**BIODIVERSITY RESEARCH**

# Effects of slope and riparian habitat connectivity on gene flow in an endangered Panamanian frog, *Atelopus varius*

Corinne L. Richards-Zawacki*

Department of Ecology and Evolutionary Biology, University of Michigan Museum of Zoology, 1109 Geddes Ave., Ann Arbor, MI 48109 and Department of Integrative Biology, University of California, Berkeley, 3101 Valley Life Sciences Building, Berkeley, CA 94720, USA

## ABSTRACT

**Aim** Understanding how heterogeneous landscapes shape genetic structure not only sheds light on processes involved in population divergence and speciation, but can also guide management strategies to promote and maintain genetic connectivity of populations of endangered species. This study aimed to (1) identify barriers and corridors for gene flow among populations of the endangered frog, *Atelopus varius* and (2) assess the relative contributions of alternative landscape factors to patterns of genetic variation among these populations in a hypothesis testing framework.

**Location** This study took place in western Panama and included all nine of the remaining known populations of *A. varius* at the time of study.

**Methods** The influence of landscape variables on gene flow among populations was examined by testing for correlations between alternative landscape-resistance scenarios and genetic distance. Fifteen alternative hypotheses about the influence of (1) riparian habitat corridors, (2) steep slopes, and (3) climatic suitability on patterns of genetic structure were tested in a causal modelling framework, using Mantel and partial-Mantel tests, along with an analysis of molecular variation.

**Results** Only the hypothesis attributing resistance to dispersal across steep slopes (genetic isolation by slope distance) was fully supported by the causal modelling approach. However, the analysis of molecular variance and the paths of least-slope among populations suggest that riparian habitat connectivity may influence genetic structure as well.

**Main conclusions** These results suggest that patterns of genetic variation among *A. varius* populations are affected by the slope of the landscape such that areas with steep slopes act as barriers to gene flow. In contrast, areas of low slope, such as streams and mountain ridges, appear to be important corridors for gene flow, especially among high elevation populations. These results engender important considerations for the management of this critically endangered species.

*Correspondence: Corinne L. Richards-Zawacki, University of Michigan Museum of Zoology, 1109 Geddes Ave., Ann Arbor, MI 48109 and University of California, Berkeley, 3101 Valley Life Sciences Building, Berkeley, CA 94720, USA.
E-mail: coririchards@berkeley.edu

## INTRODUCTION

Understanding how landscape heterogeneity affects genetic structure can provide insight into important biological processes, such as metapopulation dynamics, the formation of species distributions, population divergence and speciation (Manel *et al.*, 2003; Storfer *et al.*, 2007). The ever-growing number of landscape genetic studies, which identify how landscape variables influence patterns of gene flow and genetic variation, has contributed to this understanding for a variety of taxa, including plants (Liepelt *et al.*, 2002; Fievet *et al.*, 2007), crustaceans (Michels *et al.*, 2001), insects (Keyghobadi *et al.*, 1999, 2005a,b; Finn *et al.*, 2006), molluscs (Pfenninger, 2002; Arnaud, 2003), birds (Piertney *et al.*, 1998), mammals (Roach *et al.*, 2001; Cegelski *et al.*, 2003; Scribner *et al.*, 2005; Vignieri, 2005), fish (Poissant *et al.*, 2005) and amphibians (Funk *et al.*,

2005; Spear *et al.*, 2005; Lowe *et al.*, 2006; Giordano *et al.*, 2007). Because landscape genetic analyses can identify specific barriers or corridors for gene flow and predict the effects of alternative management strategies on connectivity, they have an applied value as well (Storfer *et al.*, 2007), especially for endangered species (Stevens *et al.*, 2006; Wilmer & Wilcox, 2007).

Several aspects of the life histories of amphibians suggest that landscapes are likely to have strong effects on dispersal and genetic structure (Funk *et al.*, 2005; Spear *et al.*, 2005). For example, their generally low vagility (Blaustein *et al.*, 1994) and, in many cases, high philopatry (Duellman & Trueb, 1994) can lead to low levels of gene flow, even among geographically proximate populations (Garcia-Paris *et al.*, 2000; Shaffer *et al.*, 2000; Monsen & Blouin, 2003; Spear *et al.*, 2005). In addition, their biphasic life cycles, consisting in most cases of an aquatic larval phase and a terrestrial adult phase, suggest that both aquatic and terrestrial landscape features could play a role in the evolution of genetic structure (Spear *et al.*, 2005). While these aspects of amphibian biology suggest a strong role for landscape features in structuring genetic diversity, the relative contributions of different types of landscape heterogeneity to patterns of population connectivity and isolation remain largely unclear. Because amphibians are often hard to detect outside of their aquatic breeding periods, terrestrial habitat use and movement patterns are difficult to measure directly and remain poorly understood for most species (MacKenzie *et al.*, 2002; Semlitsch, 2003). Landscape genetic analyses are, therefore, an important tool for understanding the relative influences of alternative landscape factors on patterns of amphibian divergence and gene flow (Spear *et al.*, 2005). Additionally, because amphibian species are declining world-wide (Stuart *et al.*, 2004), this information will be useful in designing effective conservation and management strategies.

The goals of this study were to (1) identify important barriers and corridors for gene flow among remaining populations of the endangered frog, *Atelopus varius* (Lichtenstein & Martens, 1856) and (2) measure the relative contributions of these landscape factors to patterns of genetic variation among these populations in a hypothesis testing framework. Understanding how landscape characteristics affect evolutionary processes is particularly urgent for these frogs, as *Atelopus* is among the most imperilled of all amphibian genera – thus far, 62 of 77 described species have been classified by the IUCN as extinct or critically endangered (La Marca *et al.*, 2005; Pounds *et al.*, 2006; Lötters, 2007). Declines and extinctions in this group have been attributed to the frogs' extreme sensitivity to environmental perturbations (Lötters, 1996), an emerging fungal disease called chytridiomycosis (La Marca *et al.*, 2005) and global warming (Pounds *et al.*, 2006). In Panama, *A. varius* is in critical danger of extinction due to these same threats in addition to over-collection for the illegal pet trade (Zippel, 2002; Pounds *et al.*, 2008). This species was historically found throughout much of montane Costa Rica and western Panama (Savage, 1972), but has disappeared from most of its range over the past decade (Pounds & Crump, 1994; Lips, 1998; Zippel *et al.*, 2006).

*Atelopus varius* exhibits extreme morphological variation, even among geographically proximate populations (Richards & Knowles, 2007). However, in contrast to their morphological diversity, life-history traits vary less among populations. The species breeds in and lives around swiftly flowing streams in lowland rain forest and humid montane forest habitat (Savage, 1972; Lötters, 1996). As adults, both sexes have well-defined home ranges along these streams and show site fidelity, indicating that the dispersal of this life stage is probably low (Crump, 1986; Lötters, 1996). While little is known about the movement patterns of juvenile *A. varius*, the adult frogs' strong association with riparian habitat, coupled with the fact that larval movement would necessarily be restricted to streams, suggests that streams are likely important corridors for dispersal. Although their exact climatic tolerances are unknown, given the genus's apparent sensitivity to climatic changes (Rivero, 1963; Pounds *et al.*, 2006), regional climatic differences may influence the distribution and movement patterns of *A. varius* as well. Finally, the abrupt changes in elevation that characterize this species' habitat may also affect dispersal and gene flow. The potential for limited gene flow in this species is supported by a previous study (Richards & Knowles, 2007), which found a strong pattern of isolation by distance among populations.

In this study, I quantify the effect of landscape heterogeneity on gene flow and genetic differentiation among endangered *A. varius* populations by comparing the strengths of correlations between alternative landscape-resistance scenarios and genetic distance among nine populations from western Panama (Fig. 1). To test the hypothesis that gene flow among *A. varius* populations has been facilitated by riparian habitat corridors but impeded by steep slopes and areas of less-suitable climate, correlations between landscape distances, Euclidian
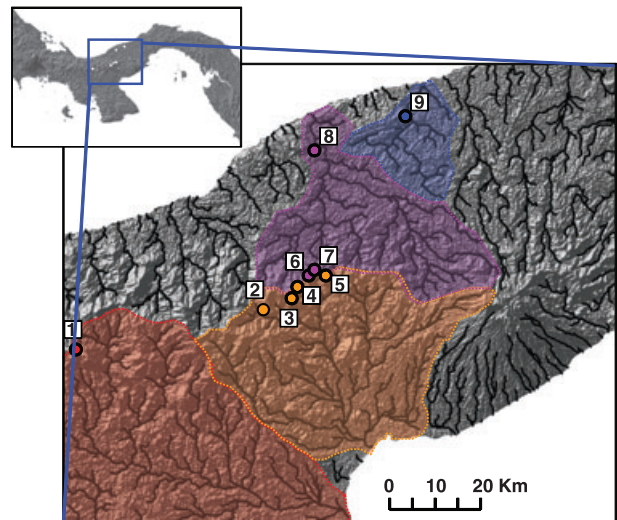


**Figure 1** Study area and sampled populations. The four sampled drainage basins are indicated by different coloured areas on the shaded relief map (refer to inset for the position of the sampling area within Panama).

| Population | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | |
| 2 | **0.417*** | | | | | | | |
| 3 | **0.411*** | **0.275*** | | | | | | |
| 4 | **0.366*** | **0.223*** | **0.269*** | | | | | |
| 5 | **0.356*** | 0.130* | 0.131* | 0.132* | | | | |
| 6 | **0.246*** | **0.127*** | **0.122*** | 0.041 | 0.042 | | | |
| 7 | **0.299*** | 0.094* | 0.082 | 0.071 | −0.039 | 0.009 | | |
| 8 | **0.438*** | **0.403*** | **0.380*** | **0.251*** | **0.337*** | 0.091 | **0.251*** | |
| 9 | **0.600*** | **0.637*** | **0.619*** | **0.423*** | **0.510*** | **0.218*** | **0.383*** | −0.041 |

Table 1 Pairwise $F_{ST}$-values. Significant values are marked with an asterisk (*); however, only values in bold remain significant after sequential Bonferroni correction ($\alpha = 0.05$).

(straight-line) distance and the genetic distance among mitochondrial DNA haplotypes were compared using Mantel tests and an analysis of molecular variance (AMOVA). Partial-Mantel tests and a causal modelling framework were used to evaluate 15 alternative hypotheses about the relationship between landscape factors and gene flow. Given that the number of healthy A. varius populations has continued to dwindle since the initiation of this study (Richards-Zawacki, unpublished data), the decision may someday be made to reintroduce individuals from captively managed populations to Panama (Zippel, 2002). As such, this study not only contributes to our understanding of how landscape heterogeneity influences the formation of genetic structure, but also has potential applications for the future management of these highly endangered frogs.

## METHODS

### Sample collection and sequencing

Toe-clip tissue samples were collected from all known A. varius populations throughout their extant range in Panama (Fig. 1). These frogs were historically found throughout much of montane Costa Rica and western Panama, but by the time of collection (February to August, 2004), their extant range had been reduced to the nine western Panamanian populations sampled here, which includes parts of the Veraguas, Coclé and Colón provinces. All animals were captured by hand and released at the point of capture within 5 min of data collection. The latitude and longitude for each population were recorded in the field using a hand-held GPS unit (Garmin e-Trex H, 3-m accuracy). The sampled populations ranged in elevation from 92 to 1124 m and spanned four drainage basins as well as the continental divide. Tissues were preserved in a salt-saturated DMSO and EDTA solution (see Appendix S1 in Supporting Information) in the field and stored at room temperature until the time of DNA extraction.

A 755-base pair fragment of CytB and 630 base pairs of COI were sequenced from the mitochondrial genome for each of the 76 individuals in this study. Sample sizes ranged from 6 to 10 individuals per population, with a median sample size of eight individuals per population. Extraction of genomic DNA, primers, polymerase chain reaction conditions and sequencing

were as described in Richards & Knowles (2007). All genetic data included in this study were previously published elsewhere (Richards & Knowles, 2007) and deposited in GenBank.

### Genetic analyses

The sequence data from the two mitochondrial genes were concatenated into a single haplotype for each individual (GenBank Accession numbers for CytB: EF494922–EF494948 and COI: EF494967–EF494995). Population pairwise $F_{ST}$-values (Table 1) were estimated in ARLEQUIN version 2.0 (Schneider et al., 2000). An AMOVA with individuals nested by (1) population and (2) drainage basin was performed in ARLEQUIN 2.0 (Excoffier et al., 1992; Schneider et al., 2000) using 1000 permutations, and a Kimura 2-Parameter distance matrix with $\Gamma = 0.2226$.

### Landscape friction gradients

Given what is known about the frogs' natural history, three landscape factors – climatic suitability, changes in slope and the distribution of riparian habitat – were identified as potentially influencing the structure of A. varius populations. Based on these predictions, a series of landscape friction surfaces were generated to account for the difficulty A. varius would potentially face in dispersing through (1) changes in slope, (2) suboptimal climates, and (3) non-riparian habitat. Because little information about the ecology and movement behaviour of the species was available from which to empirically derive or estimate relative friction values for each landscape factor, alternative friction surfaces representing different levels of resistance to dispersal were investigated.

#### Slope and riparian habitat

Friction maps for slope and riparian habitat were built from a 90-m resolution digital elevation map (DEM) produced by the CGIAR Consortium for Spatial Information (http://csi.cgiar.org). The slope at each cell within the study area was calculated using the 'slope' function in the Spatial Analyst extension of ArcGIS Desktop 9.2. To convert the slope data into a series of friction gradients, slope values were standardized so that areas with zero slope had a friction value of one and friction

increased linearly with slope. Slope gradients were developed with maximum friction values ranging from 5 to 100. Stream data for the study area were generated from the DEM using the 'stream definition' function (stream threshold = 50) in the Arc Hydro 1.1 extension of ArcGIS Desktop 9.2. Stream friction gradients were developed from these data by assigning all stream cells a friction value of one and all non-stream cells a consistent, larger friction value, which varied from 5 to 100 among alternative gradients.

### Climate

Climate suitability gradients were developed using the output of a species distribution model generated in Maxent version 3.0.4-beta (Phillips *et al.*, 2006). The species distribution model was developed using a series of 19 bioclimatic layers (see http://www.worldclim.org/bioclim.htm, for variable descriptions) at a spatial resolution of 30 arc-seconds (Hijmans *et al.*, 2005; see also http://www.worldclim.org/) and 93 localities where *A. varius* is known to have occurred (see Appendix S2 in Supporting Information). These localities were compiled from 11 museum collections and the nine populations sampled for this study. Performance of the species distribution model was evaluated using receiver operating characteristic (ROC) analysis. For this analysis, 25% of species occurrence records were randomly selected as test data, and 10,000 randomly selected pixels from the study area were used as background points. The area under the ROC curve for the test data was 0.874, indicating good discrimination between the presence and absence of the species (Phillips *et al.*, 2006). The species distribution modelling algorithm in Maxent uses the set of climate layers and species occurrences to predict the climatic suitability of each cell of the study area. These suitabilities are reported in the form of a GIS layer with cell values ranging from zero to one, one being most and zero being least suitable. To convert the Maxent output layer to a series of climate suitability gradients, these output values were algebraically transformed so that the most suitable climate areas had a friction value of one and friction increased linearly with decreasing climatic suitability. A series of climate suitability gradients was developed, with maximum friction values ranging from 5 to 100.

### Isolation by distance

For each landscape friction gradient, the least-cost distances and Euclidian, or straight-line distances between populations, were calculated using Pathmatrix (Ray, 2005) in ArcView 3.3. The least-cost distance is calculated as the sum of the friction values for each cell along the least-cost path between populations. If landscape heterogeneity impacts dispersal ability, then these least-cost distances are expected to provide a more realistic measure of genetic isolation among populations than straight-line, or Euclidian distances. The strength of the association between the log of each landscape factor's resulting least-cost path distance and the log of genetic distance

(measured by $F_{ST}$) was compared across multiple friction levels using Mantel tests (Mantel, 1967) in IBD version 1.5 (Bohonak, 2002) with $10^4$ randomizations. Negative values in the genetic distance matrix were set to 0.0001 prior to log transformation and Mantel testing. The strength of the association between Euclidian (straight-line) and genetic distances was assessed in the same way. Since Euclidian distance does not take into account landscape heterogeneity, the strength of the pattern of isolation by Euclidian distance served as a null model against which the performance of alternative landscape gradients was compared – only landscape factors that showed tighter correlations with genetic distance than did Euclidian distance were considered as potential influences on genetic structure. For each landscape factor (slope, climate and streams), the friction level that resulted in the largest Mantel correlation coefficient ($r$) was used for further analysis and hypothesis testing as it best explains the pattern of among population genetic structure.

## Landscape factors and hypothesis testing

To assess the relative support for each of the three landscape factors as drivers of genetic structure, the strength of the association between genetic distance and Euclidian distance was compared with those of genetic distance and the alternative landscape distances. Because each landscape factor could affect gene flow independently, in concert with others, or not at all, 15 alternative patterns of causality were possible. Following Cushman *et al.* (2006), each of these were tested as separate hypothesis (Table 2) and causal modelling (Legendre & Troussellier, 1988; Legendre, 1993) was used to identify the landscape hypothesis with the strongest support. Each hypothesis has a corresponding set of diagnostic, statistical predictions (Table 2) regarding the relationship between genetic distance and alternative landscape distances. Under this framework, only the hypothesis with the strongest support will have all of its predictions upheld. To test each prediction, the strength of the association between two distance matrices (e.g. log of genetic and log of Euclidian distances) after removing the effect of a third (e.g. the log of the least-cost distance for the slope gradient, or 'slope distance') was measured using a partial-Mantel test. These tests were carried out in IBD version 1.5 (Bohonak, 2002) with $10^4$ randomizations. The significance of the partial correlations was calculated by comparing the actual statistic with a distribution of $r$-scores derived from random permutations of the genetic distance matrix (see Raufaste & Rousset, 2001; Castellano & Balletto, 2002; and Rousset, 2002, for a discussion of potential biases to these significance values under certain scenarios).

## RESULTS

### Performance of alternative friction gradients

The strength of the pattern of isolation by distance was significant across 21 of the 22 alternative landscape gradients

**Table 2** Description of the 15 alternative landscape genetic hypotheses tested. The statistical predictions are a list of the partial-Mantel tests used to evaluate each hypothesis and the expected pattern of significance if the model is correct. G = genetic distance; D = log of Euclidian distance; Sl = 'slope distance', the log of the least-cost distance with changes in slope as the source of friction; C = 'climate distance', the log of the least-cost distance with poor climatic suitability as the source of friction; St = 'stream distance', the log of the least-cost distance with movement outside of streams as the source of friction. A period (.) separates the main distance matrices (on the left) from the covariate matrix (on the right), whose effect is removed in the partial-Mantel tests (e.g. DG.Sl is the partial-Mantel test between log Euclidian and genetic distance after removing the effect of 'slope distance').

| Hypothesis (Genetic isolation by...) | Statistical predictions | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Positive correlation ($r > 0$) | | | No correlation ($r =$ n.s.) | | |
| Distance | DG.Sl | DG.C | DG.St | SlG.D | CG.D | StG.D |
| Slope | SlG.D | SlG.C | SlG.St | DG.Sl | StG.Sl | CG.Sl |
| Climate | CG.D | CG.Sl | CG.St | DG.C | SlG.C | StG.C |
| Stream | StG.D | StG.C | StG.Sl | DG.St | CG.St | SlG.St |
| Distance and slope | DG.Sl | DG.St | SlG.C | StG.D | StG.Sl | CG.D |
| | DG.C | SlG.D | SlG.St | CG.Sl | | |
| Distance and climate | DG.Sl | DG.St | CG.Sl | SlG.D | SlG.C | StG.C |
| | DG.C | CG.D | CG.St | StG.D | | |
| Distance and stream | DG.Sl | DG.St | StG.C | SlG.D | SlG.St | CG.St |
| | DG.C | StG.D | StG.Sl | CG.D | | |
| Slope and climate | SlG.D | SlG.St | CG.Sl | DG.Sl | DG.C | StG.C |
| | SlG.C | CG.D | CG.St | StG.Sl | | |
| Slope and stream | SlG.D | SlG.St | StG.C | DG.Sl | DG.St | CG.St |
| | SlG.C | StG.D | StG.Sl | CG.Sl | | |
| Climate and stream | CG.D | CG.St | StG.C | DG.C | DG.St | SlG.C |
| | CG.Sl | StG.D | StG.Sl | SlG.St | | |
| Distance, slope and climate | DG.Sl | DG.C | DG.St | StG.D | StG.C | StG.Sl |
| | SlG.D | SlG.C | SlG.St | | | |
| | CG.D | CG.Sl | CG.St | | | |
| Distance, slope and stream | DG.Sl | DG.C | DG.St | CG.D | CG.Sl | CG.St |
| | SlG.D | SlG.C | SlG.St | | | |
| | StG.D | StG.C | StG.Sl | | | |
| Distance, climate and stream | DG.Sl | DG.C | DG.St | SlG.D | SlG.C | SlG.St |
| | CG.D | CG.Sl | CG.St | | | |
| | StG.D | StG.C | StG.Sl | | | |
| Slope, climate and stream | SlG.D | SlG.C | SlG.St | DG.Sl | DG.C | DG.St |
| | CG.D | CG.Sl | CG.St | | | |
| | StG.D | StG.C | StG.Sl | | | |
| Distance, slope, climate and stream | DG.Sl | DG.C | DG.St | | | |
| | SlG.D | SlG.C | SlG.St | | | |
| | CG.D | CG.Sl | CG.St | | | |
| | StG.D | StG.C | StG.Sl | | | |

(Table 3). The only landscape gradient that had a non-significant correlation with genetic distance was the stream-distance gradient with a maximum friction value of 100 ($St_{100}$, $P = 0.0082$). When the 22 distance matrices were ranked by Mantel correlation coefficient ($r$), Euclidian distance ranked 12th and only stream and slope distance matrices ranked higher (Table 3, Fig. 2). The strongest correlation between genetic distance and stream distance was found when the friction value for non-stream habitats was set to 10 ($St_{10}$, Fig. 2). However, several of the slope-distance matrices had stronger correlations with genetic distance than did $St_{10}$. The strongest correlation between any distance matrix and genetic distance was obtained when a slope gradient was used and the maximum friction value was set to 100 ($Sl_{100}$, Figs 2 & 3). The distance matrices for climate gradients were never more strongly correlated with genetic distance than was Euclidian distance (Fig. 2). These results were robust to jack-knife re-sampling analyses (CLR, unpublished data).

## Support for alternative landscape hypotheses

Only one of the 15 alternative hypotheses – genetic isolation by slope distance – had all of its statistical predictions upheld (Table 4). This indicates that gene flow among *A. varius* populations is influenced by changes in slope with no significant, independent relationships with Euclidian distance,

**Table 3** Mantel test results for 22 alternative distance matrices, ranked by correlation coefficient (*r*). All but one (St$_{100}$) were significant after Bonferroni correction (α = 0.05). Abbreviations follow Table 2. Subscripted numbers indicate the maximum friction value of the landscape gradient.

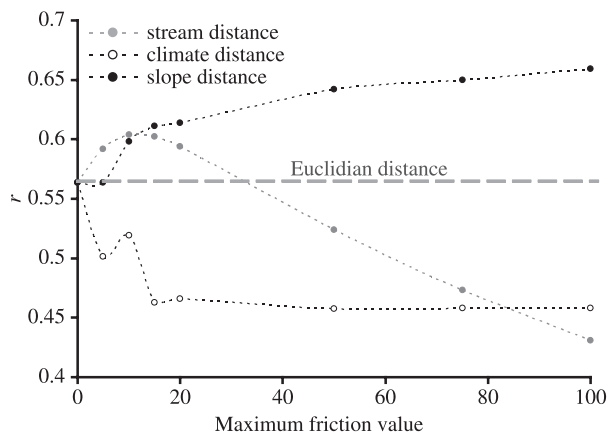| Rank | Distance matrix | *r* |
|---|---|---|
| 1 | Sl$_{100}$ | 0.659 |
| 2 | Sl$_{75}$ | 0.650 |
| 3 | Sl$_{50}$ | 0.642 |
| 4 | Sl$_{20}$ | 0.614 |
| 5 | Sl$_{15}$ | 0.611 |
| 6 | St$_{10}$ | 0.604 |
| 7 | St$_{15}$ | 0.602 |
| 8 | Sl$_{10}$ | 0.598 |
| 9 | St$_{20}$ | 0.594 |
| 10 | St$_5$ | 0.592 |
| 11 | Sl$_5$ | 0.564 |
| 12 | Euclidian | 0.564 |
| 13 | St$_{50}$ | 0.524 |
| 14 | C$_{10}$ | 0.519 |
| 15 | C$_5$ | 0.501 |
| 16 | St$_{75}$ | 0.473 |
| 17 | C$_{20}$ | 0.466 |
| 18 | C$_{15}$ | 0.463 |
| 19 | C$_{75}$ | 0.458 |
| 20 | C$_{100}$ | 0.458 |
| 21 | C$_{50}$ | 0.457 |
| 22 | St$_{100}$ | 0.431 (n.s.) |



**Figure 2** Mantel correlation coefficients (*r*) across landscape gradients and friction ranges. The *x*-axis indicates the maximum friction value for the variable.

riparian habitat or climate. The routes between populations that minimize changes in slope (i.e. the least-cost paths) tend to follow streams and rivers, as well as mountain ridges, suggesting that these landscape features are important corridors for gene flow. The AMOVA with individuals nested by (1) population and (2) drainage basin further supports the role of streams as important corridors for gene flow, as a significant proportion of genetic variation was found among drainage basins (Table 5).

## DISCUSSION

It is well recognized that the most robust inferences about population genetic structure come from studies that include data from multiple genetic loci. For this reason, microsatellites are often the markers of choice for landscape genetics and population genetics. However, one of the major drawbacks with microsatellite markers is that they are highly species specific and developing a new set of loci is not only expensive, but also time consuming – perhaps prohibitively so if there are pressing conservation needs at hand. In the case of *A. varius*, my attempt to develop a set of microsatellite loci was ultimately unsuccessful, not for lack of money or time, but because the resulting data were largely non-reproducible. I found a number of microsatellite sequences with similar or almost identical flanking regions suggesting that microsatellite repeat units have evolved in association with repetitive elements in the *A. varius* genome, as has been reported in lepidopterans (Meglecz *et al.*, 2004; Zhang, 2004) and other insect species (Arthofer *et al.*, 2007), as well as in plants (Tero *et al.*, 2006). Because *A. varius* microsatellites occur in families, primers often amplified microsatellites from multiple loci at once, rendering them unusable for this study. Several nuclear genes were screened for use in this study as well, but none exhibited sequence polymorphism among the studied populations. The mitochondrial data alone showed sufficient polymorphism among *A. varius* populations to infer genetic structure. However, as this study relies upon a single, maternally inherited locus, the inferences presented here would likely be improved with the addition of one or more additional genetic markers, should suitable ones be found.

Half of the 22 alternative landscape resistances investigated resulted in a pattern of isolation by landscape distance stronger than the null model of isolation by Euclidian, or straight-line, distance (Table 3). Only landscape gradients attributing resistance to (1) dispersal across non-riparian habitats (stream distances) or (2) dispersal across changes in slope (slope distances) explained more of the genetic variation than did Euclidian distance.

Slope distances explained more of the genetic variation than did Euclidian distance, regardless of the range of friction values used (i.e. the rate at which resistance to movement increased with slope). However, for streams, only the landscape gradients attributing low friction values (5–20) to travel through non-riparian habitat resulted in a stronger correlation with genetic distance than did Euclidian distance. The fact that the ability of stream distances to out-perform Euclidian distances as a predictor of genetic variation depended upon the friction value attributed to movement through non-riparian habitats highlights the importance of considering a range of relative friction values when calculating landscape distances using least-cost path analyses. This technique is especially useful when little information about the ecology and movement behaviour of the

**Table 4** Evaluation of alternative landscape hypotheses. Abbreviations and partial-Mantel test nomenclature follow Tables 2 and 3. Predictions upheld by the partial-Mantel tests are in bold. Values in parentheses are partial-Mantel correlation coefficients ($r$) for tests that were significant after Bonferroni correction for experiment-wise error rates ($\alpha = 0.05$). Non-significant tests are denoted by (n.s.).

| Hypothesis (predictions upheld/total) | Statistical predictions | | | | | |
|---|---|---|---|---|---|---|
| | Positive correlation ($r > 0$) | | | No correlation ($r$ = n.s.) | | |
| Distance (2/6) | DG.Sl$_{100}$ (n.s.) | DG.C$_{10}$ (n.s.) | DG.St$_{10}$ (n.s.) | Sl$_{100}$G.D (0.527) | **C$_{10}$G.D (n.s.)** | **St$_{10}$G.D (n.s.)** |
| Slope (6/6) | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.C$_{10}$ (0.609)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | **DG. Sl$_{100}$ (n.s.)** | **St$_{10}$G. Sl$_{100}$ (n.s.)** | **C$_{10}$G.Sl$_{100}$ (n.s.)** |
| Climate (2/6) | C$_{10}$G.D (n.s.) | C$_{10}$G.Sl$_{100}$ (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | **DG.C$_{10}$ (n.s.)** | Sl$_{100}$G.C$_{10}$ (0.609) | **St$_{10}$G.C$_{10}$ (n.s.)** |
| Stream (2/6) | St$_{10}$G.D (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | **DG.St$_{10}$ (n.s.)** | **C$_{10}$G.St$_{10}$ (n.s.)** | Sl$_{100}$G.St$_{10}$ (0.545) |
| Distance and slope (7/10) | DG.Sl$_{100}$ (n.s.) | DG.St$_{10}$ (n.s.) | **Sl$_{100}$G.C$_{10}$ (0.609)** | St$_{10}$G.D (n.s.) | **St$_{10}$G.Sl$_{100}$ (n.s.)** | **C$_{10}$G.D (n.s.)** |
| | DG.C$_{10}$ (n.s.) | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | **C$_{10}$G. Sl$_{100}$ (n.s.)** | | |
| Distance and climate (2/10) | DG.Sl$_{100}$ (n.s.) | DG.St$_{10}$ (n.s.) | C$_{10}$G. Sl$_{100}$ (n.s.) | Sl$_{100}$G.D (0.527) | Sl$_{100}$G.C$_{10}$ (0.609) | **St$_{10}$G.C$_{10}$ (n.s.)** |
| | DG.C$_{10}$ (n.s.) | C$_{10}$G.D (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | **St$_{10}$G.D (n.s.)** | | |
| Distance and stream (2/10) | DG.Sl$_{100}$ (n.s.) | DG.St$_{10}$ (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | Sl$_{100}$G.D (0.527) | Sl$_{100}$G.St$_{10}$ (0.545) | **C$_{10}$G.St$_{10}$ (n.s.)** |
| | DG.C$_{10}$ (n.s.) | St$_{10}$G.D (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | **C$_{10}$G.D (n.s.)** | | |
| Slope and climate (7/10) | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | C$_{10}$G. Sl$_{100}$ (n.s.) | **DG.Sl$_{100}$ (n.s.)** | **DG.C$_{10}$ (n.s.)** | **St$_{10}$G.C$_{10}$ (n.s.)** |
| | **Sl$_{100}$G.C$_{10}$ (0.609)** | C$_{10}$G.D (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | **St$_{10}$G.Sl$_{100}$ (n.s.)** | | |
| Slope and stream (7/10) | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | St$_{10}$G.C$_{10}$ (n.s.) | **DG.Sl$_{100}$ (n.s.)** | **DG.St$_{10}$ (n.s.)** | **C$_{10}$G.St$_{10}$ (n.s.)** |
| | **Sl$_{100}$G.C$_{10}$ (0.609)** | St$_{10}$G.D (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | **C$_{10}$G.Sl$_{100}$ (n.s.)** | | |
| Climate and stream (2/10) | C$_{10}$G.D (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | **DG.C$_{10}$ (n.s.)** | **DG.St$_{10}$ (n.s.)** | Sl$_{100}$G.C$_{10}$ (0.609) |
| | C$_{10}$G.Sl$_{100}$ (n.s.) | St$_{10}$G.D (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | Sl$_{100}$G.St$_{10}$ (0.545) | | |
| Distance, slope and climate (6/12) | DG.Sl$_{100}$ (n.s.) | DG.C$_{10}$ (n.s.) | DG.St$_{10}$ (n.s.) | **St$_{10}$G.D (n.s.)** | **St$_{10}$G.C$_{10}$ (n.s.)** | **St$_{10}$G.Sl$_{100}$ (n.s.)** |
| | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.C$_{10}$ (0.609)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | | | |
| | C$_{10}$G.D (n.s.) | C$_{10}$G.Sl$_{100}$ (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | | | |
| Distance, slope and stream (6/12) | DG.Sl$_{100}$ (n.s.) | DG.C$_{10}$ (n.s.) | DG.St$_{10}$ (n.s.) | **C$_{10}$G.D (n.s.)** | **C$_{10}$G.Sl$_{100}$ (n.s.)** | **C$_{10}$G.St$_{10}$ (n.s.)** |
| | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.C$_{10}$ (0.609)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | | | |
| | St$_{10}$G.D (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | | | |
| Distance, climate and stream (0/12) | DG.Sl$_{100}$ (n.s.) | DG.C$_{10}$ (n.s.) | DG.St$_{10}$ (n.s.) | Sl$_{100}$G.D (0.527) | Sl$_{100}$G.C$_{10}$ (0.609) | Sl$_{100}$G.St$_{10}$ (0.545) |
| | C$_{10}$G.D (n.s.) | C$_{10}$G.Sl$_{100}$ (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | | | |
| | St$_{10}$G.D (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | | | |
| Slope, climate and stream (6/12) | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.C$_{10}$ (0.609)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | **DG. Sl$_{100}$ (n.s.)** | **DG.C$_{10}$ (n.s.)** | **DG.St$_{10}$ (n.s.)** |
| | C$_{10}$G.D (n.s.) | C$_{10}$G.Sl$_{100}$ (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | | | |
| | St$_{10}$G.D (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | | | |
| Distance, slope, climate and stream (3/12) | DG.Sl$_{100}$ (n.s.) | DG.C$_{10}$ (n.s.) | DG.St$_{10}$ (n.s.) | | | |
| | **Sl$_{100}$G.D (0.527)** | **Sl$_{100}$G.C$_{10}$ (0.609)** | **Sl$_{100}$G.St$_{10}$ (0.545)** | | | |
| | C$_{10}$G.D (n.s.) | C$_{10}$G.Sl$_{100}$ (n.s.) | C$_{10}$G.St$_{10}$ (n.s.) | | | |
| | St$_{10}$G.D (n.s.) | St$_{10}$G.C$_{10}$ (n.s.) | St$_{10}$G.Sl$_{100}$ (n.s.) | | | |

**Table 5** Results of the AMOVA with individuals grouped by drainage basin and population.

| Source of variation | d.f. | Φ-Statistic | Percentage of total variance | $P$-value |
|---|---|---|---|---|
| Among drainage basins | 3 | $\Phi_{CT} = 0.184$ | 18.44 | 0.012 |
| Among populations within drainage basins | 5 | $\Phi_{SC} = 0.146$ | 11.91 | < 0.001 |
| Among individuals within a population | 68 | $\Phi_{ST} = 0.303$ | 69.65 | < 0.001 |

CT, variance among groups of populations; SC, variance among populations within groups; ST, variance among the individuals within a population.

study organism is available from which to empirically derive or estimate these relative friction values (Ray et al., 2002; Ray, 2005), as is the case with A. varius.

The strongest correlations with genetic distance were found when the resistance to dispersal across steep slopes was high (Sl$_{100}$, Fig. 2). For these slope gradients, least-cost paths between populations followed streambeds and rivers almost exclusively, moving over land only for short distances, most

often along mountain ridges (Fig. 3). This was especially true at high elevations, where the majority of A. varius populations are found. Paths among low elevation populations, where changes in slope are not as dramatic, tended not to follow streams and ridges as closely. The least-cost paths between populations represent the pathways that offer the least resistance to movement (i.e. path of least resistance). However, this does not necessarily imply that individual frogs followed
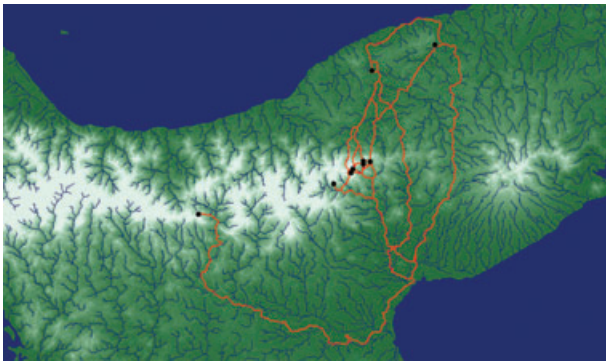
**Figure 3** Map of the least-cost paths between populations (denoted by red lines) for the landscape friction gradient most strongly correlated with genetic distance (slope$_{100}$). The background is an elevation map (light colours indicate high elevation).

these paths to physically move from one population to another. These paths are better interpreted as the routes among populations with the greatest predicted gene flow (over many generations of frogs and thousands of years) given the effect of landscape heterogeneity on movement (McRae, 2006).

Causal modelling also supported the hypothesis that gene flow among *A. varius* populations is influenced predominately by the slope of the landscape. No significant, independent relationships with stream distance, climate distance or Euclidian distance were found. However, the fact that the least-slope paths among populations tend to follow streams and rivers suggests that these features, in addition to mountain ridges, represent important corridors for gene flow among populations. The AMOVA results further highlight the importance of contiguous riparian corridors by demonstrating that a significant proportion of genetic variation is found among drainage basins, which are by definition not connected by riparian habitat (Table 5). Conversely, only a small fraction of the total genetic variation is found among populations within the same drainage basin. Taken together, these results are consistent with the idea that patterns of genetic variation among *A. varius* populations are affected by changes in elevation such that areas with steep slopes act as barriers to gene flow. In contrast, areas of low slope, such as streams and mountain ridges, appear to be important corridors for gene flow, especially among high elevation populations.

Steep slopes have been found to isolate populations of temperate amphibian species as well (Funk *et al.*, 2005; Spear *et al.*, 2005; Lowe *et al.*, 2006; Giordano *et al.*, 2007). However, because most of these studies have focused on pond-breeding amphibians, the role of riparian habitat corridors as facilitators of gene flow among amphibian populations has not often been explored. A notable exception to this was the study of Columbia spotted frogs (*Rana luteiventris*) by Funk *et al.* (2005), which found the pattern of isolation by river distance to be stronger than that of Euclidian distance over some parts of the species' range. The distribution of riparian habitat has been linked to patterns of gene flow among other, stream-associated taxa, such as the Pacific jumping mouse (*Zapus*

*trinotatus*) (Vignieri, 2005). However, this is not always the case (e.g. an alpine stream fly: Finn *et al.*, 2006).

For the nine *A. varius* populations, landscape distances attributing resistance to movement through suboptimal climates (climate distances) were less strongly correlated with genetic distance than was Euclidian distance (Table 3, Fig. 2). This result is consistent with the idea that regional climatic variation has little or no effect on gene flow among *A. varius* populations. However, it is also possible that climatic heterogeneity does influence gene flow, but that the species distribution model used to quantify relative climatic suitability failed to capture its effect. Unlike changes in elevation and the locations of riparian corridors, which have remained relatively stable in the region for thousands of years, the climate of western Panama may not have been consistent enough for a measurable correlation with the mitochondrial genetic distances among populations to have developed. The WorldClim bioclimatic variables used to build the climatic suitability gradients were derived from temperature and precipitation measurements averaged over the time period from 1950 to 2000. It is possible that patterns of gene flow are more strongly correlated with the climate landscape of earlier time periods (e.g. during the Pleistocene) than with present climate heterogeneity. Similar influences of past climatic landscapes on patterns of genetic connectivity have been found in other Neotropical riparian species (e.g. white piranha: Hubert *et al.*, 2007) as well as a host of temperate taxa (e.g. frogs: Green *et al.*, 1996; birds: Avise & Walker, 1998; spiders: Ayoub & Riechert, 2004; grasshoppers: Knowles & Richards, 2005 and fish: Stepien *et al.*, 2007).

## CONCLUSION

The landscape genetic approach of this study not only identified landscape factors affecting gene flow among *A. varius* populations, but also permitted the relative contributions and interactions of these factors to be evaluated in a hypothesis testing framework. Mantel tests, causal modelling and AMOVA uniformly support the strong role of areas of low slope, such as streams and mountain ridges, as conduits for gene flow among populations.

These findings not only contribute to our understanding of the ongoing evolutionary processes shaping variation among *A. varius* populations, but also engender important considerations for the management and conservation of this critically endangered species. For example, because gene flow appears to depend, to some extent, upon riparian connectivity, the construction of dams, introduction of potentially predatory fish, contamination of streams by agricultural runoff and other anthropogenic changes to streams and rivers are likely to affect the future evolution and ecology of these organisms. Likewise, the fragmentation of habitat along mountain ridges may adversely affect the connectivity of *A. varius* populations. Understanding how these factors affect dispersal and gene flow will be critical if, down the line, the decision is made to reintroduce captively raised *A. varius* to

Panama. Not only can this information help guide the selection of suitable locations for re-introductions, given the captively bred frogs' populations of origin, but it will also allow for informed predictions of the pattern of recolonization the frogs are likely to exhibit.

## REFERENCES

Arnaud, J.-F. (2003) Metapopulation genetic structure and migration pathways in the land snail *Heliz aspersa*: influence of landscape heterogeneity. *Landscape Ecology*, **18**, 333–346.

Arthofer, W., Schlick-Steiner, B.C., Steiner, F.M., Avtzis, D.N., Crozier, R.H. & Stauffer, C. (2007) Lessons from a beetle and an ant: coping with taxon-dependent differences in microsatellite development success. *Journal of Molecular Evolution*, **65**, 304–307.

Avise, J.C. & Walker, D. (1998) Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society B: Biological Sciences*, **265**, 457–463.

Ayoub, N.A. & Riechert, S.E. (2004) Molecular evidence for Pleistocene glacial cycles driving diversification of a North American desert spider, *Agelenopsis aperta*. *Molecular Ecology*, **13**, 3453–3465.

Blaustein, A.R., Wake, D.B. & Sousa, W.P. (1994) Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology*, **8**, 60–71.

Bohonak, A.J. (2002) IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.

Castellano, S. & Balletto, E. (2002) Is the partial Mantel test inadequate? *Evolution*, **56**, 1871–1873.

Cegelski, C., Waits, L.P. & Anderson, N.J. (2003) Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. *Molecular Ecology*, **12**, 2907–2918.

Crump, M.L. (1986) Homing and site fidelity in a Neotropical frog, *Atelopus varius* (Bufonidae). *Copeia*, **1986**, 438–444.

Cushman, S.A., McKelvey, K.S., Hayden, J. & Schwartz, M.K. (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modelling. *The American Naturalist*, **168**, 486–499.

Duellman, W.E. & Trueb, L. (1994) *The biology of amphibians*. Johns Hopkins University Press, Baltimore, MD.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes – application to human mitochondrial-DNA restriction data. *Genetics*, **131**, 479–491.

Fievet, V., Touzet, P., Arnaud, J.-F. & Cuguen, J. (2007) Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris* ssp. *maritima*) populations: do marine currents shape genetic structure? *Molecular Ecology*, **16**, 1847–1864.

Finn, D.S., Theobald, D.M., Black, W.C. & Poff, N.L. (2006) Spatial population genetic structure and limited dispersal in a Rocky Mountain alpine stream insect. *Molecular Ecology*, **15**, 3553–3566.

Funk, W.C., Blouin, M.S., Corn, P.S., Maxell, B.A., Pilliod, D.S., Amish, S. & Allendorf, F.W. (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, **14**, 483–496.

Garcia-Paris, M., Good, D.A., Parra-Olea, G. & Wake, D.B. (2000) Biodiversity of Costa Rican salamanders: implications of high levels of genetic differentiation and phylogeographic structure for species formation. *Proceedings of the National Academy of Sciences USA*, **97**, 1640–1647.

Giordano, A.R., Ridenhour, B.J. & Storfer, A. (2007) The influence of altitude and topography on genetic structure in the long-toed salamander (*Ambystoma macrodactylum*). *Molecular Ecology*, **16**, 1625–1637.

Green, D.M., Sharbel, T.F., Kearsley, J. & Kaiser, H. (1996) Postglacial range fluctuation, genetic subdivision and speciation in the western North American spotted frog complex, *Rana pretiosa*. *Evolution*, **50**, 374–390.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hubert, N., Duponchelle, F., Nunez, J., Rivera, A., Bonhomme, F. & Renno, J.-F. (2007) Isolation by distance and Pleistocene expansion of the lowland populations of the white piranha *Serrasalmus rhombeus*. *Molecular Ecology*, **16**, 2488–2503.

Keyghobadi, N., Roland, J. & Strobeck, C. (1999) Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). *Molecular Ecology*, **8**, 1481–1495.

Keyghobadi, N., Roland, J., Matter, S.F. & Strobeck, C. (2005a) Among- and within-patch components of genetic diversity respond at different rates to habitat fragmentation: an

empirical demonstration. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 533–560.

Keyghobadi, N., Roland, J. & Strobeck, C. (2005b) Genetic differentiation and gene flow among populations of the alpine butterfly *Parnassius smintheus* (Papilionidae). *Molecular Ecology*, **14**, 1897–1909.

Knowles, L.L. & Richards, C.L. (2005) Importance of genetic drift during Pleistocene divergence as revealed by analyses of genomic variation. *Molecular Ecology*, **14**, 4023–4032.

La Marca, E., Lips, K.R., Lötters, S., Puschendorf, R., Ibáñez, R., Ron, S., Rueda-Almonacid, J.V., Schulte, R., Marty, C., Castro, F., Manzilla-Puppo, J., Garcia-Perez, J.E., Bustamante, M.R., Coloma, L.A., Merino-Viteri, A., Toral, E., Bolaños, F., Chaves, G., Pounds, A. & Young, B. (2005) Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). *Biotropica*, **37**, 190–201.

Legendre, P. (1993) Spatial autocorrelation: trouble or new paradigm? *Ecology*, **74**, 1659–1673.

Legendre, P. & Troussellier, M. (1988) Aquatic heterotrophic bacteria: modelling in the presence of spatial autocorrelation. *Limnology and Oceanography*, **33**, 1055–1067.

Lichtenstein, M.H.C. & Martens, E. (1856) *Nomenclator reptilium et amphibiorum Musei zoologici berolinensis*. Namenverzeichniss der in der zoologischen sammmlung der Königlichen universität zu Berlin aufgestellten arten von reptilien und amphibien nach ihren ordnungen, familien und gattungen. Buchdruckerei der Königlichen akademie der wissenschaften, Berlin.

Liepelt, S., Bialozyt, R. & Ziegenhage, B. (2002) Wind-dispersed pollen mediates postglacial gene flow among refugia. *Proceedings of the National Academy of Sciences USA*, **99**, 14590–14594.

Lips, K.R. (1998) Decline of a tropical montane amphibian fauna. *Conservation Biology*, **12**, 106–117.

Lötters, S. (1996) *The neotropical toad genus Atelopus*. M. Vences & F. Glaw Verlags GbR, Koln, Germany.

Lötters, S. (2007) The fate of harlequin toads – help through a synchronous approach and the IUCN 'Amphibian Conservation Action Plan? *Mitteilungen aus dem Museum für Naturkunde in Berlin – Zoologische Reihe*, **83**(Suppl.), 69–73.

Lowe, W.H., Likens, G.E., McPeek, M.A. & Buso, D.C. (2006) Linking direct and indirect data on dispersal: isolation by slope in a headwater stream salamander. *Ecology*, **87**, 334–339.

MacKenzie, D.I., Nichols, J.D., Lachman, G.B., Droege, S., Royle, J.A. & Langtimm, C.A. (2002) Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, **83**, 2248–2255.

Manel, S., Schwartz, M.K., Luikart, G. & Taberlet, P. (2003) Landscape genetics: combining landscape ecology and populations genetics. *Trends in Ecology and Evolution*, **18**, 189–197.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

McRae, B.H. (2006) Isolation by resistance. *Evolution*, **60**, 1551–1561.

Meglecz, E., Petenian, F., Danchin, E., D'Acier, A., Rasplus, J.-Y. & Faure, E. (2004) High similarity between flanking regions of different microsatellites detected within each of two species of Lepidoptera: *Parnassius apollo* and *Euphydryas aurinia*. *Molecular Ecology*, **13**, 1693–1700.

Michels, E., Cottenie, K., Neys, L., De Gelas, K., Coppin, P. & De Meester, L. (2001) Geographical and genetic distances among zooplankton populations in a set of interconnected ponds: a plea for using GIS modelling of the effective geographical distance. *Molecular Ecology*, **10**, 1929–1938.

Monsen, K.J. & Blouin, M.S. (2003) Genetic structure in a montane ranid frog: restricted gene flow and nuclear-mitochondrial discordance. *Molecular Ecology*, **12**, 3275–3286.

Pfenninger, M. (2002) Relationship between microspatial population genetic structure and habitat heterogeneity in *Pomatias elegans* (O.F. Muller 1774) (Caenogastropoda, Pomatiasidae). *Biological Journal of the Linnean Society*, **76**, 565–575.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modelling of species geographic distributions. *Ecological Modeling*, **190**, 231–259.

Piertney, S.B., MacColl, A.D.C., Bacon, P.J. & Dallas, J.F. (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology*, **7**, 1645–1654.

Poissant, J., Knight, T.W. & Ferguson, M.M. (2005) Non-equilibrium conditions following landscape rearrangement: the relative contribution of past and current hydrological landscapes on the genetic structure of a stream-dwelling fish. *Molecular Ecology*, **14**, 1321–1331.

Pounds, J.A. & Crump, M.L. (1994) Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. *Conservation Biology*, **8**, 72–85.

Pounds, J.A., Bustamonte, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Maraca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sanchez-Azofeifa, G.A., Still, C.J. & Young, B.E. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, **439**, 161–167.

Pounds, J.A., Puschendorf, R., Bolaños, F., Chaves, G., Crump, M., Solis, F., Ibáñes, R., Savage, J., Jaramillo, C., Fuenmayor, Q. & Lips, K. (2008) *Atelopus varius*. *2008 IUCN red list of threatened species*. Available at: http://www.iucnredlist.org (last accessed 17 March 2009).

Raufaste, N. & Rousset, F. (2001) Are partial mantel tests adequate? *Evolution*, **55**, 1703–1705.

Ray, N. (2005) PATHMATRIX: a geographical information system tool to compute effective distances among samples. *Molecular Ecology Notes*, **5**, 177–180.

Ray, N., Lehmann, A. & Joly, P. (2002) Modeling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. *Biodiversity and Conservation*, **11**, 2143–2165.

Richards, C.L. & Knowles, L.L. (2007) Tests of phenotypic and genetic concordance and their application to the conservation of Panamanian golden frogs (Anura, Bufonidae). *Molecular Ecology*, **16**, 3119–3133.

Rivero, J.A. (1963) Five new species of *Atelopus* from Colombia, with notes on other species from Colombia and Ecuador. *Caribbean Journal of Science*, **3**, 103–124.

Roach, J.L., Stapp, P., Horne, B.V. & Antolin, M.F. (2001) Genetic structure of a metapopulation of black-tailed prairie dogs. *Journal of Mammalogy*, **82**, 946–959.

Rousset, F. (2002) Partial Mantel tests: reply to Casstellano and Balletto. *Evolution*, **56**, 1874–1875.

Savage, J.M. (1972) The harlequin frogs, genus *Atelopus*, of Costa Rica and western Panama. *Herpetologica*, **28**, 77–94.

Schneider, S., Roessli, D. & Excoffier, L. (2000) *Arlequin ver. 2.0: a software for population genetics data analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Scribner, K.T., Blanchong, J.A., Gruggemen, D.J., Epperson, B.K., Lee, C.-Y., Pan, Y.-W., Shorey, R.I., Williams, B.W., Prince, H.H., Winterstein, S.R. & Luukkonen, D.R. (2005) Geographical genetics: conceptual foundations and empirical applications of spatial genetic data in wildlife management. *Journal of Wildlife Management*, **69**, 1434–1453.

Semlitsch, R.D. (ed.) (2003) Conservation of pond-breeding amphibians. *Amphibian conservation*, pp. 8–23. Smithsonian Institution Press, Washington, DC.

Shaffer, H.B., Fellers, G.M., Magee, A. & Voss, S.R. (2000) The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite toad, *Bufo canorus* (Anura, Bufonidae) based on single-strand conformation polymorphism analysis (SSCP) and mitochondrial DNA sequence data. *Molecular Ecology*, **9**, 245–257.

Spear, S.F., Peterson, C.R., Matocq, M.D. & Storfer, A. (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology*, **14**, 2553–2564.

Stepien, C.A., Murphy, D.J. & Meadestrange, R. (2007) Broad- to fine-scale population genetic patterning in the smallmouth bass *Micropterus dolomieu* across the Laurentian Great Lakes and beyond: an interplay of behaviour and geography. *Molecular Ecology*, **16**, 1605–1624.

Stevens, V.M., Verkenne, C., Vandewoestijne, S., Wesselingh, R.A. & Baguette, M. (2006) Gene flow and functional connectivity in the natterjack toad. *Molecular Ecology*, **15**, 2333–2344.

Storfer, A., Murphy, M.A., Evans, J.S., Goldberg, C.S., Robinson, S., Spear, S.F., Dezzani, R., Delmelle, E., Vierling, L. & Waits, L.P. (2007) Putting the 'landscape' in landscape genetics. *Heredity*, **98**, 128–142.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. & Waller, R.W. (2004) Status and trends of amphibian declines and extinctions worldwide. *Science*, **306**, 1783–1786.

Tero, N., Neumeier, H., Gudavalli, R. & Schlotterer, C. (2006) *Silene tatarica* microsatellites are frequently located in repetitive DNA. *Journal of Evolutionary Biology*, **19**, 1612–1619.

Vignieri, S.N. (2005) Streams over mountains: influence of riparian connectivity on gene flow in the Pacific jumping mouse (*Zapus trinotatus*). *Molecular Ecology*, **14**, 1925–1937.

Wilmer, J.W. & Wilcox, C. (2007) Fine scale patterns of migration and gene flow in the endangered mound spring snail, *Fonscochlea accepta* (Mollusca: Hydrobiidae) in arid Australia. *Conservation Genetics*, **8**, 617–628.

Zhang, D.-X. (2004) Lepidopteran microsatellite DNA: redundant but promising. *Trends in Ecology and Evolution*, **19**, 507–509.

Zippel, K. (2002) Conserving the Panamanian golden frog: Proyecto Rana Dorada. *Herpetological Review*, **33**, 11–12.

Zippel, K.C., Ibáñez D., R., Lindquist, E.D., Richards, C.L., Jaramillo A., C.A. & Griffith, E.J. (2006) Implicaciones en la conservación de las ranas doradas de Panamá, asociadas con su revisión taxonómica. *Herpetotropicos*, **3**, 29–39.

Editor: Bruce Patterson

## SUPPORTING INFORMATION

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

**Appendix S1** Room temperature tissue preservative.

**Appendix S2** Museum records and locality data used in species distribution modelling.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.