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Aquatic insects in a sea of desert: population genetic structure is shaped by limited dispersal in a naturally fragmented landscape

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Habitat requirements and landscape features can exert strong influences on the population structure of organisms. For aquatic organisms in particular, hydrologic requirements can dictate the extent of available habitat, and thus the degree of genetic connectivity among populations. We used a landscape genetics approach to evaluate hypotheses regarding the influence of landscape features on connectivity among populations of the giant water bug *Abedus herberti* (Hemiptera: Belostomatidae). *Abedus herberti* is restricted to naturally-fragmented, perennial stream habitats in arid regions of North America. This species is exceptional because it is flightless at all life stages. Thus, we hypothesized a high degree of population genetic structure in *A. herberti* due to hydrologic constraints on habitat and low dispersal ability of the organism. A total of 617 individuals were sampled from 20 populations across southeastern Arizona, USA and genotyped at 10 microsatellite loci. We used a Bayesian clustering method to delineate genetic groups among populations. To determine which of six landscape variables (representing hypotheses of landscape-level connectivity) has the strongest association with genetic connectivity in *A. herberti*, we used information-theoretic model selection. Strong population structure was evident among *A. herberti* populations, even at small spatial scales. At a larger scale, *A. herberti* populations were hierarchically structured across the study region, with groups of related populations generally occurring in the same mountain range, rather than in the same major watershed. Surprisingly, stream network connectivity was not important for explaining among-population patterns. Only the *Curvature* landscape variable was identified as having an association with genetic connectivity in *A. herberti*. The *Curvature* variable hypothesizes that gene flow tends to occur where local topography is concave, such as within stream drainages and dry gullies. Thus, our results suggest that population connectivity may depend on the shape of local overland topography rather than direct connectivity within stream drainage networks.

Habitat structure can have major ecological and evolutionary consequences for organisms (Robinson et al. 1992, Keyghobadi 2007, Stutchbury 2007). For many species, the movement of individuals between populations is infrequent when large distances separate fragmented habitat patches or when the landscape between patches is hostile. Under such circumstances, local populations may become demographically and genetically isolated from each other over time. Stochastic processes in small, isolated populations can result in losses of genetic diversity and may lead to local extinction (Frankham 2005). Conversely, isolation can facilitate the adaptation of populations to local conditions, depending on the relative rates of among-population gene flow vs local selective pressures (Kawecki and Ebert 2004). For aquatic organisms, the requirement of a specific habitat type, such as flows that are perennial (always present), intermittent (seasonally-present when the water table rises due to rainfall), or ephemeral (present only during flood events), may further influence population dynamics at the landscape level. Furthermore, recent studies suggest that the dendritic branching nature of streams may be important for explaining patterns of genetic connectivity among populations (Fagan 2002, Campbell Grant et al. 2007).

Rates of gene flow among populations arise from the interaction of a species' dispersal ability, the spatial distribution of its habitat, and the resistance to dispersal offered by the landscape between habitat patches. Highly vagile organisms, such as birds or flying insects, may maintain high rates of gene flow despite habitat fragmentation (Bohonak 1999). Genetic differentiation among populations (i.e. population structure) of these organisms tends to be minimal, sometimes at very large spatial scales (Oomen et al. 2011, Ridley et al. 2011). Species with limited dispersal ability, such as amphibians, tend to show strong population structure at multiple scales (Blouin et al. 2010, Milá et al. 2010).

Aquatic insects demonstrate a wide range of dispersal modes and abilities (Bilton et al. 2001). Accordingly, the pattern and scale of population structure vary among them (Hughes et al. 2009). For stream-dwelling taxa, the most common mode of dispersal is adult flight, rather than

the passive drift of flightless larvae (Hughes et al. 2008). Aquatic insects that disperse via flight generally have population structures that show little correlation with the spatial structure of their freshwater habitats and often lack a pattern of genetic isolation by distance (IBD; Freeland et al. 2003, Múrria et al. 2010). Flightless or weak-flying species tend to be more restricted by the spatial arrangement of their habitats (Preziosi and Fairbairn 1992, Finston and Peck 1995, Caterino and Chatzimanolis 2008, McCulloch et al. 2009) and also exhibit IBD (Miller et al. 2002). Thus, the population structures of these insects may bear strong signatures of habitat structure.

The field of landscape genetics has emerged from studies that investigate the influences of landscape features on gene flow (Sork and Waits 2010). Landscape genetics capitalizes on the increasing availability of high resolution spatial data (e.g. GIS data from satellite imagery) and multilocus genetic data. Genetic data are summarized to obtain estimates of functional connectivity between populations – i.e. realized rates of gene flow (Brooks 2003). Landscape features determine structural connectivity by acting as pathways or barriers to gene flow (Brooks 2003). Landscape genetics methods are useful for identifying the landscape features that are most strongly correlated with functional connectivity – i.e. the features that likely provide structural connectivity. This approach provides a powerful alternative to tracking individual movements via mark–recapture or telemetry, particularly when studying dispersal in species for which the latter methods are impractical.

In this study, we used high resolution genetic and landscape data to evaluate multiple hypotheses regarding the influence of landscape features on functional connectivity among populations of a flightless aquatic insect, the giant water bug *Abedus herberti* (Hemiptera: Belostomatidae). *Abedus herberti* is an indicator species of perennial freshwater habitats (Bogan and Lytle 2011). As such, it serves as an excellent model to investigate the population structure and landscape genetics of other aridland freshwater organisms such as fish, amphibians, and other invertebrates. *Abedus herberti* is restricted to perennial streams and stream pools in arid regions of North America, although its ability to breathe air allows it to survive in the terrestrial environment for periods of time. *Abedus herberti* is able to persist in small, isolated populations that evolve in response to local conditions (Lytle et al. 2008, Finn et al. 2009). Because of these factors, a high degree of population structure is expected in this species (Finn et al. 2007). *Abedus herberti* is also able to climb over steep terrain during 'flood escape behavior', when it uses rainfall as a cue to crawl into riparian areas to escape flash floods, which can cause high mortality in populations (Lytle 1999). Based on this biological information, we formulated specific hypotheses concerning how these biological factors might interact with the landscape to generate population genetic patterns.

We addressed two main questions in this study: 1) what is the population structure of *A. herberti* in the study region and how do the species' dispersal ability and habitat requirements influence this structure? To address this question, we used an individual-based Bayesian clustering method to delineate the hierarchical genetic divisions among the populations in our dataset. 2) Which of six landscape variables (related to vegetation, topography, and the distribution of freshwater habitats) has the strongest association with functional connectivity in *A. herberti*? To address this question, we used an information-theoretic model selection procedure to identify the landscape variables that appear to have the greatest influence on connectivity in *A. herberti*.

Methods

Study organism and landscape

The giant water bug *Abedus herberti* is a predatory hemipteran insect that lives in perennial streams and pools in the arid southwest of the U.S. and northern Mexico. *Abedus herberti* requires freshwater habitat year-round, as each of its life stages is completely dependent on water (Smith 1976). Unlike most aquatic insects, *A. herberti* is flightless, and so dispersal in this species is limited to movement within the streams and occasionally over dry land (Lytle 1999). Individuals rarely leave the water, except during heavy rainfall events when they crawl up canyon walls in order to escape floods (Lytle et al. 2008), a behavior that may sometimes result in movement of individuals over headwater divides. A phylogeographic study of this species examining mitochondrial DNA (mtDNA) suggested that population connectivity over headwater divides may be stronger than connectivity of the stream drainage network (Finn et al. 2007).

The freshwater habitat of *A. herberti* is distributed across the Madrean Sky Island region of North America and exhibits a high degree of natural fragmentation. Most streams and pools in this region are restricted to the higher elevations of disjunct mountain ranges, and these mountains are isolated from each another by a 'sea' of lowland desert. During the late Pleistocene epoch (ending 10 000 yr ago), mesic habitats were more widespread in the region and there was presumably greater connectivity among freshwater streams at all elevations (Holmgren et al. 2003).

Field sampling

We collected *A. herberti* on multiple field trips between 2002 and 2011. The time differences among our samples represent at most a few generations of this long-lived (>2 yr) insect. In several localities, samples collected in different years showed no significant genetic differentiation (results not shown). Thus, we assumed that our sampling did not bias our results. We sampled individuals from each of 20 localities (median sample size $= 27$, total of 617 individuals) distributed across four 'Sky Island' mountain ranges in southeastern Arizona: the Santa Rita, Huachuca, Whetstone, and Mule mountains (Fig. 1; Table 1). We also sampled several populations occurring in lowland rivers and cienegas (perennial spring-fed marshes). We collected whole specimens of adult *A. herberti* and preserved them in 95% ethanol.

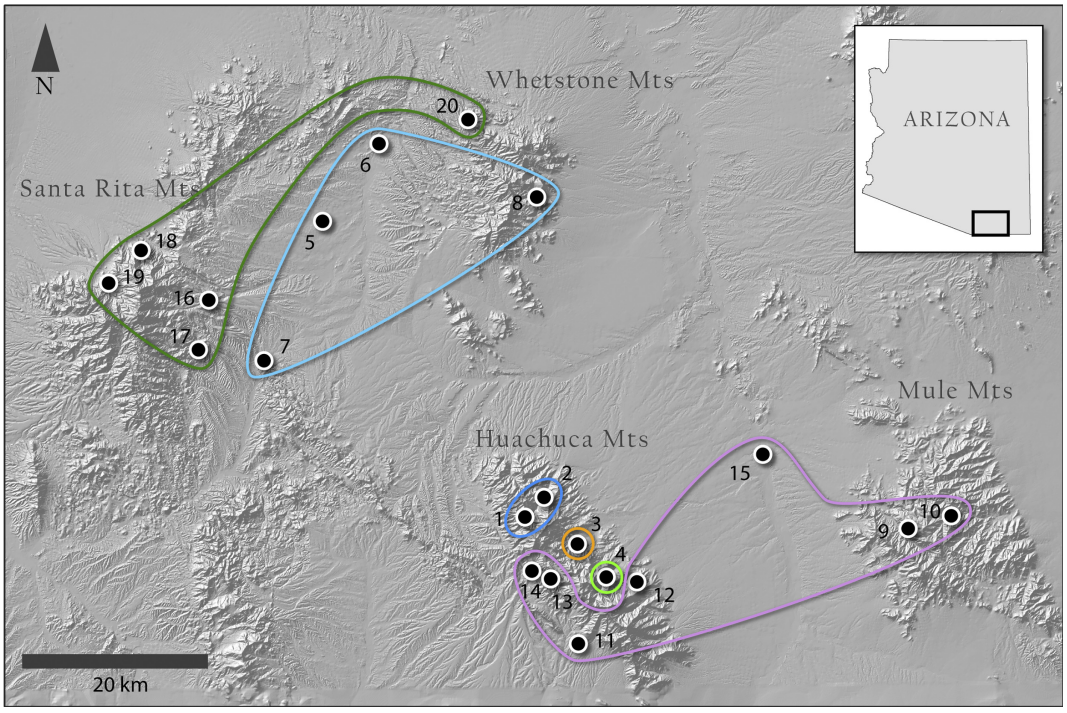

Figure 1. Map of sampling localities for *Abedus herberti* in southeastern Arizona (USA). Inset map shows the extent of the study area. Localities are numbered and represented by black circles. Localities are distributed across four 'Sky Island' mountain ranges (e.g. Santa Rita Mts). Polygons surrounding groups of localities delineate several of the major genetic groups within *A. herberti* that were identified in the STRUCTURE analysis.

DNA extraction and genotyping

From each specimen, we used muscle tissue from the coxal segment of one middle leg as a source of DNA. Whole genomic DNA was extracted from the legs using DNeasy Blood and Tissue Kits (QIAGEN). To generate a multilocus genotype for each individual, we amplified the genomic DNA at 10 microsatellite loci developed for *A. herberti* (Supplementary material Appendix 1; Daly-Engel et al. 2012). These loci were multiplexed for amplification via polymerase chain reaction (PCR) using Multilplex PCR kits (QIAGEN). Reaction conditions were unmodified from those given in the kit instructions. We ran the amplified products on an ABI 3730 sequencer (Applied Biosystems) and genotyped them using the software GENEMAPPER 4.1 (Applied Biosystems).

Table 1. Population sampling localities, sample sizes (n), geographic coordinates (UTM E and UTM N), and population metrics for *Abedus herberti* in southeastern Arizona, USA. Significant F_{IS} values are marked with an asterisk. In some cases, metrics were not calculated for a population due to small sample size.

Population	n	Name	UTM E	UTM N	*AR*	H_o	H_e	F_{IS}
1	13	Upper Huachuca	556091	3485119	3.56	0.52	0.55	0.07
2	31	Lower Huachuca	558150	3487247	4.52	0.57	0.68	0.17*
3	94	Garden	561636	3482226	4.38	0.59	0.68	0.14*
4	63	Ramsey	564805	3478682	4.13	0.52	0.62	0.15*
5	38	Empire	534311	3517018	3.64	0.46	0.62	0.26*
6	25	Mattie	540423	3525326	4.67	0.54	0.71	0.24*
7	20	Sonoita	528021	3501881	3.64	0.48	0.56	0.14
8	18	French Joe	557333	3519583	3.89	0.46	0.64	0.30*
9	24	Chulo	597363	3483941	3.15	0.49	0.55	0.09
10	28	Dixie	602038	3485196	3.96	0.53	0.63	0.16*
11	30	Cave	561920	3471390	3.92	0.50	0.62	0.19*
12	9	Carr	567953	3478122	1.99	0.46	0.32	NA
13	21	Sunnyside	558836	3478428	4.54	0.50	0.68	0.26*
14	31	Scotia	556866	3479306	4.46	0.54	0.66	0.18*
15	14	San Pedro	581608	3491573	4.14	0.46	0.66	0.31*
16	50	Gardner	522072	3508411	4.84	0.57	0.69	0.17*
17	11	Big Casa Blanca	520858	3503064	4.8	0.53	0.72	0.26*
18	45	Florida	514762	3513801	4.23	0.57	0.63	0.10*
19	49	Madera	511320	3510270	4.06	0.52	0.64	0.18*
20	3	Wakefield	549987	3527499	NA	NA	NA	NA

Population genetic data analysis

We used FSTAT 2.93 (Goudet 2001) to test for deviations from Hardy–Weinberg and linkage equilibrium within populations. To test for evidence of null alleles and other causes of deviation from Hardy–Weinberg equilibrium (HWE), we used MICROCHECKER (Van Oosterhout et al. 2004). FSTAT was also used to calculate expected heterozygosity (H_e) and allelic richness (*AR*; rarefied to a sample size of 14) for each population. These measures of genetic diversity were averaged over all loci.

We estimated the effective size (N_e) of each population and tested them for evidence of bottlenecks in order to obtain a more complete understanding of how the interaction of genetic drift and gene flow influences population structure in *A. herberti*. To estimate N_e, we used the sibship assignment method implemented in the program COLONY (Wang 2009). In this method, a sibship assignment analysis is performed first, to estimate the frequencies of full or half siblings in the sample. An estimate of N_e is then calculated from the sibship frequencies. Settings for the analysis in COLONY were as follows: full likelihood method, medium run lengths, medium likelihood precision, random mating, no inbreeding, and assuming polygamy for both sexes. The latter assumption was based on previous behavioral and genetic studies of *A. herberti* and related species that suggested that polygamous mating is common in these insects (Smith 1979, Inada et al. 2011). For comparison, we also estimated N_e using the linkage disequilibrium method in the program LDNe (Waples and Do 2008).

We tested for recent reductions in N_e using the Wilcoxon signed-rank test in the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996, Piry et al. 1999). A population with a significant number of loci exhibiting heterozygosity excess can be identified using this test. Such heterozygosity excess – relative to a stable population with the same allelic diversity – is expected following a recent population bottleneck (Cornuet and Luikart 1996). In the coalescent simulations of BOTTLENECK, we used the two-phase microsatellite mutation model (TPM), with a 0.95 proportion of stepwise mutations (and thus a 0.05 proportion of multistep mutations) and a 12% variance in the number of mutational steps for the multistep phase (the values recommended by Piry et al. 1999).

Although the heterozygosity-based test in BOTTLENECK is suitable for uncovering evidence of recent, moderate population bottlenecks, the *M*-ratio method (Garza and Williamson 2001) is better for detecting bottlenecks that occurred in the more distant past (Williamson-Natesan 2005). Rare alleles are likely to be lost when a population goes through a bottleneck, such that the number of alleles in the population (k) should decline more rapidly than the range in allele size (r; Garza and Williamson 2001). The *M*-ratio ($M = k/r$) of a bottlenecked population tends to be smaller than that of a population at equilibrium (i.e. not bottlenecked). The signature of a bottleneck as measured by *M* can remain in a population for hundreds of generations, making this test appropriate for investigating historical bottlenecks (Garza and Williamson 2001). In practice, *M* is compared to a critical *M* value (M_c), which marks the lower 5% of the *M*-ratio distribution obtained for 10 000 simulated populations at equilibrium. We used the programs M_P_Val and Critical_M to calculate *M* and M_c, respectively (Garza and Williamson 2001). Critical_M requires four input parameters that define the TPM model to be simulated. We kept the mutation rate (μ) at the recommended value of 5.0×10^{-4} and the average size of mutations greater than one repeat (Δ_g) at 3.5 (also recommended by Garza and Williamson 2001). We estimated M_c separately using two values for the proportion of two-step mutations ($p_g = 0.1$ and 0.3) and for two estimates of pre-bottleneck genetic diversity ($\theta = 0.4$ and 2, based on $N_e = 200$ and 1000, respectively).

Exact tests in FSTAT were used to test the significance of pairwise genetic differentiation between populations. Population 20 was excluded from pairwise comparisons (including landscape genetics analysis; see below) due to its small sample size ($n = 3$; other populations ranged in size from 9 to 94 individuals). We used a Mantel test (Mantel 1967) with 10 000 permutations in the software GenAlEx (Peakall and Smouse 2006) to test for an IBD relationship among the populations. IBD is a signature of a stepping-stone pattern of population structure, where gene flow is highest between neighboring populations and decreases as the geographic distance between populations increases (Wright 1943). The Mantel test was performed on log-transformed genetic (*cGD*, see below) and topographically-adjusted geographic distances.

As a measure of genetic distance between pairs of populations, we used conditional genetic distance (*cGD*), derived from a population graph (Dyer et al. 2010). A population graph is a network (with nodes linked by edges) that models the covariance structure of the genetic data (Dyer and Nason 2004). A pair of populations connected by gene flow will be linked in the graph by an edge. Conversely, pairs of populations that are isolated from each other by a lack of gene flow will not be linked in the graph. The length of an edge between two populations in the graph is inversely proportional to the genetic covariance between the populations. The more edges that link directly to a population in a graph, the more genetically well-connected that population is inferred to be. The *cGD* between a pair of populations is the shortest path that connects them through the population graph. Dyer et al. (2010) demonstrated that *cGD* may outperform traditional measures of genetic distance, such as F_{ST}, for describing spatial genetic structure. For detailed explanations of graph construction and theory, see Dyer and Nason (2004) and Dyer et al. (2010). We used the software GeneticStudio (Dyer 2009) to construct the population graph and to derive *cGD* for all pairs of populations.

We used the program STRUCTURE (Pritchard et al. 2000) to determine the pattern of hierarchical population genetic structure among the *A. herberti* individuals sampled across the study region. STRUCTURE employs a Bayesian clustering algorithm to sort individual genotypes into groups that best conform to Hardy–Weinberg and linkage equilibrium. For each value of *K* (the hypothesized number of distinct genetic groups in the dataset) from 1–21,

we performed 10 independent runs of STRUCTURE under the correlated allele frequencies model allowing admixture. Each run had 2×10^5 iterations with a burn-in of 1×10^5 iterations. For each value of K, we calculated the mean and standard deviation of ln Pr(X|K) (the estimated likelihood of K) across the 10 runs. We used these values to apply the ΔK method of Evanno et al. (2005) to identify the most likely number of genetic groups. Our first STRUCTURE analysis used the entire dataset of 617 individuals. This allowed us to identify the top-most level of hierarchical population structure in the dataset. We then carried out separate analyses on the major groups identified by the first analysis (for values of K from 1 to 10), applying the method of Evanno et al. (2005) in each analysis. We iterated this process until all subgroups were partitioned, such that at the finest hierarchical level, each distinct genetic group included only one or two sampling localities.

To describe patterns of recent gene flow (over the last several generations), we used BayesAss ver. 3.0.1 (Wilson and Rannala 2003). This program uses a Bayesian Markov Chain Monte Carlo (MCMC) approach that relies on multilocus gametic disequilibrium information to estimate asymmetrical migration rates (and their respective 95% credible sets) between population pairs. The method does not assume that populations are in Hardy–Weinberg equilibrium. We ran BayesAss for 5 million iterations, discarding the first 2 million as burn-in, and sampled the MCMC every 2000 iterations.

Landscape data

We acquired landscape data layers from the Arizona State Land Dept (<www.land.state.az.us>) and used ArcGIS 9.3 (Environmental Systems Research Inst.) to catalog and manipulate the data. We used a digital elevation model (10 m resolution) to calculate topographically-adjusted Euclidean distances between all pairs of populations.

From the GIS data, we generated pixelated maps (i.e. rasters) for each of six variables that we hypothesized to have an influence on gene flow in *A. herberti*. See Table 2 and below for explanations of these variables and their hypothesized relationships with gene flow. Each map was used as input for the program CIRCUITSCAPE (McRae 2006), which uses circuit theory to model gene flow among populations in a given landscape. CIRCUITSCAPE calculates the resistance of the landscape to gene flow between each pair of populations (analogous to electrical resistance in a circuit diagram), allowing for multiple pathways between the populations. This pairwise resistance is a summation of the resistances of individual pixels in the input map; pixels with high values are hypothesized to offer high resistance to movement, and vice versa. Thus, pairwise resistances from CIRCUITSCAPE model the structural connectivity of populations, based on the landscape/habitat feature represented by the input map.

We used the raw values of the map pixels to assign resistance values to our maps. Although the ways in which

Table 2. Details of the six landscape variables included in multiple regression models. Importance values were calculated from model weights (w_i) as described in the methods.

Variable name	Importance	Hypothesized relationship to gene flow in *Abedus herberti*	Explanation
Canopy cover	0.11	Dense canopy cover from trees and shrubs provides a relatively cool and moist microenvironment that increases the chance of survival for dispersers.	Pairwise resistances between populations based on low resistance of map pixels with high percent cover and high resistance of pixels with low percent cover.
Curvature	1.0	Dispersal is highest in areas with strongly concave topography. These areas tend to be canyons, gullies, and saddle points between drainages. They are relatively cool and moist and are the places where water tends to flow. Bugs exhibiting flood escape behavior are likely to follow these routes into adjacent canyons. Dispersal is lowest across areas with strongly convex topography. Ridgelines that separate drainages tend to have convex topography.	Pairwise resistances between populations based on low resistance of map pixels with concave topography and high resistance of pixels with convex topography.
Elevation	0.39	Flood escape behavior causes water bugs to climb up the steepest canyon walls during heavy rainfall events. Dispersal occurs when bugs continue to climb and eventually end up in an adjacent stream, where they subsequently reproduce.	Pairwise resistances between populations based on low resistance of map pixels at higher elevations and high resistance of pixels at lower elevations.
Perennial habitats	0.16	Perennial freshwater habitats, which exist as fragmented patches in the study region, act as stepping stones for dispersal among populations.	Pairwise resistances between populations based on low resistance of map pixels in patches of perennial freshwater habitats and high resistance of pixels in the matrix between these patches.
Stream-resistance	0.23	Dispersal is easiest within the stream/river network, but can also occur over land. However, resistance to dispersal is relatively high over land due to decreased chance of survival for dispersers.	Pairwise resistances between populations based on low resistance of map pixels in the stream/river network and high resistance of pixels out of the network.
Stream-strict	0.16	Dispersal occurs only within the stream/river network. *Abedus herberti* is aquatic and requires freshwater habitat for all stages of its life cycle. *Abedus herberti* is flightless and rarely leaves the stream habitat.	Pairwise least-cost paths between populations that strictly follow the stream/river network. Only one path exists between any pair of populations.

dispersing animals perceive the landscape most certainly do not correspond to the pixel values of the maps, using the raw pixel values should be more conservative than arbitrarily assigning relative costs of landscape features based on expert opinion (a practice that some have questioned; Spear et al. 2010). We transformed the raw values of the maps so that they were all on the same scale (1 for lowest resistance, 10 000 for highest resistance; results were qualitatively similar for different values of highest resistance) before using them in CIRCUITSCAPE. We performed a separate CIRCUITSCAPE analysis for five of our six variables, generating independent data sets of all pairwise resistance distances as output. See Fig. 2 for an example of CIRCUITSCAPE output and how this relates to the underlying landscape variable. The sixth variable, *Stream-strict* (Table 2; Fig. 3), was generated via a least-cost path analysis in ArcGIS, rather than using CIRCUITSCAPE. Thus, only one pathway connects each pair of populations for the *Stream-strict* variable, and this pathway is restricted to the stream network. Data for all six variables were used as independent variables in multiple regression models (see Landscape genetics analysis section). We did not include Euclidean distance as a separate variable in our models because it is already accounted for in each of the other variables because resistance in CIRCUITSCAPE always increases as Euclidean distance increases.

Landscape genetics analysis

We evaluated a set of landscape genetics hypotheses, each represented by a statistical model that includes a subset of the six landscape variables (Table 2). The *Canopy cover* variable represents the hypothesis that dispersal should occur through forested areas; *Curvature* hypothesizes that dispersal occurs along concave corridors such as stream beds, dry gullies, or low saddle points along mountain ridges; *Elevation* suggests dispersal occurs over steep terrain; *Perennial* suggests that networks of perennial habitats serve as dispersal stepping stones; *Stream-resistance* and *Stream-strict* both hypothesize that drainage network geometry is important for dispersal, with the former allowing for multiple dispersal pathways between population pairs and the latter allowing only a single 'least cost' pathway. Figure 3 depicts hypothetical gene flow scenarios for each of the landscape variables. The paths of gene flow in each scenario are determined by the hypothesized resistance to dispersal associated with the given landscape variable.

For this analysis, we used an information-theoretic approach (Burnham and Anderson 2002) to select the model(s) that were most supported by our data. Model selection using the information-theoretic approach is an alternative to traditional null hypothesis testing that has gained widespread use in the ecological sciences (Hobbs and Hilborn 2006, Grueber et al. 2011) and has been applied recently in the field of landscape genetics (Pavlacky Jr et al. 2009, Wang 2009, Murphy et al. 2010, Garroway et al. 2011).

There were 47 candidate models in the set, representing all combinations of the six predictor variables, but excluding

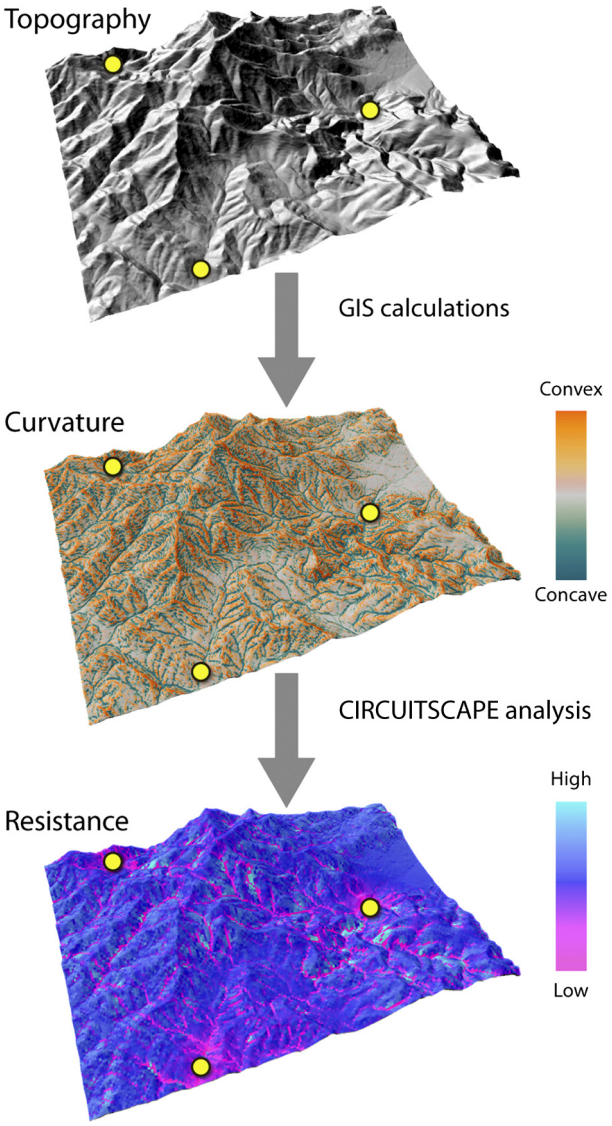

Figure 2. Example of the relationship between an underlying landscape variable and resistance (as calculated using CIRCUITSCAPE). A digital elevation model in the GIS environment defines topography (top of the figure). Three populations located in this hypothetical landscape are shown as yellow circles. The curvature of the landscape (concave or convex) is calculated using the GIS software. Convex areas of the landscape are shown in orange while concave areas are shown in blue. Ridgelines tend to be convex; stream drainages and gullies tend to be concave. A map of curvature is used as input to the CIRCUITSCAPE program. CIRCUITSCAPE applies circuit theory to calculate the landscape resistance between each pair of populations. Multiple pathways of low resistance can exist between any pair of populations. At the bottom of the figure, these pathways are shown in pink.

models that included both the *Stream-strict* and *Stream-resistance* variables, as we see these as representing mutually exclusive hypotheses about the paths of gene flow between *A. herberti* populations. The response variable in these models was *cGD*. We fit each multiple linear regression models in R (R Development Core Team) then derived the log-likelihood for each model. From the log-likelihoods, we calculated Akaike's information criterion (AIC; Akaike 1973, Burnham and Anderson 2002), and ranked the

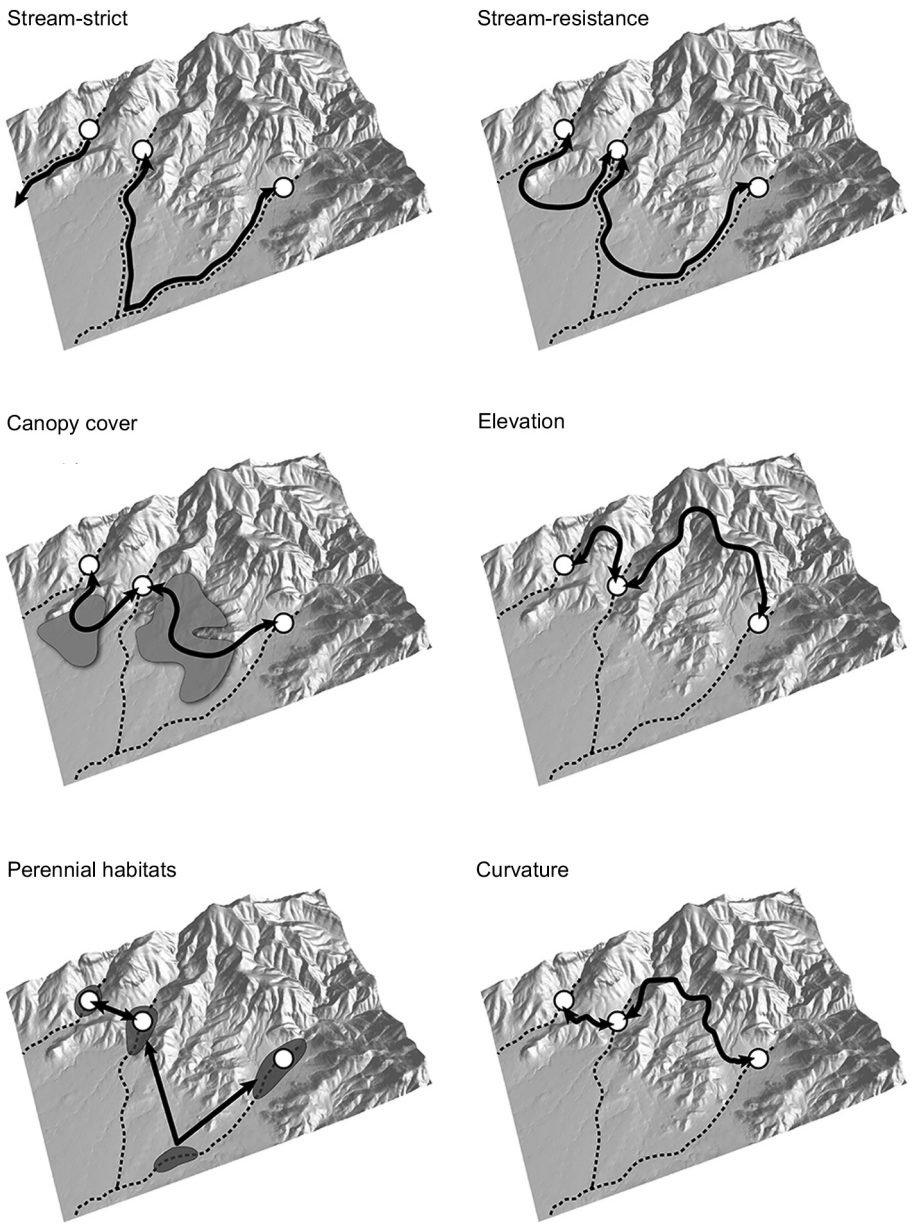

Figure 3. Hypothetical scenarios of gene flow based on each of the six landscape variables. In each scenario, the locations of three hypothetical populations are shown as white circles in a generic mountain landscape. Streams are depicted by dotted lines and thick black lines with arrowheads represent bi-directional gene flow between pairs of populations. The paths of gene flow in each scenario are determined by the hypothesized resistance to dispersal associated with the given landscape variable. In the *Canopy cover* scenario, for example, the underlying hypothesis is that dispersal is easiest where canopy cover is densest. Thus, gene flow is expected to be highest through areas with dense canopy cover (shown as grey polygons in the figure). See the Table 2 for more detail on each of the landscape variables.

models from lowest to highest AIC. Once the models were ranked, additional information-theoretic metrics could be calculated. The difference between the AIC of a particular model and the AIC of the estimated best-fitting model (i.e. the model with the lowest AIC) is ΔAIC. Models with $\Delta AIC > 2$ are generally treated as having very little support (Burnham and Anderson 2002), so we discarded such models before conducting subsequent analyses.

For each of the remaining models, we calculated an Akaike weight (w_i), which is the probability that the model is actually the best-fitting of the candidate models (Burnham and Anderson 2002). The sum of Akaike weights across the models is 1.0. When the weight of the model with the lowest AIC is not close to 1.0, there is evidence of model selection uncertainty. It is prudent to make inferences using the entire set of candidate models when model selection uncertainty is evident. In this study we use multimodel inference to calculate the importance of each landscape variable represented among the final set of models. The importance of a variable is the sum of w_i across all the models that contain the variable.

Note that we did not seek to estimate parameter values for the regression models – our goal was simply to identify which of the six landscape variables are associated

with functional connectivity in *A. herberti*. In other words, we did not develop predictive models. This allowed us to minimize issues of multicollinearity (Supplementary material Appendix 2) and the non-independence of our pairwise distance data. The multiple regression approach we used allowed us to fit each model and calculate AIC. This step in the procedure is the same as in multiple regression using distance matrices (MRDM), a statistical approach that can be used to estimate parameters in models based on pairwise data (Legendre et al. 1994).

Results

Basic genetic characteristics

Genetic diversity within populations of *A. herberti* was low to moderate: H_e ranged from 0.32 to 0.72 and *AR* ranged from 1.99 to 4.84 (Table 1). No pairs of loci exhibited consistent linkage disequilibrium in multiple populations and there were no loci that deviated from HWE in more than a few populations. Thus, we retained all 10 loci in our analyses. Most of the populations were out of HWE in multilocus tests (all but populations 1, 7 and 9; 12 and 20 were not tested due to low sample sizes), with significantly positive F_{IS} values (Table 1). However, these deviations appeared to be driven by only one or two loci in each population. In none of the populations did every locus have a positive F_{IS}. This suggests that the cause of HWE deviations is technical (e.g. due to null alleles), rather than biological (e.g. inbreeding or Wahlund effect). Indeed, results from MICROCHECKER indicated evidence of null alleles at 6 loci (A10, A114, B9, A103, D6, and A106). We used the program FreeNA (Chapuis and Estoup 2007) to estimate null allele frequencies and to generate corrected F_{ST} estimates. Corrected and uncorrected F_{ST} values were very similar (results not shown), suggesting that the effects any null alleles on our results should be minimal. FreeNA does not generate corrected *cGD* values, and the effect of null alleles on this distance metric has not been evaluated to our knowledge. There is no assumption of HWE or any mutation model underlying *cGD*, so deviations from HWE may be less problematic for *cGD* than for F_{ST}.

Effective population size estimates from the sibship assignment method in COLONY ($N_{e(SA)}$) were all less than 70 (median = 28; Supplementary material Appendix 3). Linkage disequilibrium estimates ($N_{e(LD)}$) were roughly similar to $N_{e(SA)}$, but more variable (Supplementary material Appendix 3). The Wilcoxon sign-rank tests in BOTTLENECK were all non-significant (Supplementary material Appendix 3). However, the *M*-ratio method detected bottleneck signatures in all of the populations when the simulation parameters were $\theta = 0.4$ or 2.0 and $p_g = 0.1$. Under the most conservative parameter combination ($\theta = 2$, $p_g = 0.3$), nine populations (1–4, 8, 13–16) still showed evidence of bottlenecks. The *M*-ratio method is more sensitive to signatures of historical bottlenecks and is robust to violations of its underlying assumptions, whereas the Wilcoxon sign-rank test of BOTTLENECK is less sensitive in general and better suited for detecting recent bottlenecks (Garza and Williamson 2001, Spear et al. 2006, Shama et al. 2011).

Population genetic structure

All exact tests of pairwise differentiation between populations were significant after applying a Bonferroni correction for

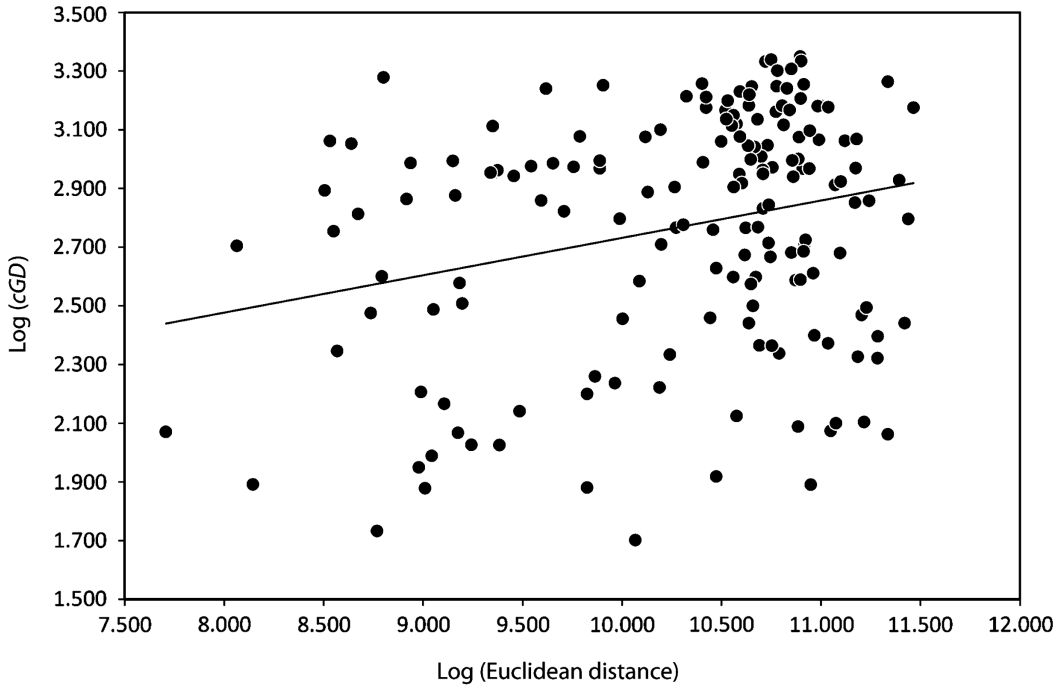

Figure 4. Plot of pairwise, log-transformed conditional genetic distance (*cGD*) vs pairwise, log-transformed Euclidean distance. Distances were calculated between each pair of *A. herberti* populations. The two distance measures are significantly correlated (Mantel $r = 0.26$, $p = 0.004$), suggesting a population structure pattern of isolation-by-distance (IBD).

multiple comparisons. Even populations separated by a Euclidean distance of only a few kilometers were significantly differentiated. A significant Mantel test of the correlation between *cGD* and Euclidean distance revealed evidence of weak IBD (Fig. 4; Mantel $r = 0.26$, $p = 0.004$). There was a high degree of scatter in the IBD relationship. IBD was most apparent when the geographic distance used was pairwise Euclidean distance; the relationship was weaker when the geographic distances were measured only along streams connected in the stream network (Mantel $r = 0.16$, $p = 0.047$).

Pairwise conditional genetic distances (*cGD*) derived from the population graph (Fig. 5) ranged from 5.49 to 28.50 (Supplementary material Appendix 4). Pairwise F_{ST} ranged from 0.03 to 0.37 (Supplementary material Appendix 4). The global F_{ST} was 0.12 ($p < 0.0001$). Interestingly, *cGD* and F_{ST} were not correlated (Mantel $r = -0.023$, $p = 0.407$) and F_{ST} was not correlated with Euclidean distance (Mantel $r = 0.026$, $p = 0.403$). These results highlight the utility of *cGD* as a potentially more powerful measure of genetic differentiation between populations (Dyer et al. 2010). The improved sensitivity of *cGD* for detecting spatial genetic patterns likely results from the conditioning of this distance measure on the genetic covariance of the full set of populations, rather than on just two isolated populations at a time. Also, *cGD* for a set of populations may approach migration-drift equilibrium at a faster rate than F_{ST}, as shown by Dyer et al. (2010).

When data from all 617 individuals was analyzed using STRUCTURE, the number of genetic groups with the highest support was two ($K = 2$; Fig. 6). One of these groups consisted of four adjacent sampling localities on the east side of the Huachuca Mountains (populations 1–4). Most of the remaining populations clearly belonged to the second group. However, several of the sampling localities

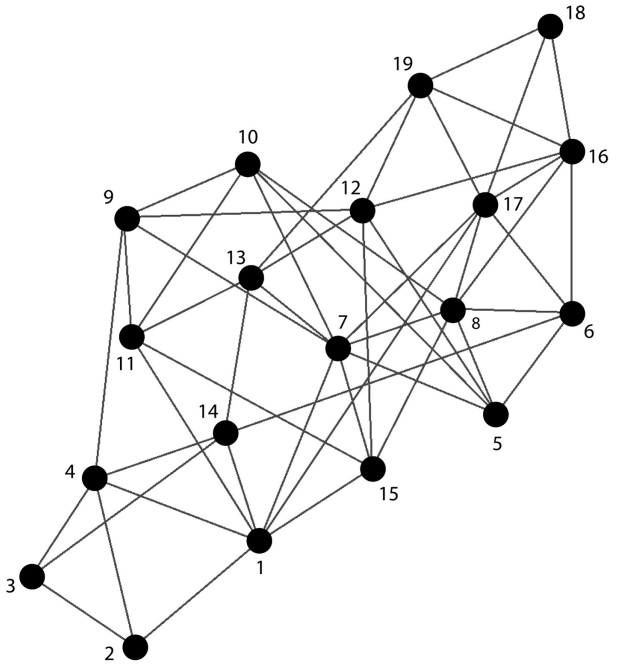

Figure 5. The population graph constructed with GeneticStudio. The graph had 50 edges connecting the 19 population nodes (population 20 not included due to small sample size). A saturated population graph for this number of populations, with none of the non-significant edges removed, would have 171 edges (all pairwise connections). The arrangement of population nodes within the graph generally reflects the geographic locations of the sampled populations.

(2, 13, 14, 18, and 20) included significant proportions of individuals representing both major genetic groups. For example, individuals from locality 18 were evenly divided between the two groups. The result for this locality is

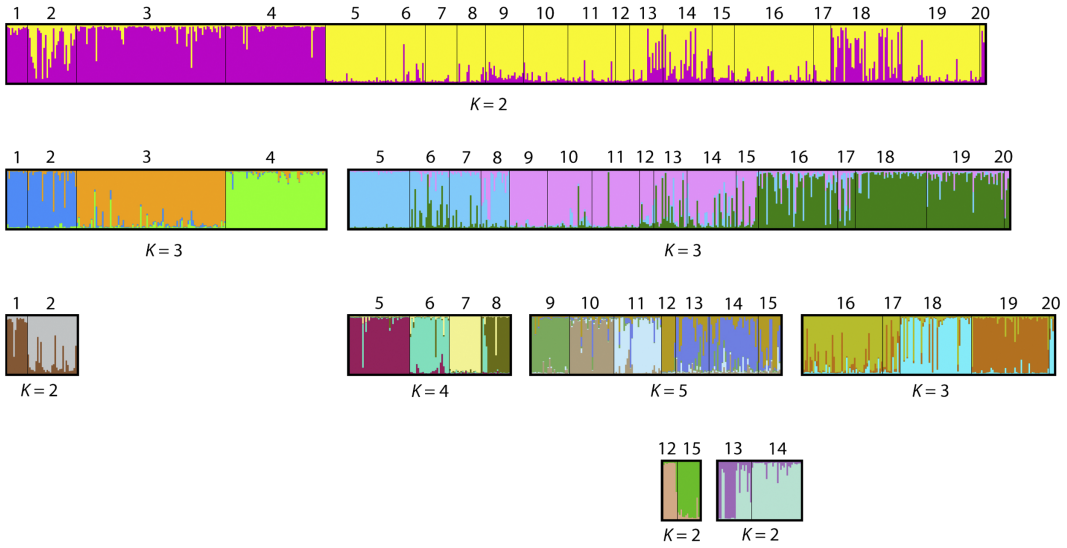

Figure 6. Plots of population assignment of *Abedus herberti* individuals based on the hierarchical STRUCTURE analysis applying the method of Evanno et al. (2005). Each sampling locality is designated by its number code (Fig. 1; Table 1). Results for the analysis using the entire dataset (617 individuals from 20 sampling localities) are shown in the top bar. At this topmost level of hierarchical population structure, two major genetic groups were identified (i.e. $K = 2$ had the most support). The two groups are shown in different shades of grey (colour online). Further analyses were run on each of these two groups and their subgroups. At the finest levels of structure, each sampling locality was identified as representing a single population (with the exceptions of population pairs 12/15, and 18/20).

difficult to interpret, given the large geographic distance between locality 18 and the localities consisting mostly of group 1 individuals.

Subsequent, independent analyses of these two major groups revealed further hierarchical structure. Most of the groups identified at intermediate levels of structure consisted of individuals from neighboring localities, although a few groups included geographically distant localities. For example, population 20 is ~40 km from the other populations in its group (at the second level of hierarchical structure). At the finest level of structure, most individual sampling localities were identified as single populations.

Recent migration rates estimated using BayesAss differed from zero (i.e. the 95% credible set did not include zero) in only seven out of 171 population pairs (Supplementary material Appendix 5). All of these non-zero rates were < 0.20 and represent asymmetrical gene flow between neighboring populations (1 and 2, 14 and 13) as well as more distantly-separated populations (12, 14, and 15; 6 and 16; 8 and 10).

Landscape genetics

Out of 47 candidate models, only nine had ΔAIC less than the cutoff value of two (Supplementary material Appendix 6). The models in this reduced set included from one to four landscape variables. All of the six variables are represented among the final models, but only *Curvature* was present in all of the models. The model with the lowest AIC, and thus the candidate model with the highest likelihood of being the best approximating model, included only the *Curvature* variable. However, the distribution of Akaike weights (w_i) among the final nine models indicated that no single model was clearly the best. To account for this model selection uncertainty, we calculated importance values for each of the six variables (Table 2). *Curvature* had the highest importance value, 1.0. The second highest importance value was 0.38 for *Elevation*. There is a significant correlation between *Curvature* and *Elevation* (Mantel $r = 0.78$, $p = 0.001$). As a post hoc analysis, we performed a partial Mantel test (Smouse et al. 1986) between pairwise *Curvature* resistances and *cGD*, controlling for Euclidean distance. The test result was very close to statistical significance (Mantel $r = 0.18$, $p = 0.056$), suggesting that *Curvature* has an important influence on gene flow that does not depend on Euclidean distance. Similar partial Mantel tests on the other landscape variables were all non-significant (p ranged from 0.186 to 0.87). Note, however, that the partial Mantel test can have inflated type I error with sample sizes less than ~50 (Legendre and Fortin 2010).

Discussion

Population structure

Strong population structure is clearly evident among the *Abedus herberti* populations in our study. Most of the populations are genetically distinct, even at small spatial scales, and we found little evidence of recent gene flow among them. For example, populations 1 and 2 are both within the same stream drainage and are only 3.2 km apart, yet they show significant genetic differentiation. These populations exist in two distinct perennial reaches that are separated by an intermittent reach that is dry for several months a year (Jaeger and Olden 2011). Interestingly, this population pair was also one of the few that showed evidence of recent gene flow (Supplementary material Appendix 5), with downstream migration occurring from population 1 to 2.

The distinctness of populations explains some of the results of Lytle et al. (2008), which found evolved differences in flood escape behavior among populations of *A. herberti*, attributable to differences in flood regime among canyons. In common garden behavioral experiments, populations from mid-sized canyons (strong correlation between rainfall events and subsequent flash floods) were quick to exit the stream, responding after approximately 20 min of heavy rainfall. Conversely, populations from small canyons (few floods) or large canyons (floods not predictable from rainfall cues) required much longer periods of rainfall to trigger flood escape behavior, or did not respond at all. Such fine-scale adaptation to the local environment is only attainable when gene flow is minimal (or when selection is very strong), as is documented here.

At the highest level of hierarchical population structure, populations 1–4 in the Huachuca Mountains formed a genetic group to the exclusion of all other populations. While we were not surprised that these four populations are genetically similar, it is unclear why they should be so distinct from the other populations in our study. Ramsey and Garden canyons (which harbor populations 3 and 4, respectively) contain the largest patches of perennial stream habitat in the study region. Our field surveys indicate that the *A. herberti* populations in these streams are correspondingly large. A possible explanation for the genetic distinctness of populations 1–4 is that because they occupy large, stable habitat patches, they experience fewer of the bottlenecks that result from climatic fluctuations and flash flooding (Finn et al. 2009). Another possibility is that these populations have been able to maintain genetic diversity and cohesiveness as a relatively large, interconnected group (i.e. metapopulation), while other populations in the region have diverged randomly due to genetic drift. However, populations 1–4 did show evidence of historical bottlenecks in the *M*-ratio tests, suggesting that they may not have stable across the time scale of hundreds of generations.

Weak IBD relationships with a high degree of scatter, as we found in *A. herberti*, may be characteristic of insects with limited dispersal (Peterson and Denno 1998). Populations of the stream-dwelling, flightless waterstrider *Aquarius remigis* show a high degree of genetic differentiation at small spatial scales and a lack of IBD – patterns attributed to very low gene flow combined with the effects of genetic drift and bottlenecks (Preziosi and Fairbairn 1992). A similar scenario, in which populations are not at migration-drift equilibrium due to strong genetic drift (Hutchison and Templeton 1999), is likely for *A. herberti*.

The evidence of IBD in our data and the similarity of populations that exist in the same mountain range corroborate the findings of Finn et al. (2007), who used a mtDNA marker to show that *A. herberti* population structure conforms to the 'Headwater Model.' This model predicts that gene flow occurs across low mountain divides separating

neighboring headwater populations, rather than within the dry washes that form drainage networks. Thus, what little gene flow there is among populations of *A. herberti* appears to occur over land. This may seem counterintuitive in light of this species' need for water. However, gene flow through the drainage network may be less likely because these drainages are intermittent or ephemeral except during catastrophic floods and are thus unlikely to provide suitable movement corridors for *A. herberti* at larger distances. Furthermore, mortality from flooding is exceptionally high for aquatic insects inhabiting these streams (Lytle 2000), so it is unlikely that individuals would be able to survive being washed downstream in order to crawl back up to an adjacent tributary. Thus, short overland pathways between perennial headwater streams may offer the best compromise between distance and landscape resistance to dispersal.

Landscape genetics

Of the six landscape variables included in our set of competing multiple regression models, only *Curvature* was identified as having an association with functional connectivity in *A. herberti*. Although *Curvature* was positively correlated with Euclidean distance, *Curvature* still appeared to be correlated with genetic distance after Euclidean distance was accounted for (using a partial Mantel test). The *Curvature* variable reflects the structural connectivity of populations provided by areas with concave local topography. Such areas include stream drainages and dry gullies – with the latter far outnumbering the former in this study region – as well as low saddle points located at drainage divides separating adjacent canyons. Compared to areas with convex topography, concave areas are relatively moist, shady, and cool. These conditions should favor the survival of dispersing individuals that are traveling overland in an arid environment. Between any pair of populations, there are multiple pathways that follow gullies and drainages.

Flood escape behavior is another biological characteristic of *A. herberti* that the *Curvature* variable may account for, at least partially. These insects leave the water and climb up canyon walls when there is heavy rainfall, and typically choose the steepest route away from the active stream channel. Although most individuals of this flightless species return to the same stream within 24 h post-flood, it is conceivable that flood escape behavior could result in gene flow if individuals sometimes crawl into adjacent drainages, rather than back to where they originated. If *A. herberti* that are escaping floods tend to follow concave areas such as gullies, the *Curvature* variable may capture some of the effect that this behavior has on gene flow in this species. The *Elevation* variable was also included in our regression models as an attempt to model the effects of flood escape behavior on gene flow. This variable had the second highest importance value based on multimodel inference. However, *Elevation* was not significantly correlated with genetic distance after the influence of Euclidean distance was removed using a partial Mantel test.

The distribution of *A. herberti*'s perennial freshwater habitat is likely to change significantly in the future. Climate models for the 21st century predict that rainfall will decrease and temperatures will rise in the southwestern United States (Seager et al. 2007). Populations would become even more fragmented under such conditions and local extinctions would be more likely. In fact, population 8 in the present study – from French Joe Canyon in the Whetstone Mountains – went extinct in 2005 after a prolonged drought (1999–2005) resulted in the complete drying of the stream (Bogan and Lytle 2011). The stream now flows only intermittently, and *A. herberti* has not recolonized it. The Wilcoxon sign-rank bottleneck test came closer to statistical significance for this now extinct population than for any other population (Supplementary material Appendix 3).

While there is strong evidence that restricted dispersal in *A. herberti* has resulted in substantial population structure, our conclusions regarding the association of landscape curvature and genetic (i.e. functional) connectivity in this species must be more tentative. Because the *Curvature* and *Elevation* variables are correlated, it is difficult to disentangle their effects. And while *Curvature* was clearly the most important of the six variables in our models, even the best-fitting model (lowest AIC score) did not explain very much of the variance in genetic distance ($R^2 = 0.062$). Landscape genetic analyses are often confounded by noisy data (Storfer et al. 2006). One possible source of noise in our data is high genetic variance among populations due to strong genetic drift. Small populations that suffer repeated bottlenecks (due to flash floods or climatic fluctuations) are likely to become differentiated through drift. The resulting differences among populations should have a mostly random spatial pattern if gene flow is very low, as our data suggest for *A. herberti*. Our data also indicate that population bottlenecks may have occurred in the last several hundred generations. In this situation, the noise of genetic drift may swamp out the signal from gene flow and landscape genetics analyses may have reduced power to detect relationships between landscape features and gene flow. This is likely a common problem in landscape genetics studies of species with small populations and/or populations subject to bottlenecks. One possible method of accounting for the confounding influences of historical demographic events in landscape genetics was presented by Dyer et al. (2010).

Two other caveats regarding our results need to be added. First, deviations from HWE (homozygote excess) were common among our samples and may have biased our results (Selkoe and Toonen 2006). Deviations were not consistent across loci or populations, suggesting that null alleles may be the underlying cause. The presence of null alleles can lead to the overestimation of F_{ST} (Chapuis and Estoup 2007). However, we expect that any bias in the genetic distances introduced by null alleles is negligible, for reasons stated in the results section. In the presence of null alleles, STRUCTURE may cluster individuals into more than the true number of populations (Falush et al. 2003). Nevertheless, nearly every one of our sampling sites was identified in our hierarchical STRUCTURE analysis as representing a single genetic cluster (at the finest level of the hierarchy), and no 'extra' populations were identified. The second caveat is related to the use of pairwise distances in an AIC-based model-selection framework. We caution

that the validity of this approach has yet to be evaluated. The primary issue is sample size: in our dataset, there are 171 pairwise distances for the 19 populations. It has not been established how best to adjust for the inflation of sample size that occurs when non-independent, pairwise measures are used.

The results of the present study allow us to reduce the number of hypotheses to consider in future research on *A. herberti*. For example, our results combined with those of Finn et al. (2007) give us reason to rule out the possibility that gene flow in *A. herberti* occurs primarily within the dendritic stream network, a result that runs contrary to the pattern predicted for many other aquatic organisms (Fagan 2002, Campbell Grant et al. 2007).

Most aquatic insects are capable of flight – and thus aerial dispersal – as adults. Insects with aerial dispersal tend to have high gene flow among populations and weak population structure as a consequence, sometimes at large spatial scales (Freeland et al. 2003, Ridley et al. 2011). *Abedus herberti* is at the opposite extreme of the gene flow spectrum because it is flightless at all life stages. Flightless species show greater genetic differentiation among populations than their winged counterparts (Preziosi and Fairbairn 1992, Finston and Peck 1995). Thus, we predict that population structure in *A. herberti* will be strong compared to that of co-occurring insects with aerial dispersal. Future comparisons among the population structures and landscape genetics of species with different modes of dispersal and life histories will shed more light on the interaction of dispersal ability and landscape resistance in freshwater invertebrates.

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Supplementary material (Appendix ECOG-00002 at <www.oikosoffice.lu.se/appendix>). Appendix 1–6.

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