

ORIGINAL
ARTICLE



Effects of landscape and history on diversification of a montane, stream-breeding amphibian

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ABSTRACT

Aim The aim of this study was to understand the roles of landscape features in shaping patterns of contemporary and historical genetic diversification among populations of the Andean tree frog (*Hypsiboas andinus*) across spatial scales.

Location Andes mountains, north-western Argentina, South America.

Methods Mitochondrial DNA control region sequences were utilized to assess genetic differentiation among populations and calculate population pair-wise genetic distances. Three models of movement, namely traditional straight-line distance and two effective distances based on habitat classification, were examined to determine which of these explained the most variation in pair-wise population genetic differentiation. The two habitat classifications were based on digital vegetation and hydrology layers that were generated from a 90-m resolution digital elevation model (DEM) and known relationships between elevation and habitat. Mantel tests were conducted to test for correlations between geographic and genetic distance matrices and to estimate the percentage variation explained by each type of geographic distance. To investigate the location of possible barriers to gene flow, we used Monmonier's maximum difference algorithm as implemented in BARRIER 2.2.

Results At both geographic scales, effective distances explained more variation in genetic differentiation than did straight-line distance. The least-cost distances based on the simple classification performed better than the more detailed habitat classification. We controlled for the effects of historical range fragmentation determined from previous nested clade analyses, and therefore evaluated the effect of different distances on the genetic variation attributable to more recent factors. Effective distances identified populations that were highly divergent as a result of isolation in unsuitable habitats. The proposed locations of barriers to gene flow identified using Monmonier's maximum difference algorithm corresponded well with earlier analyses and supported findings from our partial Mantel tests.

Main conclusions Our results indicate that landscape features have been important in both historical and contemporary genetic structuring of populations of *H. andinus* at both large and small spatial scales. A landscape genetic perspective offers novel insights not provided by traditional phylogeographic studies: (1) effective distances can better explain patterns of differentiation in populations, especially in heterogeneous landscapes where barriers to dispersal may be common; and (2) least-cost path analysis can help to identify corridors of movement between populations that are biologically more realistic.

Keywords

Andes, effective distance, frog, habitat distribution, *Hypsiboas andinus*, landscape genetics, mitochondrial DNA, partial Mantel test, population genetics, spatial analyses.

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INTRODUCTION

Investigations of the historical impact of landscape features on genetic diversity and differentiation have a long history in evolutionary ecology (e.g. Gentry, 1982; Capparella, 1991; Gascon *et al.*, 2000; Costa, 2003). The discipline of landscape genetics, however, is quite new and as of yet its perspectives are under-utilized in studies of natural populations (Manel *et al.*, 2003). For example, recently developed assays of variation in high-resolution molecular markers combined with novel spatial statistical approaches can provide significant insight into the causes that underlie the distribution of genetic and adaptive diversity across spatial and temporal scales spanning a few metres or generations to entire species' ranges or millennia (reviewed in Manel *et al.*, 2003; Storfer *et al.*, 2006). Such analyses can identify how contemporary and historical landscape features have interacted to influence individual movements, and thus ultimately gene flow. Studies of spatial patterns in genetic diversity, however, have often attributed a primary (sometimes exclusive) role to historical processes, such as vicariance, and complementary assessments of contemporary landscape-level processes are urgently needed to gain a full understanding of the generation and maintenance of genetic diversity (Vucetich & Waite, 2003).

Response to landscape features depends on the life-history characteristics of a species. Highly vagile species (as adults or larvae) typically have little genetic structuring owing to their ability to disperse over long distances (e.g. eels, Avise *et al.*, 1986; sea cucumbers, Uthicke & Benzie, 2003; pearl oysters, Yu & Chu, 2006; coral reef fish, Haney *et al.*, 2007), whereas more sedentary species show genetic differentiation even at small spatial scales (e.g. frogs, Loughheed *et al.*, 1999; butterflies, Keyghobadi *et al.*, 2005; snails, Holland & Cowie, 2007). Although potential dispersal ability is an important prerequisite for gene flow, other life-history characteristics may constrain its effect. For example, many species undergo long-distance migrations, and hence have great dispersal capability, but some are highly philopatric, returning to breed at or near the site where they were hatched or born; in other words, the distance travelled by an individual during its annual cycle is much greater than the distance travelled by its gametes (e.g. Wenink *et al.*, 1996; Alvarado Bremer *et al.*, 2005; Vähä *et al.*, 2007). Modelling the movement of animals is an important contribution to interpreting patterns of genetic variation (contemporary and historical) across landscapes.

Isolation by distance (Wright, 1943), one of the best studied spatial genetic relationships, is often calculated based on matrices of straight-line distances between sampling locations. Genetic differentiation among populations may not be well explained by such measures if the species' required habitat does not occur in a direct line between the two sites. Recent explicit considerations of landscape features in a topographically heterogeneous region have demonstrated the importance of measuring biologically meaningful dispersal pathways (Keyghobadi *et al.*, 1999; Funk *et al.*, 2005; Spear *et al.*, 2005). Past studies, however, have tended to look at the

influence of landscape at one of two levels without explicitly linking the two: either (1) coarsely at a large scale (> 100 km) in a biogeographical or phylogeographic context, or (2) in detail at a fine scale (hundreds of metres to several kilometres), where contemporary processes are more prominent than historical ones. Here we examine the effect of incorporating different levels of detail regarding habitat distribution at different scales to explain patterns of genetic diversity. Our study addresses the following questions. Do analyses using distance metrics based on intricate habitat distribution matter only at smaller scales? Is such detail required at larger scales, where differentiation may be more likely to be the result of historical fragmentation than of isolation by distance? Does a simultaneous consideration of phylogeographic and landscape genetic perspectives provide insights unavailable to either in isolation?

Study area

General biomes and ecoregions of South America are described in Cabrera & Willink (1980). Contemporary north-western Argentina possesses great topographic diversity and habitat complexity associated with the Andes Mountains leads to great spatial variation in precipitation and temperature (Handford, 1988). The area is generally arid to semi-arid; however, heavy precipitation from humid air masses travelling from the north-east in the summer falls primarily on the eastern slopes of the Andean ranges, resulting in longitudinal strips of forest that stretch along these eastern slopes south from Bolivia to the province of Catamarca (c. 28° S). These montane forests are essentially continuous except for a narrow invasion of arid thorn scrub habitat in the province of Salta (c. 25° S, Fig. 1), which separates the southernmost tip of this forest peninsula (Brown *et al.*, 2001). Away from these mesic east-facing slopes rainfall is much lower, resulting in the semi-arid chaco on the flat and slightly tilted terrain to the east, and in the arid interior valleys and high plateau to the west (Czajka & Vervoort, 1956; Cabrera, 1976; Vervoort, 1982; Handford, 1988). These arid habitats are possible dispersal barriers for taxa dependent on mesic environments.

This complex distribution of habitats must affect present-day connectivity among populations. Furthermore, historical changes in elevation and climate probably changed the connectivity among habitats, and hence among populations, which may have promoted the diversification of taxa in this region. In particular, the area between c. 24° and 26° south currently contains narrow bands of rain forest (Sierras de Metán, Lumbrera, Santa Barbara) that are separated by chacoan arid thorn scrub vegetation, effectively isolating the southernmost extension of moist Andean forest. During dry glacial periods the arid thorn scrub almost certainly would have extended its range, probably completely severing the connection between the southern (26°–27° S) and the northern (< 24° S) moist forests. Koscinski *et al.* (2008) used a suite of phylogeographic approaches, including nested clade analysis, and attributed marked genealogical differentiation among

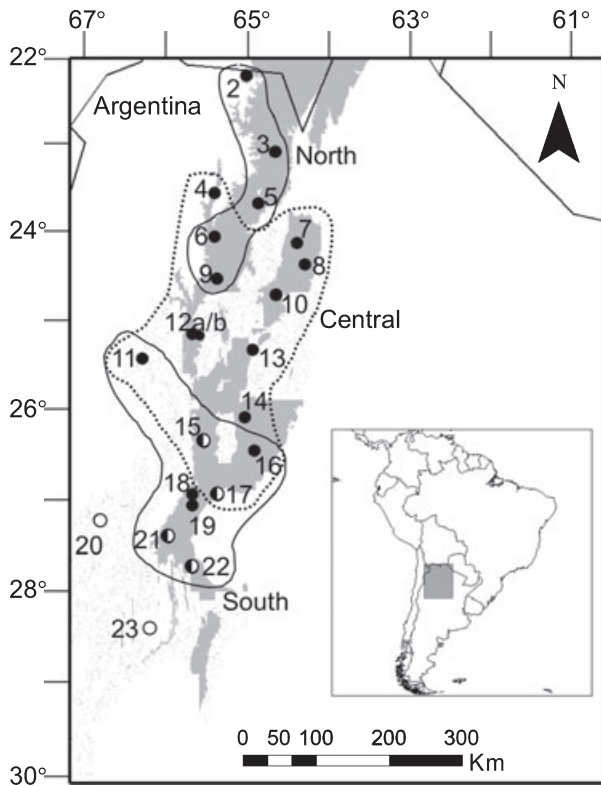


Figure 1 Distribution of distinct mitochondrial control region lineages of *Hypsiboas andinus* across north-western Argentina (Koscinski *et al.*, 2008). Unfilled circles, lineage no. 2 only; black circles, lineage no. 1 only; mixed black-and-white circles, both lineages. The distributions of the three subclades within lineage no. 1 across populations are indicated with solid and dotted lines. The north and south clade do not overlap with each other, but both overlap with the central clade. Shaded regions represent suitable habitat for *H. andinus*.

populations of tree frogs [*Hypsiboas andinus* (Müller, 1924)] primarily to historical factors – range fragmentation and expansion. Since patterns of population genetic differentiation and diversity may be caused by either historical or contemporary factors, an important task is to disentangle the effects of each in order better to understand the processes shaping these patterns. Controlling for the effects of historical differentiation found by Koscinski *et al.* (2008), the aim of our study is to use new landscape genetic approaches to investigate the roles of landscape features in shaping contemporary genetic diversification among populations at various geographical scales.

MATERIALS AND METHODS

Given their limited vagility, diverse fecundities, breeding biology and breeding habitats, frogs offer excellent possibilities for examining the factors involved in evolutionary divergence, diversification and speciation. *Hypsiboas andinus* Müller, 1926 (formerly *Hyla andina*; Faivovich *et al.*, 2005; Wiens *et al.*, 2005) is a moderate-sized tree frog (50–60 mm) distributed along the Andes Mountains from the province of Catamarca,

Argentina (28° S) to northern Bolivia (16° S). It is found mostly near water along the streams, ditches or flooded areas of the deeply incised river valleys (Ceï, 1980; Duellman *et al.*, 1997; personal observation) in various habitats, from lowland humid montane forests (500 m a.s.l.) to montane grasslands (> 1500 m a.s.l.), but is not found in the semi-arid chaco to the east nor in high-altitude Monte and puna habitats (Duellman *et al.*, 1997). *Hypsiboas andinus* eggs are deposited in masses containing c. 600 eggs (M. Vaira, unpublished data) secured to submerged vegetation in streams (Ceï, 1980; personal observation). Larvae are aquatic and develop at the site of oviposition. Generation time for the species is not known but is probably 1 year, as for the closely related *Hypsiboas pulchellus* (Basso & Kehr, 1992).

Detailed methods for sample collection and molecular procedures are described in Koscinski *et al.* (2008). A total of 247 individuals from 23 populations are included in the analyses presented below (Table 1). We used a 340-bp fragment of the mitochondrial control region to assess genetic differentiation among populations (GenBank accession numbers EU403157–EU403413). To calculate population pair-wise F_{ST} , we used the Kimura-2-Parameter (K2P) genetic distance,

Table 1 Sample sizes for mtDNA control region analyses for each population of *Hypsiboas andinus* and the type of habitat found at the site. Site numbers refer to those in Fig. 1. Samples were collected by D.K. and/or S.C.L. and/or P.H. in 1987, 2001, 2004, 2005 or 2006.

Site	<i>n</i>	Habitat type
2	2	Grassland
3	10	Yungas
4	11	Grassland
5	10	Yungas
6	19	Yungas
7	6	Yungas
8	10	Transition Forest
9	7	Yungas
10	5	Yungas
11	3	Monte
12a	19	Yungas
12b	12	Yungas
13	5	Chaco serrano
14	7	Yungas
15	11	Yungas
16	20	Yungas
17	14	Yungas
18	19	Grassland
19	20	Yungas
20	4	Monte
21	16	Grassland
22	21	Transition Forest
23	6	Monte

which corrects for multiple substitutions and different rates of substitution between transitions and transversions, as implemented in ARLEQUIN 2.000 (Schneider *et al.*, 2000).

Well-supported mitochondrial lineages of *H. andinus* across the study area showed strong differentiation that we attributed to historical range fragmentation based on phylogenetic and nested clade analyses (Fig. 1; Koscinski *et al.*, 2008). At the deepest level, two divergent lineages appeared to be in secondary contact in the southern portion of the species range. Within the widespread lineage no. 1, recent (< 2 Ma) fragmentation has resulted in three subclades distributed in a north-to-south series (Fig. 1; Koscinski *et al.*, 2008). To further investigate potential causes of these divergence events and the locations of possible barriers to gene flow, here we employ Monmonier's maximum difference algorithm as implemented in BARRIER 2.2 (Manni *et al.*, 2004; Manni & Guérard, 2004). We calculated one barrier for the entire study area (all individuals in all populations). The analyses were then repeated (two barriers were calculated) for individuals in populations containing lineage no. 1 only (excluded: all individuals at site nos 20 and 23, and some individuals at site nos 15, 17, 21 and 22).

Given that the vegetation types of north-western Argentina are strongly related to temperature and precipitation, and hence to elevation, latitude and aspect, we utilized a 90-m resolution digital elevation map (DEM) and the 'reclass' function of the ArcGIS 9.1 Spatial Analyst extension (ESRI, 2005) to create a digital vegetation layer based on published detailed distributions of habitat types (Czajka & Vervoort, 1956; Cabrera, 1976; Vervoort, 1982; Handford, 1988) and on personal observations of the authors (55+ cumulative years of experience in the region). Because there were no digital layers depicting the surface hydrology of the region available, we used the same 90-m DEM to estimate the locations of all streams and rivers in the region using the ArcHydro 9.1 extension in ArcGIS 9.1 (ESRI, 2005). Based on these created vegetation and hydrology layers, as well as on the ecological requirements (Ceï, 1980; Duellman *et al.*, 1997) and known locations of *H. andinus*, we defined suitable habitat as shown in Table 2. In the chaco serrano and Monte, the frogs would be restricted to movement along waterways (within streams only) and adjacent habitat, as the habitat outside the riparian zone would be unsuitable.

We examined three models of dispersal to test which explained the most variation in pair-wise population genetic differentiation: (1) straight-line distance, (2) simple effective distance, and (3) detailed effective distance. Isolation by distance has traditionally been examined using straight-line (or Euclidean) distance between sites and is used here as the null model. Straight-line distance between sampled populations of *H. andinus* was calculated using the ArcGIS 9.1 Spatial Analyst extension. Neither landscape structure nor elevation was included in this measure.

We used a least-cost approach (Michels *et al.*, 2001; Adriaensen *et al.*, 2003) to calculate two effective distances – simple and detailed. These differ in the amount of detail used

Table 2 Cost values used for each habitat in the least-cost path analysis for each type of effective distance measured. Higher values indicate increased cost to movement owing to less suitable habitat. In the chaco serrano and Monte, the frogs are restricted to movement only within waterways as the habitat outside the riparian zone is unsuitable. In the detailed classification, streams were assigned cost values based on the type of habitat they were found within.

Habitat	Suitability	Simple	Detailed	
		Cost values	Habitat cost values	Stream cost values
Chaco	No	–	–	–
Transition forest	Yes	1	30	20
Chaco serrano	Within streams only	1	–	80
Yungas	Yes	1	10	10
Alisos Forest	Yes	1	40	30
Grassland	Yes	1	45	35
Monte	Within streams only	1	–	80
Puna	No	–	–	–

to define classes of habitats and therefore in their resistance-to-movement or 'cost' values. The effective distances were calculated by first developing least-cost grids for each of the sampling sites. Cost values for surrounding pixels were assigned based on habitat suitability such that decreased suitability related to greater cost of movement through the pixel. Completely unsuitable habitats (i.e. habitats in which *H. andinus* cannot survive) were considered absolute barriers to movement and were assigned impassable cost values. Cost values were assigned in relative terms to each other (i.e. one habitat is twice as costly to travel through as another) based on our experience of the abundance of the species in each type of habitat and on published reports (Ceï, 1980). This least-cost surface was then utilized to generate the paths of 'least-cost' between all sampling sites. The lengths of these paths were then calculated as the effective distance. For the simple distance we coded the cost of movement through all suitable habitat to be equal (two categories: suitable vs. not suitable), whereas for the detailed distance we partitioned the suitable habitat into more categories to define cost values for each vegetation type separately (Table 2).

Partial Mantel tests (Mantel, 1967) were performed to look for correlations between each of the three geographic distance matrices and the K2P genetic distance matrix, as well as percent of the variation explained by each type of geographic distance. Significance of correlations was tested using 2000 random permutations. All partial Mantel tests were performed in FSTAT 2.9.3 (Goudet, 2001). We performed the analyses at two scales to look for differences in explanatory power at different scales: (1) for the entire data set (23 populations, *c.* 760 km north to south) and (2) for a subset of populations containing only lineage no. 1 with pair-wise Euclidean distances of 100 km or

less (total of 36 pairs, Table S1). Because removal of values greater than 100 km resulted in incomplete matrices, we unfolded the matrices and assessed significance of correlations using vector permutation of the genetic distance column as implemented in PERMUTE! 3.4a9 (Legendre *et al.*, 1994; Casgrain, 2001) using 999 permutations.

To examine the effect of landscape features in explaining historical differentiation we used a matrix of clade identity between populations based on which genetic lineages were present in each compared population (0: same lineages present in both populations, 1: different lineages, 0.5: share one lineage). Analyses were therefore run with the clade identity matrix as the first explanatory variable before the addition of geographic distances. The analyses were run for the entire data set (identifying the two deeply divergent lineages no. 1 and no. 2) as well as for the smaller data set based on the three subclades within mitochondrial lineage no. 1.

RESULTS

Genetic differentiation

Population pair-wise F_{ST} values for the subset data set ranged from 0 to 0.92 (Table S1), and for the entire data set from 0 to 1.00 (Table S2). For the entire range, Monmonier's maximum difference algorithm, implemented in BARRIER, identified a sharp genetic discontinuity in the southern portion of the range, suggesting a possible barrier to gene flow (Fig. 2a).

Within lineage no. 1 only, two potential barriers were identified across the range (Fig. 2b), which correspond well with the subclades identified using a phylogeographic approach (Koscinski *et al.*, 2008).

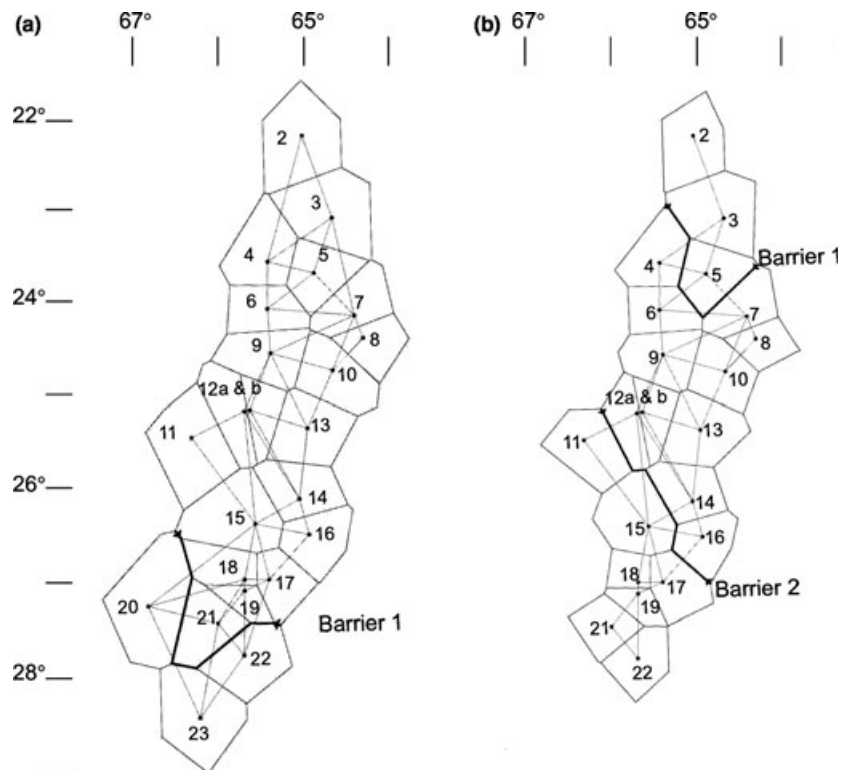
Entire data set

Although all three geographic distances were highly correlated (both effective distances to Euclidean: $r = 0.88$, $P < 0.001$), effective distances for some pair-wise comparisons were much greater in magnitude than the Euclidean distances (Fig. 3). In particular, pair-wise comparisons involving populations 11, 20 and 23 showed the greatest difference (arrow in Fig. 3, paths for 11–20–12a shown in Fig. 4). Shorter pair-wise Euclidean distances appear to be more similar for all measures.

Effective geographic distances explained more variation in the genetic data than did Euclidean distance (Table 3). Effective distances had significant partial correlations after controlling for the effect of straight-line distance. Using the best model, the simple classification for the effective geographic distance, 36% of the variation in pair-wise population F_{ST} was explained.

The clade identity matrix was significantly correlated with pair-wise F_{ST} ($r = 0.38$, $r^2 = 0.14$, $P < 0.0001$). All distances maintained significant partial correlations with pair-wise F_{ST} when the clade identity matrix was added to the analyses (Table 3). The variation explained increased only in the model with Euclidean distance alone (20–24%).

Figure 2 Locations of sharp genetic discontinuities (solid black lines) in *Hypsiboas andinus*: (a) across the entire study area and (b) across a portion of the study area corresponding to populations containing individuals with lineage no. 1 haplotypes as identified using mitochondrial control region sequences. Population pair-wise F_{ST} values were analysed using Monmonier's maximum difference algorithm as implemented in BARRIER (Manni *et al.*, 2004). The genetic discontinuities probably correspond to barriers to gene flow among the populations. Thin pale grey lines, Delaunay triangulation; thicker grey lines, Voronoï tessellation; black points, sampling sites.



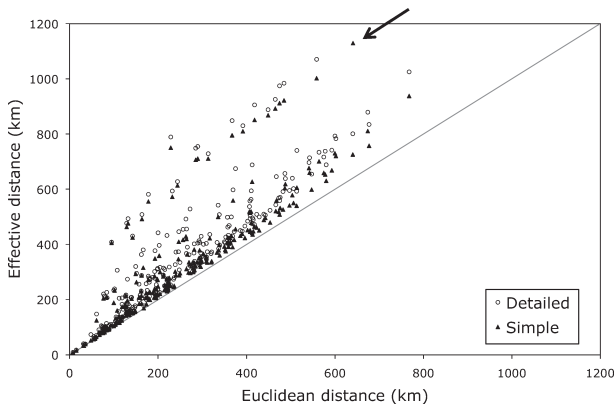


Figure 3 Relationship between pair-wise simple and detailed habitat effective distances and Euclidean distance. The grey line is the line of equality. The arrow indicates pair-wise effective distances that are much larger than Euclidean distances; see text for more details.

Subset of data set

Euclidean geographic distance was not significantly correlated with the matrix of genetic distances at the <100 km scale and explained only 5% of the total variation; therefore we did not include it when testing effective distances (Table 4). Only the simple effective distance was significantly correlated with genetic distance, explaining 20% of the variation. The clade identity matrix for lineage no. 1 was significantly correlated with genetic distance ($r = 0.51$, $r^2 = 0.26$, $P = 0.001$) and

improved the percentage variation explained for all three distances. Only the simple effective distance had a significant partial correlation after addition of the clade identity matrix.

DISCUSSION

At both scales, effective distances explained more variation in pair-wise genetic differentiation than did straight-line geographic distance. The least-cost distances, based on the simple classification, performed better than the more detailed habitat classification. We were also able to control for the effects of historical fragmentation, and therefore evaluate the effect of different distances on the remaining genetic variation. Our results suggest that landscape features play a prominent role in historical and contemporary genetic structuring of populations of *H. andinus* at both large and small scales.

At the larger scale, the main historical genetic discontinuity between the two lineages is found in the southern portion of the range (Fig. 2a). Populations 20 and 23 contain lineage no. 2 only, whereas population 22 has both lineages present in equal proportions. Although two other populations contain both lineages the majority of individuals have only lineage no. 1 haplotypes. The location of the putative barrier suggests that population nos 15, 17, 21 and 22 are probably sites of secondary contact between these two divergent lineages.

Barriers, such as prominent topographic features and rivers, have been found to correspond to the genetic divergence of populations on either side (e.g. Bermingham & Avise, 1986; Loughheed *et al.*, 1999; Keyghobadi *et al.*, 1999; Funk *et al.*,

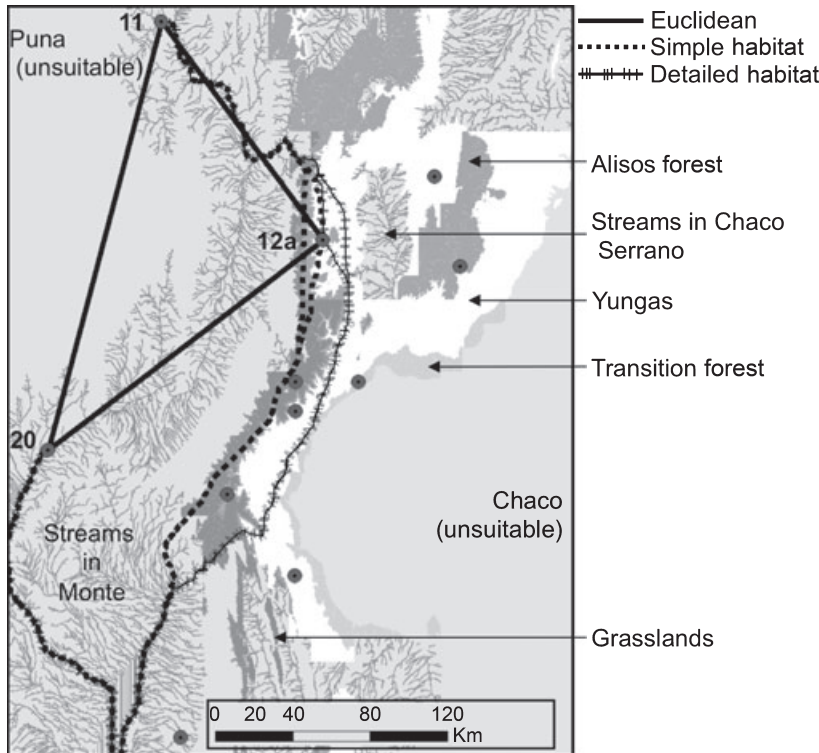


Figure 4 Routes between population nos 11–12a–20 of *Hypsiboas andinus* using Euclidean distance, and simple and detailed habitat effective distances. Shades of grey indicate the type of habitat according to the detailed habitat classification (see Table 2). The simple classification weights all suitable habitats equally.

Table 3 Correlations between genetic distance and various geographic distances using partial Mantel tests for the entire data set. Each model was tested with and without the clade identity matrix.

Route	Variable	Partial correlation (r)	Variance explained (r^2)	Clade identity matrix	
				Partial correlation (r)	Variance explained (r^2)
Null straight line	Euclidean distance***	0.45	0.20	0.31	0.24
Simple classification	Euclidean distance***	-0.45	0.36	-0.31	0.36
	Simple habitat distance***	0.40		0.35	
Detailed classification	Euclidean distance***	-0.45	0.34	-0.31	0.34
	Detailed habitat distance***	0.37		0.32	

The significance of correlations is indicated as follows: * < 0.05 , ** < 0.01 , *** < 0.001 .

Table 4 Correlations between genetic distance and various geographic distances using partial Mantel tests at the smaller scale (< 100 km). Each model was tested with and without the clade identity matrix. Because Euclidean distance was not significantly correlated with genetic distance, it was not included in the effective distance models.

Route	Variable	Partial correlation (r)	Variance explained (r^2)	Clade identity matrix	
				Partial correlation (r)	Variance explained (r^2)
Null straight line	Euclidean distance	0.21	0.05	0.13	0.28
Simple classification	Simple habitat distance	0.45**	0.20	0.29*	0.33
Detailed classification	Detailed habitat distance	0.32	0.10	0.18	0.29

The significance of correlations is indicated as follows: * < 0.05 , ** < 0.01 , *** < 0.001 .

2005). Funk *et al.* (2005) found that mountain ridges, which do not have suitable habitat, strongly affected the population differentiation of Columbia spotted frogs (*Rana luteiventris*). Populations that had a short straight-line distance but were on opposite sides of ridges showed high genetic divergence, but populations not separated by ridges were genetically similar over longer geographic distances (Funk *et al.*, 2005). Because ridges are clearly acting as barriers, the effective or ecological distance between populations is much longer than the straight-line distance, as frogs would need to travel a much larger distance to 'go around' the ridge. The presence of unsuitable habitat in our study similarly resulted in much longer pair-wise distances, as movement was modelled to avoid these areas. If these habitats serve as barriers to movement and hence the populations show higher genetic differentiation, then models using effective distance should better explain the variation in pair-wise population F_{ST} .

Indeed, we found that populations surrounded largely by unsuitable habitat (e.g. nos 11, 20, 23) had effective distances to other populations that were much larger than the Euclidean pathways (Figs 3 & 4). These populations were usually found where movement would necessarily be restricted to riparian habitat along streams. Such patterns are found in many aquatic species that move exclusively through specific corridors such as waterways (e.g. Turner & Trexler, 1998; Fetzner & Crandall, 2003; Spear *et al.*, 2005; Vähä *et al.*, 2007). Our findings suggest that peripheral populations typically found in the most environmentally extreme parts of the species range are most likely to be genetically differentiated from other populations but also most subject to underestimation of population pair-

wise geographic distances when using Euclidean distance. This bias may have consequences for detecting patterns of isolation by distance, and for distinguishing historical from contemporary processes (see the review by Eckert *et al.*, 2008). Vucetich & Waite (2003) found peripheral populations of grassland birds to have lower N_e than core populations, suggesting that genetic drift might have a much more pronounced effect in populations along edges of the species range. Differentiation in peripheral populations of *H. andinus* could be the result of historical isolation, but contemporary processes such as genetic drift may also play a key role through the loss of genetic diversity.

To examine the effect of historical differentiation between the two major mitochondrial lineages in *H. andinus*, we used a design matrix to identify relationships among populations based on which lineages were present in each population. The clade identity matrix was significantly correlated with pair-wise F_{ST} , as would be expected given that it describes differences among populations resulting from historical processes, and it alone explained 14% of the total variation in the genetic data set. Although all geographic distances were still significantly correlated with pair-wise F_{ST} after controlling for history, only the Euclidean distance model showed an improvement in percentage variation explained. Effective distances appear to account for the variation resulting from historical factors even without the addition of the clade identity matrix. Because the effective distance routes avoid currently unsuitable habitat, they probably better encompass not only contemporary but also historical connectivity among populations.

We also found a significant correlation between the clade identity matrix for lineage no. 1 and the genetic differentiation at the smaller scale (< 100 km). The clade identity matrix alone explained 26% of the variation and increased the percentage variation explained for all three geographic distances. Given what we know of the history of the region, unsuitable habitat was probably more extensive during glacial maxima, isolating populations in the remaining moist refugia. Two contemporary well-defined areas, at *c.* 22° S and *c.* 27° S, of moist habitat may have retained their mesic character during glacial dry periods and thus may have supported montane forests and dependent populations of taxa. These mesic forests are separated by arid chacoan scrub that probably expanded during dry glacials, severing the connection between these mesic forests. Assuming that contraction of moist habitats at mid-altitudes occurred during the Pleistocene, the current distribution of the yungas may closely represent suitable habitat for *H. andinus* during glacial periods. Such a model predicts severing of mesic habitats between site nos 12a/b and 15 – which may represent the barrier between the north and south refugia for the subclades in our study. Under this model, the Sierra de Santa Barbara mountain chain (site nos 7, 8, and 10) would also be isolated and may have served as the refugium for the central subclade. Putative barriers identified in this study using landscape genetic approaches support our previous findings of differentiated subclades across the study area.

At the smaller scale, only the simple effective distance was significantly correlated with genetic distance, which suggests that habitat features may be driving differentiation rather than distance alone. Previous studies of salamanders (Spear *et al.*, 2005; Giordano *et al.*, 2007) and frogs (Rowe *et al.*, 2000; Funk *et al.*, 2005) have suggested that features such as water bodies, forests, meadows and mountains rather than geographic distance alone may affect the movement of individuals and hence population differentiation. Even at the smaller scale in our study, populations separated by unsuitable habitat may be driving the correlation between genetic differentiation and distance. Some pair-wise effective distances were greater than 200 km (twice the Euclidean distance) owing to the avoidance of unsuitable habitat. Patterns of connectivity and isolation at this scale may result in differentiation owing to contemporary processes such as limited gene flow among populations separated by unsuitable habitat, as well as to the historical isolation described above.

Effects of scale

All distances were significantly correlated at the larger scale, but only the simple effective distance showed a statistically significant relation at the small scale (with or without the clade identity matrices). The difference in patterns suggests that different processes may drive genetic differentiation at different scales. Several studies have found that a pattern of isolation by distance is not as evident at very small scales (review in

Newman & Squire, 2001; Stepien *et al.*, 2007; Wilmer & Wilcox, 2007). Structure at smaller scales may be strongly affected by metapopulation and extinction/recolonization dynamics (Rowe *et al.*, 1998; Newman & Squire, 2001; Wilmer & Wilcox, 2007), by differences in effective population size (Vucetich & Waite, 2003) and by behavioural traits such as site fidelity (Stepien *et al.*, 2007; Vähä *et al.*, 2007). At larger scales, historical processes such as fragmentation and range expansion may leave a stronger signature in genetic differentiation among populations.

Effects of habitat classification

Although both effective distances performed better than the Euclidean one, the simple habitat effective distance explained the most variation at both scales. The critical determinant of population differentiation appears to be the availability of *any* suitable habitat rather than differences in suitability. Although many studies use indices (e.g. genetic differentiation) to infer movement of organisms, the interaction between the organism and the landscape is rarely quantified at suitable scales because of the logistical limitations of sampling at sufficient density (but see Keyghobadi *et al.*, 2005). Effective distances among populations and levels of connectivity will vary from group to group depending on life-history attributes (D'Eon *et al.*, 2002), but little is known about many species in the Neotropics. Our findings may suggest that dispersal in *H. andinus* depends on factors other than the fine-scale differences in habitat we included in the detailed classification, or that historical changes in phytogeography, which do not match patterns of contemporary habitats, have affected genetic differentiation. Further investigation of movement patterns for this (and other) species will shed light on the processes responsible for genetic diversification in this region.

Given our understanding of the facets of life history that might shape movement patterns of *H. andinus*, it is not surprising that effective distances better explain patterns of genetic differentiation. Our study demonstrates that using effective distance can better explain patterns of differentiation in populations, especially in heterogeneous landscapes where barriers to dispersal may be common, and that combining phylogeographic and landscape genetic perspectives provides more comprehensive insights into the causes of the present-day distribution of genetic diversity. Using a least-cost path analysis may identify corridors of movement between populations that are biologically more realistic and more relevant to understanding the landscape-level processes that are critical for conservation planning.

ACKNOWLEDGEMENTS

Provincial Governments and the National Parks of Argentina kindly permitted us to work in the north-west, and we wish to thank everyone who has assisted with field collections and laboratory work over the years. A special thank you to M.-A.

Lachance for providing expertise and laboratory space. Thank you also to Z. Chen for assistance with GIS analyses. Funding for the project was provided by NSERC grants to S.C.L. and P.H.; NSERC scholarships, OGSST scholarships and various University of Western Ontario awards to A.G.Y. and D.K.; and Sigma Xi Grants in Aid of Research and a Gaije Award from the Society of Ichthyologists and Herpetologists to D.K.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Population pair-wise F_{ST} values used in the subset analyses (total of 36 pairs).

Table S2 Population pair-wise F_{ST} values used in the entire data set analyses.

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Editor: Brett Riddle