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Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (*Ambystoma californiense*)

IAN J. WANG, WESLEY K. SAVAGE† and H. BRADLEY SHAFFER
Department of Evolution and Ecology and Center for Population Biology, 1 Shields Avenue, University of California, Davis, CA 95616, USA

Abstract

A major goal of landscape genetics is to understand how landscapes structure genetic variation in natural populations. However, landscape genetics still lacks a framework for quantifying the effects of landscape features, such as habitat type, on realized gene flow. Here, we present a methodology for identifying the costs of dispersal through different habitats for the California tiger salamander (*Ambystoma californiense*), an endangered species restricted to grassland/vernal pool habitat mosaics. We sampled larvae from all 16 breeding ponds in a geographically restricted area of vernal pool habitat at the Fort Ord Natural Reserve, Monterey County, California. We estimated between-pond gene flow using 13 polymorphic microsatellite loci and constructed GIS data layers of habitat types in our study area. We then used least-cost path analysis to determine the relative costs of movement through each habitat that best match rates of gene flow measured by our genetic data. We identified four measurable rates of gene flow between pairs of ponds, with between 10.5% and 19.9% of larvae having immigrant ancestry. Although *A. californiense* is typically associated with breeding ponds in grassland habitat, we found that dispersal through grassland is nearly twice as costly as dispersal through chaparral and that oak woodland is by far the most costly habitat to traverse. With the increasing availability of molecular resources and GIS data, we anticipate that these methods could be applied to a broad range of study systems, particularly those with cryptic life histories that make direct observation of movement challenging.

Keywords: gene flow, GIS, landscape genetics, least-cost path analysis, microsatellite, population structure

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Introduction

The accurate assessment of how genes flow in real populations across real landscapes is a key element of ecological and conservation genetics. Gene flow significantly affects population genetic differentiation in the majority of animal species in nature (Bohonak 1999) and plays an important role in evolution both as a constraint (by preventing local adaptation) and by promoting the spread of beneficial alleles (Slatkin 1987). Gene flow is inherently tied to dispersal and represents a key link between ecological traits, local environment, and micro-evolution.

Examining the role that landscapes play in dispersal and gene flow can reveal much about the effect of environmental variation on the distribution of genetic variation in natural populations (Arnaud 2003; Manel *et al*. 2003; Geffen *et al*. 2004; Funk *et al*. 2005; Spear *et al*. 2005; Stevens *et al*. 2006; Storfer *et al*. 2007). The developing field of landscape genetics has provided a framework for this activity. Although substantial progress has been made, efforts have largely focused on analyses dealing with linear distance correlations (Storfer 1999; Manel *et al*. 2003; Spear *et al*. 2005) and spatial autocorrelation (Smouse & Peakall 1999; Barbujani 2000; Trenham *et al*. 2001; Manel *et al*. 2003; Storfer *et al*. 2007). While this isolation-by-distance approach has been useful, it ignores landscape heterogeneity that may play an important role in

Correspondence: Ian J. Wang, Fax: 1 (530)752-1449;
E-mail: ijwang@ucdavis.edu
†Present address: Department of Biological Sciences, Lehigh University, Bethlehem, PA 18015, USA.

the way that habitats are actually traversed in nature. Landscape features and habitat connectivity are known to influence dispersal patterns and genetic structure among natural populations (Michels *et al*. 2001; Spear *et al*. 2005; Giordano *et al*. 2007). Especially for animals that interact continuously with their environments, such as small vertebrates, the distribution of landscape features can substantially influence how these animals move across a landscape (Schippers *et al*. 1996; Cushman *et al*. 2006). Thus, accounting for landscape heterogeneity and the precise distribution of features on a landscape can contribute critical insights into our understanding of gene flow and population structure (Spear *et al*. 2005; Cushman *et al*. 2006; Giordano *et al*. 2007; Storfer *et al*. 2007).

Unfortunately, for many species, direct observation of individuals moving among patches of suitable breeding habitat may be virtually impossible, especially for those that are small, nocturnal, seasonally limited, subterranean, or otherwise difficult to mark, track, and observe. An alternative approach for these taxa lies in the integration of GIS-based tools with population genetic analyses (Spear *et al*. 2005; Cushman *et al*. 2006; Storfer *et al*. 2007). Although GIS data are the most sophisticated and informative landscape data available, few studies in landscape genetics have fully integrated GIS technology (Spear *et al*. 2005; Storfer *et al*. 2007). GIS cost-weighted analyses, such as least-cost path analysis (LCPA), which account for variation in the ease of movement across regions of a heterogeneous landscape, are particularly promising as a way of evaluating the most likely paths by which genes flow across landscapes (Storfer *et al*. 2007). In LCPA, 'costs' are assigned to different landscape features, such as habitat type or elevation. These 'costs' generally reflect some understanding of resistance or mobility through a landscape feature for a species, but other factors associated with movement (for example thermal stress, predation risk, or energy expenditure) can also contribute to the 'cost' of traversing a landscape. The landscape is then searched for the path between two points that minimizes the total cumulative cost of moving between those points (Storfer *et al*. 2007).

Several studies have shown that some form of least-cost path distance fits patterns of genetic structure more closely than geographic distance (Michels *et al*. 2001; Coulon *et al*. 2004; Stevens *et al*. 2006), demonstrating the value of the cost-weighted approach. However, in these and other studies, costs were predetermined based upon observational records and *a priori* expectations, and the ways in which landscape quality is perceived by a particular species may not correspond to our assumptions (With *et al*. 1997; Wiens 2001; Cushman *et al*. 2006). So what can we do if we have no expectation, or want to explicitly test our expectations, for the costs of movement through different habitats? Spear *et al*. (2005) take a major step by utilizing least-cost path analysis, but discuss the difficulty of developing cost parameters for habitat types when no data are available for determining cost assignments.

In this study, we explore a strategy that utilizes a genetic assignment method of estimating gene flow between populations, GIS landscape data to construct least-cost paths of dispersal between those sites, and a model fitting process to infer the costs of traversing each habitat type. Using this approach, we can make qualitative comparisons of cost schemes (as in Michels *et al*. 2001; Coulon *et al*. 2004; Stevens *et al*. 2006, and Cushman *et al*. 2006) but we can also use genotype data and the distribution of habitat types on a landscape to quantitatively inform us of the costs of movement through these habitats. We present our analysis of gene flow between breeding ponds and the relative costs of movement through different habitat types in the federally endangered California tiger salamander, *Ambystoma californiense*. Amphibians are especially suitable for studies of landscape genetics because they often form metapopulations around breeding ponds, have easily distinguishable cohorts, and have fairly low vagility (Beebee & Rowe 2000; Newman & Squire 2001; Jehle & Arntzen 2002; Funk *et al*. 2005; Smith & Green 2005; Spear *et al*. 2005; Stevens *et al*. 2006; Zamudio & Wieczorek 2007). In many cases, little knowledge exists about upland habitat use in amphibians, and the behaviour of many species (Spear *et al*. 2005), including the California tiger salamander (Trenham & Shaffer 2005; Searcy & Shaffer 2008), makes direct observation of habitat use extremely challenging.

Several studies have demonstrated the importance of retaining connectivity among amphibian breeding pond networks (Newman & Squire 2001; Jehle & Arntzen 2002; Andersen *et al*. 2004; Trenham & Shaffer 2005; Stevens *et al*. 2006), and others have identified the need to understand the effects of habitat fragmentation and the distribution of habitat patches on gene flow to understand the requirements for population persistence (Gibbs 1998; Guerry & Hunter 2002; Funk *et al*. 2005; Spear *et al*. 2005; Rittenhouse & Semlitsch 2006). Because amphibians are often sensitive to anthropogenic habitat alteration (Guerry & Hunter 2002) and are facing local and global declines (Fisher & Shaffer 1996; Collins & Storfer 2003), studies providing information on the importance of different habitat types to sustaining amphibian communities also play a critical role in conservation strategies (Zamudio & Wieczorek 2007).

We use our methodology to determine the importance of different habitats in retaining population connectivity. Our goals were to estimate gene flow between populations based on microsatellite genotypic data, to use these estimates to measure the costs of dispersal through three habitat types (grassland, chaparral, and oak woodland), and to identify the likely dispersal corridors on the landscape using least-cost path analysis. Finally, we examined whether the inferred habitat costs matched expectations based upon natural-history observations. Our results quantify the ecological importance of habitat heterogeneity to a species for which little is known about the costs of movement through these three fundamental habitat types and suggest that our approach

can shed light on habitat-associated dispersal in other taxa for which direct observation is difficult.

Materials and methods

Study system

The California tiger salamander, *Ambystoma californiense*, is a federally listed, pond-breeding amphibian endemic to central California (Loredo *et al*. 1996; Shaffer & Trenham 2005). *Ambystoma californiense* breed in seasonal and permanent ponds that are free of fish and other introduced predators (Shaffer & Trenham 2005). Aquatic larvae grow in these pools for 3–6 months, at which time they metamorphose and disperse onto the surrounding terrestrial landscape. Adult and juvenile salamanders routinely travel at least 1 km from breeding ponds (Trenham *et al*. 2001; Trenham & Shaffer 2005; Searcy & Shaffer 2008); except for a few weeks of breeding activity, they are primarily fossorial. They reside in small mammal (primarily California ground squirrel, *Spermophilus beecheyi* and Botta's pocket gopher, *Thomomys bottae*) burrows for most of their lives, which provide protection against predation and dessication (Loredo *et al*. 1996; Shaffer & Trenham 2005; Trenham & Shaffer 2005; Searcy & Shaffer 2008). Although *A. californiense* may live for up to 11 years, they generally breed only once or twice during their lifetimes (Trenham *et al*. 2000). The single long-term mark–recapture study available indicates that interpond dispersal occurred regularly, on a relatively xeric landscape in central California (Hastings Reservation); approximately 30% of first-time breeders migrated to a different breeding pond from where they were born, and about 30% of second-year breeders moved to a new breeding pond (Trenham *et al*. 2001).

We conducted our research at the Fort Ord Natural Reserve, in Monterey County, California. Fort Ord is a protected area managed by the University of California that contains an intact set of natural vernal pools, many of which are used as breeding sites by *A. californiense*. There are three distinct vegetation types surrounding the vernal ponds on this reserve: grassland, maritime chaparral, and oak woodland. The grasses in the reserve are a mixture of California natives and invasives, including ryegrasses (*Leymus* spp.), needlegrasses (*Nassella* spp.) and hair-grasses (*Deschampsia* spp.). The Monterey Bay maritime chaparral is a distinctive community dominated by manzanitas (*Arctostaphylos* spp.) and California lilac (*Ceanothus* spp.). The oak woodland contains stands of coast live oaks (*Quercus agrifolia*) with toyon (*Heteromeles arbutifolia*) and western poison oak (*Toxicodendron diversilobum*) in the understory (Hickman 1993). A dozen breeding ponds in the northern part of the reserve are separated from four southern ponds by a low range of hills. However, within each of these two regions, the total vertical displacement does not exceed 25 m (National Elevation Dataset, http://ned.usgs.gov/). The northern Fort Ord breeding ponds comprise a relatively isolated set of vernal pools that form a closed network covering approximately 10 km^2, with no additional known breeding sites to the north, west or east; the four breeding ponds to the south are largely introgressed with non-native invasive *Ambystoma tigrinum* genes (Fitzpatrick & Shaffer 2007) and are isolated by both distance (~4 km) and elevation from the northern pools at our study site.

Population sampling and pond characterization

California tiger salamander populations were sampled from all 16 vernal pools at the Fort Ord Natural Reserve during June 2004 (Fig. 1; Table 1). Tissues were collected as tail-clips from larval salamanders and were preserved in 95% ethanol.

Because California tiger salamanders are primarily terrestrial as adults and breed more or less synchronously in

Table 1 Geographical coordinates of breeding ponds in the Fort Ord Natural Reserve, Monterey County, CA. Acronyms correspond to those used in the figures; *N* represents the number of samples included in microsatellite genotyping for each population

Population	Acronym	Latitude	Longitude	*N*
Ostracod Pond	OC	36.6386	–121.7529	46
Henniken West Pond	HW	36.6449	–121.7594	23
Henniken East Pond	HE	36.6460	–121.7564	46
Lower Machine Gun Flats	LM	36.6379	–121.7432	46
Upper Machine Gun Flats	UM	36.6355	–121.7461	45
Leslie Pond	LE	36.6427	–121.7508	45
West Twin Pond	WT	36.6466	–121.7478	44
East Twin Pond	ET	36.6460	–121.7454	46
Far East Pond	FE	36.6457	–121.7389	47
Chaparral Pond	CH	36.6340	–121.7662	23
Mudhen Lake	MH	36.6288	–121.7317	10
10α Vernal Pool	TA	36.6263	–121.7656	23
Impossible Rd × Mercury Rd	IM	36.5957	–121.7599	10
Riso Rd × Eucalyptus Rd	RE	36.5960	–121.7794	25
Barloy Canyon	BC	36.6057	–121.7467	24
Laguna Seca	LS	36.5923	–121.7664	11

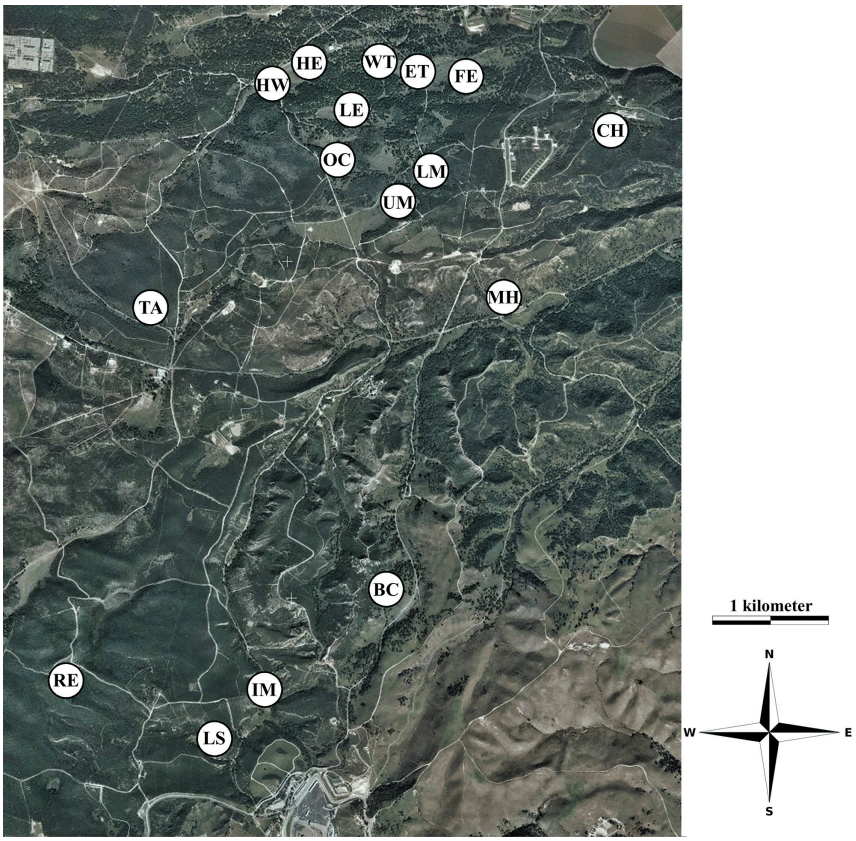

Fig. 1 Satellite image of the Fort Ord UC Natural Reserve, Monterey County, California, with localities for 16 California tiger salamander breeding ponds sampled for this study. Population acronyms correspond to those used in Table 1.

vernal pools from November to May (Shaffer & Trenham 2005), our samples constitute a single breeding cohort at each pond.

To ensure that ponds were of similar quality, we counted small mammal burrows along 100-m transects extending from the pond edges in each of the four cardinal directions. Density of small mammal burrows is the only metric of pond quality that has correlated with *A. californiense* abundance in previous studies (Trenham 2001; Trenham & Shaffer 2005). We performed chi-squared tests on each pair of ponds, comparing the number of burrows observed by each to the number expected if burrows were distributed evenly between the two ponds.

Genotyping

Tissues were digested in lysis buffer with Proteinase K, and genomic DNA was purified using a standard ethanol precipitation. Extracted samples were diluted to 10 ng/μL and used as template in polymerase chain reactions (PCRs) for 13 tetranucleotide microsatellite loci (*Acal*B136, *Acal*B142, *Acal*B148, *Acal*D019, *Acal*D021, *Acal*D032, *Acal*D036, *Acal*D065, *Acal*D071, *Acal*D073, *Acal*D082, *Acal*D098, *Acal*D108, Savage, 2008). Forward primers for each PCR were labelled with a 5′ fluorescent tag (6-FAM, NED, or HEX) for visualization. We amplified loci individually and ran PCR products, in sets of three loci, on an ABI 3100 Capillary Electrophoresis Genetic Analyzer (Applied Biosystems) at the UC Davis College of Biological Sciences DNA Sequencing Facility (http://dnaseq.ucdavis.edu/). Fragments were sized with ROX-500 size standard and collected with GeneScan version 3.1, and scoring and binning was performed with Genotyper version 2.5 (Applied Biosystems). We used Micro-Checker version 2.2.3 (van Oosterhout *et al*. 2004) to check for potential scoring errors and the presence of null alleles.

Population detection

We investigated the number of genetic clusters in our sampled landscape using BAPS version 4.14 (Corander *et al*. 2003). BAPS is distinct from other population identification software in that it treats populations, instead of individuals, as units. Thus, BAPS determines which populations have different allele frequencies, rather than partitioning individuals based upon Hardy-Weinberg equilibrium. Additionally, BAPS can accommodate geographic data associated with sampling as prior information (Pearse & Crandall 2004; Corander *et al*. 2008). We performed the 'admixture based on pre-defined populations' analysis in BAPS, using the population of sample origin as prior information. Although the name suggests that population assignments are static, BAPS can merge two predefined populations into one if it fails to

detect significant allele frequency differences. We used 1000 iterations to estimate the admixture coefficient for each sample and 10 iterations to estimate the admixture coefficient for 20 reference individuals. We then used the genetic clusters identified in BAPS in an analysis of molecular variance (AMOVA) implemented in GenAlEx version 6.3 (Peakall & Smouse 2006) to quantify the fraction of the total genetic variance among the genetic populations.

Gene flow estimation

We used a genetic assignment method, implemented in BayesAss+ version 1.3 (Wilson & Rannala 2003), to identify larvae with immigrant ancestry among our breeding populations. Unlike coalescent-based estimates of gene flow, genetic assignment methods are suitable for estimating recent rates of gene flow (Berry *et al*. 2004; Pearse & Crandall 2004). BayesAss+ uses Markov chain Monte Carlo resampling to estimate asymmetrical rates of gene flow between populations and also calculates a confidence interval for results that would be returned from uninformative data (Wilson & Rannala 2003; Pearse & Crandall 2004). Genetic assignment methods are a common tool in landscape genetics and have been shown to deliver accurate results when sampling sufficient individuals across a range of unlinked loci (Paetkau *et al*. 2004), even when interpopulation dispersal is common (Berry *et al*. 2004). We averaged the results from three independent runs with 6 million generations each, discarding 2 million as burn-in, sampling the chain every 2000 generations, and using default parameter settings. Because we sampled larvae, which do not disperse, the results of these analyses represent estimates of true gene flow, or dispersal followed by successful breeding; in this study, we use the term dispersal to describe individual interpopulation movements which result in gene flow.

Least-cost path analysis

We performed a GIS least-cost path analysis to identify dispersal corridors and determine the cost of movement through different habitat types (grassland, chaparral, oak woodland). To infer the appropriate costs for each habitat type, we calculated least-cost distances over a range of values and compared them to those predicted by our genetic analyses. There were essentially three steps to our analysis: (i) assign hypothetical costs to each habitat type, (ii) calculate the least- cost distances between ponds using those costs, and (iii) compare these least-cost distances to those predicted by gene flow estimates. We performed the matching analysis [step (iii)] under the logical assumption that the rate of gene flow between two populations is inversely proportional to the cost of moving between them (i.e. when the cost is high, gene flow should be low).

First, we constructed a detailed habitat map based upon satellite imagery and field surveys. This raster covered the area surrounding the northern 12 ponds, including all of those for which measurable rates of gene flow were detected. We excluded the southern four ponds because we did not want to sacrifice map resolution and because no gene flow was detected among them or with northern ponds. The final habitat raster was constructed by scoring each cell with a value corresponding to either vernal pool or one of the three habitat types: grassland, chaparral, or oak woodland. The raster contained square cells with 1-m dimensions, which should adequately capture fine-scale patterns of habitat distribution. We excluded elevation as a layer in our GIS analysis because the resolution (30-m horizontal resolution and 10-m vertical resolution) and error (7–15 m in elevation) in the available data are high relative to the overall range in elevation (National Elevation Dataset, available: http://ned.usgs.gov).

Using the Spatial Analyst extension in ArcGIS version 9.2 (ESRI), we performed a least-cost path analysis. In the LCPA, a 'cost raster' was first created by assigning values to each of the three habitat types. A 'cost distance raster' was then created by giving a value to each cell equal to the cumulative cost of reaching it from the source. From this cost distance raster, ArcGIS then identifies the path resulting in the lowest cost to reach a target pond from the source. We recorded this path and the total cost of moving along it, which is known as the least-cost path distance.

We conducted LCPAs on a wide range of cost combinations. Because costs are relative, one habitat type was always set to 1 and the other two were assigned every combination, in 0.1 unit increments, from 1 to 10 (low to high). This resulted in a total of 24 843 least-cost path analyses run on different combinations of costs. Our goal was to find combinations of cost values that would result in least-cost path distances predicted by the gene flow estimates. Essentially, we assumed that the rates of gene flow inferred from our genetic data reflected the cost of movement between different breeding ponds such that higher rates of gene flow indicated relatively less costly dispersal. For instance, if a pair of ponds (A_1–A_2) had twice the interpond gene flow of another pair (B_1–B_2), then we would expect the least-cost distance between pond-pair A_1–A_2 to be half that between pond-pair B_1–B_2. We found the relative rates of gene flow between pairs of ponds from the estimates in our molecular analysis. Because BayesAss+ (Wilson & Rannala 2003) provides a 95% confidence interval in addition to the mean of gene flow between ponds, we were able to establish the 95% confidence interval of relative rates predicted from our molecular data.

For each of the 24 843 LCPAs, we took the least-cost path distances and compared them to the distances expected by the rates of gene flow resulting from our molecular analysis. If all of the least-cost distances fell within their expected ranges, based on the 95% confidence interval, then we

accepted the habitat cost values used to generate those paths as reflecting biologically accurate costs of dispersal. This resulted in a range of values for which the habitat costs matched expectations.

As a hypothetical example, consider the scenario in which the confidence intervals for the rates of gene flow detected between two pairs of ponds are 0.10 to 0.15 (A_1–A_2) and 0.20 to 0.30 (B_1–B_2). We would expect the cost of dispersing in A_1–A_2 to be greater than in B_1–B_2, because the rate of gene flow is lower. Thus, the least-cost distance between A_1–A_2 could be as high as 3times (0.30/0.10 = 3.00) or as low as 1.33 times (0.20/0.15 = 1.33) the least-cost distance between B_1–B_2. If there are two habitat types on this landscape (*X* and *Y*), we can assign hypothetical costs to these habitats until we find a range of values that fit our expectations for least-cost distances. If we set *X* =1 and *Y* = 2, the least-cost distance between A_1–A_2 might equal 16 and between B_1–B_2 might equal 8. This would result in a relative least-cost distance of 2 (16/8= 2), which falls within our confidence interval for expected relative distances of 1.33 to 3. Thus, we would accept *X* = 1 and *Y* = 2 as realistic values for habitats *X* and *Y*. If, on the other hand, values of *X* = 2 and *Y* = 1 resulted in least-cost distances of 5 for A_1–A_2 and 6 for B_1–B_2, then we would reject these values because they result in a relative distance (5/6 = 0.83) outside of the confidence interval.

To visually and geographically identify dispersal corridors between breeding ponds, we then plotted the least-cost paths on our habitat map. This enabled us to identify the particular routes used in interpond dispersal. Our approach is summarized by a flow chart showing the intersection of molecular genetic and GIS techniques in Fig. 2.

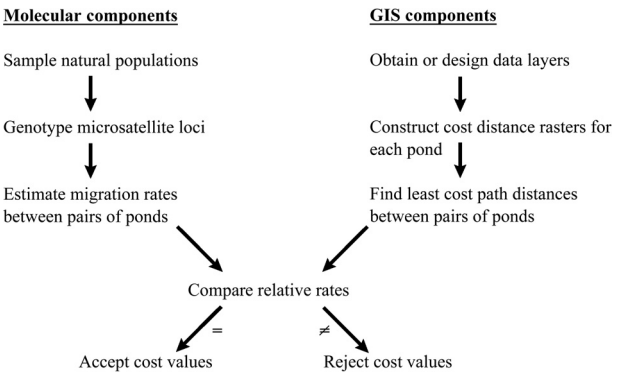

Fig. 2 Flow chart showing the steps in our methodology for estimating the costs of dispersal across different landscape features. The technique integrates molecular genetic analyses and GIS least-cost path analyses.

Results

Genotypic data

All 13 of our microsatellite loci were highly polymorphic, ranging from 7 to 19 alleles, with an average of 11.6 alleles, at each locus (Table 2). Micro-Checker did not indicate the presence of null alleles or scoring error. We were unable to unambiguously score 2.1% of the genotypes and coded these as missing data.

Population detection

BAPS results recognize a large number of individuals with admixed ancestry, but support the presence of 15 separate populations (Fig. 3). BAPS recognized 15 of 16 sampling

Table 2 Number of alleles at each microsatellite locus used in genotyping

Locus	No. of alleles
B1-42	7
D036	11
D071	13
B1-36	7
D098	19
D108	12
D073	10
D082	14
D019	10
D065	14
B1-48	12
D021	13
D032	9

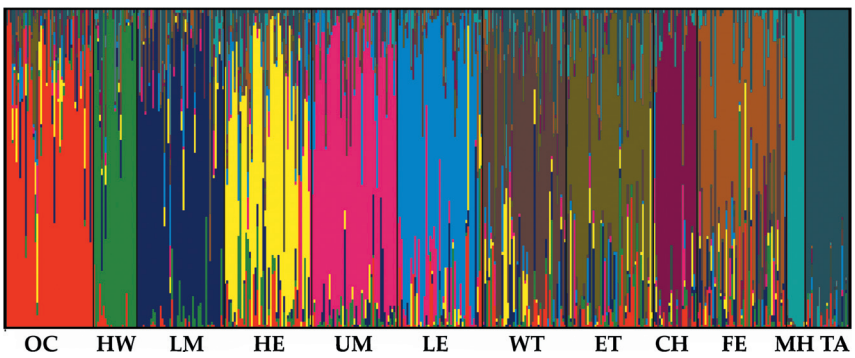

Fig. 3 Population assignment based on Bayesian population clustering implemented in BAPS version 4.14 (Corander *et al*. 2003). Each vertical bar represents an individual, and bars are divided into proportions based upon the probability of assignment to each population. Individuals are clustered by collecting locality. BAPS recognized 15 subpopulations, corresponding to the original collecting localities

Table 3 Relative gene flow, direct distances, and cost distances between the four breeding ponds used in the least-cost path analysis. All measures are set relative (rate or distance = 1.00) to the shortest distance or lowest rate. 'Cost distance' is the expectation that would result from the given dispersal rates if interpond dispersal is a reflection of the relative ease of movement across a landscape. For dispersal rate and cost distance, the mean value is first and the 95% confidence interval is in parentheses. Population acronyms correspond to those used in Table 1

	HE–WT	HE–ET	UM–LE	WT–LM
Gene flow	1.25 (0.82–1.60)	1.00 (0.80–1.23)	1.90 (1.39–2.60)	1.17 (0.88–1.52)
Cost distance	1.52 (0.77–3.19)	1.90 (1.00–3.256)	1.00 (0.473–1.15)	1.62 (0.81–2.96)
Direct distance	1.00	1.35	1.23	1.31

Table 4 Inferred costs of dispersal through different habitat types, resulting from least-cost path analysis. All costs are relative to dispersal through chaparral, which is measured to be least costly. Values are the relative costs of movement over equivalent distances between different habitat types

	Grassland	Chaparral	Woodland
Lower bound	1.7	1.0	4.6
Upper bound	2.2	—	5.3

ponds as largely differentiated, and collapsed the Riso × Eucalyptus (RE) population into others, primarily Impossible × Mercury (IM). Thus, both visually and statistically, BAPS recognized our 16 ponds as somewhat admixed, but with sufficient fine-scale differences between breeding ponds to recognize 15 separate subpopulations. Consistent with the BAPS results, our AMOVA test of these 15 subpopulations attributed 12% of the total molecular variance to among-population variation and 88% to within-population variation.

Gene-flow estimation

Genetic assignment in BayesAss+ indicated that most of the interpond rates of gene flow in our analysis are indistinguishable from those generated by uninformative data. However, for four breeding pond pairs (HE–WT, HE–ET, WT–LM, and LE–UM), the estimated dispersal rate fell outside of the confidence interval provided for comparison with uninformative data. These represent 'significant' or 'measurable' dispersal rates given our genotypic data and are the four interpond dispersal rates used in our LCPA (Table 3). These four rates are relatively high, ranging from 10.5 to 19.9% of the target population explained by gene flow from the source, indicating that interpond movement can be common. We found no significant differences in the density of small mammal burrows around each pond for each pair ($P > 0.20$ in all cases), suggesting that gene flow was not substantially related to this important component of pond quality. We did not survey burrow density across the entire study area. If burrow density varied in the different habitat types, then any benefit that it provided to *Ambystoma californiense* would have been factored into the overall cost of traversing that habitat type.

Least-cost path analysis

Our least-cost path analysis indicates that dispersal through chaparral is the least costly to *A. californiense*, and that movement through grassland is approximately twice, and through oak woodland roughly five times as costly as movement through chaparral (Table 4). A small range of costs values (1.7–2.2 for grassland and 4.6–5.30 for oak woodland) produced cost distances that fell within the 95% confidence interval inferred from our genetic data (Table 4). The inferred dispersal corridors are plotted on our habitat map as least-cost paths in Fig. 4.

Discussion

Population structure and gene flow

Our finding of subtle differences in allele frequencies between breeding ponds of *Ambystoma californiense* appears consistent with similar results of population substructure in other ambystomatid salamanders (Spear *et al*. 2005; Giordano *et al*. 2007; Zamudio & Wieczorek 2007). Both for the microsatellites discussed here and for regional variation in mitochondrial DNA (Shaffer *et al*. 2004), substantial variation exists among ponds at a local level (12% for microsatellites data in this fine-scale study, and 18–31% for mitochondrial DNA at a larger regional level). Despite this variation, key differences between breeding sites remain. BAPS analysis, which included prior information on population of origin, identified 15 distinct subpopulations in our network of 16 breeding ponds. Thus, this system is an interesting case where gene flow maintains connectivity between populations, but does not overwrite genetic signatures of among-pond differentiation.

Given the observed moderate-low degree of microsatellite variation between ponds, our observations of fairly high levels of gene flow (nearly 20% in one case) between certain ponds appear reasonable. Another study of a relatively distantly related ambystomatid salamander (Zamudio &

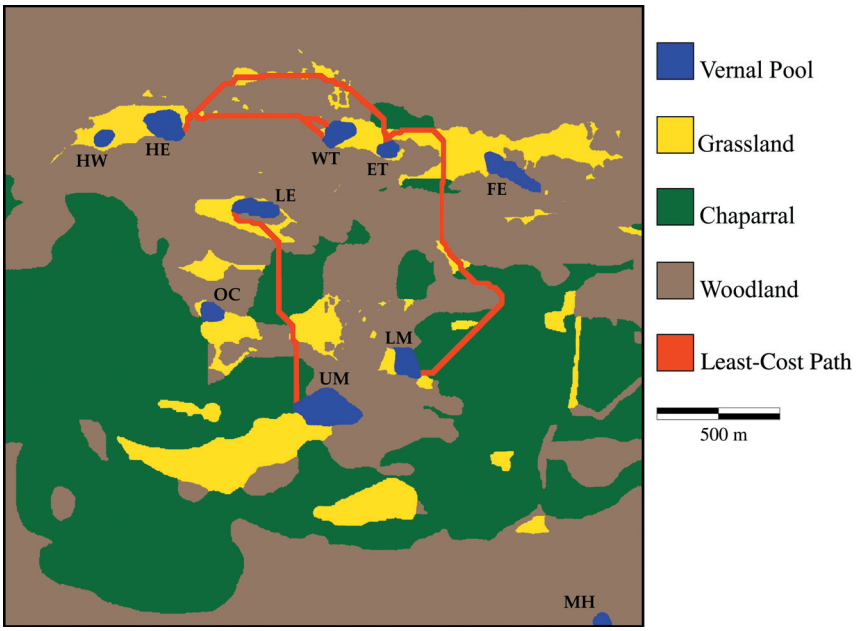

Fig. 4 Habitat map showing least-cost paths between breeding ponds for four interpopulation dispersal corridors. The least-cost paths shown are those that most closely fit the relative mean dispersal rates. The paths were calculated as having one cell width, but are drawn as wider for visualization. Population acronyms correspond to those used in Table 1.

Wieczorek 2007) found similarly high levels (up to 25%) of gene flow across a larger, more mesic landscape. California, with its Mediterranean climate of hot, dry summers may appear to be a difficult place for overland amphibian dispersal, but several field studies on California tiger salamanders indicate that overland dispersal among ponds 600 m apart is fairly common (Trenham *et al*. 2001) and that individuals may move at least 1 km, and perhaps up to 3 km from breeding sites (Trenham & Shaffer 2005; Searcy & Shaffer 2008). Clearly, geographically proximate breeding ponds form important interconnected networks, and understanding the landscape factors promoting or limiting gene flow is critical to understanding the viability of populations on a landscape (Shaffer *et al*. 2000; Trenham *et al*. 2001; Couvet 2002; Spear *et al*. 2005; Stevens *et al*. 2006; Zamudio & Wieczorek 2007).

Costs of dispersal through different habitats

Our least-cost path analysis returned the surprising result that dispersal through grassland is nearly twice as costly to *A. californiense* as dispersal through chaparral. *Ambystoma californiense* is typically associated with grassland habitat, where it uses small mammal burrows for protection from predators and temperature extremes (Loredo *et al*. 1996; Trenham 2001; Trenham *et al*. 2001; Shaffer & Trenham 2005; Trenham & Shaffer 2005). However, while chaparral is often regarded as being a harsh, difficult habitat for movement, our results indicate that this may not be the case. In fact, our results are broadly compatible with a study in the related blotched tiger salamander, *Ambystoma tigrinum* (Spear *et al*. 2005), which found that open shrub habitat (a habitat that is structurally similar to chaparral) was correlated with decreased population differentiation in the dry, mountainous region of Yellowstone National Park. Our observation of a fivefold greater cost of traversing oak woodland compared to chaparral was also initially surprising, given the open canopy and grassland understory of California oak habitat. Whether these differential costs reflect biological differences in vegetation, microhabitat humidity, predator accessibility, small mammal burrow density, or a number of other factors are intriguing hypotheses that will require additional field studies.

Although our results disagree with the natural-history expectation that grassland should be the preferred habitat of *A. californiense*, they do not directly contradict any empirical data on habitat costs or movement preferences for this species. Although there are substantial data suggesting that *A. californiense* prefer to reside in grassland, there are no data on the habitat preferences that these salamanders have when moving between ponds (Trenham 2001; Trenham *et al*. 2001; Trenham & Shaffer 2005). Animals may prefer different habitats for residence vs. dispersal, during different times in their life cycles, or during different season. In addition, the single radio-tracking study for this species (Trenham 2001) found that upland habitat use by California tiger salamanders in the drier, less coastal habitat of Hastings Reserve preferentially overexploited grassland and isolated oaks compared to continuously wooded oak patches. Although the data of Trenham (2001) are based on the frequency of adult salamanders in their terrestrial retreats and ours reflect gene flow between breeding sites, both indicate that oak woodland is avoided (more costly) compared to grassland (unfortunately, there was no chaparral in the habitat followed by Trenham 2001).

Obviously, the extent to which our method and results can, and should, play a part in conservation management

will depend on the local landscape and the repeatability of our results across the range of the species. As in any system, corroborating evidence from multiple sources, including direct field observations and measurements, are necessary to understand the effects of different habitats to dispersal in *A. californiense* and to truly test the efficacy of our proposed methodology. Additionally, the possibility remains that spatial differences at an even finer scale, such as subtle terrain contours, contribute to the cost of traversing a landscape. We hope that with increasingly detailed spatial data, these fine-scale patterns can be fully resolved, just as the presently available advances in GIS data have enabled detailed studies of habitat differences on a landscape such as this. At the least, our data indicate the importance of considering habitat types to understanding the movement of animals across landscapes.

Conclusions

Traditionally, landscape genetics approaches have examined landscapes as uniform space (Storfer 1999; Barbujani 2000; Manel *et al*. 2003; Storfer *et al*. 2007). However, a deeper understanding of the processes governing the movement of genes on landscapes necessitates the use of both complex landscape and genetic data (Barbujani 2000; Manel *et al*. 2003; Spear *et al*. 2005; Cushman *et al*. 2006; Storfer *et al*. 2007). Our results join a small set of empirical analyses that demonstrate the feasibility and importance of incorporating landscape characteristics into a richer understanding of population genetic processes. Furthermore, our least-cost path analysis methodology considers the specific distribution of landscape features and their relation to natural movement among populations, allowing us to make precise inferences about the value of different habitat types and the particular dispersal corridors that exist on a landscape.

With the increasing availability of GIS data and the ability of users to generate their own, site-specific layers, the methods discussed here could become broadly applicable to a wide range of taxa in the very near future. These methods can be easily adapted to utilize a variety of other landscape features, including geological formations, humidity levels, the presence of roads or rivers, urban structures, temperature, and elevation, for the eventual development of fully detailed, three-dimensional landscape data. Finally, if genetic data are unavailable for dispersal rate estimates, or if ongoing gene flow using assignment-based methods cannot be estimated, mark–recapture data may serve in their place.

With the knowledge that organisms interact in intricate ways with their landscapes (Slatkin 1987; Trenham *et al*. 2001; Spear *et al*. 2005), the incorporation of detailed landscape data into landscape genetic analyses seems like a logical step. Landscape genetics is playing an increasingly important role in population genetics by providing a framework for quantitatively modelling the effects of landscapes on gene flow, population substructure, and genetic variation (Manel *et al*. 2003; Storfer *et al*. 2007), and least-cost path analysis provides an important tool for improving our understanding of landscape effects.

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Ian Wang studies the role of landscapes and environments on population structure, gene flow, and adaptation in amphibians. Brad Shaffer and his laboratory group have maintained a long-standing interest in the evolution, ecology, and conservation biology of ambystomatid salamanders, with a strong recent focus on long term studies of the endangered California tiger salamander.
