

# Demographic Stability Metrics for Conservation Prioritization of Isolated Populations

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**Abstract:** Systems of geographically isolated habitat patches house species that occur naturally as small, disjunct populations. Many of these species are of conservation concern, particularly under the interacting influences of isolation and rapid global change. One potential conservation strategy is to prioritize the populations most likely to persist through change and act as sources for future recolonization of less stable localities. We propose an approach to classify long-term population stability (and, presumably, future persistence potential) with composite demographic metrics derived from standard population-genetic data. Stability metrics can be related to simple habitat measures for a straightforward method of classifying localities to inform conservation management. We tested these ideas in a system of isolated desert headwater streams with mitochondrial sequence data from 16 populations of a flightless aquatic insect. Populations exhibited a wide range of stability scores, which were significantly predicted by dry-season aquatic habitat size. This preliminary test suggests strong potential for our proposed method of classifying isolated populations according to persistence potential. The approach is complementary to existing methods for prioritizing local habitats according to diversity patterns and should be tested further in other systems and with additional loci to inform composite demographic stability scores.

**Keywords:** *Abedus herberti*, climate change, demographic stability, evolutionary process, habitat stability, headwater streams, isolation, population genetics

Medidas de Estabilidad Demográfica para la Priorización de la Conservación de Poblaciones Aisladas

**Resumen:** Los sistemas de parches de hábitat aislados geográficamente albergan especies que ocurren naturalmente como poblaciones pequeñas y separadas. Muchas de esas especies son de preocupación para la conservación, particularmente bajo las influencias interactuantes del aislamiento y el cambio global. Una estrategia de conservación potencial es la priorización de poblaciones con mayor probabilidad de persistencia y que funjan como fuentes para futuras recolonizaciones de localidades menos estables. Proponemos un método para clasificar la estabilidad a largo plazo (y, probablemente, potencial de persistencia en el futuro) con medidas demográficas compuestas derivadas de datos estándares de genética poblacional. Las medidas de estabilidad se pueden relacionar con medidas simples del hábitat para un método directo de clasificación de localidades para informar al manejo de la conservación. Probamos estas ideas en un sistema de arroyos aislados en desiertos con datos de secuencia mitocondrial de 16 publicaciones de un insecto acuático no volador. Las poblaciones exhibieron un amplio rango de valores de estabilidad, que fueron pronosticados significativamente por el tamaño del hábitat acuático en la época de sequía. Esta prueba preliminar sugiere un fuerte potencial para nuestro método de clasificación de poblaciones aisladas de acuerdo con su potencial de persistencia. Este método es complementario de los métodos existentes para la priorización de hábitats locales con base en los patrones de diversidad y debería ser probado en otros sistemas y con loci adicionales para conformar valores de estabilidad demográfica compuestos.

**Palabras Clave:** *Abedus herberti*, aislamiento, arroyos, cambio climático, estabilidad demográfica, estabilidad de hábitat, genética de poblaciones, proceso evolutivo

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## Introduction

Small patches of habitat distributed intermittently throughout nonhabitat space are often key conservation foci, particularly given the strong beta diversity that can be generated by among-patch isolation. Examples include desert springs (Hershler et al. 2005; Kodric-Brown & Brown 2007), mountaintop islands (Finn et al. 2006; Holycross & Douglas 2007), headwater streams (Finn & Poff 2005; Finn et al. 2007; Meyer et al. 2007), and caves (Sharratt et al. 2000). Isolation across such spatially structured systems promotes strong local specialization and potential endemism and can contribute to strong intraspecific population genetic structure.

Ideally, entire systems of isolated habitat patches would be protected. Nevertheless, given growing tension between human resource needs and conservation issues, prioritization has become a key objective in conservation policy and management (Faith 1992; Moritz 2002). As such, managers require scientifically supported prioritization schemes to protect existing diversity maximally, and, perhaps more important, to preserve the ecological and evolutionary processes maintaining diversity (Crandall et al. 2000; Moritz 2002; Mace & Purvis 2008).

Researchers often are left to infer process from more readily measurable genetic, phenotypic, or ecological pattern, and several such strategies exist that can provide information toward prioritization. The phylogenetic diversity approach, for example, suggests prioritizing localities that contain taxonomic assemblages representing the greatest diversity of evolutionary lineages (cf. Faith 1992), thereby maximizing the protection of evolutionary "feature" diversity. Phylogenetic diversity can be calculated at the inter- or intraspecific levels. Also meaningful at the intraspecific level are spatially correlated patterns of neutral genetic divergence and potentially adaptive phenotypic or ecological differences among populations (e.g., Desmet et al. 2002; Holycross & Douglas 2007; Richards & Knowles 2007). The rapidly developing field of population genomics also has begun to provide advances on the frontlines of assessing genetic patterns related to adaptive differences (Bonin et al. 2007). Each of these approaches could be useful for prioritizing a set of populations representing the widest range of observed genetic or adaptive differences.

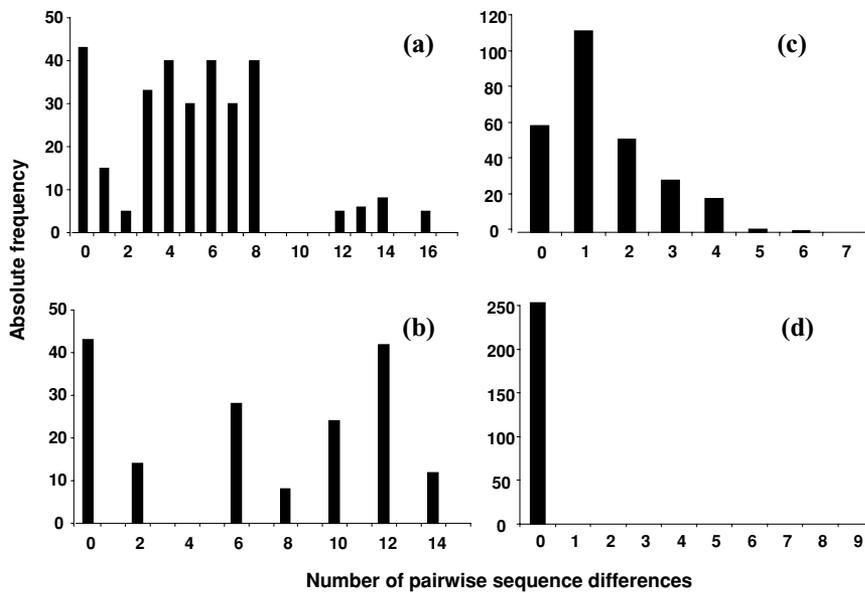
Although single- or multilocus genetic data are often collected during population studies, their value in inferring not only spatial diversity patterns but also local demographic history (e.g., Rogers & Harpending 1992; Cornuet & Luikart 1996) is often overlooked. Differences in demographic history among populations suggest differences in the important processes of population size fluctuation versus stability throughout recent evolutionary history. Such differences might reflect varying capacities of local populations to withstand environmental fluctuations that have occurred across ecologically meaningful

timescales and may indicate potential to persist through future environmental change. Populations showing evidence of long-term stability may therefore be top conservation priorities.

Demographic history can be inferred from allele or haplotype distribution patterns of well-sampled local populations. A comparison of mismatch distributions (Rogers & Harpending 1992), for example, can reveal striking differences among populations. Figure 1 shows a range of empirical mismatch distributions for four populations of a headwater stream insect spanning a broad array of demographic histories. Several statistics have been developed to test whether observed mismatch distributions depart significantly from null hypotheses of population stability or growth in effective size (Rogers et al. 1996; Rozás et al. 2003). Beyond these, there is a suite of conceptually similar statistical tests for DNA sequence data (e.g., Tajima 1989; Fu 1997) and microsatellites (Cornuet & Luikart 1996; Garza & Williamson 2001). Barring evidence of selection, these tests essentially weigh evidence of population expansion (e.g., excesses of singletons or star-shaped, short-branched phylogenies) versus a bottleneck (e.g., excessive nucleotide diversity or absence of singletons).

These tests typically are used independently of one another and on relatively inclusive clades sampled over wide geographic extents. Tajima's  $D$  (Tajima 1989), for example, is commonly combined with phylogeographic analyses to ask whether major clades show evidence of recent expansion or contraction in effective size (Mahoney 2004; Carstens et al. 2005). Ideally, a composite metric that incorporates the strengths and averages out the weaknesses of a range of the many available tests would provide the most robust inference regarding population demographic history. Furthermore, these tests can be suitable for use at the finer spatial scale of single populations, provided there is evidence that populations are isolated (i.e., migration is minimal and drift, mutation, and response to environmental fluctuation are the key processes driving local genetic patterns). A comparative evaluation of the resulting composite metrics among a series of populations could then inform conservation management.

These composite metrics also could be used to assess the influence of local habitat characteristics on population history. Populations that show long-term size stability or maintain historical evidence of expansion (both of which suggest demographic stability over an ecologically meaningful time period) are likely to occur in stable habitat patches, whereas populations with evidence of recent bottlenecks or founder events likely occupy less stable habitats (e.g., Domínguez-Domínguez et al. 2007). Henceforth, *stability* in a habitat patch or local population refers to persistence without major fluctuation throughout recent evolutionary history. In most ecological systems, there is likely to be some key abiotic stressor



*Figure 1. Mitochondrial mismatch distributions for four headwater stream populations of *Abedus herberti* in the southwestern United States and northern Mexico: (a) Gardner Canyon, (b) French Joe Canyon, (c) Cajon Bonito, (d) East Whitetail Creek. Refer to “Class 1: Mismatch Distributions” for detailed interpretation.*

that influences habitat stability and, potentially, long-term population stability. These stressors—such as fire, drought, or other disturbances—often occur at different frequencies or intensities in different locations. Variance in intermittency among ponds, for example, likely influences local-scale differences in demographic stability among frog populations, with more-permanent ponds housing more-stable populations (Newman & Squire 2001). Identifying the habitat characteristics that confer long-term population stability provides further information relevant to conservation prioritization.

Our main objective is to introduce an approach to combine several types of statistical tests on genetic sequence data for inferring demographic stability of naturally isolated populations. Composite metrics can be used to inform conservation prioritization directly, as well as to explore ecological links between local habitat characteristics and demographic stability. We advocate this additional ecological approach for two reasons. First, this approach can generate mechanistic hypotheses regarding observed differences in population stability, and second, habitat characteristics often can be quantified more easily in the field than can population genetics and may serve as useful surrogates for prioritization. Finally, we tested this composite-metric approach in populations of a flightless aquatic insect species in arid western North America.

## Methods

### Candidate Demographic Statistics

We considered a set of six statistical tests appropriate for use with mitochondrial or other nonrecombining DNA sequence data. Mitochondrial DNA has been well developed as a population genetic tool, and mtDNA sequence

analysis is straightforward and widely accessible. Similar tests can be run with other markers such as microsatellites (referenced earlier). Each of the following tests has a unique approach and set of assumptions but in general can be categorized into one of three major classes, following Ramos-Onsins and Rozás (2002). We suggest selecting at least one test per class when developing composite population scores.

### CLASS 1: MISMATCH DISTRIBUTIONS

A mismatch distribution plots the frequencies of observed number of nucleotide differences for all pairs of individuals in a population (Fig. 1). The distribution is expected to be multimodal for a population that has maintained a long-term stable effective population size (Fig. 1a). Multimodality reflects the stochastic nature of gene trees under neutral evolution at a stable demographic equilibrium (Slatkin & Hudson 1991; Rogers & Harpending 1992). Under a recent bottleneck in population size, the multimodality of the mismatch distribution becomes more pronounced owing to random loss of intermediate haplotypes (Fig. 1b). By contrast, a population that has undergone an expansion in effective size typically has a smooth unimodal distribution resulting from the gradual but continual accumulation of new, closely related haplotypes via mutation (Fig. 1c). A signature of expansion can be retained in the mismatch distribution for thousands of generations, with the wave in the distribution gradually moving away from the y axis. A monomorphic population, in contrast, has no true distribution because all individuals are identical (Fig. 1d).

Although a mismatch plot is convenient for visual interpretation of demographic history, there are limited statistics for testing hypotheses about its shape, and most of these simply test a null hypothesis of unimodality. We

considered the raggedness index (rg) and the mean absolute error (MAE). Raggedness (Harpending 1994) is a measure of the smoothness of the distribution, and its value is expected to decrease with the increased smoothness expected under a hypothesis of expansion. The MAE describes the difference between the observed mismatch distribution and that generated from a simulated expansion event (Rogers et al. 1996). Similar to rg, MAE is expected to decrease with increased probability of expansion.

## CLASS 2: HAPLOTYPE FREQUENCIES

We considered a single statistic in this category: Fu's (1997)  $F_S$  compares observed haplotype frequency distribution in a sample population with that expected under the Ewens (1972) distribution, which assumes the infinite alleles model, neutral evolution, and mutation-drift equilibrium in the local population. Fu's  $F_S$  is based on the probability of obtaining a Ewens-simulated population with greater than or the same total number of haplotypes observed in the natural population. The test statistic is the logistic function of this probability and is negative when the probability is low and positive when it is high. Thus, negative  $F_S$  is expected following population expansion, which tends to produce an abundance of new, rare haplotypes. Conversely, bottlenecked populations should yield a positive  $F_S$  given loss of rare haplotypes and decreased haplotype diversity.

## CLASS 3: RELATIONSHIPS BETWEEN SEGREGATING SITES AND NUCLEOTIDE DIVERSITY

Populations that have undergone recent bottlenecks tend to have an excessive nucleotide diversity (average genetic distance among individuals) compared with that expected given the number of segregating sites (DNA sites that are polymorphic across individuals), whereas the opposite is expected under population growth. We considered three statistics in this category. The commonly used Tajima's  $D$  (Tajima 1989) compares population  $\theta$  estimates generated using segregating sites versus nucleotide diversity models. Under stable population size, these  $\theta$  are expected to be equal, and they vary in opposite directions under population growth versus bottleneck. Tajima's  $D$  is 0 under demographic stability, negative under growth, and positive following a bottleneck. We also considered the  $R_2$  statistic of Ramos-Onsins and Rozás (2002), which includes a term for the number of singleton mutations present in the sample. More singletons are expected during and shortly following population growth. The  $R_2$  statistic varies such that lower values are expected following growth and higher values following a bottleneck. Finally, we included the  $D^*$  statistic of Fu and Li (1993), which also incorporates a term for number of singletons present. Fu and Li's  $D^*$ , like Tajima's  $D$ ,

is expected to be negative under population growth and positive following a bottleneck.

## Composite Stability Metrics

Among these statistics, a range of strengths and weaknesses can be expected (Ramos-Onsins & Rozás 2002; Depaulis et al. 2003). For example,  $R_2$  was developed specifically for detection of growth, and its bottleneck-detection capacity is uncertain. In addition, mathematical properties of individual statistics sometimes can result in a misinterpretation of recent demography (e.g., Tajima [1989] provides examples of when Tajima's  $D$  can be significantly negative even when a bottleneck has occurred). To make full use of the range of information provided, while averaging out potential weaknesses across statistics, we considered composite metrics.

We took two approaches to this objective. We discretely assigned a population to one of several categories according to compiled results across the range of statistics considered, and then we developed a continuous variable to describe where a population lies along a multivariate continuum of population stability scores. A key benefit of assigning multivariate scores is that these values can be used in regression analyses. For example, inferred population stability can be regressed on quantitative measures of relevant habitat characteristics. A drawback, however, is that the exact value of a multivariate axis score has little direct ecological meaning, and population scores will vary depending on the number and scores of other populations included in the analysis. Categorical assignment, by contrast, may result in less statistical power for further hypothesis testing; however, discrete categories are ecologically meaningful. A categorical approach may be more helpful to address management issues where specific conservation directives can be assigned for each demographic category. The following case study illustrates both approaches.

## Case-Study System and Species

In arid western North America perennial stream reaches are sparsely distributed, which can lead to strong isolation of the resident organisms (Bogan & Lytle 2007). These permanent habitats are essential to allow strictly aquatic species to persist through the dry season. Spatial isolation of habitat patches is most important to organisms with poor dispersal ability, such as the giant water bug *Abedus herberti*. *A. herberti* is flightless, and although individuals have the ability to crawl temporarily from the stream, they only do so during monsoon rainstorms that occasionally interrupt the midsummer dry season (Lytle 1999; Lytle & Smith 2004). Crawling from the stream during these storms is an adaptive response to avoid flood-induced mortality (Lytle et al. 2008). These relatively rare crawling events appear to be the only

mechanism for among-stream dispersal, but they are infrequent enough that most populations are significantly genetically divergent from one another (Finn et al. 2007). *A. herberti* is a top predator and requires permanent aquatic habitat due to its lengthy (>1 year) life span and lack of a diapause stage. These attributes make *A. herberti* an indicator for the presence of perennial aquatic systems harboring unique communities (Bogan & Lytle 2007).

A key environmental stressor for low-mobility aquatic species is habitat stability (i.e., the long-term probability of habitat failure). An issue of concern in western North America is the current climate trajectory toward increased warmer and drier summers and increased drought frequency (IPCC 2007; Shamir et al. 2007). Indeed, a recent 5-year extreme drought in Arizona (Phillips & Thomas 2005) appears to have caused a shift from perennial flow to intermittency in several streams. *A. herberti* has been extirpated in these localities (D.A.L. and M.T.B., unpublished data). Even streams where *A. herberti* currently persist probably have been variably susceptible to drought throughout the climatically dynamic longer-term past. As a simple contemporary measure of historic habitat stability, we focused on dry-season aquatic habitat size because smaller habitats likely have a greater long-term probability of failure than do larger habitats. We hypothesized a positive relationship between size of permanent aquatic habitat and genetically determined long-term population persistence.

### Genetic Data and Demographic Inference

To calculate the six population demographic statistics, we used previously collected mitochondrial sequence data from a series of *A. herberti* populations occupying small mountain ranges known as the Madrean Sky Islands, as described in detail by Finn et al. (2007). Here we used a southern subset of 16 of the 24 originally described populations because detailed habitat information (see below) was lacking for the remaining eight. Previously obtained summary data on sample size and haplotype and nucleotide diversity are reiterated in columns 2–5 of Table 1. All COI–COII sequences were deposited in GenBank as listed by Finn et al. (2007).

We calculated demographic statistics for 14 of these 16 populations. The remaining two populations (East Whitetail and West Whitetail) were monomorphic and thus had no variability for statistical analysis. We used DnaSP (Rozás et al. 2003) for all statistical tests, and significance was assessed for each with 1000 replicate coalescent simulations conducted with observed number of segregating sites held constant. When possible, we used two-tailed tests to identify significantly low or high values with  $\alpha = 0.05$ .

We used the six demographic statistics to generate both categorical and continuous composite metrics. For the categorical approach, we defined five categories of

population stability according to a set of rules based on statistical results, with categories ordered numerically according to increasing inferred stability. Monomorphic populations were placed in category 1. Although no statistics could be generated for these populations, monomorphism suggests severe demographic instability resulting from a strong, recent bottleneck or local extinction followed by a recent founder event. For the remaining populations, we used patterns of significance among the demographic statistics  $F_S$ , Fu's  $F_S$ , Tajima's  $D$ ,  $R_2$ , and Fu and Li's  $D^*$  for assignment to the other four categories. These assignment rules are summarized in Table 2.

For the continuous approach, we used a Bray–Curtis (B-C, or “polar”) ordination of the six demographic statistics calculated for the 14 polymorphic populations (PC-ORD version 5.0, McCune & Mefford 2006). Bray–Curtis ordination has the particular strength of separating multivariate points between two conceptual end points (McCune & Grace 2002). Although still distributing populations in multivariate space, the B-C approach attempts to reduce most of the variation into a single dimension. Our objective was to use axis scores along this primary dimension as the composite metric for each population. Prior to analysis, we relativized all data such that the sum of each column in the multivariate matrix equaled 1, providing equivalent weighting among independent statistics. We used the variance–regression end point selection method, which negates the influence of potential outliers (McCune & Grace 2002). The end points selected in this procedure had suites of statistical values that suggested long-term growth (Cajon Bonito; see Table 1) at one extreme and a recent strong bottleneck (Price Canyon) at the opposite. The remaining 12 populations were therefore fit into the ordination between the two end points along B-C axis 1.

### Habitat Measures

Total dry-season habitat area was calculated by mapping wetted reaches in each of the 16 streams between 1300- and 2200-m elevation (typical range for *A. herberti*). Upstream of known populations, we searched all tributaries maintaining dry-season wetted habitat, and downstream we mapped only the mainstem extending to 1300-m elevation. We approximated wetted area of flowing water by marking with a hand-held GPS unit the upper and lower extents of flow, recording stream width at 10-m intervals, and computing total area as average width by total length of the reach. At streams with >2 km of continuous flow, we recorded stream width at 100-m intervals. For wetted habitat occurring as isolated pools, we measured surface area of each pool to the nearest 0.1 m<sup>2</sup>. Because depth varies minimally in these small streams, habitat surface area is a useful proxy for total habitat volume.

Twelve of the 16 streams were measured in June–early July 2004, at the conclusion of a 5-year drought, whereas

Table 1. Sample size, habitat area, population genetic information, and demographic statistics and inference for each *Abedus herberti* population.

Site/population name	n	Habitat area (m <sup>2</sup> )	No. of haplotypes	Nucleotide diversity	Individual demographic statistics <sup>a</sup>				MAE	rg	F <sub>S</sub>	D	D*	R2	B-C axis 1 score <sup>b</sup>	Demographic category <sup>c</sup>
					F <sub>S</sub>	D	D*	R2								
Cajon Bonito	24	8895	10	0.001	0.51	0.098	-5.6*	-1.74*	-1.37	0.078	0	5				
Chulo Canyon	24	53	2	0.002	0.943	0.72*	5.36*	2*	1.08*	0.23*	0.193	2				
Dixie Canyon	13	693	4	0.005	0.932	0.21	4.07	0.59	1.19*	0.18	0.107	3				
East Turkey Creek	40	571	3	0.008	1.48	0.53*	15.6*	1.09	1.73*	0.16	0.2	3				
East Whitetail Creek	23	33	1	0	na	na	na	na	na	na	na	1				
French Joe Canyon	19	12	4	0.007	1.13	0.3*	7.29*	1.87*	1.53*	0.22*	0.155	2				
Florida Canyon	23	76	5	0.004	1.06	0.27	3.71	-0.44	0.65	0.11	0.091	4				
Garden Canyon	21	2270	5	0.001	0.394	0.13	-0.14	-0.45	-0.177	0.114	0.047	4				
Gardner Canyon	25	89	7	0.005	0.685	0.043	2.45	-0.72	-1.34	0.12	0.045	4				
Madera Canyon	21	95	5	0.004	1.17	0.57*	3.63	-0.73	-1.95	0.14	0.116	3				
North Fork Cave Creek	21	796	3	0.003	1.06	0.17	4.46	-2.36*	-3.74*	0.21*	0.054	3				
Price Canyon	19	28	2	0.01	1.54	0.76*	18.6*	3.12*	1.61*	0.26*	0.276	2				
Ramsey Canyon	24	250	5	0.004	1.32	0.64*	3.75	-0.46	1.26*	0.115	0.14	3				
Rucker Canyon	41	1029	9	0.01	1.41	0.105	9.4*	1.42	0.11	0.16	0.127	3				
West Turkey Creek	20	598	9	0.01	1.18	0.069	3.8	1.03	0.19	0.17	0.095	4				
West Whitetail Creek	18	15	1	0	na	na	na	na	na	na	na	1				

<sup>a</sup>The six statistics used for demographic inference; MAE, mean absolute error; rg, raggedness; F<sub>S</sub>, Fu's F<sub>S</sub>; D, Tajima's D; D\*, Fu and Li's D\*; R2, Ramos-Onsins and Rozás's growth statistic. Any values significant at  $\alpha = 0.05$  (two-tailed) are marked with an asterisk (\*).

<sup>b</sup>Bray-Curtis ordination (B-C).

<sup>c</sup>Demographic category placement according to rules in Table 2.

**Table 2.** Summary of the categorical demographic assignment strategy for *Abedus herberti* populations according to results of six statistical tests (Table 1) and resulting inference for each category.

Demographic category	Rule of assignment	Inference
1	monomorphic	recent founder event or extreme bottleneck
2	all measured statistics significantly high	recent strong bottleneck
3	some statistics significantly high	historic bottleneck
4	no significant statistics	long-term demographic stability
5	some statistics significantly low*	long-term growth signature

\*No populations had significantly low values for all calculated statistics.

the remaining four (North Fork Cave Creek [NFCC], Price Canyon, East and West Whitetail) were quantified in June 2007. For comparable values of habitat area between years, we measured one stream (East Turkey Creek) in both years and adjusted all 2007 values downward by the measured proportional difference (15%).

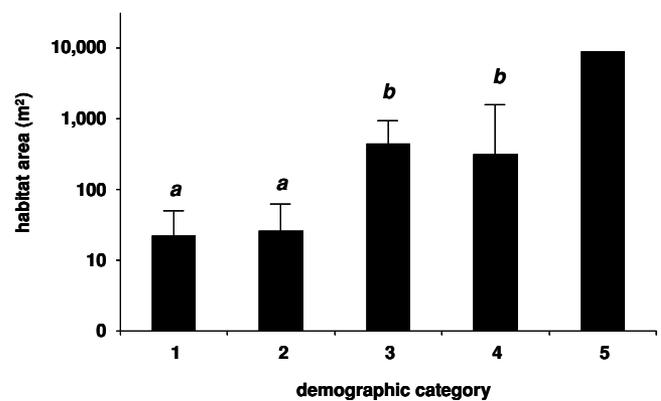
## Results

Statistical results varied widely among populations, which suggests there were a range of local demographic histories (Table 1), and the six statistics tended to be positively correlated (Table 3). Many individual statistics were significant, and in the majority of such cases two-tailed tests revealed significance at the upper (bottleneck) ends of the distributions (Table 1). Significantly, low values occurred in only two populations: Cajon Bonito for results in two different statistical classes (Tajima's  $D$  and Fu's  $F_S$ ) and NFCC, which showed significantly low values of two statistics within class 3 (Tajima's  $D$  and Fu and Li's  $D^*$ ). The value of the remaining class-3 statistic ( $R_2$ ) for NFCC was significantly high, and statistics in classes 1 and 2 trended toward high values. This population was

**Table 3.** Correlations (Pearson's  $r$ ) among individual demographic statistics and the Bray-Curtis (B-C) ordination axis 1 score used as the continuous population demographic score for *Abedus herberti* populations.

	$rg$	$R_2$	$D$	$D^*$	$F_S$	B-C axis 1
MAE	0.54	0.50	0.54	0.40	0.83*	0.77*
$rg$		0.44	0.47	0.44	0.55	0.81*
$R_2$			0.67*	0.26	0.67*	0.71*
$D$				0.79*	0.73*	0.84*
$D^*$					0.51	0.68*
$F_S$						0.89*

\*Significant values of  $r$  at  $\alpha = 0.01$ .



**Figure 2.** Mean (+2 SE) dry-season habitat area for each demographic category (described in Table 2) of the 16 *A. herberti* populations of the Sky Islands. Categories are ranked by increasing demographic stability. For category 5,  $n = 1$ ; therefore, variance could not be calculated and the category could not be included in post hoc tests. Letters above error bars indicate homogenous groups at  $\alpha = 0.05$  according to Tukey's b test after an analysis of variance including only the first four categories ( $df = 3$ ,  $F = 7.03$ ,  $p = 0.007$ ).

the only one to reveal significantly conflicting inferences from two or more of the statistical results.

Assigning populations to demographic categories suggested that the more stable categories occupied larger habitats (Fig. 2). Habitat area ranged from 12 m<sup>2</sup> at French Joe Canyon to 8895 m<sup>2</sup> at Cajon Bonito (Table 1). The ANOVA revealed significant differences in mean habitat area among demographic categories ( $p < 0.002$ ). Means for the monomorphic and significantly bottlenecked categories (1 and 2, respectively) were not different. Similarly, categories 3 and 4 were homogeneous, but both were significantly greater than category 1 and 2 means. Finally, category 5 could not be included in a post hoc test because it contained only one population (Cajon Bonito), but its habitat area (8895 m<sup>2</sup>) was much greater than the second-largest habitat (2270 m<sup>2</sup> at Garden Canyon, category 4) and an order of magnitude greater than the means of categories 3 (572 m<sup>2</sup>) and 4 (758 m<sup>2</sup>).

The B-C ordination of population demographic statistics explained 64% of the total variation along the primary axis. Axis 2 explained an additional 19%. Axis-2 values were primarily associated with variation in the raggedness index, although raggedness was also correlated with axis 1 (Table 3). The high explanatory value of axis 1, compared with axis 2, supports our use of axis-1 values as composite stability scores. These continuously distributed scores are inversely related to inferred stability because higher values of each of the statistics indicate stronger evidence for recent bottleneck. These stability scores were significantly predicted by dry-season habitat

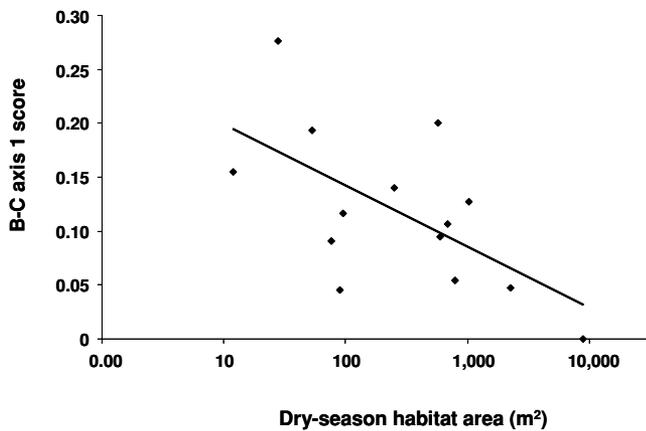


Figure 3. Axis 1 score from Bray–Curtis ordination of the six demographic statistics versus habitat area for the 14 polymorphic populations of *A. herberti* ( $R^2 = 0.38$ ;  $p = 0.02$ ). Axis-1 score is negatively associated with demographic stability.

area, with populations occupying more extensive habitats having lower axis-1 scores ( $R^2 = 0.38$ ,  $p = 0.02$ ; Fig. 3).

Although sample size for statistical inference varied from 13 to 41 individuals per population (Table 1), this variation did not have a detectable influence on individual statistical values or on composite stability scores. Sample size was not correlated with the reciprocal of B-C axis-1 score ( $r = -0.15$ ). Furthermore, the two populations with least and greatest sample sizes fell into the middle stability category (3, Table 1).

## Discussion

Using an ecologically important indicator species of perennial headwater streams, we evaluated a new approach toward classifying isolated populations according to demographic persistence potential. Our results suggested a wide variability in long-term demographic stability among 16 isolated *A. herberti* populations. We also demonstrated a potential mechanistic link between genetically inferred population stability metrics and a surrogate measure of long-term habitat stability. This information is particularly relevant to conservation management. Stable populations or those retaining a signature of past expansion are more likely to persist through future environmental fluctuation, provided their habitats are protected from further anthropogenic disturbance. During rapid global change, isolated populations likely will not have the capacity to respond by shifting their ranges. Arguably then, it is the historically most persistent populations that will have the best chance of allowing persistence of the species as a whole.

Use of a composite demographic metric that combined results of six independent statistical tests provided novel insight. Many individual test results were positively correlated, as expected. Nevertheless, the NFCC population demonstrated that different statistics can yield discordant conclusions about a single population's demographic history. The disagreement among the tests resulted from an odd haplotype distribution and highlights the benefit of combining results of multiple types of statistical test for strongest demographic inference.

We developed both categorical and continuous composite metrics. In both cases there was a significant effect of dry-season habitat size on the demographic score (Figs. 2 & 3). These results suggest that although the score itself ultimately provides the most information about population history and persistence potential, it has a solid link to an easily measured habitat proxy in this system.

Although the continuous-scoring approach revealed a gradual increase in population stability with increasing dry-season habitat size, the categorical approach suggested some potentially important habitat-size thresholds that may prove useful for conservation management. Categories 1 and 2 (monomorphic and severely bottlenecked populations) had similar mean dry-season habitat area, and categories 3 and 4 (slightly bottlenecked and stable) also were similar and had greater habitat area than categories 1 and 2. Category 5 habitat size could not be tested statistically because  $n = 1$  (Cajon Bonito). This population, however, clearly showed the potential for long-term persistence through further environmental change because it had retained a signature of historic growth and occupied a habitat that was an order of magnitude larger than the second-largest habitat in this study. Given these results, it is possible that a binary, stable–unstable answer to the question of long-term demographic stability may be useful for preliminary conservation planning for *A. herberti*. Category 1 and 2 populations were unstable and may be sink-like populations, relying on periodic recolonization from nearby source populations (potentially stable categories 3–5) to compensate for severe bottlenecks or local extinction. Thus, streams that maintain a dry-season habitat area greater than the mean of categories 3–4 may be conservation priorities if the objective is to maintain stable source populations over time. Within this group, it also would be useful to consider the proximity of sink locations because the most likely routes of recolonization following local extinction are via overland, headwater-to-headwater dispersal (Finn et al. 2007).

Cajon Bonito was unique not only as the sole category-5 population but also as the demographic growth end point in the ordination. Although a statistical signal of growth can be interpreted as a period of recovery following a bottleneck (Cheng et al. 2005), we interpret the current result as retention of a growth signal through a lengthy population history and hence high persistence potential. The distinct unimodality of the mismatch distribution

(Fig. 1c) and the relatively slow mutation rate expected for insect mitochondrial COI-COII (2.2% per million years; Gaunt & Miles 2002) suggest a long-term signature of demographic expansion, on the order of tens of thousands of years (see Rogers 1995). A history of this magnitude dates to well before the Pleistocene-Holocene switch to ongoing warmer climatic patterns, significantly predating events of current ecological significance.

Conversely, even within the current 10,000-year interglacial period, there have been several ecologically relevant climatic fluctuations, including the Medieval warm period, when epic droughts occurred in the southwestern United States (Meko et al. 2007). This type of event might have caused the inferred bottlenecks in less stable *A. herberti* populations (i.e., those occupying smaller habitats more prone to failure during exceptional drought years). The populations that have maintained stability through these more recent periods of warmer temperatures and drought thus provide evidence that they could persist through future climatic shifts.

## Conclusions

There is a need for concise decision making about where to invest finite conservation resources to maximize the benefit. Particularly in an era of rapid climate change, the causes of which are well beyond the scope of finer-scale management action, conservation biologists face an urgent imperative to identify landscape units that will maximize overall representation and persistence of genetic diversity (e.g., Moritz 2002; Desmet et al. 2002). Significant progress has already been made in addressing diversity representation (e.g., Faith 1992). Here, we introduced a relatively simple empirical approach to identifying populations that are most likely to continue to weather environmental fluctuations, thereby addressing the issue of diversity persistence. It undoubtedly will be a union of these two general strategies that will provide the most robust approach to assigning meaningful priority to local habitats for overall conservation goals. In the case study system, for example, Finn et al. (2007) demonstrated deep phylogenetic divergences across several populations. A potential synthetic conservation strategy would include prioritizing the most stable populations from each of several major clades in the region.

Future research directions include validation of this approach in other systems and incorporation of multiple types of loci, such that the genome will be well represented and additional statistical tests can be incorporated into the composite stability scores. A potential drawback of the approach is that it will be most powerful in systems of strongly isolated populations, such as the desert headwater streams in the case study. Strong local demographic inference from the suite of statistics discussed here can be made only when migration is minimal com-

pared with the processes of drift and mutation. In many cases, however, it is these systems of highly isolated populations that are of serious conservation concern under rapid global change.

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