

Geographic Determinants of Gene Flow in Two Sister Species of Tropical Andean Frogs

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Abstract

Complex interactions between topographic heterogeneity, climatic and environmental gradients, and thermal niche conservatism are commonly assumed to indicate the degree of biotic diversification in montane regions. Our aim was to investigate factors that disrupt gene flow between populations and to determine if there is evidence of downslope asymmetric migration in highland frogs with wide elevational ranges and thermal niches. We determined the role of putative impediments to gene flow (as measured by least-cost path (LCP) distances, topographic complexity, and elevational range) in promoting genetic divergence between populations of 2 tropical Andean frog sister species (*Dendropsophus luddeckei*, $N = 114$; *Dendropsophus labialis*, $N = 74$) using causal modeling and multiple matrix regression. Although the effect of geographic features was species specific, elevational range and LCP distances had the strongest effect on gene flow, with mean effect sizes (Mantel r and regression coefficients β), between 5 and 10 times greater than topographic complexity. Even though causal modeling and multiple matrix regression produced congruent results, the latter provided more information on the contribution of each geographic variable. We found moderate support for downslope migration. We conclude that the climatic heterogeneity of the landscape, the elevational distance between populations, and the inability to colonize suboptimal habitats due to thermal niche conservatism influence the magnitude of gene flow. Asymmetric migration, however, seems to be influenced by life history traits.

Key words: Andes, asymmetric migration, elevational range, least-cost path distances, migration rate, topographic complexity

The increase of biological diversity at lower latitudes is a well-known biogeographic pattern (Pianka 1966; Rohde 1992). A closer look reveals, however, that tropical hotspots of biodiversity are not randomly distributed, but are mainly associated with continental highlands (Myers et al. 2000; Orme et al. 2005; Jansson and Davies 2008). Compared with lowland areas, montane regions contain exceptionally high diversity relative to their area (Körner 2007).

Most studies that have tested hypotheses of montane speciation in the tropics use phylogenetic approaches that compare either rates of speciation (Hey and Nielsen 2004; Wiens et al. 2006) or the association of tree topology with highland colonization and/or diversification (Hall 2005; Roberts et al. 2006). Even though phylogenetic approaches may answer questions about biogeographic processes such as colonization of the highlands by lowland species (Lynch 1999; Hall 2005; Roberts et al. 2006), landscape genetic analyses are perhaps

more appropriate to explain the effects of complex landscapes on the early stages of speciation of montane lineages. Determining how gene flow is associated with particular features of the landscape, therefore, can provide fine-grain information about which geographic factors effectively restrict gene flow among populations, promoting their divergence.

Associating gene flow with the characteristics of the landscape is complex because of the difference in scale between the horizontal (latitude/longitude) and vertical (altitudinal) components of topography. For instance, in phylogeographic studies of montane species (e.g., Funk et al. 2005; Guarnizo et al. 2009; Kebede et al. 2007), the maximum horizontal distances that separated populations were ~60, 216, and 770 km respectively, whereas the maximum vertical distances were ~2.0, 1.5, and 1.1 km. Because horizontal distances may be orders of magnitude larger than vertical distances, the effect of the vertical component of the landscape on gene flow is likely to be underestimated.

The vertical component of a topographic surface may influence population connectivity. For example, an organism, especially an ectotherm, moving across a steep terrain will encounter different conditions than one on a flat terrain. Even though the thermal physiology of some ectotherms is conservative relative to other groups (Angilletta 2002), the abrupt change in temperature associated with even the slightest changes in elevation exposes individuals to extremely different physiological and environmental regimes (Navas 2006). In general, an organism in complex topographies must traverse environmental gradients several times during ascent and descent, which may restrict the long-term rate of movement. Organisms with narrow niches, however, would be restricted to zones or isoclines (presumably horizontal) of suitable habitat. Moving along such habitat isoclines would require traversing greater geographic distances. This presumably represents an extra energy expenditure that may reduce migration rate.

The magnitude and direction of gene flow may be influenced by steep topography. Given that water flows downhill, we expect to find asymmetric migration favoring downward movement in species that are dependent on water (e.g., bull kelp [Collins et al. 2010], salmon [Consuegra et al. 2005], and sticklebacks [Bolnick et al. 2008]).

Tropical Andean frogs are an ideal group for studying diversification in montane regions because anurans generally have restricted migration abilities (Smith and Green 2005), their metabolism is affected by environmental variables associated with elevational gradients (Feder and Burggren 1992), and montane clades of frogs tend to have higher rates of diversification (Smith et al. 2007). We used the sister species *Dendropsophus labialis* and *Dendropsophus luddeckei* (Hylidae) to determine how topography influences the amount and direction of gene flow in species with wide elevational distributions.

Specifically, we determined the role of least-cost path (LCP) distance, topographic complexity, and elevational range as determinants of gene flow. We hypothesized that 1) topographic complexity and LCP distances will display the strongest association with gene flow because these measures are associated with the climatic heterogeneity of regions connecting populations/individuals; 2) elevational distance between populations will display the weakest association with gene flow because of the

small amount of vertical distance relative to horizontal distance; and 3) gene flow will be asymmetric in a downslope direction because eggs and larvae (tadpoles) are displaced downhill by water flow.

Materials and Methods

Sampling and DNA Sequencing

Tissues were obtained from *D. labialis* and *D. luddeckei* captured around small ponds (10–15 m in diameter) along the western slope of the Eastern Andes of Colombia (Table 1; Figure 1). High abundance and elevational ranges of at least 2000 m characterize these 2 species; most species of Andean frogs have narrower elevational ranges, usually between 400 and 600 m (Navas 2002). We selected species with wide elevational distributions to facilitate analysis of the effect of elevational distance on gene flow. We obtained tissues by clipping the toe of adult frogs or the tail tips of larvae (Institutional Animal Care and Use Committee protocol number 07101901). To avoid sampling siblings (which biases allelic diversity), adults were preferred over larvae. In cases in which larvae were collected, different sizes of larvae (presumably indicating matings of different pairs) from various regions of the pond were used. After recording the geographic coordinates of each individual, tissues were immediately transferred to plastic tubes of 97% ethanol and were stored at -80 °C until DNA extraction.

We estimated gene flow using DNA sequences from the fast-evolving control region of mitochondrial DNA (600 bp), the nuclear proopiomelanocortin (POMC) gene (480 bp), and exons 2 and 3 of the cellular myelocytomatosis (c-myc) gene (800 bp). Genomic DNA was extracted using the Viogene Blood and Tissue Genomic DNA Extraction Miniprep following the manufacturer's protocols. Three DNA fragments were amplified using published primers for the control region (controlJ2-L: 5'-GCATTACGTTTCACGAAGWTGG-3', controlP-H: 5'-GTCCATAGATTCASTTCCGTCAG-3'; [Goebel et al. 1999]), POMC (PomcR1: 5'-GGCRTTYTTGAAWAGAGTCAITAGWGG-3', PomcF1: 5'-ATATGTCATGASCCAYTTCGCTGGAA-3'; [Vicentes et al. 2007]), and c-myc (cmcy1U: 5'-GAGGACATCTGGAARAARTT-3'; cmcy3L: 5'-GTCTTCCTC

Table 1 Sampling sites, map codes, geographic coordinates, elevation (above sea level), and the number of individuals analyzed for each locus

Species	Population	Code	Coordinates (lat N, long W)	Elevation (m)	n	Number of individuals sequenced
<i>Dendropsophus luddeckei</i>	La Cueva	A	6°24'15.20", 72°22'55.90"	3729	14	14 (control region), 11 (POMC), 04 (c-myc)
	Guina*	B	6°05'51.90", 72°52'45.84"	3288	18	18 (control region), 18 (POMC), 06 (c-myc)
	Paipa*	C	5°44'50.60", 73°08'01.60"	2642	20	20 (control region), 19 (POMC), 01 (c-myc)
	Arcabuco*	D	5°45'14.22", 73°26'40.30"	2575	20	20 (control region), 19 (POMC), 13 (c-myc)
	Villa de Leyva*	E	5°38'55.20", 73°31'56.10"	2125	13	13 (control region), 12 (POMC), 04 (c-myc)
<i>Dendropsophus labialis</i>	Chiquinquir�	F	5°36'27.70", 73°46'10.92"	2542	17	17 (control region), 16 (POMC), 09 (c-myc)
	Las Pilas*	G	5°07'0.130", 74°07'37.10"	2313	6	06 (control region), 06 (POMC), 01 (c-myc)
	Pantano*	H	5°00'14.40", 74°10'32.20"	3192	15	15 (control region), 12 (POMC), 01 (c-myc)
	Guadalupe*	I	4°35'44.20", 74°01'49.76"	3276	18	18 (control region), 18 (POMC), 04 (c-myc)
	Las Brisas*	J	4°25'58.62", 73°55'13.19"	1970	18	18 (control region), 17 (POMC), 05 (c-myc)

Populations within each species are ordered from north to south. Asterisks indicate localities used in the asymmetric migration calculations.

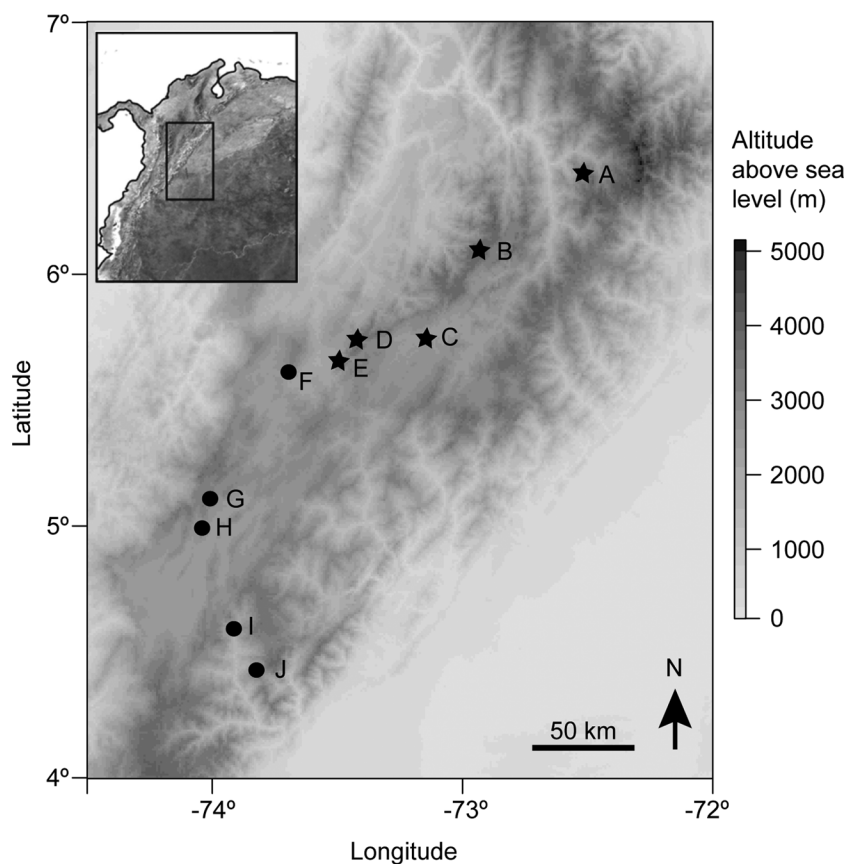


Figure 1. Map of the Colombian Eastern Andes showing the sampling localities for each species. Stars: *Dendropsophus luddeckei*, dots: *Dendropsophus labialis*.

TTGTCRTTCTCYTC-3'; [Crawford 2003]). Amplifications were performed with a reaction mix containing 2.5 μ L of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.5), 2.5 μ L of 8 μ M dNTP's, 13.875 μ L of dH₂O, 1.0 μ L of MgCl₂, 0.125 μ L of DNA polymerase, 1.25 μ L of each primer (10 μ M), and 2.5 μ L of the DNA extract for a final volume of 25 μ L.

The PCR cycle for the control region included an initial denaturing step of 2 min at 94 °C, followed by 35 amplification cycles (30 s at 94 °C, 30 s at 48 °C, 1 min at 72 °C), and a final extension of 7 min at 72 °C. For POMC, we used the PCR cycle protocol from Guarnizo et al. (2009). For c-myc, we used the PCR cycle protocol from Crawford (2003). PCR products were purified using a Viogene Gel Purification Kit. Clean PCR products were sequenced at the ICMB Core Research Facility at the University of Texas at Austin using BigDye v3.1 cycle sequencing chemistry, and the Sanger method using ABI 3730 DNA sequencers. To determine the 2 alleles present on each heterozygous individual (identified as having 2 chromatogram peaks of the same intensity at a base position) for POMC and c-myc, we used the PHASE algorithm (Stephens et al. 2001) in DNAsp 5.1 (Librado and Rozas 2009). We used 100 iterations and a confidence probability threshold of >90%. Heterozygous sites were treated as missing when confidence intervals were <90%.

Estimation of Genetic Distances and Geographic Variables

We estimated genetic distances between individuals with pairwise Kimura-2 parameter distance (K2P) (Kimura 1980) using MEGA 5.0 (Tamura et al. 2007). Genetic distances between individuals were used as a proxy for gene flow (Rousset 2000; Selonen et al. 2010). The K2P model was chosen to facilitate comparison with other studies on frogs (Rowe 2006; Kosciński et al. 2009). K2P distances were calculated using the mitochondrial control region, given that its faster substitution rate provides higher intraspecific resolution than the 2 nuclear loci (Kurabayashi et al. 2010).

LCP distances, which are paths between 2 points that minimize the accumulated landscape feature costs across a matrix, were estimated with the Spatial Analyst Extension in ARCGIS V9.2 (ESRI). In heterogeneous landscapes, LCP distances, as measured here, are expected to be considerably larger than Euclidean distances, since topographic features such as mountains or deserts obstruct the movement of individuals across the landscape and on average increase the LCPs. In contrast, in homogeneous landscapes, LCP distances are expected to be closer to Euclidean distances, since individuals can move without impediments across the landscape. To generate the cost layer needed to estimate the LCP

distances, we followed Wang and Summers (2010). Their method assumes that regions in which the species has a low probability of occurrence have high costs of migration. We performed the distribution probability analysis for each species with the 19 bioclimatic layers with 30 arc-second resolution (Hijmans et al. 2005) using MAXENT V3.3.1 (Phillips et al. 2006). MAXENT predicts the environmental suitability of each cell in a layer using values that range from 0 to 1, where higher scores indicate a more suitable habitat for a species. We used the inverse of these values, because lower suitability should have a higher migration cost. Because the 19 bioclimatic variables reflect precipitation and temperature, LCP distances contain coarse ecological information.

To estimate the inverse distribution probability of each species (used as cost layers), we included 21 localities for *D. luddeckei* and 20 for *D. labialis*, based on our data and data from the museum of the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia. We assessed the accuracy of the distribution probabilities generated by MAXENT with a receiver operating characteristic (ROC) analysis (Phillips et al. 2006), randomly selecting 25% of occurrence records and 10000 cells as background points. We confirmed the model by comparing its output with data from museum distribution records.

To estimate topographic complexity along vectors, we modified an oceanographic technique to estimate rugosity (Zawada et al. 2010). We estimated all possible pairwise contour vectors (which take topographic relief into account; Figure 2) among populations using the 3D Analyst of ARCGIS 9 (ESRI) with a 250 m resolution STRM digital elevation model (<http://srtm.csi.cgiar.org/>). We then divided each contour distance by the corresponding Euclidean distance between each pair of populations (Figure 2); this ratio is always ≥ 1 . The larger the ratio, the more complex is the landscape. A smaller ratio indicates a less complex (flatter) terrain.

The elevational distance between populations was estimated as the Euclidean distance perpendicular to the horizontal plane.

Causal Modeling

We used causal modeling (Legendre and Troussellier 1988; Cushman et al. 2006; Richards-Zawacki 2009; Cushman and Landguth 2010) to determine which combination of spatial factors influences genetic distance (a proxy for gene flow) between individual haplotypes. Causal modeling is more effective at correctly identifying contributing factors and rejecting spurious correlations than simple correlation analysis (Legendre and Troussellier 1988; Cushman and Landguth 2010). We formulated 7 models that describe the effect of various combinations of geographic variables (LCP distances, topographic complexity, and elevational distances) on the genetic differentiation of populations (Table 2). Each model is characterized by a unique combination of partial correlations, and therefore, supported only if all its conditions are met.

To test the causal models, we performed partial Mantel tests (using the program *zt* version 1.0 [Bonnet and Van de Peer 2002]) between a target matrix and a genetic distance matrix while controlling for the effect of a third covariate matrix. A Bonferroni correction was applied to adjust the significance level given that multiple tests were performed.

Multiple Matrix Regression With Randomization

Partial Mantel tests have been recently criticized because of their high type-I error rate and low power (Raufaste and Rousset 2001; Harmon and Glor 2010; Guillot and Rousset 2013; but see Legendre and Fortin 2010). Mantel tests, however, are still widely used landscape genetics and phylogeography mainly because of the absence of alternative methods than can detect the effect of nonindependent geographical variables on gene flow (Guillot and Rousset 2013). An alternative method, multiple matrix regression with randomization (MMRR) was introduced by Wang (2013), which tests the significance of a multiple regression using a randomized permutation procedure to account for the potential nonindependence between variables. The main advantages of this method are that it produces appropriate levels of type-I error

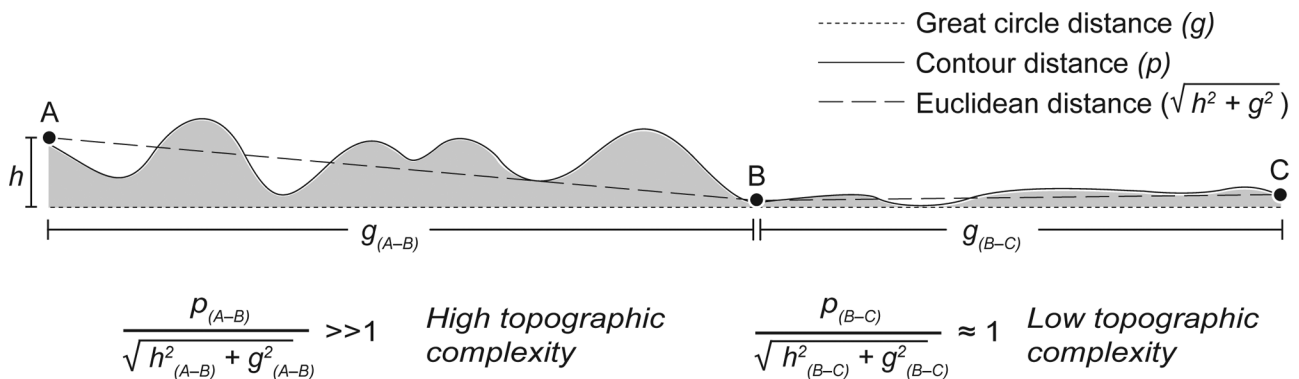


Figure 2. Left box: hypothetical high topographic complexity scenario between the populations A and B, where the contour distance (dotted line) is larger than Euclidean distance (dashed line), making the ratio greater than 1. Right box: low topographic complexity scenario between populations B and C, where the contour distance is similar to the Euclidean distance, making the ratio close to 1. h = altitudinal difference between each pair of populations. Euclidean distance is calculated as the hypotenuse of the right triangle whose sides are h and g .

Table 2 Causal model of the expected relationship between geographic variables and genetic distances

Model	Correlation	Controlling for:	Expected significance
A (LCP)	LCP × G	Elev	Sig
	LCP × G	TC	Sig
	Elev × G	LCP	NS
	TC × G	LCP	NS
B (Elev)	Elev × G	LCP	Sig
	Elev × G	TC	Sig
	LCP × G	Elev	NS
	TC × G	Elev	NS
C (TC)	TC × G	LCP	Sig
	TC × G	Elev	Sig
	LCP × G	TC	NS
	Elev × G	TC	NS
D (LCP and Elev)	LCP × G	Elev	Sig
	LCP × G	TC	Sig
	Elev × G	TC	Sig
	Elev × G	LCP	Sig
E (LCP and TC)	TC × G	LCP	NS
	TC × G	Elev	NS
	LCP × G	Elev	Sig
	LCP × G	TC	Sig
F (Elev and TC)	Elev × G	TC	NS
	Elev × G	LCP	NS
	TC × G	LCP	Sig
	TC × G	Elev	Sig
G (Elev, TC, and LCP)	LCP × G	Elev	Sig
	LCP × G	TC	Sig
	Elev × G	TC	Sig
	Elev × G	LCP	Sig
H (none)	TC × G	LCP	Sig
	TC × G	Elev	Sig
	LCP × G	Elev	NS
	LCP × G	TC	NS
	Elev × G	TC	NS
	Elev × G	LCP	NS
	TC × G	LCP	NS
	TC × G	Elev	NS

The combination of variables supported by each model is enclosed in parentheses. The “×” indicates a correlation between 2 matrices. Sig: Correlation significantly different from 0 at 0.05; NS: correlation nonsignificant at $P = 0.05$. Elev, elevational distances; G, K2P genetic distances; TC, topographic complexity.

(Wang 2013), and it uses multiple regression, assessing the independent contribution of each variable in the model. We performed the MMRR method using the R function from Wang (2013) with 10 000 permutations, and compared its results with the partial Mantel tests from the causal models.

Migration Asymmetry

To measure upslope versus downslope migration, we selected for each species a subset of population pairs that

are geographically close to each other (less than 50 km apart) but occur at different elevations (Table 1). Migration rate was estimated under a coalescent framework using isolation with migration (Hey and Nielsen 2004; Hey 2005), allowing migration (m) to vary in both directions. For this analysis, we used POMC, c-myc, and the mitochondrial control region.

Given that IM assumes no intragenic recombination (Hey and Nielsen 2004), we examined the nuclear genes, POMC and c-myc, for evidence of recombination using IMgc (Woerner et al. 2007), which uses the 4-gamete test (Hudson and Kaplan 1985) to identify the largest recombination-free block of sequences for each locus. We then used the longest contiguous nonrecombining block for all further analyses. We were interested in comparing relative and not absolute migration rates within each species, and therefore we did not scale parameters with mutation rate or generation time.

Preliminary IM runs with large priors were conducted to determine prior maxima on the migration parameter. Priors were reduced in subsequent runs until their distribution was optimized and minimized zero probability tail lengths. We estimated the parameters m_1 (maximum migration rate from population 1 to population 2), m_2 (maximum migration rate from population 2 to population 1), and θ_1 , θ_2 (theta, the product of 4 times the effective population size and mutation rate of populations 1 and 2). To detect asymmetric migration, we compared the proportion of the Markov Chain Monte Carlo samples in which the product between the effective number of gene copies and the per gene copy migration rate ($2N_m$) was larger in the upslope direction ($2N_1m_1 > 2N_2m_2$) than the downslope direction ($2N_2m_2 > 2N_1m_1$).

Migration rate estimates were based on a Markov chain of 10^7 generations that was run until the effective sample size values of parameter correlations were greater than 200, following a 100 000 step burn-in. We used the HKY85 substitution model and an inheritance scalar of 0.25 for the mitochondrial control region and 1.0 for the 2 nuclear loci (given that the effective population size of a mitochondrial gene size is one-fourth that of a nuclear gene). We used 10 coupled chains with a linear heating increment of 0.05. Three independent runs with different random seeds were used to evaluate congruence of the results. The Bayesian confidence interval was determined using the 90% highest posterior density, estimated from the shortest span on the X-axis that contains 90% of the posterior probability for each parameter.

Results

We sequenced 114 individuals of *D. luddeckei* and 74 of *D. labialis* (Genbank accession numbers in Supplementary Material online). Not surprisingly, for each species the nucleotide diversity per site (π) of the 2 nuclear fragments was on average an order of magnitude lower relative to the mitochondrial control region (*D. luddeckei*: control region $\pi = 0.0450$, POMC $\pi = 0.0032$, c-myc $\pi = 0.0023$; *D. labialis*: control region $\pi = 0.0234$, POMC $\pi = 0.0025$, c-myc $\pi = 0.0015$).

The area under the ROC curve in the MAXENT analysis used to estimate the LCP distances was 0.915 ± 0.053 (standard deviation) in *D. luddeckei* and 0.931 ± 0.044 in *D. labialis*. Our results indicated that each model had a good fit to the distribution of each species based on our sampled localities and museum locality records.

The *D. labialis* causal model D (Table 3) had the strongest support after Bonferroni correction; in other words, both LCP and elevational distances were negatively associated with genetic distances, but topographic complexity did not have an effect. In *D. luddeckei*, however, causal model B was fully supported, with only elevational distances having a negative association with genetic distances (Table 3).

A multiple regression with randomization was run to predict genetic distances from topographic complexity, LCP distances, and elevation distances. In both species each of these variables predicted genetic distances (*D. labialis*: $F = 3229.8$, $P < 0.0005$; *D. luddeckei*: $F = 56.6$, $P < 0.0005$). However, the effect of each variable on gene flow depended on the species. In *D. labialis*, LCP distances had the highest regression coefficient (β) followed by elevation; however, the effect of each variable was significant (LCP distance: $\beta = 0.75$, $P < 0.0005$; elevation distance: $\beta = 0.37$, $P < 0.0005$; topographic complexity: $\beta = -0.15$, $P < 0.0005$). In *D. luddeckei*, the only variable that contributed significantly to genetic distance was

elevational distance (elevation distance: $\beta = 0.24$, $P = 0.003$; LCP distance: $\beta = -0.033$, $P = 0.75$; topographic complexity: $\beta = 0.01$, $P = 0.83$).

We found that in all cases migration rate was larger in the downslope direction (Figure 3). We observed, however, that in the MCMC run, the population migration rate that supported the downslope direction ($2N_2m_2 > 2N_1m_1$) was found only in the Guina–Paipa, and Guadalupe–Las Brisas population pairs (Table 4).

Discussion

Elevation Distance and Gene Flow

Wiens and Donoghue (2004) argued that the sum of all factors that prevent species from colonizing suboptimal habitats determines the geographic distribution of species. Such factors may be intrinsic features of the organism, such as physiology, behavior, and genetics, and/or extrinsic such as population dynamics, competition, and predation (Gaston 2009). The existence of suboptimal regions within the geographic range of a species can restrict the amount of gene flow between subpopulations, and if the inability to colonize suboptimal regions persists through time the probability for allopatric divergence and speciation increases.

We found in both *D. labialis* and *D. luddeckei* that the elevational distance between populations was negatively associated with genetic distances (we assume that genetic distances are a proxy for gene flow). This rejects our hypothesis that elevational distances would not affect the genetic differentiation of individuals given the small ratio of vertical to horizontal distance. It seems, then, that the ecological differences caused by the altitudinal gradient restrict the abilities of populations of these 2 species to colonize new elevations, and that the elevational difference between populations is a barrier to gene flow, especially between pairs of populations at lower elevations.

Interestingly, the fact that the elevation gradient increases the genetic differentiation of populations provides a paradox: why are there populations at so many different elevations in these 2 species given individuals seem to avoid moving along the elevational gradient? Certainly different mechanisms influence the geographic distribution of populations within species. It is also clear that the climatic history of the Andes has not been constant through time (Hooghiemstra and Van der Hammen 2004). From the point of view of niche conservatism, organisms in the tropical Andes may have tracked shifting Pleistocene climates, moving uphill and/or downhill (following their optimal temperatures) more readily than evolving a new range of climate tolerances (Wiens and Graham 2005). Some authors suggest, however, that the rapid Pleistocene climate change may have pushed populations to their limits of adaptation, thus influencing the appearance of adaptations to novel temperatures, and therefore, novel elevations for the species (Davis and Shaw 2001). In other words, genetic adaptation due to rapid climate change may have allowed for the formation of montane species with wide elevational distributions in spite of the fact that within such species each population is adapted to a

Table 3 Partial Mantel correlation coefficients (Mantel's r) and P values from the causal model

Species	Partial Mantel scheme	r	P value
<i>Dendropsophus luddeckei</i>	TC $\times$ G. LCP	0.006	0.437
<i>Dendropsophus luddeckei</i>	TC $\times$ G. Elev	-0.004	0.450
<i>Dendropsophus luddeckei</i>	LCP $\times$ G. TC	0.106	***
<i>Dendropsophus luddeckei</i>	LCP $\times$ G. Elev	-0.012	0.390
<i>Dendropsophus luddeckei</i>	Elev $\times$ G. TC	0.146	***
<i>Dendropsophus luddeckei</i>	Elev $\times$ G. LCP	0.101	***
<i>Dendropsophus labialis</i>	TC $\times$ G. LCP	0.055	0.028
<i>Dendropsophus labialis</i>	TC $\times$ G. Elev	-0.198	***
<i>Dendropsophus labialis</i>	LCP $\times$ G. TC	0.801	***
<i>Dendropsophus labialis</i>	LCP $\times$ G. Elev	0.797	***
<i>Dendropsophus labialis</i>	Elev $\times$ G. TC	0.473	***
<i>Dendropsophus labialis</i>	Elev $\times$ G. LCP	0.428	***

The " $\times$ " indicates a correlation between 2 matrices, and the period indicates a third covariable that was controlled. Correlations significantly different from 0 after Bonferroni correction depicted in bold. Elev, elevational distances; G, genetic distances; TC, topographic complexity.

*** $P < 0.0001$.

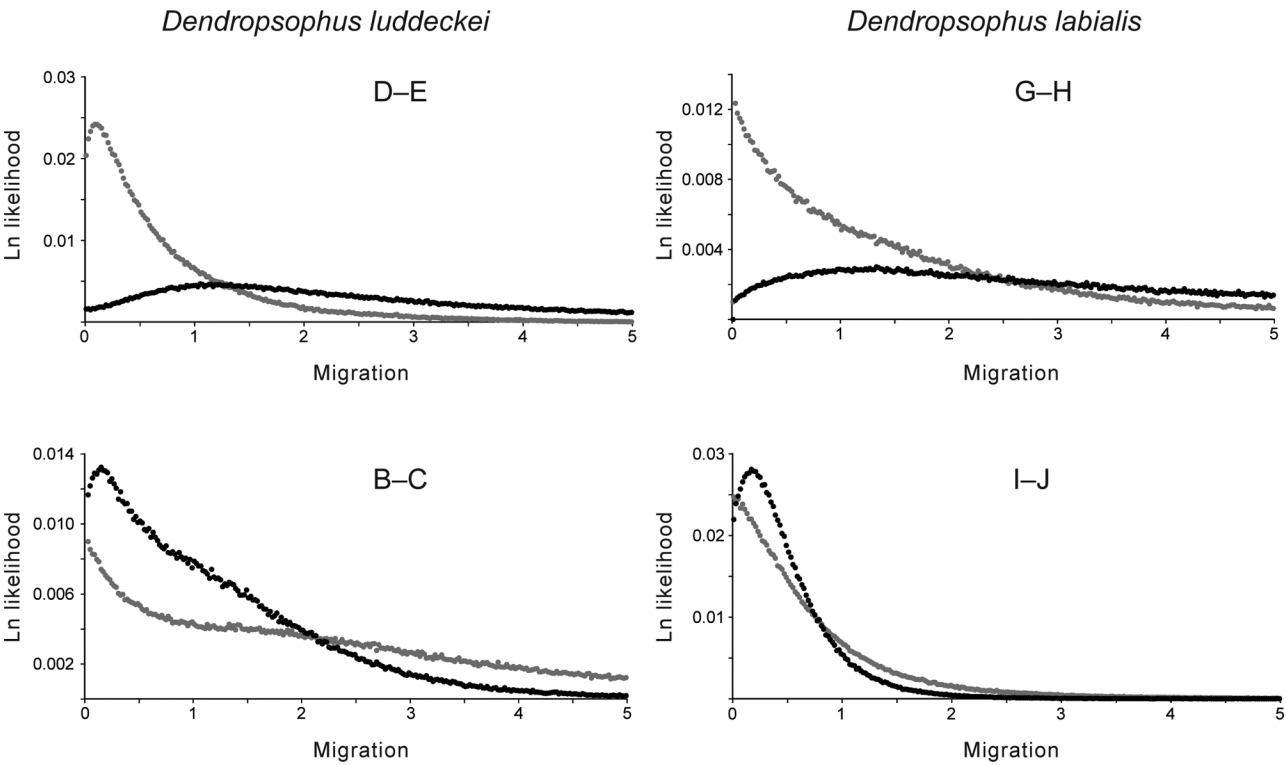


Figure 3. Posterior probability densities for migration rate in *Dendropsophus luddeckei* and *Dendropsophus labialis*. Dark curves: downslope direction; gray curves: upslope direction. Capital letters indicate the population map codes in Table 1.

Table 4 Demographic parameters (estimated with the peak probability distributions after smoothing) for *Dendropsophus luddeckei* and *Dendropsophus labialis*

Species	Population upslope	Population downslope	m_1 (upslope)	m_2 (downslope)	Proportion of time $2N_2m_2 > 2N_1m_1$
<i>Dendropsophus luddeckei</i>	D	E	0.090 (0.010–1.810)	1.130 (0.010–11.690)*	0.426
	B	C	0.010 (0.010–6.770)*	0.130 (0.010–2.630)	0.584
<i>Dendropsophus labialis</i>	I	J	0.010 (0.010–1.690)	0.150 (0.010–1.030)	0.727
	G	H	0.010 (0.010–9.190)*	1.310 (0.010–15.770)*	0.315

Capital letters correspond to the map codes in Table 1. m_1 and m_2 are the maximum migration rates from population 1 to population 2 and vice versa. The 90% upper posterior density intervals are in parentheses. Asterisks indicate multiple peaks along the interval. The last column is the proportion of time during the course of the MCMC run in which the population migration rate ($2N_m$) was higher in the downslope direction.

narrow elevation range. If the latter hypothesis is correct, allopatric speciation in the Andes along the horizontal axis would be more common than ecological speciation along the elevational axis. Some authors suggest this is the case (Lynch et al. 1997; Lynch 1999; Dingle et al. 2006; Guarnizo et al. 2012), suggesting that niche conservatism would be more common than niche divergence.

Why has elevational distance not been proposed more frequently as a factor that influences gene flow in montane species? Janzen (1967) proposed that tropical organisms should have narrow thermal tolerances and therefore low levels of gene flow along the elevation gradient. Conversely, in temperate regions, where seasonality in temperature exists, organisms should display wide thermal tolerances and high gene

flow along the elevation gradient. Recent studies have validated some of the main predictions of the Janzen hypotheses (Ghalambor et al. 2006; Cadena et al. 2012). We suggest then, that the potentially strong effect of elevational distance on gene flow in the tropics has not been detected yet because the majority of phylogeographic and landscape genetic studies with amphibians has been performed in temperate regions. The general significance of elevational distances across tropical montane species, therefore, remains to be tested.

LCP Distances and Gene Flow

We found that in *D. labialis* LCP distances (that are dependent on temperature and precipitation) were strongly negatively

associated with gene flow. This supports our hypothesis that LCP distances would display the strongest association with gene flow because LCP distance indirectly quantifies the presence of geographic features that are outside the species' physiological tolerances, and therefore, may behave as geographic barriers. We believe the reason that LCP distances and not elevational distances are correlated more closely with genetic distances is that LCP distance includes an isolation-by-distance component that elevational distances lack (since LCP and Euclidean distances are correlated, but elevation and Euclidean distances are not). *Dendropsophus labialis*, therefore, exemplifies a case in which both axes (horizontal and vertical) can be independently associated with genetic differentiation.

We hypothesized that topographic complexity (as measured by rugosity) would significantly reduce gene flow, given the abundant evidence from other vertebrates such as mammals (P rez-Espona et al. 2008; Neaves et al. 2009), birds (Cadena et al. 2007; Graham et al. 2010; Fjelds  et al. 2012), and amphibians (Funk et al. 2005; Giordano et al. 2007; Wollenberg et al. 2008; Kosciński et al. 2009; Murphy et al. 2010; Wang 2012). We found, however, a weak effect of topographic complexity on genetic distances. We believe the reason that LCP distance (and not topographic complexity) is more closely associated with gene flow is because topographic complexity was estimated along straight line vectors, whereas organisms likely do not disperse in straight lines between populations. In complex topographies such as the Andes a more appropriate way to estimate topographic complexity is to use the minimum convex polygons that encompass sampled populations instead of vectors, since polygons are more likely to identify the true impediments to gene flow across the landscape. In a recent paper, Guarnizo and Cannatella (2013) estimated topographic complexities using polygons (not vectors) in several frog species. The potential drawback of using polygons, however, is that only a single one topographic complexity estimate can be obtained per species. The advantage of using vectors is that multiple topographic complexity estimates can be obtained between locality pairs within a single species. Therefore, vectors may be more useful for intraspecific studies and polygons for interspecific ones.

Comparison Between Causal Modeling and MMRR

We found congruence between the causal modeling results and MMRR. However, MMRR provided more information about the independent effect of each variable on gene flow. For example, the causal model identified the variables that were significantly associated with the genetic distances, but it does not rank them in terms of their relative effect. MMRR, however, provided regression coefficients that rank the effect of each variable. Moreover, even though both methods correctly identified the strongest variable affecting genetic distances on each species (LCP distance in *D. labialis* and elevation distance in *D. luddeckei*), MMRR, and not causal modeling, was able to find a significant effect of some variables, even when these variables contributed less to the regression, such as topographic complexity in *D. labialis*. This may be a

consequence of the presumed low power of partial Mantel tests (Legendre and Fortin 2010). MMRR is a novel approach and therefore needs to be further scrutinized. However, we find it to be a useful alternative to Mantel tests.

Migration Asymmetry and Life History

Our results indicate that migration seems to be asymmetric in both *Dendropsophus* species since in all population pairs the maximum migration parameter was larger in the downslope direction. However, the evidence is not very strong, since the population mutation rate was larger in the downslope direction in only 50% of the runs (Table 4). Moreover, our posterior probability distributions are rather broad, implying a reduced certainty about inferences of asymmetric gene flow. Nevertheless, the fact that the population pair separated by the largest elevational distance (Las Brisas—Guadalupe) had the largest support for downslope dispersal suggests that it is more likely to detect the asymmetric dispersal pattern when the differences in elevation between the 2 populations are large.

Our finding of downslope migration might be due to the fact that 1) these 2 sister species lay eggs in ponds from which water overflow moves downhill, making eggs and/or larvae more likely to passively migrate to low elevations than the reverse; and 2) populations at lower elevations seem to have narrower thermal tolerances in locomotor performance than populations at higher elevations (Navas 2006), presumably, because thermal variation is larger at higher elevations. Therefore, from the perspective of thermal physiology, individuals from high elevations can more easily colonize the lowlands than the reverse.

Given that most of the polymorphic sites used to estimate migration asymmetry are from a mitochondrial gene (control region), the asymmetric migration rate estimates might be female biased. Nevertheless, there is evidence that both males and females of *D. labialis* are highly philopatric (L ddecke and Am zquita 2010), decreasing the possible migration rate differences between males and females.

Although our population and genetic sampling are limited, the important effects of the horizontal and vertical landscape components on the amount and direction of the migration rate are evident. Identifying the most relevant mechanisms promoting diversity in tropical mountains should be a priority, as high mountain ecosystems are the most vulnerable to extinctions due to climate change (Still et al. 1999; Moritz et al. 2008).

Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>.

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References

- Angilletta M. 2002. The evolution of thermal physiology in ectotherms. *J Thermal Biol.* 27:249–268.
- Bolnick D, Caldera E, Matthews B. 2008. Evidence for asymmetric migration load in a pair of ecologically divergent stickleback populations. *Biol J Linnean Soc.* 94:273–287.
- Bonnet E, Van de Peer Y. 2002. zt: a software tool for simple and partial Mantel tests. *J Stat Softw.* 7:1–12.
- Cadena CD, Klicka J, Ricklefs RE. 2007. Evolutionary differentiation in the Neotropical montane region: molecular phylogenetics and phylogeography of *Buarremon* brush-finches (Aves, Emberizidae). *Mol Phylogenet Evol.* 44:993–1016.
- Cadena CD, Kozak KH, Gómez JP, Parra JL, McCain CM, Bowie RC, Carnaval AC, Moritz C, Rahbek C, Roberts TE, et al. 2012. Latitude, elevational climatic zonation and speciation in New World vertebrates. *Proc Biol Sci.* 279:194–201.
- Collins CJ, Fraser CI, Ashcroft A, Waters JM. 2010. Asymmetric dispersal of southern bull-kelp (*Durvillaea antarctica*) adults in coastal New Zealand: testing an oceanographic hypothesis. *Mol Ecol.* 19:4572–4580.
- Consuegra S, Verspoor E, Knox D, Garcia de Leaniz C. 2005. Asymmetric gene flow and the evolutionary maintenance of genetic diversity in small, peripheral Atlantic salmon populations. *Conserv Genet.* 6:823–842.
- Crawford AJ. 2003. Huge populations and old species of Costa Rican and Panamanian dirt frogs inferred from mitochondrial and nuclear gene sequences. *Mol Ecol.* 12:2525–2540.
- Cushman SA, Landguth EL. 2010. Spurious correlations and inference in landscape genetics. *Mol Ecol.* 19:3592–3602.
- Cushman SA, McKelvey KS, Hayden J, Schwartz MK. 2006. Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *Am Nat.* 168:486–499.
- Davis MB, Shaw RG. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science.* 292:673–679.
- Dingle C, Lovette IJ, Canaday C, Smith TB. 2006. Elevational zonation and the phylogenetic relationships of the *Henicorhina* wood-wrens. *Auk.* 123:119–134.
- ESRI. 2011. ArcGIS Desktop: Release 10. Redlands (CA): Environmental Systems Research Institute.
- Feder M, Burggren W. 1992. Environmental Physiology of the Amphibians. Chicago (IL): University of Chicago Press.
- Fjeldså J, Bowie RC, Rahbek C. 2012. The role of mountain ranges in the diversification of birds. *Annu Rev Ecol Evol Syst.* 43:249–265.
- Funk WC, Blouin MS, Corn PS, Maxell BA, Pilliod DS, Amish S, Allendorf FW. 2005. Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Mol Ecol.* 14:483–496.
- Gaston KJ. 2009. Geographic range limits: achieving synthesis. *Proc Biol Sci.* 276:1395–1406.
- Ghalambor CK, Huey RB, Martin PR, Tewksbury JJ, Wang G. 2006. Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr Comp Biol.* 46:5–17.
- Giordano AR, Ridenhour BJ, Storfer A. 2007. The influence of altitude and topography on genetic structure in the long-toed salamander (*Ambystoma macrodactylum*). *Mol Ecol.* 16:1625–1637.
- Goebel AM, Donnelly JM, Atz ME. 1999. PCR primers and amplification methods for 12S ribosomal DNA, the control region, cytochrome oxidase I, and cytochrome b in bufonids and other frogs, and an overview of PCR primers which have amplified DNA in amphibians successfully. *Mol Phylogenet Evol.* 11:163–199.
- Graham CH, Silva N, Velásquez-Tibatá J. 2010. Evaluating the potential causes of range limits of birds of the Colombian Andes. *J Biogeogr.* 37:1863–1875.
- Guarnizo CE, Amézquita A, Bermingham E. 2009. The relative roles of vicariance versus elevational gradients in the genetic differentiation of the high Andean tree frog, *Dendropsophus labialis*. *Mol Phylogenet Evol.* 50:84–92.
- Guarnizo CE, Escallón C, Cannatella DC, Amézquita A. 2012. Congruence between acoustic traits and genealogical history reveals a new species of *Dendropsophus* (Anura: Hylidae) in the high Andes of Colombia. *Herpetologica.* 68:523–540.
- Guarnizo CE, Cannatella DC. 2013. Genetic divergence within frog species is greater in topographically more complex regions. *J Zool Syst Evol Res.* doi:10.1111/jzs.12027.
- Guillot G, Rousset F. 2013. Dismantling the Mantel test. *Methods Ecol Evol.* 4:336–344.
- Hall J. 2005. Montane speciation patterns in *Ithomiola* butterflies (Lepidoptera: Riodinidae): are they consistently moving up in the world? *Proc R Soc Lond B Biol Sci.* 272:2457–2466.
- Harmon LJ, Glor RE. 2010. Poor statistical performance of the Mantel test in phylogenetic comparative analyses. *Evolution.* 64:2173–2178.
- Hey J. 2005. On the number of New World founders: a population genetic portrait of the peopling of the Americas. *PLoS Biol.* 3:e193.
- Hey J, Nielsen R. 2004. Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. *Genetics.* 167:747–760.
- Hijmans R, Cameron S, Parra J, Jones P, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol.* 25:1965–1978.
- Hooghiemstra H, Van der Hammen T. 2004. Quaternary Ice-Age dynamics in the Colombian Andes: developing an understanding of our legacy. *Philos Trans R Soc Lond B Biol Sci.* 359:173–80; discussion 180.
- Hudson RR, Kaplan NL. 1985. Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics.* 111:147–164.
- Jansson R, Davies TJ. 2008. Global variation in diversification rates of flowering plants: energy vs. climate change. *Ecol Lett.* 11:173–183.
- Janzen D. 1967. Why mountain passes are higher in the tropics. *Am Nat.* 101:233–249.
- Kawecki TJ, Holt RD. 2002. Evolutionary consequences of asymmetric dispersal rates. *Am Nat.* 160:333–347.
- Kebede M, Ehrlich D, Taberlet P, Nemomissa S, Brochmann C. 2007. Phylogeography and conservation genetics of a giant lobelia (*Lobelia giberroa*) in Ethiopian and Tropical East African mountains. *Mol Ecol.* 16:1233–1243.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 16:111–120.
- Körner C. 2007. The use of 'altitude' in ecological research. *Trends Ecol Evol.* 22:569–574.
- Koscinski D, Yates AG, Handford P, Lougheed SC. 2009. Effects of landscape and history on diversification of a montane, stream-breeding amphibian. *J Biogeogr.* 36:255–265.
- Kurabayashi A, Yoshikawa N, Sato N, Hayashi Y, Oumi S, Fujii T, Sumida M. 2010. Complete mitochondrial DNA sequence of the endangered frog

- Odorrana ishikawae* (family Ranidae) and unexpected diversity of mt gene arrangements in ranids. *Mol Phylogenet Evol.* 56:543–553.
- Legendre P, Troussellier M. 1988. Aquatic heterotrophic bacteria: modeling in the presence of spatial autocorrelation. *Limnol Oceanogr.* 33:1055–1067.
- Legendre P, Fortin MJ. 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Mol Ecol Resour.* 10:831–844.
- Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 25:1451–1452.
- Lüddecke H, Amézquita A. 2010. Populational short and long term fluctuations in the high Andean frog *Dendropsophus labialis*. *Rev Ecol Latinoam.* 15:1–6.
- Lynch JD, Ruiz-Carranza PM, Ardila-Robayo MC. 1997. Biogeographic patterns of Colombian frogs and toads. *Rev Acad Colomb Cienc.* 21:237–248.
- Lynch JD. 1999. Ranas pequeñas, la geometría de evolución, y la especiación en los Andes colombianos. *Rev Acad Colomb Cienc.* 23:143–159.
- Moritz C, Patton JL, Conroy CJ, Parra JL, White GC, Beissinger SR. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. *Science.* 322:261–264.
- Murphy MA, Dezzani R, Pilliod DS, Storfer A. 2010. Landscape genetics of high mountain frog metapopulations. *Mol Ecol.* 19:3634–3649.
- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature.* 403:853–858.
- Navas CA. 2002. Herpetological diversity along Andean elevational gradients: links with physiological ecology and evolutionary physiology. *Comp Biochem Physiol A Mol Integr Physiol.* 133:469–485.
- Navas CA. 2006. Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integr Comp Biol.* 46:82–91.
- Neaves LE, Zenger KR, Prince RI, Eldridge MD, Cooper DW. 2009. Landscape discontinuities influence gene flow and genetic structure in a large, vagile Australian mammal, *Macropus fuliginosus*. *Mol Ecol.* 18:3363–3378.
- Orme CD, Davies RG, Burgess M, Eigenbrod F, Pickup N, Olson VA, Webster AJ, Ding TS, Rasmussen PC, Ridgely RS, et al. 2005. Global hotspots of species richness are not congruent with endemism or threat. *Nature.* 436:1016–1019.
- Pérez-Espona S, Pérez-Barbería FJ, McLeod JE, Jiggins CD, Gordon IJ, Pemberton JM. 2008. Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Mol Ecol.* 17:981–996.
- Phillips S, Anderson R, Schapire R. 2006. Maximum entropy modeling of species geographic distributions. *Ecol Model.* 190:231–259.
- Pianka E. 1966. Latitudinal gradients in species diversity: a review of concepts. *Am Nat.* 100:33–46.
- Pringle JM, Blakeslee AM, Byers JE, Roman J. 2011. Asymmetric dispersal allows an upstream region to control population structure throughout a species' range. *Proc Natl Acad Sci USA.* 108:15288–15293.
- Raufaste N, Rousset F. 2001. Are partial mantel tests adequate? *Evolution.* 55:1703–1705.
- Richards-Zawacki CL. 2009. Effects of slope and riparian habitat connectivity on gene flow in an endangered Panamanian frog, *Atelopus varius*. *Divers Distrib.* 15:796–806.
- Roberts J, Brown J, May R, Arizabal W, Schulte R, Summers K. 2006. Genetic divergence and speciation in lowland and montane peruvian poison frogs. *Mol Phylogenet Evol.* 41:149–164.
- Rohde K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. *Oikos.* 65:514–527.
- Rousset F. 2000. Genetic differentiation between individuals. *J Evol Biol.* 13:58–62.
- Rowe G, Harris DJ, Beebe TJ. 2006. Lusitania revisited: a phylogeographic analysis of the natterjack toad *Bufo calamita* across its entire biogeographical range. *Mol Phylogenet Evol.* 39:335–346.
- Selonen V, Hanski IK, Painter JN. 2010. Gene flow and natal dispersal in the Siberian flying squirrel based on direct and indirect data. *Conserv Genet.* 11:1257–1264.
- Smith M, Green D. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography.* 28:110–128.
- Smith SA, de Oca AN, Reeder TW, Wiens JJ. 2007. A phylogenetic perspective on elevational species richness patterns in Middle American treefrogs: why so few species in lowland tropical rainforests? *Evolution.* 61:1188–1207.
- Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet.* 68:978–989.
- Still CJ, Foster PN, Schneider SH. 1999. Simulating the effects of climate change on tropical montane cloud forests. *Nature.* 398:608–610.
- Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol.* 24:1596–1599.
- Vieites DR, Min MS, Wake DB. 2007. Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proc Natl Acad Sci USA.* 104:19903–19907.
- Wang IJ. 2012. Environmental and topographic variables shape genetic structure and effective population sizes in the endangered Yosemite toad. *Divers Distrib.* 18:1033–1041.
- Wang IJ. 2013. Examining the full effects of landscape heterogeneity on spatial genetic variation: a multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution.* doi:10.1111/evo.12134
- Wang IJ, Summers K. 2010. Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Mol Ecol.* 19:447–458.
- Wiens JJ, Donoghue MJ. 2004. Historical biogeography, ecology and species richness. *Trends Ecol Evol.* 19:639–644.
- Wiens JJ, Graham CH. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu Rev Ecol Evol Syst.* 36:519–539.
- Wiens JJ, Graham CH, Moen DS, Smith SA, Reeder TW. 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hyliid frogs: treefrog trees unearth the roots of high tropical diversity. *Am Nat.* 168:579–596.
- Woerner AE, Cox MP, Hammer MF. 2007. Recombination-filtered genomic datasets by information maximization. *Bioinformatics.* 23:1851–1853.
- Wollenberg KC, Vieites DR, van der Meijden A, Glaw F, Cannatella DC, Vences M. 2008. Patterns of endemism and species richness in Malagasy cophyline frogs support a key role of mountainous areas for speciation. *Evolution.* 62:1890–1907.
- Zawada DG, Piniak GA, Hearn CJ. 2010. Topographic complexity and roughness of a tropical benthic seascape. *Geophys Res Lett.* 37:L14604.

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