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Genetic divergence within frog species is greater in topographically more complex regions

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Abstract

Most global hotspots of biodiversity and endemism are in montane regions. One explanation is that montane regions have intrinsically higher speciation rates than lowland regions because complex mountain topography and climate variation facilitate genetic isolation among populations. Here, we ask from an intraspecific perspective whether frog species whose haplotypes are connected by topographically/climatically complex regions display strong genetic isolation (greater scaled genetic distances), compared with species whose haplotypes are connected by less complex regions. We analysed published DNA sequences of several frog species from tropical Central and South America for the mitochondrial *cob*, *cox1* and *16S* rRNA genes. Pairwise genetic distances among haplotypes within each species were scaled to the geographic distances between each pair of haplotypes. Topographic complexity was positively correlated with scaled genetic distances, and isolation-by-resistance was supported only in species from more topographically complex regions. This suggests that heterogeneous topographies increase landscape resistance, which in turn favours the appearance of isolation-by-resistance. Moreover, we found that the potential barriers that restrict gene flow within species are more closely related to factors associated with temperature and topography than to precipitation.

Key words: Topographic complexity – landscape resistance – mountains – genetic divergence – speciation

Introduction

Regions of very high species diversity may reveal not only the general mechanisms that promote genetic divergence but also those biotic and abiotic factors that are relevant for biological diversification in geographic regions. One such region is the tropical Andes, a global hotspot of species richness and endemism for plants and vertebrates (Myers et al. 2000; Orme et al. 2005). Historically, researchers have compared Andean diversity patterns for a multitude of species with patterns of the surrounding lowlands. For example, Duellman (1999) found beta diversity levels for amphibians in the tropical Andes (753 species, 95% endemic) much higher than in the Amazon–Guyana lowlands (335 species, 82% endemic) or the Chocó (126 species, 42% endemic). Highland regions with high diversity and endemism are widespread in other tropical regions such as the Great Rift Valley in Africa (Orme et al. 2005), the Guyana highlands (Rull 2005), the Indian subcontinent highlands (Roelants et al. 2004) and the Panamá and Costa Rica highlands in Central America (Mittermeier et al. 1998).

Two non-exclusive hypotheses may explain some of these highland diversity patterns. The first is that highland diversity is due to multiple dispersals from the lowlands. In contrast, the second is that montane regions are characterized by intrinsically higher rates of speciation (and/or lower rates of extinction) compared with the lowlands. Currently, multiple lines of evidence suggest that the latter hypothesis is better supported in some regions such as the Andes, because its diversity largely evolved *in situ* (Kroner et al. 2005; Hughes and Eastwood 2006; Santos et al. 2009; Sedano and Burns 2010).

Explicit mechanisms that explain local diversity patterns within the tropical highlands include the accumulation of species through time. Wiens et al. (2006) suggested that the high diversity of mid-elevation hyliid frogs of Central America is explained better by the old colonization times of these regions than by differences in rates of diversification between lowlands and high-

lands. In some African, Middle Eastern and South American lineages, however, climatic cycles combined with historical refugia have been suggested as the main promoter for species diversification in the mountains (Gvoždík et al. 2010; Lawson 2010; García-R et al. 2012). In contrast, Gonzales-Voyer et al. (2011) argued that within tropical highlands, it is not clade age or climatic cycles what explain among-clade variation in species richness, but rather a combination of morphological and ecological traits. Ecological traits (smaller body sizes) were also attributed to influence genetic variation patterns in mountain frogs of Madagascar (Pabijan et al. 2012).

As opposed to studies in temperate regions that treat intraspecific divergence (Martin and McKay 2004), the factors that promote genetic divergence within the tropics have been examined primarily from an interspecific perspective. In particular, factors that contribute to intraspecific divergence across different species in tropical montane regions have not been considered. Studies that focus on the intraspecific genetic variation of populations are important for understanding early stages of speciation. For instance, in some cases it has been demonstrated that if intraspecific genetic divergence is reinforced by ecological divergence, it is more likely that speciation will occur in spite of various degrees of gene flow (Irwin et al. 2001). Therefore, montane regions, which are topographically heterogeneous and display a gradient of ecological conditions, should be more prone to show genetic divergence among populations compared with topographically homogeneous regions. Evidence from different taxonomic groups supports this scenario (Moritz et al. 2000 [vertebrates], Friedlander et al. 2008 [humans], Wollenberg et al. 2008 [frogs], Kosciński et al. 2009 [frogs]).

Topographically homogeneous regions, however, often display heterogeneous environments (high ecological diversity) that might provide different levels of resistance to gene flow among populations (McRae 2007). Such regions usually are underestimated in studies focusing on topographic complexity as the single variable to which gene flow is compared, which is why it is desirable to consider landscape resistance (McRae 2007).

Here, we use tropical frogs from Central and South America as models to determine how intraspecific genetic divergence is

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affected by both the species topography (measured as topographic complexity) and the climatic environment (measured as landscape resistance). Specifically, we tested whether genetic differentiation per unit geographic distance was greater in species whose individuals were scattered along regions characterized by high topographic (resistance) heterogeneity, relative to species whose individuals were scattered along more homogenous regions. Frogs are ideal for this purpose given their small home range and high physiological susceptibility to the external environment compared with other vertebrates (Zeisset and Beebe 2008).

Materials and Methods

DNA sequence retrieval

DNA sequences from eight families of tropical frogs in Central and northern South America were obtained from GenBank (See Table S1 for geographic coordinates of the haplotypes and the GenBank sequences authors). We used phylogeographic studies that explicitly indicated the geographic coordinates for each sequence or provided a map with sampling localities that could be georeferenced. GenBank sequences of the mitochondrial genes cytochrome b (*cob*), cytochrome oxidase I (*cox1*) and 16S ribosomal RNA genes were selected because the abundance of these allows comparative analysis. Sequences were aligned with MUSCLE (Edgar 2004) and then trimmed to the same length.

Given that the differences in the number of sampled localities across species potentially might bias genetic patterns, we tested the effect of species with either a very large or very small number of sampled localities by excluding these species, following Quintero and Wiens (2012). We found, however, that the exclusion of these species did not affect the main relationships (not shown), and therefore, all the results include these species.

Intraspecific genetic divergence

The overall genetic divergence characteristic of each species was quantified in two ways: using the maximum-scaled genetic distance (the largest scaled genetic distance found across all haplotype pairs within a single species) and the mean-scaled genetic distance. The scaling was performed dividing each genetic distance by the great-circle distance (the shortest distance between two points on the surface of a sphere) connecting each haplotype pair (Martin and McKay 2004). This scaling yielded comparable genetic distances across pairwise comparisons, as organisms with low vagility such as frogs generally display isolation-by-distance (Slatkin 1993; Mønsen and Blouin 2004; Fouquet et al. 2007).

We are aware that topography-corrected distances have been used to determine more accurately the actual distance that organisms would move among populations. We preferred great-circle distances over topography-corrected distances for two reasons: First, topography-corrected distances are meaningful at small geographic scales, when horizontal and vertical (elevation) distances between populations are of comparable scale. In our study, however, great-circle distances between populations are on the order of tens of thousands of meters, but changes in elevation are on the order of hundreds of meters at most. Therefore, using topography-corrected distances instead of great-circle distances would not effectively change the scaling of genetic distances. Second, we did not include topography-corrected distances as it might be misleading to correct geographic distances along a vector by topography and then correlate such topography-corrected distances with the topographic complexity (rugosity) of an area (as will be described below).

The rationale for estimating maximum-scaled genetic distances is based on the variety of sampling designs that characterize the GenBank sequences. For instance, assuming isolation-by-distance, a sample of multiple individuals per geographic locality would display a higher proportion of 0.0 genetic distances per kilometre compared with samples scattered across the landscape. Thus, mean estimates for genetic divergence are biased if multiple haplotypes from the same locality are analysed. Maximum genetic distances per kilometre do not suffer from this bias as they indicate the highest scaled genetic divergence that occurs

across all individuals sampled within each species, independent of sampling design. A drawback of maximum-scaled genetic distances is that they are susceptible to outliers, which is why mean-scaled genetic distances per species were also estimated.

Genetic distances between all haplotype pairs were estimated with the Kimura-2 parameter (K2P) model (Kimura 1980) using MEGA 4.0 (Tamura et al. 2007). The K2P model was chosen to facilitate comparison with other studies performed with frogs (Vences et al. 2005a; Crawford et al. 2010). In MEGA, we used the complete-deletion option, and therefore, gaps were not included in the genetic distance calculations. Great-circle distances were quantified using the R-Package (Legendre and Vaudor 1991).

Topographic complexity

The topographic complexity of habitat was estimated as rugosity, a term commonly used in oceanography to characterize surface complexity in coral reefs (Zawada et al. 2010). We divided the surface area of the minimum convex polygon encompassing all sampled localities for each species (which takes into account three-dimensional relief) by its planar area. Values close to one indicate low topographic complexity (flatter regions), and values higher than one indicate high complexity because surface area increases with increasing inclination relative to the planar area. Because this method estimates the topographic complexity of an area, it may identify landscape formations restricting gene flow that are harder to find by other methods based on topographic variation along vectors (Murphy et al. 2010). The minimum convex polygons were estimated with the Hawth's tools extension (www.spatialecology.com) for ArcGis 9.0 (ESRI). Rugosity was calculated with the 3D analyst of ArcGis 9.0 (ESRI), based on a 250 m-resolution STRM digital elevation model (<http://srtm.csi.cgiar.org/>). To test the relationship between topographic complexity and the intraspecific genetic divergence across species for each gene, we used Spearman's rank correlation coefficient.

Landscape resistance

We used circuit theory to estimate isolation-by-resistance between all pairs of individuals within a species (McRae 2007). Circuit theory simultaneously considers all pairwise paths connecting populations and estimates landscape resistance as an analogue of electrical resistance, in which movements of individuals and gene flow are analogues of electrical current (McRae 2007).

To determine how landscape resistance was associated with gene flow, we used causal modelling (Fig. 1) to test whether landscape resistances, great-circle distances, both, or none, explained genetic distances, following McRae and Beier (2007).

The resistance matrix was estimated following Wang and Summers (2009), which assumes that regions in which the species has low probability of occurrence have high costs to migration. We estimated the

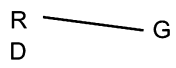
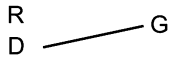
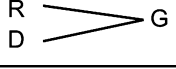
Model A		RXG.D DXG.R	Sig. N.S.
Model B		RXG.D DXG.R	N.S. Sig.
Model C		RXG.D DXG.R	Sig. Sig.
Model D		RXG.D DXG.R	N.S. N.S.

Fig. 1. Causal model scheme for the expected relationship between landscape resistances (R), great-circle distances (D) and genetic distances (G). Model A: Isolation-by-resistance. Model B: Isolation-by-distance. Model C: Both isolation-by-resistance and distance. Model D: Neither isolation-by-resistance nor distance. The 'x' indicates a correlation between two matrices. The '.' indicates the covariable that is controlled in the partial Mantel test. Sig = significant correlation, NS = non-significant correlation

distribution probability models for each species based on 19 bioclimatic layers with 30 arc-second resolution (Hijmans et al. 2005) using MAXENT V3.3.1 (Phillips et al. 2006). The same localities included in the genetic analyses were used to estimate the models. We assessed the accuracy of the distribution probabilities with a receiver operating characteristic (ROC) analysis (Phillips et al. 2006), randomly selecting 25% of occurrence records and 10 000 cells as background points.

The distribution probability models generated by MAXENT were used as inputs for the program Circuitscape (McRae and Shah 2009) to model landscape resistances. Because the 19 bioclimatic variables used to build the resistance matrix are associated with precipitation and temperature, they contain coarse ecological information associated not only with the topographic and climatic properties of the habitats that potentially behave as barriers but also with the physiological effects that restriction movement of individuals across these regions. For this analysis, we included the species that had sequences from *cob*, as this gene had the largest sample size and geographic coverage of individuals.

Rates of DNA evolution

Intraspecific genetic variation across species may depend not only on abiotic factors such as topography and climate but also on biotic ones such as differences in the rate of DNA evolution. For instance, the differences in genetic variation between species from complex and non-complex topographies might not be the result of topography itself, but rather because of the differences in the rate of DNA evolution of the species between these two geographic regions. For this reason, we tested for rate heterogeneity across species. We used a relative rate test based on the Langley-Fitch (1974) method implemented in r8s (Sanderson 2006), to determine whether the rates of evolution for *cob*, *cox1* and *16S* were uniform across the tree (null hypothesis). Significance was assessed with a chi-square test with one degree of freedom. The phylogenies used to perform this test were estimated with maximum likelihood (ML) using RAxML-VI-HPC (Stamatakis 2006) with a GTRGAMMA model of evolution and a standard heuristic search with 1000 replicates. To root the trees, we used *Microhyla rubra* (GenBank AB201224) for *cob*, *Microhyla heymonsi* (AY458596) for *cox1* and *M. rubra* (AB201192) for *16S*.

The possibility of cryptic species

Morphologically indistinguishable (cryptic), yet genetically divergent, species are well known in frogs (Vences et al. 2005a; Elmer and Cannatella 2008). Given that sequences were obtained from studies of different geographic scope, some individuals may represent cryptic species, depending on one's species criterion.

To prune highly divergent sequences that might correspond to cryptic species, we generated scatter plots of genetic distance versus geographic distance (Good and Wake 1992). If two well-defined clusters of points divergent in the genetic distance axis (y-axis) were observed to overlap in the geographic distance axis (x-axis), we generated a gene tree determining the genetic divergence between the two major clades (Figure S1). If such genetic divergences were >5% (in *cox1* and *cob*) and >2.5% (in *16S*), we assumed the two clades were two cryptic species (as they maintain a large genetic divergence in spite of the close geographic proximity) and therefore selected the clade with the greater number of haplotypes to use in the analyses. This is a conservative limit, as the proposed threshold for candidate species in frogs using *cox1* is ~10% (Vences et al. 2005a) and ~5% in *16S* (Vences et al. 2005b). If the scatter plot generated a smooth pattern with no evident gaps in the y-axis and/or a gap in the y-axis with no overlap in the x-axis (that can result from unsampled geographic regions), then we assumed the individuals belonged to a single species (Figure S1). This procedure avoids the problems caused by differing criteria of species delimitation among authors (Sites and Marshall 2004).

Results

In total, 145 *cox1* haplotypes (alignment length: 488 bp) were found for seven species, 385 *cob* haplotypes (alignment length: 337 bp) for 12 species and 258 *16S* haplotypes (alignment length: 390 bp) for nine species (Table 1). No indels were found

in either the *cob* and *cox1* gene; there were two short indels in *16S* for the species *Dendrobates pumilio*, *Dendropsophus labialis* and *Dendropsophus luddeckei* that were not included in the across-species comparisons. GenBank accession numbers and associated geographic coordinates are in Table S1. All sequences in Table S1 were used in the analyses. Identical haplotypes within species were not removed to prevent sampling bias. The scatter plots between geographic and genetic distances (after removing the potential cryptic species for each gene) are displayed in Figure S2. Because of the possibility of poaching endangered species, geographic coordinates for *A. varius* are not included, as requested by C. Richards-Zawacki. *Atelopus*, *Allobates*, *Ameerega* and *Dendrobates* were characterized by lowland distributions and small elevation ranges, except for *A. varius*, which had an elevational range of at least 1000 meters. *Craugastor* and *Physalaemus* had on average larger elevational ranges than *Atelopus*, but these species are restricted to lower elevations. Both *Dendropsophus* species had wide elevation ranges and by far reached the highest elevations (Table 2).

In terms of the relationship between topographic complexity and gene flow, we found a positive relationship between rugosity and scaled maximum K2P distances km^{-1} for each gene (Fig. 2). The correlation was marginally significant for *cob* and *cox1* and marginally not significant for *16S* (Table 3). We also found a positive and marginally significant relationship between rugosity and mean K2P distances km^{-1} for *cox1* and *16S*, but the relationship was not significant for *cob* (Figure S4, Table 3).

Regarding the relationship between resistance distances and gene flow, causal modelling fully supported model A in

Table 1. Species analysed and the number of sequences retrieved from GenBank

Family	Species	<i>cox1</i>	<i>cob</i>	<i>16S</i>
Bufonidae	<i>Atelopus flavescens</i>	–	19	–
Bufonidae	<i>Atelopus franciscus</i>	–	14	–
Bufonidae	<i>Atelopus hoogmoedi</i>	–	29	–
Bufonidae	<i>Atelopus spumarius barbotini</i>	–	9	–
Bufonidae	<i>Atelopus varius</i>	25	25	–
Craugastoridae	<i>Craugastor crassidigitus</i>	27	27	–
Craugastoridae	<i>Craugastor fitzingeri</i>	30	20	–
Craugastoridae	<i>Craugastor talamancae</i>	5	7	–
Craugastoridae	<i>Craugastor podiciferus</i>	–	–	31
Hylidae	<i>Dendropsophus labialis</i>	21	41	–
Hylidae	<i>Dendropsophus luddeckei</i>	13	37	9
Hylidae	<i>Dendropsophus leucophyllatus</i>	–	–	14
Hylidae	<i>Dendropsophus ebraccatus</i>	–	–	48
Dendrobatidae	<i>Ameerega hahneli</i>	–	–	23
Dendrobatidae	<i>Ameerega trivittata</i>	–	–	26
Dendrobatidae	<i>Dendrobates pumilio</i>	–	–	22
Dendrobatidae	<i>Allobates femoralis</i>	–	–	21
Strabomantidae	<i>Pristimantis kichwarum</i>	–	68	–
Odontophrynidae	<i>Proceratophrys boiei</i>	–	54	–
Leptodactylidae	<i>Physalaemus pustulosus</i>	24	–	–
Leptodactylidae	<i>Physalaemus petersi</i>	–	–	64

Scientific names authorities: *Atelopus flavescens* Duméril and Bibron, 1841; *Atelopus franciscus* Lescure, 1974; *Atelopus spumarius barbotini* Lescure, 1981; *Atelopus varius* Lichtenstein and Martens, 1856; *Craugastor crassidigitus* (Taylor, 1952); *Craugastor fitzingeri* (Schmidt, 1857); *Craugastor talamancae* (Dunn, 1931); *Craugastor podiciferus* (Cope, 1886); *Dendropsophus labialis* (Peters, 1863); *Dendropsophus luddeckei* Guarnizo, Escallón, Cannatella, and Amézquita 2012 *Dendropsophus leucophyllatus* (Beireis, 1783); *Dendropsophus ebraccatus* (Cope, 1874); *Ameerega hahneli* (Boulenger, 1884); *Ameerega trivittata* (Spix, 1824); *Dendrobates pumilio* Schmidt, 1857; *Allobates femoralis* (Boulenger, 1884); *Pristimantis kichwarum* Elmer and Cannatella 2008; *Proceratophrys boiei* (Wied-Neuwied, 1824); *Physalaemus pustulosus* (Cope, 1864); and *Physalaemus petersi* (Jiménez de la Espada, 1872).

Table 2. Genetic and geographic parameters for each gene/species included in the analysis

Gene	Species	Max gen dist/km	Mean gen dist/km	Rugosity	Number of localities sampled	Median elevation (m)	Min elevation (m)	Max elevation (m)
<i>cob</i>	<i>Atelopus flavescens</i>	0.005970	0.001260	1.00135	4	28	4	105
<i>cob</i>	<i>Atelopus franciscus</i>	0.012048	0.001300	1.00035	5	182	132	468
<i>cob</i>	<i>Atelopus hoogmoedi</i>	0.008982	0.000540	1.00071	6	272	161	394
<i>cob</i>	<i>Atelopus s. barbotini</i>	0.006192	0.000860	1.00013	3	217	213	314
<i>cob</i>	<i>Atelopus varius</i>	0.033346	0.001811	1.01221	7	667	102	1031
<i>cob</i>	<i>Craugastor crassidigitus</i>	0.030504	0.000657	1.00768	16	489	43	1419
<i>cob</i>	<i>Craugastor fitzingeri</i>	0.002976	0.000091	1.00631	15	217	16	1091
<i>cob</i>	<i>Craugastor talamancae</i>	0.000960	0.000398	1.00097	6	244	0	295
<i>cob</i>	<i>Dendropsophus labialis</i>	0.005970	0.000272	1.00819	13	2820	1922	3638
<i>cob</i>	<i>Dendropsophus luddeckei</i>	0.011983	0.000368	1.01451	9	2699	2197	3436
<i>cob</i>	<i>Pristimantis kichwarum</i>	0.002976	0.000083	1.00058	6	348	270	663
<i>cob</i>	<i>Proceratophrys boiei</i>	0.008982	0.000736	1.00144	3	799	591	1053
<i>cox1</i>	<i>Atelopus varius</i>	0.010225	0.000948	1.01033	9	737	58	1144
<i>cox1</i>	<i>Craugastor crassidigitus</i>	0.002781	0.000592	1.00769	14	612	155	1305
<i>cox1</i>	<i>Craugastor fitzingeri</i>	0.000975	0.000101	1.00453	20	249	22	1311
<i>cox1</i>	<i>Craugastor talamancae</i>	0.000527	0.000267	1.00513	4	108	0	528
<i>cox1</i>	<i>Dendropsophus labialis</i>	0.008150	0.000651	1.00842	9	2900	2541	3664
<i>cox1</i>	<i>Dendropsophus luddeckei</i>	0.004065	0.000446	1.01109	6	2563	2122	2960
<i>cox1</i>	<i>Physalaemus pustulosus</i>	0.002384	0.000146	1.00465	23	92	0	802
<i>16S</i>	<i>Allobates femoralis</i>	0.003048	0.000074	1.00052	15	151	56	1858
<i>16S</i>	<i>Ameerega hahneli</i>	0.006116	0.000154	1.00036	11	219	20	1858
<i>16S</i>	<i>Ameerega trivittata</i>	0.006104	0.000081	1.00039	17	219	28	1858
<i>16S</i>	<i>Craugastor podiciferus</i>	0.051194	0.003652	1.01266	8	1696	1445	2152
<i>16S</i>	<i>Dendrobates pumilio</i>	0.025690	0.000250	1.00493	19	48	13	589
<i>16S</i>	<i>Dendropsophus ebraccatus</i>	0.032362	0.000365	1.01142	15	47	16	1046
<i>16S</i>	<i>Dendropsophus leucophyllatus</i>	0.006116	0.000360	1.00016	6	78	7	286
<i>16S</i>	<i>Dendropsophus luddeckei</i>	0.025706	0.000926	1.02146	6	2667	2245	3676
<i>16S</i>	<i>Physalaemus petersi</i>	0.012277	0.000074	1.00014	22	233	102	3688

C. crassidigitus (isolation-by-resistance), model B in *D. labialis* (isolation-by-distance) and model C in both *C. fitzingeri* and *A. varius* (isolation-by-resistance and distance; Fig. 1, Table 4). The other species sampled for *cob* did not show significant correlations between genetic distances and resistance/geographic distances (Table 4).

For each gene, the relative rate test rejected the hypothesis that the genes were evolving at similar rates (Figure S3, Table S2). This result suggests that species across basal clades display differences in their rates of DNA evolution. Rate homogeneity, however, was not rejected in less inclusive clades (Table S2), which means that species within basal clades had comparable rates of DNA evolution.

Discussion

The magnitude of genetic divergence within a widespread lineage indicates the potential for its future speciation (Martin and McKay 2004). In spite of the multitude of factors that may influence intraspecific genetic divergence, such as demographic dynamics (different population sizes interacting with genetic diversity), sexual selection, life history characteristics (Summers et al. 1997; Wang and Summers 2009) and/or body size (Pabijan et al. 2012), we still found a marginally significant association between topographic complexity and intraspecific genetic variation when comparing multiple anuran species (the fact that the correlations had barely significant p-values but large correlation coefficients suggests that we had a small sample size in the number of species to properly detect the effect of topographic complexity across species). Our result implies that tropical frogs whose populations are found throughout regions with complex topographies have effectively less gene flow than frogs from less complex regions (assuming that genetic distances are a proxy for

gene flow). Interestingly, we found that even though most species in topographically complex regions were montane species (>1000 m.a.s.l), some lowland species were characterized by larger topographic complexities compared with species from mountainous regions. This suggests that intraspecific genetic divergence would be predicted more by the complexity of the landscape that separates populations rather than by a montane or lowland distribution.

Our finding from the causal model analysis that isolation-by-resistance (or the effect of climatic heterogeneity on genetic distances) is present only in the species characterized by high topographic complexities [*C. crassidigitus*, *C. fitzingeri*, and *A. varius*, (Fig. 2)] suggests that heterogeneous topographies increase landscape resistance, which in turns favours isolation-by-resistance. This suggestion is confirmed by the fact that all species with no evidence for isolation-by-resistance were from low topographic complexity regions, with the exception of *D. luddeckei*.

The fact that only the species in regions of higher topographic complexity displayed isolation-by-resistance suggests that temperature, rather than precipitation, provides more climatic resistance to the species dispersal, as topographic complexity implies elevation variance, which is directly related to temperature (although this relationship is less predictable with precipitation). We suggest that because precipitation patterns in the tropics have a strong seasonal component, the level of precipitation fluctuates annually, reducing its overall effects on gene flow. Tropical temperatures, however, are strongly dependent on topography, not seasonality, creating a temporal stability that would facilitate the role of temperature as a geographic barrier. In other words, the presence of mountains and their temperature gradients seems to be a factor that influences intraspecific genetic divergence for most species.

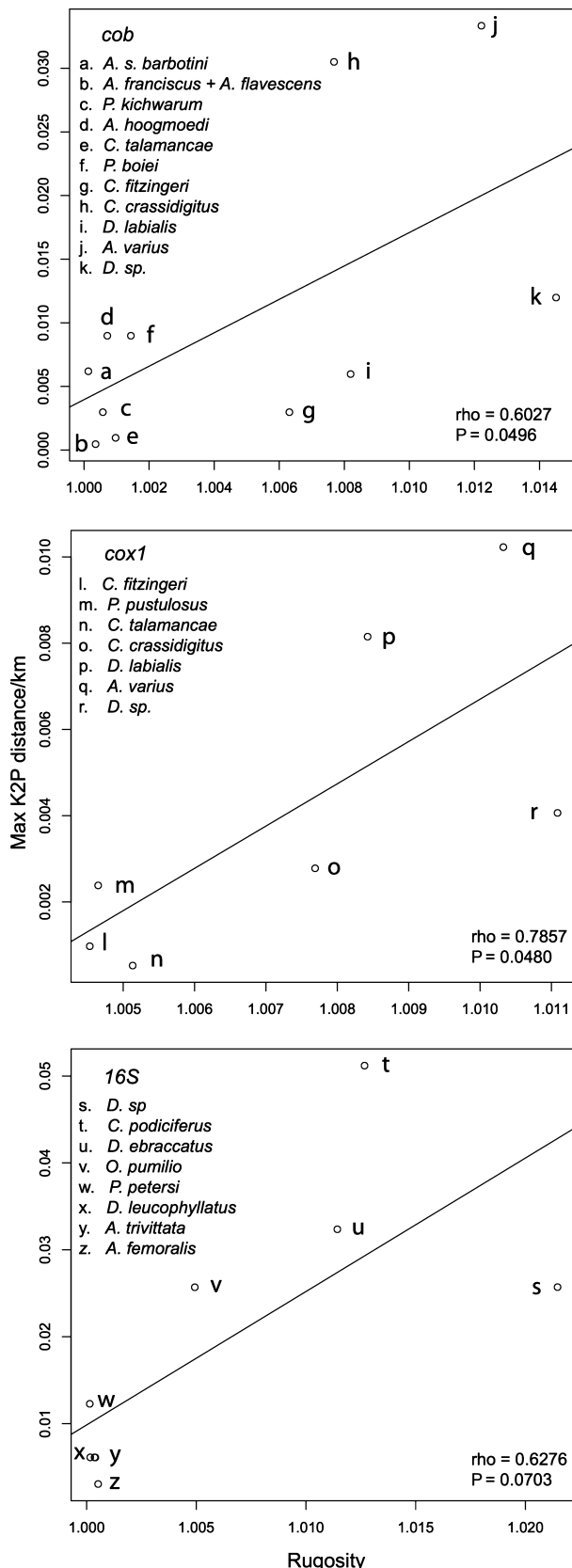


Fig. 2. Relationship between topographic complexity (rugosity) and the scaled maximum genetic distance (K2P) across species. Top: *cob*, middle: *cox1*, bottom: *16S*. The *cob* haplotypes from the species *Atelopus flavescens* and *Atelopus franciscus* were combined into a single species, as their status as different species is doubtful (Noonan and Gaucher 2005)

Table 3. Spearman's correlation coefficients between K2P genetic distances and landscape features

	Correlation (Rho)	Significance (two-tailed)
<i>cob</i>		
Maximum genetic distance $\text{km}^{-1} \times$ rugosity	0.603	0.050
Mean genetic distance $\text{km}^{-1} \times$ rugosity	0.127	0.714
<i>cox1</i>		
Maximum genetic distance $\text{km}^{-1} \times$ rugosity	0.786	0.048
Mean genetic distance $\text{km}^{-1} \times$ rugosity	0.786	0.048
<i>16S</i>		
Maximum genetic distance $\text{km}^{-1} \times$ rugosity	0.628	0.070
Mean genetic distance $\text{km}^{-1} \times$ rugosity	0.683	0.050

Significant correlations are in bold.

Table 4. Partial Mantel correlations (r) between spatial and *cob* genetic pairwise distances across species

Species	Partial Mantel test	r	p-value
<i>Atelopus flavescens</i> ,	RxG.D	-0.23547	0.38466
<i>Atelopus franciscus</i>	DxG.R	0.11025	0.56743
<i>C. fitzingeri</i>	RxG.D	-0.30697	0.01604
	DxG.R	0.55552	0.00001
<i>C. talamancae</i>	RxG.D	0.16517	0.63373
	DxG.R	0.26897	0.41183
<i>A. hoogmoedi</i>	RxG.D	-0.16110	0.63402
	DxG.R	-0.19663	0.42119
<i>P. kichwarum</i>	RxG.D	0.07569	0.58858
	DxG.R	0.03211	0.78126
<i>C. crassidigitus</i>	RxG.D	-0.20889	0.26564
	DxG.R	0.36984	0.02979
<i>A. varius</i>	RxG.D	0.80994	0.03926
	DxG.R	-0.79334	0.01666
<i>D. luddeckei</i>	RxG.D	-0.29500	0.17229
	DxG.R	0.04703	0.80301
<i>D. labialis</i>	RxG.D	-0.00265	0.88581
	DxG.R	0.39036	0.0030

RxG.D, Correlation between resistance distances (R) and genetic distances (G) controlling for great-circle distances (D); DxG.R, Correlation between great-circle distances and genetic distances, controlling for resistance distances. The haplotypes from the species *Atelopus flavescens* and *Atelopus franciscus* were combined into a single species, as their status as different species is doubtful (Noonan and Gaucher 2005). Significant correlations are in bold.

A mechanistic explanation for the effect of topography on intraspecific genetic divergence may be related to the two-fold effect of topography on species diversity (Ruggiero and Hawkins 2008): a change in elevation is accompanied by a steep change in temperature. It is well known that populations characterized by wide elevational distributions are physiologically adapted to their particular elevational ranges (Navas 2006). Therefore, complex topographies, when compared to flat regions, would not only provide more opportunities for allopatric speciation (Hoelzer et al. 2008) but also for ecological selection and parapatric reproductive isolation.

Given the seemingly important role of temperature variation as a geographic barrier to gene flow in frogs, why do they not frequently adapt to the suboptimal temperatures resulting from heterogeneous landscapes? A variety of species of frogs seems to maintain their thermal niche preferences, which is one aspect of niche conservatism (Angilletta et al. 2002; Wiens and Graham 2005; Kozak and Wiens 2010). This phenomenon is not exclusive to frogs, as a multitude of organisms seem to

maintain their thermal preferences, which is evidenced by the movement of species from different taxonomic groups to regions of increasing elevation and increasing latitude as a response to global climate change (Parmesan and Yohe 2003). Therefore, both the width of each species' physiological tolerance to novel temperatures (constrained by niche conservatism) and the degree of environmental heterogeneity may indicate whether or not a species can disperse across the landscape, which may be the key aspect contributing to allopatric separation of populations in topographically heterogeneous regions (Wiens and Graham 2005).

Temperature, then, seems to be the most important environmental variable that restricts the distribution and therefore shapes the intraspecific genetic divergence of frogs (Navas 1996, 1997; Angilletta et al. 2006).

The apparent effectiveness of topography in promoting genetic divergence in frogs might strongly depend on latitude because seasonally determined temperatures depend on the latitudinal gradient. According to the Janzen hypothesis (Janzen 1967), tropical organisms should be adapted to narrow physiological tolerances in temperature as a consequence of low seasonal (temporal) overlap in thermal regimes along elevation. Accordingly, in temperate regions, organisms should be adapted to wider temperature tolerances as a result of the greater temporal overlap in temperatures along any given elevation. The prediction is that the relationship between topographic complexity and genetic divergence will be less pronounced in temperate regions when multiple species across elevation gradients are compared. The effect of latitude on gene flow along elevational gradients has not been systematically tested (Ghalambor et al. 2006), and therefore, its effects on the early stages of speciation need further exploration.

Finally, we found evidence for rate heterogeneity within the major clades of the *cob*, *cox1* and *16S* trees. However, we do not believe that the positive relationship between topographic complexity and intraspecific genetic divergence is the result of faster rates of mtDNA evolution in species from more topographically complex regions. The reason is that subsets of species under a uniform rate of mtDNA evolution repeatedly displayed a positive association between topographic complexity and genetic divergence, suggesting that it is topographic complexity itself that is positively associated with intraspecific genetic divergence, and not the differences in rate of molecular evolution among clades. For example, in clades E and L (Figure S3), rate homogeneity was not rejected. Nevertheless, the species within those clades found in complex topographies (*Atelopus varius* and *D. pumilio*) were characterized by larger scaled genetic divergences than the species from less complex regions within those clades. In other words, the differences in scaled genetic divergences across species do not seem to reflect the rate of DNA evolution for each species.

To conclude, comparative analysis of multiple species supports the hypothesis that a combination of heterogeneous landscapes and thermal niche conservatism would facilitate intraspecific genetic divergence. The observation that the variance in total amount of genetic variation within species independent of geographic distance was large suggests to us that unmeasured factors (i.e. ecological) also have an effect on the genetic divergence of the species analysed. More studies that contrast the role of ecological factors on the patterns of intraspecific genetic variation are needed in tropical areas. Mountains in the tropics and their complex topographies, which are commonly recognized as cradles of speciation (Dick and Wright 2005), might yield important clues for understanding why lower latitudes contain greater species diversity (Wiens et al. 2006).

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Resumen

La mayoría de los hotspots globales de biodiversidad y endemismo se encuentran en regiones montañosas. Una explicación es que estas regiones poseen una tasa de especiación superior comparado con las tierras bajas, ya que la compleja topografía y la variación climática facilitan el aislamiento reproductivo y divergencia genética entre poblaciones. Nosotros analizamos desde un punto de vista intraespecífico si las especies de ranas cuyos haplotipos están conectados por regiones topográficamente/climáticamente complejas muestran un fuerte aislamiento genético (mayores distancias genéticas), en comparación con las especies cuyos haplotipos están conectados por regiones menos complejas. Analizamos las secuencias de ADN de varias especies de ranas de Centro y Sur América para los genes mitocondriales *cox1*, *cob*, y *16S* rARN publicados en Genbank.

Las distancias genéticas entre todos los pares de haplotipos dentro de cada especie fueron estandarizadas por las distancias geográficas que las separaban. Encontramos que la complejidad topográfica se correlacionó positivamente con las distancias genéticas, y el aislamiento-por-resistencia fue apoyado únicamente en las especies de regiones topográficamente más complejas. Esto sugiere que las topografías heterogéneas aumentan la resistencia al movimiento entre poblaciones, que a su vez favorece la aparición de aislamiento-por-resistencia. Por otra parte, se encontró que los posibles obstáculos que restringen el flujo genético dentro de las especies están más estrechamente relacionados a la temperatura ambiental y topografía que a los patrones de precipitación.

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Supporting Information

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

Figure S1. Methodology used to identify the potential presence of cryptic species from the sequences retrieved from GenBank (see text).

Figure S2. Scatter plots of geographic and genetic distances

across species. Top box: *cob*, middle box: *cox1*, bottom box: *16S*.

Figure S3. Maximum likelihood gene genealogies used to estimate rate heterogeneity across taxa. Left: *cob*, middle: *cox1*, right: *16S*. Capital letters on top of branches are associated with Table S1. Numbers on top of branches are bootstrap values. Outgroups are described in the text.

Figure S4. Relationship between topographic complexity (rugosity) and the mean-scaled genetic distance (K2P) per species. Top: *cob*, middle: *cox1*, bottom: *16S*.

Table S1. List of all the mitochondrial haplotypes included in the analysis, their Genbank accession numbers and geographic coordinates.

Table S2 Relative rate test for each gene estimated using r8s.