be expected of low-dispersal terrestrial species occupying the adjacent habitat (e.g. alpine butterflies, DeChaine & Martin, 2004; grasshoppers, Knowles, 2001); that is, landscape-scale genetic variance should partition significantly according to shared islands of higher-elevation terrestrial habitat within which headwater streams are nearby enough to allow some overland gene flow (Finn *et al.*, 2006). We refer to this framework as the 'headwater model' (HM), basic predictions of which are compared with the SHM and DVM in Fig. 1.

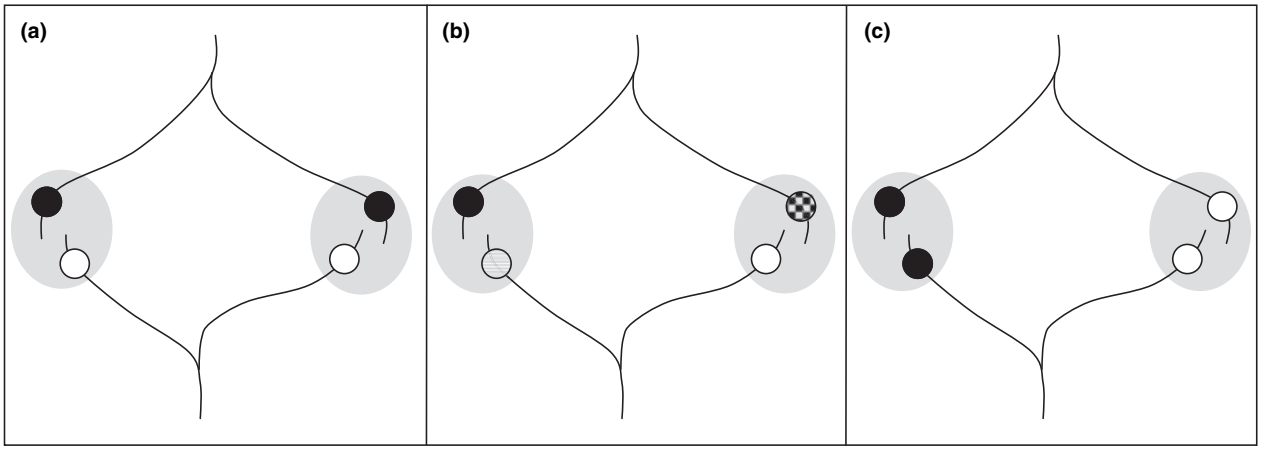

Fig. 1 Conceptual diagrams with predictions of (a) the stream hierarchy model, (b) the Death Valley model and (c) the headwater model, each acting in identical simplified stream network configurations including adjacent major drainages, headwaters of which originate in one of two higher-elevation terrestrial localities (grey shading). Small circles indicate local aquatic populations, and colours reflect predicted partitions of genetic variance. (a) Stream hierarchy model predicts higher gene flow within than among major drainage basins; therefore genetic diversity can be partitioned significantly among basins; (b) Death Valley model assumes complete isolation of all populations, regardless of location on the landscape; therefore no imposed group structure will explain significant genetic variance, and local drift is the dominant process leading to strong differentiation among populations; (c) Headwater model suggests that for headwater specialists with some overland dispersal ability, habitat specialization does not allow gene flow to occur throughout the drainage network, and rather limited short-distance terrestrial dispersal yields significant partitioning of genetic variance among terrestrial islands of preferred headwater habitat.

Because a key component of the HM is the presumed concordance of population genetic patterns of headwater stream species and the terrestrial species specializing in the intervening landscape, an ideal location to test it would be one in which terrestrial species also have been well-studied. In such a system, we would have the ability not only to evaluate stream network-related population genetic patterns for a focal aquatic species but also to compare broader-scale phylogeographic patterns of the focal species to those of terrestrial counterparts.

The Madrean Sky Islands (henceforth: 'Sky Islands') of southeastern Arizona, U.S.A. and neighboring areas of New Mexico, U.S.A. and northern Mexico consist of remnant woodlands associated with small mountain ranges separated by a sea of lower-elevation desert scrub and grassland (Brown, 1994; see Fig. 2). The terrestrial system has been well-studied in both biogeographic (e.g. Lomolino, Brown & Davis, 1989; Coblentz & Riitters, 2004; Bogan & Lytle, 2007) and phylogeographic (e.g. Barber, 1999; Masta, 2000, Downie 2004; Smith & Farrell, 2005a; Tennessen & Zamudio, 2007) contexts due in part to this well-defined patchiness, as well as to the interesting Pleistocene history of the region. Climatic cycles driven by regularly-spaced Pleistocene glacial/interglacial cycles (e.g. see Williams *et al*., 1998) resulted in concordant expansions and contractions of the extent of woodland habitat. Indeed, paleoecological studies suggest that as recently as *c*. 10 000 years ago woodlands covered most of the region (Betancourt, Van Devender & Martin, 1990; Thompson & Anderson, 2000). Correspondingly, evolutionary studies of terrestrial species currently associated with the woodland habitat show evidence of population fragmentation into isolated groups (e.g. Barber, 1999; Maddison & McMahon, 2000; Masta, 2000; Smith & Farrell, 2005a).

A pertinent aside, however, is that phylogeographic patterns across the Sky Islands tend to be more complex than would be expected under the hypothesis of fragmentation of a once-panmictic biotic assem-

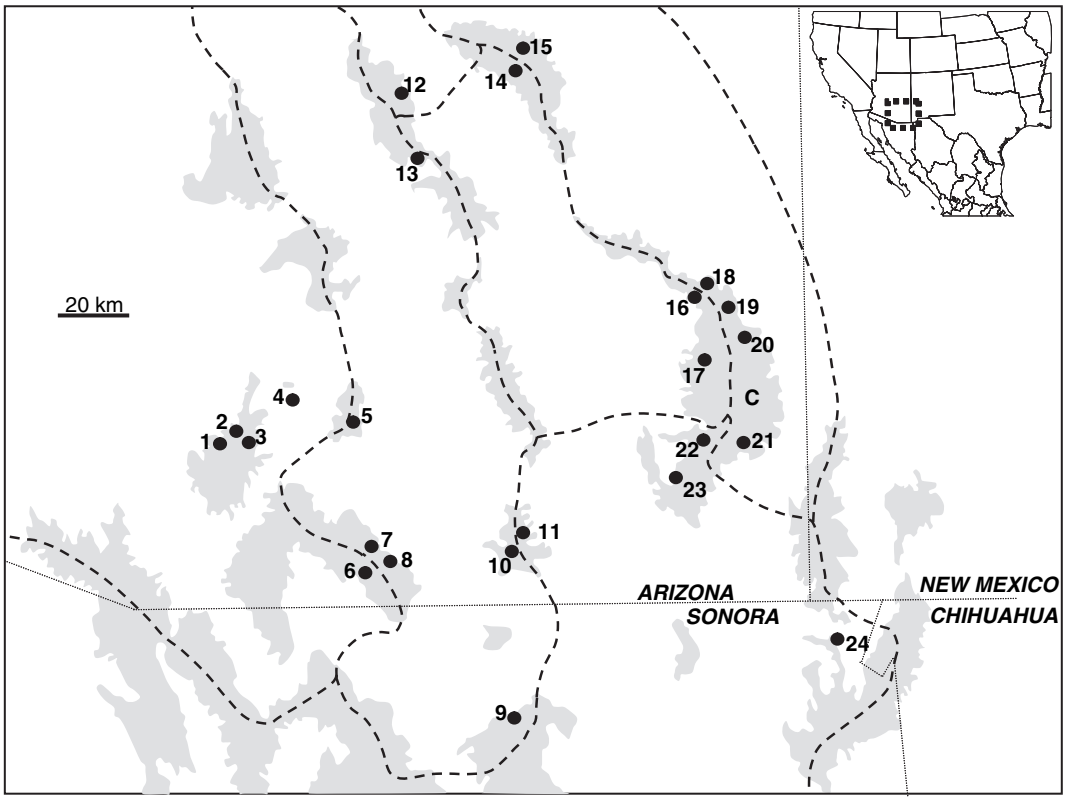

Fig. 2 Locations of Madrean Sky Islands, according to extent of oak and conifer woodland (in grey). Inset map locates this region in southwestern N. America. Black circles indicate sample streams with ID numbers (see Table 1 for specifics), and bold broken lines indicate major drainage boundaries. Sky island marked 'C' indicates the Chiricahua mountain range.

blage extending across the region. In general, the Sky Islands region is a biological interface where Rocky Mountain (to the north) and Sierra Madre Occidental (to the south) faunal and floral assemblages show some degree of overlap (Brown, 1994; Omernik, 1987). Some recent studies have revealed a contact zone for both major species assemblages and phylogroups in the eastern Sky Islands, particularly in the vicinity of the relatively extensive Chiricahua mountain range (see Fig. 2). As such, substantial phylogeographic discontinuities have been demonstrated in this area (e.g. Barber, 1999; Masta, 2000; Parker & Rissing, 2002; Smith & Farrell, 2005a,b).

The terrestrial patchiness evidenced by Sky Islands woodlands extends to the streams of the region, many of which are perennial on higher-elevation mountain slopes but are typically dry or seasonally intermittent washes at lower elevations (Bogan & Lytle, 2007). Hence, these streams contain many specialist aquatic species, a majority of them insects, whose distribution is restricted to the higher-elevation perennial stream reaches. Sky Islands stream insects support general biogeographic expectations, with an exceptionally high diversity due in large part to the coexistence of Rocky Mountain and Sierra Madrean-type assemblages that alternate in dominance with major seasonal hydrological shifts (Bogan & Lytle, 2007). However, although this headwater insect assemblage is unique in that it is restricted not only to the mountain islands but also within these is confined to streams or springs (thus acting to some degree as islands within islands), there has been very little research on the distribution and phylogeography of representative species.

In this study, we analysed sequence variation in the mitochondrial COI and COII genes of a representative aquatic insect *Abedus herberti* Hidalgo subsp. *herberti* collected from across the Sky Islands region. We addressed two major objectives that vary in spatial/temporal scale. First, we evaluated the strength of evidence for the headwater model of population genetic structure, in contrast to the stream hierarchy and Death Valley models (Fig. 1). This objective addresses patterns of contemporary gene flow and asks whether gene flow is related to the current structure of the landscape. Secondly, we asked if an inferred phylogeography of *A. h. herberti* is broadly concordant with pervasive patterns demonstrated for terrestrial Sky Island species. This objective focuses primarily on the deeper phylogeographic history of the species. Together, the objectives can be used to address the question of whether this representative Sky Islands stream insect exhibits contemporary and historical movement patterns similar to some well-studied local terrestrial species. To conclude, we discuss resulting conservation implications.

Methods

Study organism

Abedus herberti (Hemiptera: Belostomatidae) are (24.5–40 mm in length), predacious giant water bugs that are year-round inhabitants of perennial stream pools and springs in the southwestern US and northern Mexico. The common subspecies *A. h. herberti* is widely distributed across this range, while the rare subspecies *A. h. utahensis* is restricted to several localities in northern Arizona and southern Utah (Menke, 1960). Our study focuses on the common *A. h. herberti*, which we will refer to hereafter as *A. herberti* for brevity. All life stages are aquatic, and aquatic habitat is required for mating and brooding (Smith, 1976). *Abedus herberti* are flightless, but individuals sometimes exit the water temporarily to avoid flash floods (Lytle, 1999; Lytle & Smith, 2004). There is therefore some potential for terrestrial among-stream dispersal by crawling, but mark-recapture studies have shown that most individuals return to the same stream within 24 h of floods (Lytle, 1999, Lytle unpubl. data).

Field and laboratory methods

We collected 13–41 *A. herberti* individuals from each of 24 populations in the Sky Islands area (Fig. 2; Table 1) during the summer dry seasons of 2004–06. Populations were delimited by stream as groups of individuals occupying the isolated reaches still containing pools during the driest parts of the summer (June–early August). None of the stream-delimited populations was hydrologically connected to any other within the longitudinal range of the species. In each of two streams (19 and 22; Fig. 2) we sampled two disconnected reaches that were separated by stream distances of *c.* 2.5 and 10 km, respectively. Data from upstream and downstream reaches were combined

Table 1 List of locations and summary statistics for each population; site numbers refer to Fig. 2

Site no.	Name	Mountain range	Drainage	UTM easting	UTM northing	N	No. of haplotypes	Nucleotide diversity (π)	Gene diversity
1	Madera Canyon	Santa Rita	Santa Cruz R.	511320	3510270	21	5	0.004	0.68
2	Florida Canyon	Santa Rita	Santa Cruz R.	514942	3512864	23	5	0.004	0.56
3	Gardner Canyon	Santa Rita	Santa Cruz R.	521964	3508191	25	7	0.005	0.86
4	La Cienaga	n/a*	Santa Cruz R.	534295	3516791	19	4	0.007	0.78
5	French Joe Canyon	Whetstone	San Pedro R.	557348	3519377	19	4	0.007	0.75
6	Sunnyside Canyon	Huachuca	Santa Cruz R.	558836	3478428	20	3	0.001	0.56
7	Garden Canyon	Huachuca	San Pedro R.	561327	3481411	21	5	0.001	0.71
8	Ramsey Canyon	Huachuca	San Pedro R.	564938	3478517	24	5	0.004	0.67
9	Arroyo Claro	Sierra los Ajos	San Pedro R.	594759	3430381	19	9	0.003	0.85
10	Chulo Canyon	Mule	San Pedro R.	597363	3483941	24	2	0.002	0.46
11	Dixie Canyon	Mule	R. Yaqui	606141	3484843	13	4	0.005	0.73
12	Oak Creek	Galiuro	Aravaipa Cr.	567996	3619861	21	7	0.005	0.86
13	Wildcat Canyon	Galiuro	San Pedro R.	569955	3580674	22	8	0.004	0.8
14	Grant Creek	Pinaleño	Wilcox Playa	599734	3611531	20	7	0.003	0.79
15	Ash Creek	Pinaleño	San Simon R.	606755	3626581	15	3	0.002	0.56
16	W. Whitetail Creek	Chiricahua	Wilcox Playa	654923	3542401	18	1	0	0
17	W. Turkey Creek	Chiricahua	Wilcox Playa	655280	3526645	20	9	0.01	0.9
18	E. Whitetail Creek	Chiricahua	San Simon R.	662750	3541790	23	1	0	0
19	E. Turkey Creek	Chiricahua	San Simon R.	665630	3531568	40	3	0.008	0.63
20	Cave Creek	Chiricahua	San Simon R.	666283	3530314	21	3	0.003	0.45
21	Price Canyon	Chiricahua	San Simon R.	664818	3516292	19	2	0.01	0.51
22	Rucker Canyon	Chiricahua	R. Yaqui	654625	3514513	41	9	0.01	0.71
23	Leslie Canyon	Chiricahua	R. Yaqui	639931	3498493	20	1	0	0
24	Cajon Bonito	Sierra San Luis	R. Yaqui	690289	3462272	24	10	0.001	0.78

*Stream 4 is a lowland cienaga not located in a mountain range.

for all analyses. For each reach, we collected *A. herberti* individuals from all occupied pools along the length of the perennial reach to ensure spatially widespread sampling and avoid collecting closely related individuals more often than expected by chance. Most collections were comprised solely of adults, but in some cases we also collected juveniles to bolster sample size, in which case we collected as evenly as possible from each of the five juvenile instars, thereby minimizing the chance of collecting siblings. We immediately preserved specimens in 95% ethanol in the field.

In the lab, we refreshed the ethanol and stored specimens at −20 °C until DNA extraction, which was accomplished using a Qiagen DNEasy kit with 20–40 mg of leg muscle tissue from each specimen. We PCR-amplified a single 1032-bp mtDNA product that included slightly >1/3 the COI gene at the 3′ end, the entire tRNA-leucine, and most of the COII gene using primers C1-J-2441 (''Dick'') and C2-N-3661 (''Barbara'') (Simon *et al.* 1994). PCR reactions included 36.4 μl ddH_2O, 6 μl 10X buffer, 9.6 μl 25 mM $MgCl_2$, 1.2 μl 10 mM dNTPs, 1.2 μl each primer (10 μM), 0.4 μl *Taq* polymerase, and 4 μl template DNA at 1/10 dilution. The mixture was subjected to the following program: 95 °C for 3 min, 50 °C for 1 min, 72 °C for 1.5 min, 30 cycles of {95 °C for 1 min, 50 °C for 1 min, 72 °C for 1.5 min}, 72 °C for 4 min, final hold at 4 °C. Products were purified using the UltraClean PCR Clean-up Kit (Mo Bio Laboratories) and sequenced on an ABI Prism 3730 DNA analyzer (Applied Biosystems) using PCR primers.

Analyses

We used BIOEDIT software (Hall, 1999) to align sequences from 532 total individuals collected across the study area and used MEGA3 (Kumar, Tamura & Nei, 2004) for preliminary evaluation of haplotypes and their distribution among populations. We then used ARLEQUIN 2.000 (Schneider, Roessli & Excoffier, 2000) to calculate gene diversity (the probability that two randomly-chosen haplotypes are different) and π (nucleotide diversity as mean number of pairwise sequence differences per site) for each of the 24 sampled populations. ARLEQUIN was also used to run a global analysis of molecular variance (AMOVA) as per Weir & Cockerham (1984) to partition genetic variance into

within- versus among-population components according to *p*-distances. For this and other AMOVA analyses described below, we used 10 000 permutations of the data to assess statistical significance. Highly significant and large proportions of variance explained at the among-population level would suggest an overall high degree of isolation among streams and therefore would provide preliminary support for the Death Valley model.

We then ran two additional AMOVAs that included sequence data from the full extent of the study region: one that assigned population group structure according to major drainage basin occupied, and another imposing group structure according to mountain range. Significant genetic variance explained at group level in these analyses would provide evidence to refute the DVM assumption of zero among-population gene flow and rather provide support for either or both of the stream hierarchy model (significant structure among basins) or the headwater model (significant structure among mountain ranges). For both analyses, we initially included all 24 sampled populations, with the exception of the population from stream no. 4 for the mountain range grouping as it occurs in an atypical lowland location unassignable to a range. However, because some of the groups in both basin and range categories were comprised of only a single population (see Fig. 2, Table 1), there was a possibility that statistical power would be lacking to reveal population structure. Therefore, in a replicate set of analyses we excluded any populations that were single members of a defined group.

Following initial results revealing significant structure among mountain ranges, we ran a further AMOVA using only the seven populations within the most population-rich mountain range (the Chiricahuas) to ask whether genetic structure in this single headwater area could be further partitioned among the three drainages represented. This analysis tested for evidence that the SHM operates at the finer, within mountain range scale (see Wishart & Hughes, 2003 for an example of this type of pattern). In the absence of significant drainage basin structure at this scale, a further manifestation of terrestrial- rather than aquatic-like distribution would be revealed by a significant pattern of genetic isolation by distance (IBD; *cf*. Rousset, 1997) within the mountain range. Significant IBD according to Euclidean distance alone disregards stream network and other landscape structure and suggests that gene flow is correlated simply with spatial proximity of one population to another. This result is expected of low-dispersal terrestrial species in the adjacent habitat and would provide evidence for the HM. We used IBDWS (Jensen, Bohonak & Kelley, 2005) to perform a Mantel test of IBD using Slatkin's (1995) linearized F_{ST} versus log (geographic distance) for all pairs of Chiricahua populations. Statistical significance was assessed using 10 000 permutations of the data.

The next set of analyses included a nested clade analysis (NCA; e.g. Templeton, Routman & Phillips, 1995) and related inquiries used to infer a broad-scale phylogeography for comparison with results of prior analyses using terrestrial Sky Islands organisms. A haplotype network was constructed using TCS 1.21 software (Clement, Posada & Crandall, 2000), and network reticulations that likely were caused by high mutation rates and saturation at specific nucleotide sites were resolved following common theoretical predictions (see Crandall & Templeton, 1993; Posada & Crandall, 2001). For NCA, the network was then delineated into hierarchical clades according to rules outlined by Crandall (1996), and clades were assessed for significant geographical association using GEODIS 2.5 (Posada, Crandall & Templeton, 2000, 2006). We used the most updated key (11 Nov 2005 at http://darwin.uvigo.es) to assign historical inferences for each nested clade with significant phylogenetic–geographic relationships. There are some criticisms of the NCA method, including the inability to assign statistical confidence to key-generated inferences (e.g. Knowles & Maddison, 2002). Here we used NCA for its heuristic value in differentiating broadly between major categories of possible historical events (i.e. population contraction via fragmentation versus population expansion and gene flow).

We also used nested clades revealed in the haplotype network to visualize landscape-scale phylogeographic structure by mapping for each population the proportions represented by each of five major phylogroups. Phylogroups were defined at the four-step clade level (the most inclusive clade level in this study), except in the cases where four-step clades were comprised of relatively distantly-related three-step clades (three-step clades separated by greater than or equal to four unsampled steps in the haplotype network; see Figs 3–5), in which case

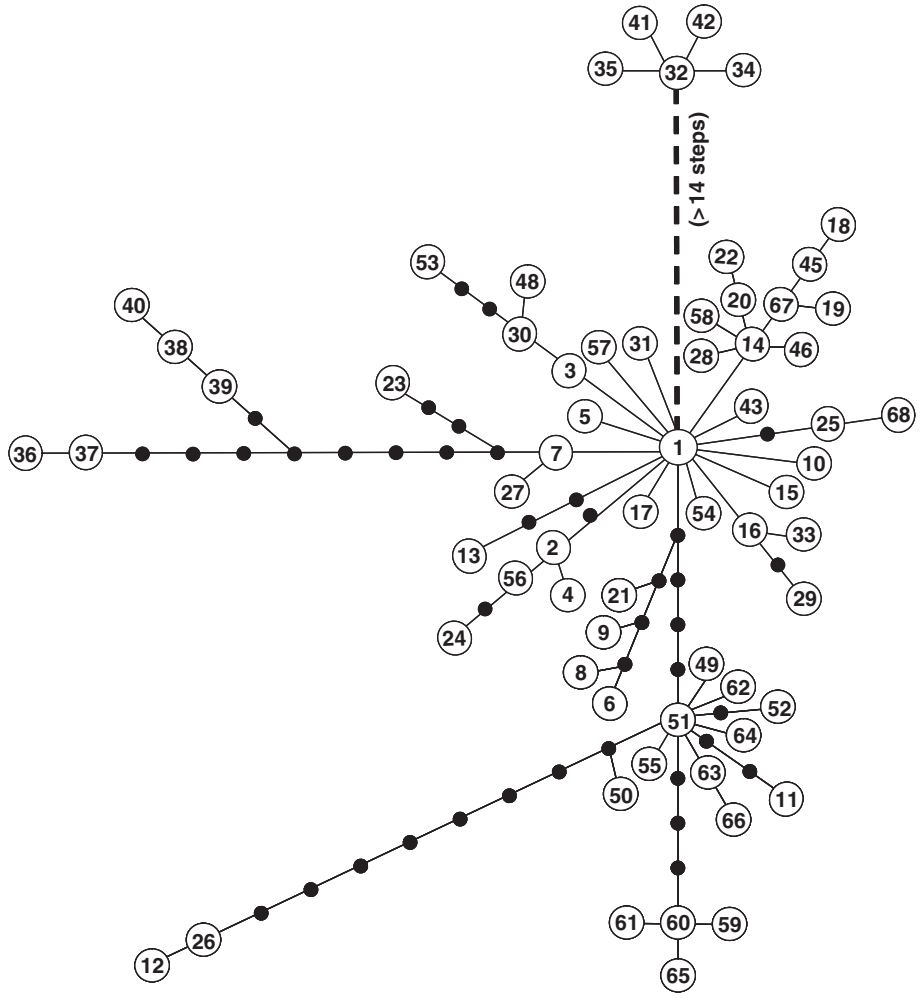

Fig. 3 Haplotype network. Each circle represents a haplotype, either sampled (white with haplotype ID) or unsampled (smaller black circles), and connector lines indicate single nucleotide substitutions. See Appendix for sample sizes.

these distant three-step clades were considered separate major phylogroups. We then used MEGA3 (Kumar *et al.*, 2004) to calculate net nucleotide divergence (average percent sequence difference standardized by average within-population percent difference) based on *p*-distance among the five major phylogroups. Significance was assigned by bootstrapping with 10 000 replicates, and we compared divergence estimates to a mean divergence rate of 2.2% Myr^{-1} for the arthropod COI gene (Gaunt & Miles, 2002) to provide relative approximations of coalescence timing.

Results

We identified a total of 66 unique haplotypes of the 1032-bp COI–COII fragment (GenBank accession numbers EF090202–EF090243; EF090245–EF090246; EF090248–EF090255; EF125996–EF126009) across 532 individuals in the 24 sampled populations. Sixteen of the populations contained at least one private haplotype (i.e. a haplotype found only in one population); indeed, 47 of the 66 haplotypes (71%) were private (see Appendix). Gene diversity varied widely among populations, from zero (monomorphic population) in three instances to 0.85–0.90 in four instances. Populations with zero diversity each were associated with the Chiricahua mountain range, while the highest-diversity populations were distributed broadly across the study area (streams 3, 9, 12 and 17; Fig. 2, Table 1), including the Chiricahua range. Nucleotide diversity (π) also varied widely, from zero in the monomorphic populations to 0.01 at three streams each in the Chiricahuas (Table 1).

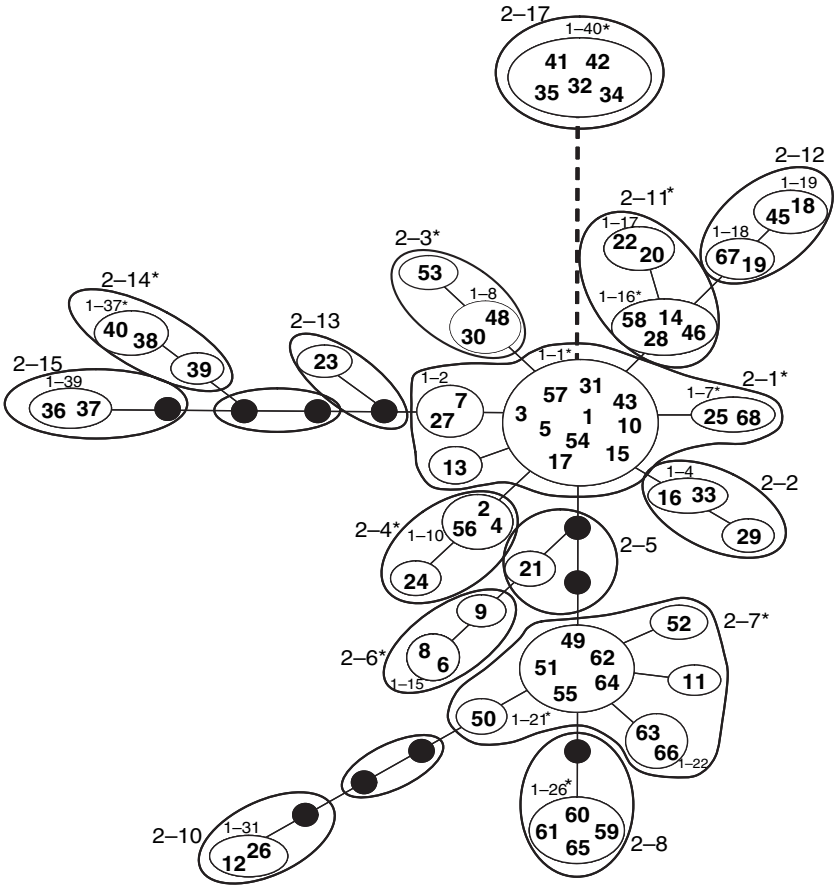

Fig. 4 One- and two-step hierarchical clade nestings for NCA. Bold numbers are haplotypes from Fig. 3 organized into one-step clades delineated by thin black circles, and into two-step clades enclosed in thicker black lines. Smaller circles with black fill indicate hypothetical one-step clades containing no sampled haplotypes. Asterisks indicate clades with significant geographic association.

An overall AMOVA to assess the partitioning of genetic variance within versus among the 24 populations revealed that 55% of total variation could be explained at the among-population level ($F_{ST} = 0.55$; $P < 0.0001$), suggesting extreme geographic isolation and very little current among-population gene flow. Populations with an exceptionally low effective population size, however, can artificially inflate the F_{ST} estimate. A second overall AMOVA in which we excluded the three monomorphic populations revealed an F_{ST} of 0.45, still large and with the same level of significance.

AMOVA further revealed that grouping all populations according to drainage basin did not explain a significant proportion of genetic variance ($F_{CT} = 0.08$; $P = 0.13$; Table 2a). Conversely, mountain range groups did account for significant variance ($F_{CT} = 0.18$; $P = 0.04$; Table 2b). A similar pattern was achieved after excluding the single-population groups (drainage basin groups: $F_{CT} = 0.09$, $P = 0.06$; mountain range groups: $F_{CT} = 0.23$; $P = 0.01$) and are not shown in the table. As expected, in both cases, significance was increased compared with when single-member groups were included, but per cent variance explained at the group level demonstrated a larger increase for the mountain groups and became significant at $\alpha = 0.01$, while for the basin groups, variance explained remained non-significant at $\alpha = 0.05$. Despite significance at the group level, however, on average a highly significant proportion of genetic variance was still explained among populations even *within* groups ($P < 0.0001$; see F_{SC} values Table 2a,b).

Within the most population-rich mountain range (the Chiricahuas), the among-basin variance component was not significantly different from zero ($F_{CT} = -0.15$, $P = 0.72$; Table 2c), revealing an absence of genetic structure according to the three major drainage basins on this single sky island. As at the full study extent, however, among-population variance was significant both within basins and across the whole mountain range (Table 2c). Isolation by Euclidean distance was also significant across Chiricahuas stream populations, according to a Mantel test ($Z = 18.20$; $r = 0.53$; $P = 0.007$).

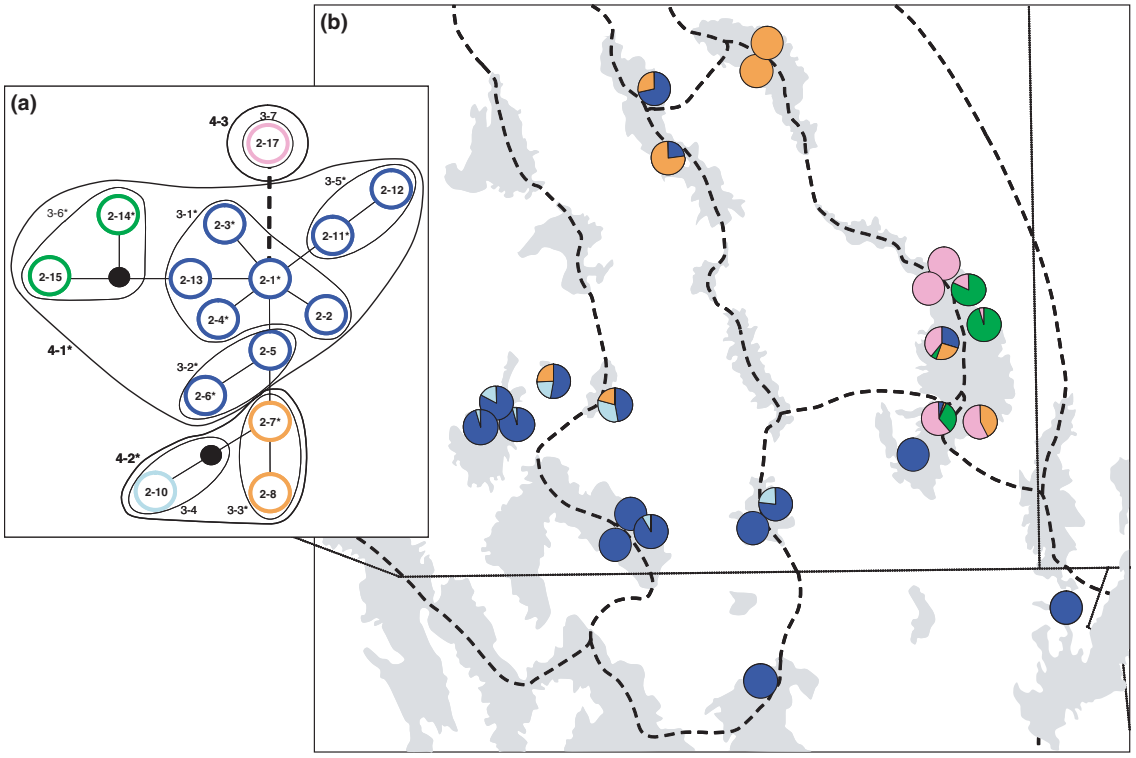

Fig. 5 (a) Three- and four-step clade nestings for nested clade analysis (NCA), with geographically significant clades marked by asterisks. Two-step clades identified in Fig. 4 are enclosed in thickened coloured circles representing major phylogroup representation. Black filled circles represent entirely unsampled two-step clades separating major phylogroups within four-step clades. (b) Map replicated from Fig. 1 with major phylogroup representation as colour-coded in (a) for each sampled stream population. Dark blue = phylogroup 1 in text; green = phylogroup 2; orange = phylogroup 3; light blue = phylogroup 4; pink = phylogroup 5.

Table 2 AMOVA results

Source of variation	d.f.	Variance components	Percentage variation	Fixation indices
(a) Streams grouped according to major drainage basin				
Among basins	5	0.43 (Va)	7.52	F_{CT} (Va/Vt) = 0.08 (P = 0.13 NS)
Among streams within basins	18	2.74 (Vb)	48.2	F_{SC} [Vb/(Vb + Vc)] = 0.52 (P < 0.0001)
Within streams	508	2.52 (Vc)	44.28	
Total	531	5.69 (Vt)		F_{ST} (1 − Vc/Vt) = 0.56 (P < 0.0001)
(b) Streams grouped according to mountain range				
Among mountain ranges	8	1.02	17.52	F_{CT} = 0.18 (P = 0.04)
Among streams within ranges	14	2.33	40	F_{SC} = 0.49 (P < 0.0001)
Within streams	490	2.48	42.47	
Total	512	5.84		F_{ST} = 0.58 (P < 0.0001)
(c) Streams within the Chiricahua range grouped according to drainage basin				
Among Chiricahua basins	2	−1.05	−14.88	F_{CT} = −0.15 (P = 0.72 NS)
Among streams within basins	5	4.63	65.34	F_{SC} = 0.57 (P < 0.0001)
Within streams	194	3.51	49.54	
Total		7.08		F_{ST} = 0.50 (P < 0.0001)

F_{CT} describes the proportion of genetic variance ascribed at the group level; F_{SC} partitions variance among populations within groups.

Although several haplotypes were private to either single populations or to mountain ranges, there were a few common haplotypes distributed more widely (see Appendix). TCS software revealed a network in which the relatively widespread haplotype no. 1 occupied a central location and was considered the

Table 3 Historical inferences from nested clade analysis

Nested clade	Inference key steps	Inferred event
1-1	1-2-3-5-6-7-yes	Restricted gene flow w/some long-distance dispersal
1-7	1-19-20-2-11-12-no	Contiguous range expansion
1-16	1-19-20-2-11-12-no	Contiguous range expansion
1-21	1-2-3-5-6-7-yes	Restricted gene flow w/some long-distance dispersal
1-26	1-2-3-4-no	Restricted gene flow w/isolation by distance
1-37	1–19-no	Allopatric fragmentation
1-40	1-2-3-4-no	Restricted gene flow w/isolation by distance
2-1	1-2-3-4-no	Restricted gene flow w/isolation by distance
2-3	1-19-20-2-11-12-no	Contiguous range expansion
2-4	1-19-20-2-11-12-no	Contiguous range expansion
2-6	1-19-20-2-11-12-no	Contiguous range expansion
2-7	1-2-3-5-6-7-yes	Restricted gene flow w/some long-distance dispersal
2-11	1-2-3-5-6-7-yes	Restricted gene flow w/some long-distance dispersal
2-14	1-2-11-12-no	Contiguous range expansion
3-1	1-2-3-5-6-7-yes	Restricted gene flow w/some long-distance dispersal
3-2	1-2-11-12-no	Contiguous range expansion
3-3	1-2-3-4-no	Restricted gene flow w/isolation by distance
3-5	1-19-20-2-3-5-15-21-no	Past gradual range expansion followed by fragmentation
3-6	1-2-no interior clades	Inconclusive
4-1	1-2-3-5-15-no	Past fragmentation
4-2	1-2-3-5-15-no	Past fragmentation
Total cladogram	1-2-3-5-6-7-yes	Restricted gene flow w/some long-distance dispersal

Only clades with significant geographical structure are listed. See Figs 4 & 5a for hierarchical clade topology.

most likely ancestral state for this group due both to its abundance – it was the second most-abundant haplotype – and to its central position in the network (Fig. 3). The most abundant haplotype encountered (no. 32) was the central node of a small sub-network that was only distantly related to the remainder of the network (Fig. 3, top), such that TCS could not connect it at the 95% connection limit of ≤14 unsampled steps. This entire sub-network was found only in the Chiricahua mountain range. In the remainder of the haplotype network, there were two additional cases where at least eight unsampled steps separated highly differentiated haplotype groups (Fig. 3).

GEODIS revealed that a large proportion of clades in all hierarchical nesting levels had significant geographical associations (Figs 4 & 5a). Table 3 reports the inferences for these significant clades according to NCA. A summary of the table suggests that limited gene flow, some long-distance dispersal, and range expansions dominated the lower-level clades, fragmentation events became more prevalent in higher-level clades (including both significant four-step clades), and limited gene flow was again inferred at the level of the total cladogram. Higher-level (and thus more inclusive) clades are expected to reveal earlier historical processes than lower-level clades, which are less inclusive and reveal more recent events (e.g. see DeChaine & Martin, 2004). The broad implication of the NCA was therefore that both the most recent and the oldest events detectable in the population genetic structure of Sky Islands *A. herberti* were of limited dispersal and/or range expansion, while there was a signal at intermediate-to-high clade levels of population contraction by fragmentation. A single outlier stood out from this general pattern: the small clade 1–37 had a fragmentation signal resulting from the segregated distribution of two closely-related haplotypes among nearby streams. At the next hierarchical clade level, 1–37 was included within clade 2–14, for which range expansion was inferred.

The five major phylogroups identified from the nested clade network (Fig. 5) were: (i) the subset of clades 3–1, 3–2 and 3–5 from four-step clade 4–1; (ii) clade 3–6, the remaining three-step clade in 4–1; (iii) clade 3–3 and (iv) clade 3–4, both from four-step clade 4–2 and (v) the distant clade 4–3. Pairwise net nucleotide divergence among these major phylogroups ranged from 0.01 to 0.03 (see Table 4), roughly suggesting 0.5–1.5 million years separating them. The group on average most distant from all others was the

Table 4 Pairwise net per cent nucleotide divergence among the five major phylogroups identified in Fig. 5b

	1	2	3	4	5
1		[0.002]	[0.002]	[0.003]	[0.004]
2	0.006		[0.003]	[0.004]	[0.004]
3	0.005	0.012		[0.003]	[0.004]
4	0.011	0.015	0.009		[0.005]
5	0.017	0.022	0.02	0.026	

Below diagonal: net divergence; above diagonal: standard error.

small sub-network containing haplotype no. 32 (clade 4–3; phylogroup 5) that was confined to the Chiricahua range (Fig. 5). Phylogroup 2 was also found only in the Chiricahuas, although net nucleotide divergence between it and phylogroup 5 was quite large at 2% (Table 4). The remaining three phylogroups were geographically more widespread. Phylogroup 1, which contained the ancestral haplotype and all closely-related radiations from it, was distributed throughout the study area, was the only group collected far south into Mexico, and was collected in all mountain ranges except the northeasternmost (the Pinaleños). In the Pinaleños, Phylogroup 3 was the only representative, and this group maintained a northern affinity, distributed across only the northern half of the study area. Phylogroup 4 consisted of only two haplotypes (see Figs 4 & 5) but was relatively widespread across the west-central sky islands.

Discussion

Models of population structure

We analysed sequence data from 24 intensively-sampled *A. herberti* populations spanning the Madrean Sky Islands region to assess the weight of evidence for each of the three models of headwater stream population structure outlined in Fig. 1. Overall, there appears to be little contemporary among-stream gene flow as evidenced by large and significant F_{ST} and F_{SC} values both across the entire study region and within a single mountain range (Table 2). Given the sparse distribution of permanent pools in this arid region and the obligatory confinement of *A. herberti* to aquatic habitat at all life stages, this result is not entirely surprising. Compared to other insect headwater stream specialists that instead possess a brief flying terrestrial adult phase, overall F_{ST} for Madrean Sky Islands *A. herberti* populations is significantly higher, even when populations with extremely low effective population sizes are excluded from analysis (e.g. compare to Finn & Adler, 2006; Finn *et al.*, 2006).

These observations lend preliminary support to the Meffe & Vrijenhoek (1988) Death Valley model (Fig. 1b) which predicts significant among-population genetic structure driven by the dominance of genetic drift in completely isolated populations. Under the zero gene flow assumption of the DVM, however, genetic variance is not expected to partition significantly according to landscape structure. In this regard, despite strong overall isolation among populations, analysis of molecular variance showed that another significant proportion of genetic variance could be explained by grouping populations according to mountain range, as predicted by the headwater model (Fig. 1c), although not according to drainage basin, as would be predicted by the stream hierarchy model (Fig. 1a). Furthermore, at the finer within-mountain-range spatial scale, there was no evidence to support the SHM. The among-basin variance component was negative (though non-significant), suggesting a tendency towards overdispersion of haplotypes among the three major Chiricahua basins. Furthermore, genetic isolation by distance across the terrestrial landscape was highly significant (a result that also counters predictions of the DVM).

These results provide support for the HM in Sky Islands *A. herberti* populations and suggest that, though limited, gene flow is most likely to occur among populations sharing the same mountain range, regardless of stream network structure. Within a mountain range, headwater streams occur closer together in space, and terrestrial conditions for among-stream movement presumably are less harsh than on the desert floor separating the isolated, island-like ranges. At the same time, however, the large proportion of genetic variance explained among populations even within mountain ranges suggests that the among-stream dispersal causing the pattern is rare. During periods of intense rainfall, individual *A. herberti* often crawl from the stream and may venture dozens of meters away, even climbing steep vertical rock walls to avoid flash floods (Lytle, 1999; Lytle & Smith, 2004). While most individuals return to the same stream post-flood, it is possible that some venture far enough to disperse into adjacent catchments. In the absence of this occasional trans-basin movement, the DVM probably

would be upheld. A similar pattern has been inferred for another headwater insect species occupying streams within a harsh terrestrial environment across which individuals were presumed to have a limited ability to fly between neighbouring headwater streams (Finn *et al*., 2006).

Clearly, each of the three models depicted in Fig. 1 represents a reasonable prediction for species dwelling in headwater streams. The degree of habitat specialization along the longitudinal stream gradient combined with a species' ability to disperse over land will likely determine which model provides the most realistic framework. For aquatic habitat generalists with no overland dispersal ability, the stream hierarchy model is realistic; for habitat specialists also lacking in a terrestrial dispersal phase, the Death Valley model is realistic; and for species with strong habitat specialization and a limited ability for overland dispersal, the headwater model is likely to provide a standard conceptual framework for predicting the distribution of genetic diversity across the landscape.

Phylogeographic concordance with terrestrial species

There are several lines of evidence revealing similar phylogeographic histories for terrestrial Sky Islands species and the strictly aquatic *A. herberti*. First, major lineages in the region are deeply divergent. The five major *A. herberti* phylogroups appear to have a history that appreciably predates the end of the last Pleistocene glacial period *c.* 10 000 years ago. While these divergence depths are striking, they are broadly concordant with estimates for major Sky Islands terrestrial species lineages (tree frogs: Barber, 1999, spiders: Masta, 2000, flightless beetles: Smith & Farrell, 2005a, lizards: Tennessen & Zamudio, 2007). In each of these species, including *A. herberti*, divergences between major mitochondrial lineages were inferred to date up to on the order of a million years or more before the present.

Secondly, NCA has revealed evidence for episodic *A. herberti* population expansion and contraction similar to what has been inferred for Sky Islands terrestrial woodlands (e.g. Betancourt *et al*., 1990). Because geographical extent of the woodland habitat follows cooler/wetter conditions during glacial cycles, the distribution of perennial waters probably follows in a similar manner. The divergent major phylogroups discussed above reflect a deeply-rooted fragmentation (contraction) event that probably occurred during an exceptionally warm/dry Pleistocene interglacial period, while the inferred episodes of increased gene flow and population expansion on either side of the fragmentation event were likely allowed by more widespread cool/wet conditions associated with the cyclical glacial periods. Ancestral polymorphisms, presumably such as those associated with phylogroup 1 (dark blue group in Fig. 5), are commonly retained across geographically widespread locations, and this pattern has been demonstrated previously in Sky Islands terrestrial fauna (e.g. Masta, 2000). The extensive distribution of phylogroup 1 led to the NCA inference of limited gene flow and long-distance dispersal at the level of the total cladogram and presumably implicates an early population expansion in which *A. herberti* colonized the Sky Islands region from the Sierra Madre Occidental to the south. Phylogroup 1 dominated in our southernmost sample sites; and it has been hypothesized that the Sierra Madre serves as the primary 'mainland' that provides dispersal propagules for the Sky Islands 'archipelago' (Brown, 1994; Prival *et al*., 2002; Smith & Farrell, 2005a).

The third and final piece of evidence demonstrating similar phylogeographic histories among Sky Islands *A. herberti* and terrestrial species is a strong concordance in the geographical affinities of major lineages. The fragmentation inferences for higher-level clades discussed above typically were made because of a lack of geographical overlap among nested clades confined to one of three subregions: the Chiricahua range, the two northernmost sky islands included in our sampling, or the remainder of the study area. Further, the phylogeographic discontinuity between the northern and central/southern ranges is less prominent than that separating the Chiricahua range from the remainder of the Sky Islands, as evidenced by the confinement of two of the five major phylogroups to the Chiricahuas. These patterns are remarkably concordant with patterns revealed in other Sky Islands species (see Barber, 1999; Masta, 2000) and reflect similar population-level responses for both aquatic and terrestrial low-dispersal species to a fluctuating environment. However, the Chiricahua discontinuity is particularly exceptional for *A. herberti*. The geographic overlap here among four of the five major phylogroups, as well as the occurrence of notably high local nucleotide diversities in several of

the Chiricahuas populations (see Table 1), indicates that this mountain range has been a unique biological melting pot over recent evolutionary timescales for a merging of divergent lineages. The northeastern orientation of the Chiricahuas has presumably allowed closer contact during glacial periods with the extensive Mogollon Rim region of the Colorado Plateau (several 10s of km north of the Sky Islands) and other mountainous regions to the east (e.g. see Barber, 1999).

Implications for conservation

Globally, small headwater streams support diverse endemic biotic assemblages but often lack national jurisdictional protection (Meyer *et al*., 2007). There is now imminent conservation concern for these systems, and there has been a call for increased scientific understanding to establish headwater conservation guidelines (e.g. Lowe & Likens, 2005). Here, we have revealed that population genetic patterns in Madrean Sky Islands streams show concordance with terrestrial species in the same system, as well as with headwater stream specialists in a disparate system (Finn *et al*., 2006), and we have proposed a conceptual model (Fig. 1c) that could be applied realistically to other headwater species with similar dispersal-related traits.

The headwater model provides a simple framework for making practical conservation-related assessments. In Sky Island *A. herberti*, for example, a wide range of local genetic diversity was revealed. However, all three monomorphic populations discovered were located within the Chiricahua range. These populations suggest local habitats that have 'sink'-like characteristics and rely on immigration from nearby 'source' populations (e.g. see Hanski & Simberloff, 1997) to be maintained or rescued from periodic local extinction. There are probably two interacting reasons explaining our discovery of multiple sink-like populations within a single mountain range. First, we increased our search effort in the Chiricahuas because of our interest in the local phylogeography, a decision that is likely to have increased the probability of stumbling upon small populations in marginal habitats here. Secondly, however, under the HM the several higher-diversity populations within the Chiricahuas probably serve as sources of immigrants to maintain nearby sink populations sharing this sky island. In the absence of nearby sources, sink habitats would remain unoccupied indefinitely following local extinction. Effectively then, under the HM local populations in smaller and more sparsely-populated mountain ranges probably have a greater long-term extinction risk.

Local extinction probability for *A. herberti* and other mountaintop-adapted species will certainly increase under the pressures of the current pace of climate change. Although we have seen evidence of the instability of climate throughout the Pleistocene epoch, the current rate of warming/drying, particularly in the western U.S.A., probably outpaces anything experienced during the evolutionary history of many species (Folland *et al*., 2001; Mote *et al*., 2005; Smerdon *et al*., 2007). Furthermore, the current steep warming trajectory is occurring in the midst of an interglacial period in which baseline conditions are already near the warm/dry peak of the natural climatic cycle. Given these climate-related pressures, it should be a conservation imperative to provide some level of protection to these and other island-like systems to provide resident species a chance to realize natural evolutionary trajectories.

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Appendix Absolute abundance of haplotypes found in each stream population (for population numbers, see Fig. 2, Table 1; for haplotype tree see Fig. 3)

Haplotype no: Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1		15				11	9		2	8	6						2					1		11
2									7															
3									1			4	1											3
4									1															
5									2															
6									2															
7							1		1															
8									1															
9	11	2				1				16			1											
10																	2							
11																	5				8	1		
12	1	4	1	4	6			1			3													
13																								1
14																							20	2
15																								2
16		1	5		7		7		2								1							1
17																								1
18												2												
19												6												
20	4		5																					
21			5																					
22			3																					
23			5																					
24							3																	
25								12																
26								1																
27				6				3																
28								7																
29					2																			
30	1			4																				
31																						1		
32																18	3	23	7	1	11	20		
33																								1
34																						1		
35																						4		
36																						2		
37																						9		
38																	1			5		2		
39																			20	15				
40																			13					
41																	3							
42																	2							
43																	1							
45												1												
46	4																							
48		1																						

Appendix (*Continued*)

Haplotype no: Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
49				5																				
50													1											
51					4							3	9											
52												3												
53												2												
54							1																	
55													4											
56																								1
57											1													1
58			1			8					3													
59														1										
60													3	7	9									
61														3										
62														1										
63														6	5									
64														1										
65															1									
66														1										
67													1											
68																			2					

