MOLECULAR ECOLOGY

Molecular Ecology (2015) **24**, 580–595 doi: 10.1111/mec.13052

Mechanistic insights into landscape genetic structure of two tropical amphibians using field-derived resistance surfaces

A. JUSTIN NOWAKOWSKI,* J. ANDREW DEWOODY,†‡ MATTHEW E. FAGAN,§ JANNA R. WILLOUGHBY† and MAUREEN A. DONNELLY*¶

*Department of Biological Sciences, Florida International University, Miami, FL 33199, USA, †Department of Forestry & Natural Resources, Purdue University, West Lafayette, IN 47907, USA, ‡Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA, §Goddard Space Flight Center, Greenbelt, MD 20771, USA, ¶College of Arts and Sciences, Florida International University, Miami, FL 33199, USA

Abstract

Conversion of forests to agriculture often fragments distributions of forest species and can disrupt gene flow. We examined effects of prevalent land uses on genetic connectivity of two amphibian species in northeastern Costa Rica. We incorporated data from field surveys and experiments to develop resistance surfaces that represent local mechanisms hypothesized to modify dispersal success of amphibians, such as habitat-specific predation and desiccation risk. Because time lags can exist between forest conversion and genetic responses, we evaluated landscape effects using land-cover data from different time periods. Populations of both species were structured at similar spatial scales but exhibited differing responses to landscape features. Litter frog population differentiation was significantly related to landscape resistances estimated from abundance and experiment data. Model support was highest for experiment-derived surfaces that represented responses to microclimate variation. Litter frog genetic variation was best explained by contemporary landscape configuration, indicating rapid population response to land-use change. Poison frog genetic structure was strongly associated with geographic isolation, which explained up to 45% of genetic variation, and long-standing barriers, such as rivers and mountains. However, there was also partial support for abundance- and microclimate response-derived resistances. Differences in species responses to landscape features may be explained by overriding effects of population size on patterns of differentiation for poison frogs, but not litter frogs. In addition, pastures are likely semi-permeable to poison frog gene flow because the species is known to use pastures when remnant vegetation is present, but litter frogs do not. Ongoing reforestation efforts will probably increase connectivity in the region by increasing tree cover and reducing area of pastures.

Keywords: connectivity, field experiments, fragmentation, gene flow, land use, microclimate

Received 30 September 2014; revision received 13 December 2014; accepted 18 December 2014

Introduction

Anthropogenic land-cover change affects the majority of the Earth's terrestrial systems (Hobbs *et al.* 2009; Barnosky *et al.* 2012). The resulting landscape mosaics, often comprised of agriculture and remnant forests, constitute novel templates for ecological and evolutionary processes. Altered landscapes modify animal distributions by disrupting habitat continuity and the functional connectivity of remnant populations (Driscoll *et al.* 2013). Widespread land uses differ in the degree to which they impede gene flow and thereby reduce allelic diversity through genetic drift, reduce the spread of adaptive

Correspondence: A. Justin Nowakowski, Fax: +305 348 1986, E-mail: Nowakowskia@gmail.com

© 2014 John Wiley & Sons Ltd

genes and increase the susceptibility to further environmental change (Pearman & Garner 2005; Manel & Holderegger 2013). Strategies to maintain connectivity of reserve networks should consider the relative resistances of common land uses as well as mechanisms underlying resistance to movement (DeClerck *et al.* 2010; Laurance *et al.* 2012).

A primary focus of landscape genetics is to describe how anthropogenic environments shape patterns of gene flow and the distribution of genetic diversity across the landscape (Storfer *et al.* 2010). Commonly used connectivity analyses (e.g. circuit models and least-cost path analyses) use resistance surfaces to represent environmental templates. Resistance surfaces are typically raster grids that contain information on the spatial configuration of landscape elements (e.g. land-use types) and the hypothesized or empirical costs associated with moving across landscape features (Spear *et al.* 2010). The majority of studies to date have relied on expert opinion or model-fitting procedures to determine cost values (Spear *et al.* 2010; Zeller *et al.* 2012). However, resistance estimates are highly sensitive to the magnitude of contrast among cost values assigned to different landscape features (Rayfield *et al.* 2010; Koen *et al.* 2012). Expert-assigned values may not reflect biologically relevant levels of habitat contrast, and model-fitting approaches, in which a parameter space is explored to find resistance values that best fit the genetic data (Spear *et al.* 2010), do not facilitate a priori hypothesis testing or mechanistic insights.

Cost values derived from field data are more likely to represent biologically important contrasts among habitats. Recent studies have used survey or movement data (e.g. from mark–recapture or radio telemetry) to develop resistance surfaces (Spear *et al.* 2010); however, very few studies have used experiments to develop cost surfaces, despite the potential to evaluate different local processes associated with landscape resistance (Stevens *et al.* 2006). When landscapes are complex, organisms perceive contrasts in habitat quality among alternative movement pathways at a local scale, which may ultimately modify dispersal rates (Baguette *et al.* 2012). Experiments can be used to provide information on species responses to specific landscape features (Knowlton & Graham 2010), such as gap-crossing behaviour (Smith *et al.* 2013) and habitat-specific survival (Rittenhouse *et al.* 2008; Hammerschlag *et al.* 2010). Given time, the cumulative effects of local dispersal risks and behaviours should scale up to population-level patterns of genetic structure. However, the influence of local processes on genetic structure will ultimately be regulated by other demographic parameters, such as effective population size (Busch *et al.* 2009; Lowe & Allendorf 2010).

For amphibians, isolating effects following landscape change may be pronounced (Jenkins *et al.* 2010) and manifest quickly in populations because of limited vagility (Smith & Green 2005), short generation times and metabolic constraints on movement (Wells 2007). Ectothermy and highly permeable skin limit activity and likely increase susceptibility to extreme microclimates in altered habitats. Amphibians exhibit high rates of water loss (Rothermel & Luhring 2005; Consentino *et al.* 2011), mortality (Rothermel & Semlitsch 2006) and behavioural avoidance in land uses with little canopy cover in temperate zones (Rittenhouse & Semlitsch 2006). Tropical ectotherms may be even more sensitive to altered microclimates than temperate species (Deutsch *et al.* 2008; Huey *et al.* 2009), in part, because ambient forest temperatures are near physiological thermal maxima for some lowland species (Catenazzi *et al.* 2013).

Here, we examined land-use effects on population structure of two ecologically dominant amphibian species in a forest-agricultural landscape in Costa Rica. Our study addresses four areas that have been identified as research priorities in the landscape genetics literature. First, field data provide objective measures of habitat contrasts, but are rarely used to develop resistance surfaces for connectivity analyses (Spear *et al.* 2010; Peterman *et al.* 2014). We used relative abundance data and results of field experiments to assign cost values to resistance surfaces. Field experiments measured mechanisms hypothesized to be important determinants of connectivity for amphibians, including desiccation risk, predation and substrate resistance to movement. Second, lag times in population-genetic responses to landscape change are often assumed but infrequently tested (Storfer *et al.* 2010; Manel & Holderegger 2013), and recent research suggests that populations can respond rapidly to contemporary landscape states (Zellmer & Knowles 2009; Landguth *et al.* 2010). We used land-cover data from multiple time periods to examine effects of past and present landscape configurations on population structure. Third, single species studies dominate the landscape genetics literature; however, multispecies studies are necessary for identifying idiosyncratic and shared responses to landscape features (Goldberg & Waits 2010; Richardson 2012). Finally, tropical species are underrepresented in the literature (Manel & Holderegger 2013), despite higher rates of forest conversion and species loss occurring in tropical zones (Stuart *et al.* 2004; Vié *et al.* 2009; Hansen *et al.* 2013).

Specifically, we first compared population-genetic structure between the direct-developing litter frog, *Craugastor bransfordii*, and the poison frog, *Oophaga pumilio*. The few population-genetic studies of direct-developing

© 2014 John Wiley & Sons Ltd

species have reported fine-scale genetic structure and limited gene flow (Lampert *et al.* 2003; Elmer *et al.* 2007). Therefore, we expected a priori that litter frogs would exhibit stronger population subdivision than poison frogs. Second, we assessed the importance of landscape effects on genetic structure of each species in comparison with simple isolation-by-distance models. Because landscape modification has occurred for over half a century in the study region, we expected that empirical resistances would be more informative than Euclidean distances. Third, relative support was determined for resistance models derived from field experiments that measured different local processes. Field observations and prior validation of resistance models using an independent abundance data set suggested that cost surfaces derived from species responses to microclimate would be important predictors of landscape genetic structure (Nowakowski *et al.* 2013, In Press). Fourth, we determined whether the strength of landscape genetic correlations varied across time using land-cover maps from three different time periods. We predicted that the strength of landscape effects would be greater for older landscape configurations, reflecting time lags in population-genetic responses.

Methods

Study system and field sampling

Our study took place in the Caribbean lowland region of northeastern Costa Rica. Wet tropical lowland forest is the dominant natural vegetation type in this area and is part of a biogeographic region that extends along the Atlantic versant from Mexico to Panama (Savage 2002). The area receives about 4 m of rain annually and has a mean annual temperature of 25 °C (Sanford *et al.* 1994). The study landscape is a heterogeneous mosaic of forest, pastures and cultivated lands. Approximately 40% of the landscape remains forested (about 12% of which is secondary regrowth); however, most forest is comprised of fragmented patches and riparian zones (Sesnie *et al.* 2008; Fagan *et al.* 2013). Beginning in the 1950s, expansion of cattle ranching caused rapid deforestation for the establishment of pasture lands (Butterfield 1994). While pastures are the dominant land use, now covering 40% of the land area, agricultural crops such as banana, pineapple and heart of palm have expanded in recent decades to cover about 10% of the landscape (Fagan *et al.* 2013).

Our study focused on the two most abundant amphibian species in the region, the poison frog, *Oophaga pumilio*, and the litter frog, *Craugastor bransfordii*. *Oophaga pumilio* is a small (17–22 mm snout-to-vent length [SVL]), brightly coloured frog that contains alkaloids in its skin, which are sequestered from arthropods in its diet (Saporito *et al.* 2007). The species is dependent upon forest resources for reproduction, such as leaf-litter oviposition sites and tadpole-rearing sites in phytotelmata (Donnelly 1989a). *Oophaga pumilio* is known to maintain small home ranges on the order of 10–30 m^2 (Donnelly 1989b). *Craugastor bransfordii* is also a small frog (18–25 mm SVL) that breeds in leaf litter. However, this species lacks chemical defences, is cryptically coloured and does not have a free-living tadpole stage. What little research exists on direct-developing eleutherodactyline frogs suggests that the group is characterized by small home ranges and limited dispersal (Elmer *et al.* 2007).

Both species reach high abundances in forest, but exhibit different responses to landscape change (Kurz *et al.* 2014). Poison frogs are known to occur in pastures, where abundances are associated with the presence of individual-remnant trees (Robinson *et al.* 2013), but are rarely observed in cultivated areas (Kurz *et al.* 2014). In contrast, litter frogs reach moderate abundances in heart-of-palm plantations, but are nearly absent in pastures. Population declines were recorded for both species in protected old growth forest over a 35-year period within the study landscape (Whitfield *et al.* 2007).

As part of a larger study, we characterized relative abundances of all amphibians at 17 forest remnants by conducting visual encounter surveys along 50 × 4 m transects (e.g. von May *et al.* 2010). In each remnant, 26 transects were randomly located and searched at night, between 18:30 and 01:00 h. Our two focal species are considered diurnal; however, both species can be readily observed at night (Kurz *et al.* 2014), and night-time surveys do not provide biased abundance estimates compared to daytime surveys (Nowakowski unpubl. data). Relative abundance data for the focal species in remnants were used in downstream statistical analyses (hereafter these data are referred to as 'local abundances'). Toe clips were collected from focal species for genetic analyses and stored in 95% ethanol and frozen. Randomized transect placement increases the likeliness that sampled individuals are representative of the genetic variation that occurs within forest remnants. However, we also augmented sample sizes with individuals encountered outside of transects.

Microsatellite genotyping and summary statistics

Tissue was digested in lysis buffer and proteinase K, and standard phenol–chloroform methods were used to extract DNA (Sambrook & Russell 2001). PCRs were conducted with multiplexed sets of 2–4 fluorescently labelled (6-FAM and HEX) primer pairs. We genotyped

© 2014 John Wiley & Sons Ltd

individuals at 11 microsatellite loci for poison frogs (Oop_B9, Oop_B8, Oop_E3, Oop_G5, Oop_H5, Oop_O1, Oop_F1, Dpum14, Dpum24, Dpum63 and Dpum44; Hauswaldt *et al.* 2009; Wang & Summers 2009) and 10 loci for litter frogs (Cbra_2, Cbra_16, Cbra_18, Cbra_28, Cbra_38, Cbra_48, Cbra_53, Cbra_61, Cbra_64 and Cbra_71; Nowakowski *et al.* 2014). We used 20 μl reaction volumes for PCR amplifications that consisted of 40 ng of template DNA, 10 mM Tris-HCl, 50 mM KCl, 0.5 mg/ml BSA, 1.5 mM of MgCl2, 0.2 mM of each dNTP and NEB Taq polymerase (1U). Amplicon sizes were determined using an ABI 3130XL and GENESCAN 4.1 (Applied Biosystems). We scored raw alleles using program GENEMAPPER v3.7 (Applied Biosystems) and binned alleles using FLEXIBIN (Amos *et al.* 2007).

We summarized allele frequencies and heterozygosity and tested for significant linkage disequilibrium (LD) and deviations from Hardy–Weinberg equilibrium (HWE) using Arlequin v3.5 (Excoffier & Lischer 2010). We specified 1 000 000 Markov chain steps with a 100 000 step burn-in for performing exact tests of HWE and 1000 permutations for tests of LD and applied Bonferroni corrections (Rice 1989). Null allele frequencies were estimated using MICROCHECKER v2.2.3 (Van Oosterhout *et al.* 2004).

Population differentiation and population size

We characterized genetic differentiation between pairs of sample sites using two measures, F_{ST} and Jost's D. Values of F_{ST} are known to be dependent upon within population diversity such that for multi-allelic markers, high expected heterozygosity (H_e) can result in low maximum F_{ST} values (e.g. max F_{ST} = 0.1; Meirmans & Hedrick 2011). However, interpretation of values is facilitated by well-understood relationships among drift, migration and population size for F_{ST} measures of differentiation. On the other hand, Jost's D (D_{EST} hereafter) better reflects differentiation of allele frequencies among populations than F_{ST} and maximum values are not restricted by within-population heterozygosity (Jost 2008; Meirmans & Hedrick 2011). However, evolutionary interpretations of D_{EST} are currently less well established than for F_{ST} measures (Whitlock 2011).

Because rate of genetic drift and patterns of differentiation are influenced by effective population size (N_e), we estimated N_e at each forest remnant using the linkage disequilibrium method implemented in program LDNe (Waples & Do 2008). We specified a random mating system within populations and excluded rare alleles with frequencies <0.01. Although theory dictates that N_e affects differentiation (Frankham *et al.* 2002), N_e estimates are often imprecise (e.g. Richardson 2012) and sensitive to model parameters; abundances should covary with N_e to the extent that local populations conform to idealized population structures. Therefore, we also analysed associations between differentiation and local abundances (i.e. abundances measured in forest remnants). Influence of variation in N_e and local abundances on patterns of differentiation was assessed using linear regression. Because the objective of this analysis was to explain variation in point estimates of N_e and abundances, we used the mean pairwise F_{ST} value for each site as an explanatory variable. In all but one forest remnant, where fewer than 30 poison frogs were encountered, relative abundances recorded on transects are independent of sample sizes used in genetic analyses. Sample sizes for laboratory analyses were standardized at 30-36 samples per site per species, and any variation in final n across sites is largely attributable to genotyping completeness (i.e. individuals with missing data were culled from the final data set).

Bayesian clustering

To compare the level of population structure between species represented as the number and geographic locations of genetic demes, we used a Bayesian clustering model implemented in program STRUCTURE (Pritchard *et al.* 2000). The STRUCTURE model determines support for a given number of genetic clusters (K) by estimating the probability of the multilocus genotype data, given a specified number of clusters, Pr(X | K). Individuals are assigned to clusters so as to minimize linkage disequilibrium and deviations from HWE.

We first conducted an exploratory analysis where K was set to all possible values ≤ total number of sample sites, n (e.g. Zamudio & Wieczorek 2007). Initially, models were run with three replicates for each value of K, a 200 000 step burn-in period, and 500 000 MCMC repetitions following burn-in. Model support for the number of distinct genetic clusters (K) was determined as the maximum value of ln Pr(X | K) before asymptote for multiple possible K values using program STRUCTURE HARVESTER (Earl & vonHoldt 2012). Because all initial runs (K = 1−n) indicated that ln Pr(X | K) plateaued at K = 4 or 5, we focused additional replication on K = 1−10 for *O. pumilio* and K = 1–9 for *C. bransfordii*. For each value of K, models were run with same burn-in length and MCMC repetitions as the exploratory analysis. After six replicates for each K value, there was little variation around mean ln Pr(X | K) within the range at which K reached asymptote; therefore, we terminated the analysis. We specified models that allowed for admixture, as local populations often have mixed ancestry and correlated-allele frequencies. We used sampling location priors, which are useful for improving population assignment when data indicate

© 2014 John Wiley & Sons Ltd

weak-to-moderate population structure; location information typically improves resolution, but does not generate artificial structure in the data (Hubisz *et al.* 2009; Pritchard *et al.* 2010). Replicate runs were aligned and averaged using CLUMP (Jakobsson & Rosenberg 2007), and *Q*-plots were constructed using program DISTRUCT (Rosenberg 2004).

Resistance surfaces

We evaluated landscape effects on pairwise population differentiation using experiment and field-survey-derived resistance surfaces. Cost surfaces were created by reclassifying cell values of land-cover data sets developed from 1986, 2001 and 2011 Landsat images (Fagan *et al.* 2013). Grid cell size was 30 m for all surfaces. Empirical costs were assigned to forests, pastures and heart-of-palm plantations (locally known as palmito), which together represent approximately 86% of the study landscape. We clipped the land-cover data sets to the extent of our 2500 km^2 study area.

To develop field-survey-derived resistance surfaces, we used relative abundance data for the two focal species collected along 400 transects surveyed in forest, pasture and palmito plots in Sarapiquí, Costa Rica (hereafter referred to as 'matrix abundances' to distinguish from 'local abundance' data; Kurz *et al.* 2014). Ten sites were surveyed in 2012, and each site included a forest plot that was paired with either an adjacent pasture or palmito plot. Four habitat categories were sampled in total, forest adjacent to pasture, pasture, forest adjacent to palmito and palmito, with 100 transects surveyed in each category and 40 transects at each site. Complete details on sampling and results are reported in Kurz *et al.* (2014).

To develop experiment-derived resistance surfaces, we used data from three separate field experiments that measured (i) desiccation risk and mortality associated with exposure to microclimates in forest, pasture and palmito land-cover types, (ii) physical resistance to movement of common substrates associated with each land-cover type and (iii) predator encounter rates in focal land-cover types. Complete details on experimental approach are reported in Nowakowski *et al.* (*In Press*). Briefly, desiccation risk and mortality were measured during field trials in which frogs were placed in individual enclosures within each land-cover type, thereby exposing them to habitat-specific microclimatic conditions. Survival and change-in-mass (water loss) were recorded over one-hour trials as measures of desiccation risk. Exposure times were determined using a pilot study; 1-h periods ensured that the majority of individuals assigned to open habitats survived trials while allowing for measurable variation in responses. Trials were conducted in replicate land uses, on multiple days, at different times of day, and under a range of weather conditions to capture spatial and temporal variation in microclimatic conditions.

Substrate resistance was measured as the time taken by individuals to traverse 2-m-long enclosures containing land-cover-specific substrates (e.g. forest leaf litter and pasture grasses). Substrates were collected from random points within replicate areas of each land-cover type. Trials were initiated by placing a frog at one end of the enclosure, and a standardized stimulus was applied to ensure continued movement across the length of the enclosure (e.g. Nowakowski *et al.* 2013). Longer travel times were assumed to indicate greater physical resistance of substrates to movement. We used a tethering experiment to record the number of predation events in each land-cover type. Tethering experiments are commonly used in marine ecology studies and can provide an index of habitat-specific predator encounter rates (Aronson *et al.* 2001; Hammerschlag *et al.* 2010). Transects were established in replicate areas of each land-cover type, and 6 frogs were tethered at 10-m intervals along each transect. Frogs were tethered for 16 h (from late afternoon to early morning), and individuals that remained tethered, but exhibited injuries consistent with a predation attempt, were scored as predator encounters. Following experiments, we released individuals at capture sites. All protocols received IACUC approval.

These empirical data sets allowed us to assign resistance values that reflect biologically relevant levels of contrast among landscape elements and to make inferences about the importance of local mechanisms in explaining landscape-scale patterns of population structure (Table S1). Empirical data were translated to cost values using response ratios, an effect size metric commonly used in meta-analyses. All forested cells were assigned a resistance value of 1, assuming low resistances of forest in relation to other land uses. Cost values for pastures and palmito were then calculated as the response ratios from experimental results and survey data. For example, the cost value (C) for pastures derived from abundance data was calculated as C_p = total abundance in forest/total abundance in pasture. Therefore, lower abundances relative to forest are assumed to reflect greater resistances of pastures. Conversely, C_p derived from water loss experiments was calculated as C_p = mean percentage weight loss in pasture/mean percentage weight loss in forest, as greater desiccation risk is assumed to be associated with higher resistances. We assigned costs to other land uses, such as banana and tree plantations, on the basis of structural similarity to measured land uses, which was determined by the presence or absence of overstory and

© 2014 John Wiley & Sons Ltd

mid-story vegetation strata. Cloud-covered cells (0–0.9% of cells) and rivers were assigned resistances equal to the average resistances for forest, palmito and pasture. Our approach for assigning costs to unmeasured land uses should have negligible influence on the analyses, as these features only represent 16% of the study landscape and values were standardized across all resistance surfaces. Experiment-derived resistance surfaces were evaluated for the 2011 landscape only (and not 1986 and 2001 landscapes) based on data exploration (see results). We also created a resistance surface of potential barriers. We assigned infinite resistances to all major rivers and areas above the known elevation limits of each species (foothills of the Cordillera Central in the southern part of the landscape) under the hypothesis that these long-standing features represent strong barriers to gene flow.

To evaluate changes in the strength of landscape genetic responses (i.e. effect sizes) across time, we produced resistance surfaces for three time periods, 1986, 2001 and 2011. We used classified land-cover data from Fagan *et al.* (2013) and assigned costs using the matrix abundance data as described above. We used abundances for analyses of temporal landscape signatures, because they represent our best independent measure of resistance – abundances likely reflect the combined influence of multiple mechanisms underlying connectivity (e.g. habitat-specific survival and behaviour).

Our simplest landscape model consisted of pairwise geographic distances among sites. Isolation-by-distance (IBD) models contain no information about landscape features and can be used as null models for evaluating support for more complex landscape models. We chose to use a IBD model, as opposed to isolation by resistance (McRae *et al.* 2008), because it represents the purely spatial component of the landscape (i.e. the arrangement of sample sites).

Circuitscape modelling

We used CIRCUITSCAPE to estimate landscape resistances among sample sites (McRae & Shah 2009). CIRCUITSCAPE combines graph theory and circuit theory to model connectivity by constructing graphs that connect focal nodes representing populations or sample sites (McRae *et al.* 2008). Graph edges are replaced by resistors to create networks analogous to electrical circuits where resistances among focal nodes are a function of cost surface grids used as input. We used pairwise resistance distances output by CIRCUITSCAPE as a connectivity measure, which reflects the combined resistance of all resistors and the redundancy of pathways. Allowing for multiple connections between pairs of nodes is more realistic and may improve performance over approaches that consider only individual movement pathways, such as least-cost path analyses (McRae & Beier 2007).

In total, we generated 8 resistance surfaces for each species; these included matrix-abundance-derived surfaces for 1986, 2001 and 2011 periods, experiment-derived surfaces for water loss, survival, substrate resistance and predation responses, and a barriers surface, which were all used as input for CIRCUITSCAPE. We modelled connectivity among 15 and 17 focal regions (forest remnants) for poison frogs and litter frogs, respectively. Cost surface cells were coded as resistances, and spatial graphs were constructed by connecting eight cell neighbours using average resistance values.

Statistical analyses of landscape genetic relationships

We performed two sets of analyses, one using multiple regression on distance matrices (MRDM; Legendre *et al.* 1994) and a second using redundancy analysis (RDA; Legendre & Legendre 2012). The MRDM analysis was used to evaluate competing models with empirically derived resistance distances as explanatory variables and genetic differentiation (F_{ST} and D_{EST}) as the response. We first fit a model with only geographic distances to serve as a null model. We then fit models with distances derived from (i) a given empirical resistance surface (matrix abundance or experiment derived), (ii) local abundances and (iii) a barrier model. Because resistance distances from experiments were ecologically and statistically nonindependent, we did not include them in the same model. Local abundances were converted to distance matrices by calculating the Euclidean distance between values at pairs of sites. Nonsignificant variables were dropped in succession until all variables in the model were significant (Zuur *et al.* 2009). Significance of the regression coefficients and R^2 was determined through 10 000 permutations of the response matrix. Reduced models were then ranked based on the change in variance explained by the landscape model in comparison with the IBD model, which was calculated as $\delta R^2_{IBD} = \mathrm{adj}^2_{Land} - \mathrm{adj}^2_{IBD}$ (Dyer *et al.* 2010). The MRDM analyses were conducted using the ECODIST package (Goslee & Urban 2007) in R (R Core Team 2014).

Redundancy analysis (RDA) is a multivariate multiple regression analysis that allows for the estimation of fractions of genetic variance explained by the spatial arrangement of sites and other predictor variables, such as resistance distances (Legendre & Fortin 2010; Legendre & Legendre 2012). The matrix of dependent variables consisted of principle coordinate vectors (PCoA) derived from the pairwise genetic differentiation matrices. Analyses were repeated separately using

© 2014 John Wiley & Sons Ltd

PCoA variables for F_{ST} and D_{EST}. The spatial matrix consisted of pairwise geographic distances that were converted into a rectangular matrix of vectors using a principle coordinates of neighbour matrices analysis (PCNM; Borcard & Legendre 2002). The default truncation distance was used, and positive eigenvectors were extracted for the RDA. Explanatory variables included local abundances from transect surveys and resistance distances from the barrier model and empirical surfaces converted to site-specific connectivity indices as follows:

$$S_i = \sum \exp -\alpha d_{ij}$$

where S_i is connectivity of a given site, i, α is a scaling factor associated with the average dispersal distance of the species, and d is the resistance distance between sites i and j. The metric stems from metapopulation ecology (Moilanen & Nieminen 2002) and is increasingly employed in landscape genetics (e.g. Pflüger & Balkenhol 2014). We used forward selection to reduce the number of variables in the environmental and spatial matrices for significant RDA models. We then partitioned explained variance into environmental (i.e. landscape effects) and spatial components using the varpart function in package VEGAN (Oksanen *et al.* 2012).

We chose to use MRDM and RDA because performance of these analyses has been evaluated in landscape genetic studies (Balkenhol *et al.* 2009). The MRDM allows for the analysis of pairwise distances, which is the typical form in which landscape genetic data are organized and allows for direct tests of hypotheses concerning effective distances. The RDA focuses on site-specific connectivity in relation to all other sites and allows for partitioning of variance into pure landscape, pure spatial and spatially structured landscape components. However, information on the landscape between sites is lost when pairwise resistances are condensed into the connectivity index. Therefore, we expected these analyses to provide complementary, rather than identical, results, and we expected strong landscape effects to be detectable across analyses.

Results

Marker screening and summary statistics

We assembled complete multilocus genotypes (no missing data) for 512 poison frog individuals and 513 litter frog individuals (Table S2, Supporting information). For poison frogs, we excluded loci Oop_H5 and Dpum_44 from analyses because they exhibited high rates of nonamplification (>0.05) and occasional extra alleles, possibly attributable to null alleles and insertions, respectively. Across the remaining 9 loci, we detected significant deviation from HWE in only 1 test (of 135) after Bonferroni correction. Significant LD was found for one pair of loci in one population. For litter frogs, departure from HWE was found in 39 (of 170) tests. Most loci did not exhibit consistent deviations from HWE. However, Cbra_71 was out of HWE in all 17 populations, which is probably attributable to high frequency of null alleles at this locus (NA> 0.2). We excluded Cbra_71 from downstream analyses, but included two loci that had null alleles at lower frequencies (Cbra_48 and Cbra_61; NA <0.2). Simulations studies show that loci with null allele frequencies ≤ 0.2 have little effect on STRUCTURE model performance (Carlsson 2008) and do not significantly bias estimation of F_{ST} when differentiation is weak (Chapuis & Estoup 2007). There was no pervasive pattern of LD across populations for litter frogs (mean of 0.76 linked pairs of loci per population). Genetic diversity was generally high for both species. For poison frogs, total number of alleles per locus ranged from 6 to 24. Across all loci and sites, mean allelic richness (AR) was 11.1 ± 1.2 (mean ± SD) and mean observed heterozygosity (H_o) was 0.81 ± 0.04 (Table S2, Supporting information). For litter frogs, total alleles per locus ranged from 23 to 41, AR was 16.3 ± 0.96, and H_o was 0.81 ± 0.03.

Population differentiation and population size

Mean pairwise estimates of F_{ST} were 0.029 ± 0.017 (mean ± SD) ranging from 0.0 to 0.07, and mean estimates of D_{EST} were 0.075 ± 0.049 with a range of 0.00-0.2 for poison frogs (Table S3, Supporting information). For litter frogs, mean F_{ST} was 0.020 ± 0.007 (range: 0.004–0.044) and mean D_{EST} was 0.166 ± 0.068 (range: 0.011–0.375). The majority of pairwise F_{ST} values were significantly greater than zero (poison frogs: 90 of 105; litter frogs: 128 of 136) after Bonferroni correction for multiple tests. Effective population size varied considerably across sites for both species; we did not compute summary statistics, as point estimates and confidence limits included infinity for multiple populations. Therefore, we binned N_e estimates and converted them to ranks for further analyses. Mean pairwise F_{ST} estimates were negatively associated with local abundances (Fig 1; $R^2 = 0.409$, $P = 0.010$) and ranked N_e estimates ($R^2 = 0.251$, $P = 0.057$) for poison frogs, indicating lower differentiation of large populations than small populations. Mean pairwise F_{ST} was not correlated with local abundances ($R^2 = 0.000$, $P = 0.990$) or rank estimates of N_e (R^2 0.097, $P = 0.222$) for litter frogs.

© 2014 John Wiley & Sons Ltd

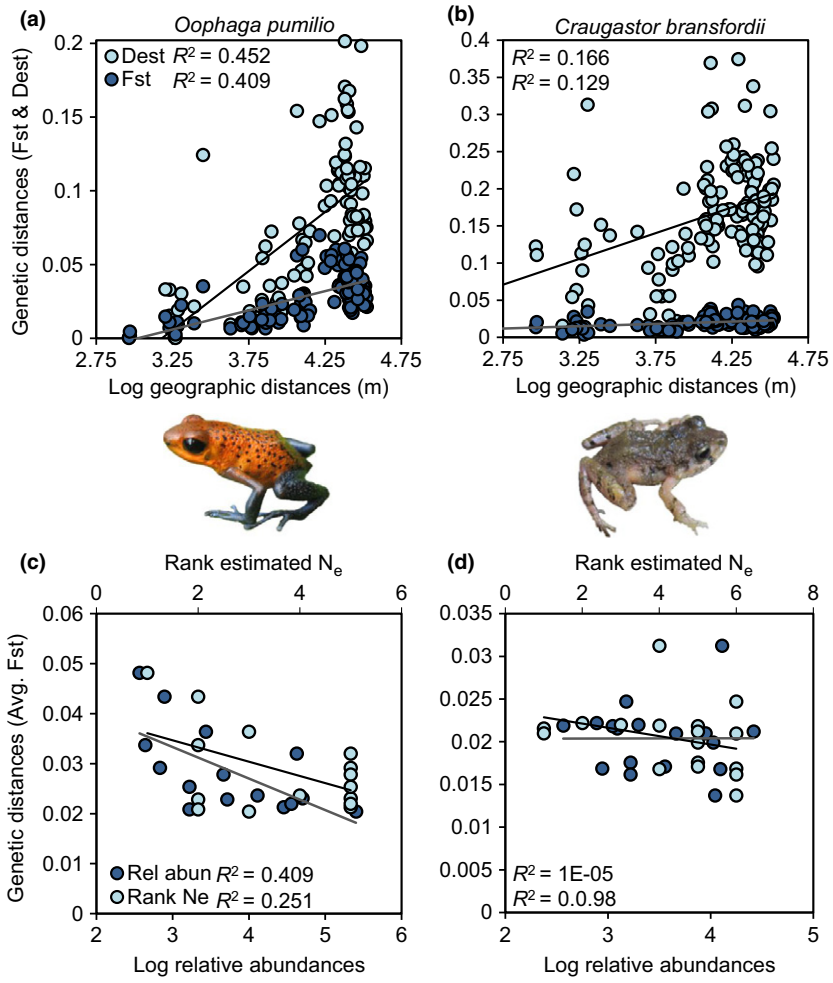

Fig. 1 Isolation-by-distance relationships for (a) *Oophaga pumilio* and (b) *Craugastor bransfordii*. Light blue circles represent pairwise Jost's *D*, and dark blue circles represent pairwise F_{ST}. Relationship between average F_{ST} for each site and population size at each site for (c) *O. pumilio* and (d) *C. bransfordii*. Rank estimates of N_e (light blue) for each population are given on the upper *x*-axis, and log local abundances (counts from transect surveys at each site; dark blue) are given on the lower *x*-axis. Note that panels a and b show pairwise values, whereas c and d show point values for each site.

Bayesian clustering

The asymptotic values of mean ln P(X | K) supported K = 5 genetic clusters for poison frogs and K = 4 clusters for litter frogs (Fig 2). Geographic patterns of population clustering were generally concordant between species, and genetic clusters tended to correspond to the spatial scale associated with groups of neighbouring sites rather than individual sites. For poison frogs, sites located in the southeastern part of the landscape comprised a single cluster, sites in the northwest formed a second cluster, and sites in the southwestern part of the landscape were grouped into a third cluster. Several sites were largely admixed between the second and third clusters, and individuals at two sites located in the northeast were assigned to two distinct clusters. Most poison frog individuals were strongly assigned to a given population (i.e. little admixture). Litter frogs, on the other hand, exhibited higher levels of admixed ancestry than poison frogs at the site level. While individuals were strongly assigned to three multi-site clusters in the southeast, northwest and southwestern parts of the landscape, individual ancestry at remaining sites was largely admixed.

Resistance distance–genetic distance relationships

Poison frogs exhibited a strong and significant isolation-by-distance relationship for both metrics of genetic differentiation (Fig 1; Table 1). For poison frogs, we did not detect consistent landscape effects across all analyses. There was a consistent and strong influence of spatial location; IBD was the best MRDM model when F_{ST} was the response variable, and was among the top three models when D_{EST} was the response. However, the RDA models suggest that poison frog populations are structured, in part, by barriers, variation in local abundances and landscape resistance, but that most of the variation explained by these variables is spatially structured (Table 1, Fig 3). It is worth-noting that resistances from barrier and water-loss surfaces were included among the top three models in three of four of the analysis–response combinations. A higher percentage of

© 2014 John Wiley & Sons Ltd

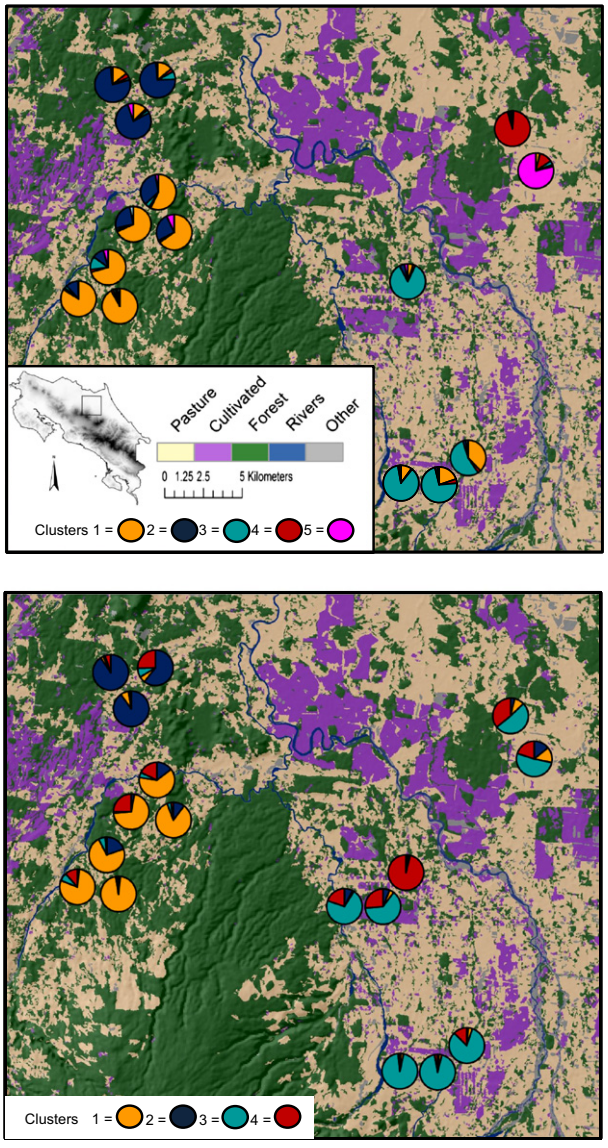

Fig. 2 Map of study area showing percentage ancestry of individuals at each site from a given cluster for *O. pumilio* (above) and *C. bransfordii* (below). Note: extent shown here is smaller than the 2500 km^2 extent used in analyses.

variation in the response was explained by 2001 and 2011 landscape configurations than the 1986 landscape; though, these resistance surfaces were not among the best supported MRDM models for poison frogs. In contrast, litter frogs exhibited significant but much weaker isolation-by-distance effects (Fig 1). There were also consistent effects of landscape features on population differentiation across all analyses (Table 1). Resistances derived from matrix abundances in combination with the 2011 landscape were the best supported models according to MRDM analyses, and survival in response to land-use-specific microclimates was best supported using RDA. For analyses of lag times, we found that R^2 increased monotonically from 1986 to 2011 for litter frogs (Fig 4, Table 1).

Discussion

The persistence of populations in remnant forests will probably depend on the exchange of migrants to counteract loss of genetic diversity through drift (Spielman *et al.* 2004). Strategies to maintain connectivity will need to identify landscape features that facilitate dispersal as well as idiosyncrasies among species. In this study, we observed similar levels of population structure for two common amphibians experiencing declines in Sarapiquí. However, the focal species exhibited different responses to landscape structure that may be attributable to differences in demography or tolerances to landscape change. Taking into account species-specific responses, we discuss recommendations to increase population connectivity.

Population structure and dispersal limitation

We expected that litter frogs would exhibit more pronounced population structure than poison frogs. However, assignment methods suggested that spatial delineation of clusters was similar between species and that both populations were largely structured at the scale of multiple adjacent sites rather than individual sample sites. We know of no direct measurements of dispersal distances for our study species. However, a review of amphibian dispersal reported an average maximum dispersal distance for anurans of 2923 m (SD = 5930 m; e.g. from mark–recapture or radio telemetry studies; Smith & Green 2005). While it is important to note the substantial variation among species, this value is similar to the mean distance among sites within multi-site genetic clusters reported here (poison frogs, 4029 m; litter frogs, 2393 m). Landscape genetic studies of amphibians in temperate zones have described population structure at similar spatial scales to our study, and genetically delineated demes in those studies also included multiple habitat patches or breeding sites (Funk *et al.* 2005; Zamudio & Wieczorek 2007).

Both species exhibited weak but significant divergence among most sampling locations according to F_{ST} estimates. However, within-population levels of H_e were high, which restricts maximum F_{ST} values (Meirmans & Hedrick 2011). An alternative measure of genetic distance, D_{EST}, indicated higher levels of differentiation for both species and a greater range of values than for F_{ST}. For poison frogs, levels of population differentiation are comparable to those reported in a previous study conducted at a larger spatial scale, in which the shortest intersite distances overlap with our longest

© 2014 John Wiley & Sons Ltd

Table 1 Model support for abundance- and experiment-derived resistance estimates using multiple regression on distance matrices and redundancy analysis. MRDM models are ranked based on the change in explained variation compared to isolation-by-distance models, calculated as $\delta R^2_{IBD} = adj^2_{Land} - adj^2_{IBD}$. The top three MRDM models are in bold for each response variable. Significant models after Bonferroni correction are indicated for corrected $\alpha = 0.05$* and 0.005**. Best RDA models after forward variable selection are listed; parentheses indicate the type of data used to generate the resistance surface, and brackets indicate that the model was fit while controlling for the spatial variables

		Craugastor bransfordii				*Oophaga pumilio*			
		F_{ST}		D_{EST}		F_{ST}		D_{EST}	
MRDM	Resistance model	Adj. R^2	δR^2_{IBD}	Adj. R^2	δR^2_{IBD}	Adj. R^2	δR^2_{IBD}	Adj. R^2	δR^2_{IBD}
Survey-derived resistances	Abundance_2011	**0.333****	**0.210**	**0.448****	**0.289**	0.256*	−0.148	0.304**	−0.143
	Abundance_2001	0.213	0.090	0.278*	0.118	0.257*	−0.147	0.317**	−0.130
	Abundance_1986	0.026	−0.097	0.079	−0.080	0.105	−0.299	0.206*	−0.241
Experiment-derived resistances	Water loss	**0.293****	**0.170**	**0.378****	**0.218**	**0.360****	**−0.043**	0.395**	−0.052
	Mort	0.250*	0.127	**0.385****	**0.226**	0.271*	−0.132	0.297**	−0.150
	Move	**0.305****	**0.182**	0.329**	0.170	**0.382****	**−0.022**	0.411**	−0.036
	Predation	0.284**	0.161	0.275**	0.116	0.303**	−0.100	0.346**	−0.101
Alternative models	Barriers	0.023	−0.100	0.058	−0.102	0.353**	−0.050	0.435**	−0.012
	Local abundance	0.007	−0.116	0.024	−0.136	0.068	−0.336	0.070	−0.377
	LogGeoDist	0.123**	0.000	0.159**	0.000	**0.403****	**0.000**	**0.447****	**0.000**
	Barriers + Water loss							**0.490****	**0.043**
	Barriers + Move							**0.491****	**0.044**

RDA	AIC	Adj. R^2	AIC	Adj. R^2
Best Model (F_{ST})	−141.12 Mort[Space]	0.2196*	−119.67 Resistance (abun) + local abun + Barriers [Space]	0.634**
Best Model (D_{EST})	−64.69 Mort[Space]	0.1429	−81.25 Resistance(abun and water) + local abun+ Barriers [Space]	0.5139*

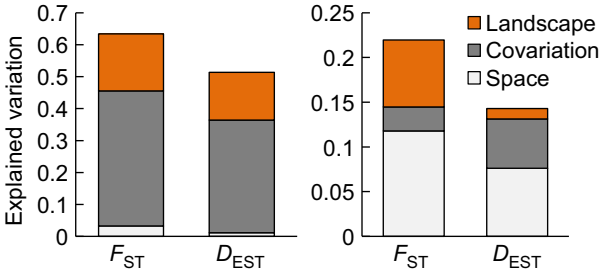

Fig. 3 Variation in genetic differentiation for *O. pumilio* (poison frogs; left panel) and *C. bransfordii* (litter frogs; right panel) explained solely by landscape variables (after controlling for space), solely by spatial arrangement of sites (after controlling for landscape variables) and by the covariation of explanatory variables with space. Best models for each species-response combination are as follows: for poison frogs, F_{ST} ~ Resistance (Abun) + Local Abun + Barriers[Space], and D_{EST} ~ Resistance (Abun and Water) + Local Abun + Barriers[Space]; for litter frogs, F_{ST} ~ Resistance(Mort)[Space], and D_{EST} ~ Resistance (Mort)[Space]. Parentheses indicate the type of data used to generate the resistance surface, and brackets indicate that the model was fit while controlling for the spatial variables.

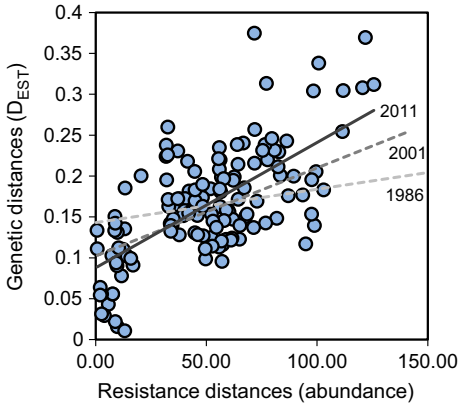

Fig. 4 Pairwise genetic distances (Jost's *D*) and resistance distances derived from matrix abundance data for litter frogs (*C. bransfordii*). Data points are shown for 2011 land-cover data only. Trendlines show change in slope and linear relationships for years 1986, 2001 and 2011.

intersite distances (Wang & Summers 2010). Here, both species exhibited significant isolation-by-distance relationships (IBD), but IBD explained considerably more

© 2014 John Wiley & Sons Ltd

of the genetic variation for poison frogs than for litter frogs (Fig 1), which indicates differences in the importance of spatially structured processes in structuring populations. A recent meta-analysis found that ectotherms generally exhibit stronger IBD relationships than other groups, possibly because of metabolic and size constraints on dispersal (Jenkins *et al.* 2010).

Evaluating empirically derived resistance models

Landscape resistance is determined by multiple local processes that can scale up to modify rates of gene flow. We analysed the effects of composite measures of resistance (i.e. abundance-derived resistance) as well as resistances derived from isolated mechanisms (i.e. experiment-derived resistances) on population structure. We predicted that strong landscape effects would be detectable for both species and responses to local microclimate would be an important mechanism underlying landscape resistance. Consistent landscape effects were found for litter frogs using empirically derived resistance surfaces. The resistance surface developed from land-use-specific (matrix) abundances was the best supported model explaining genetic variation of litter frogs according to MRDM analyses. We assumed that survey-derived resistances were inversely related to habitat quality and reflected the cumulative effects of multiple local mechanisms, such as survival and behaviour. Support for the matrix abundance model indicated that resistance to gene flow was lowest for litter frogs through forest, intermediate through palmito plantations (and similar land uses) and highest through pastures (Table S1, Supporting information).

Model support for a specific local mechanism was greatest for litter frog responses to microclimate variation across land uses (survival and desiccation risk), measured using field experiments. Responses to microclimates suggest that risk of desiccation and heat stress, as well as physiological constraints on activity, probably modify dispersal success through altered habitats (Nowakowski *et al.* 2013). Microclimate is often assumed to be an important determinant of population responses in landscape genetic studies of amphibians, but the hypothesis has rarely been evaluated with field data (this study, Peterman *et al.* 2014). There was also partial support for substrate resistance, measured as the speed that individuals moved across common substrates found in each land use. Movement speeds likely reflect the difficulty, and by extension, energetic costs associated with moving across substrates in each habitat. Model validation using abundances as an independent data set also indicated that response to microclimate was the best supported mechanism associated with landscape resistance (Nowakowski *et al.* In Press). There was relatively weak support for predation risk as a mechanism shaping population structure of litter frogs.

In contrast, geographic distance among sites was consistently among the best predictors of genetic variation for poison frogs relative to other factors, and landscape effects were not detected across all analyses. However, empirical resistance surfaces were included in the best models for some of our analyses in combination with landscape barriers and local abundances. Similar to litter frog results, there was partial support for desiccation risk and substrate resistance to movement, which suggests that these mechanisms affect structure of poison frog populations, but are not dominant factors. The lack of a consistent landscape effect for poison frogs suggests that spatially structured processes tend to override landscape genetic relationships.

Comparing species responses to landscape structure

Taken together, our analyses indicate that contemporary landscape change has been important in modifying litter frog population structure and has had a relatively weaker effect on poison frog populations in comparison with IBD. The difference in response between species may best be explained by (i) the effect of population size variation on differentiation for poison frogs, but not for litter frogs; (ii) divergent life histories between species; and 3) the ability of poison frogs to use pastures as habitat. First, large populations should take longer to respond to new dispersal barriers, because they lose alleles more slowly through genetic drift (Frankham *et al.* 2002). We observed a significant negative association between population size indices and average F_{ST} for poison frogs, indicating possible control of population size on differentiation that may outweigh contemporary landscape effects (Fig 1). Local abundances were also included in the best RDA models, but most of the explained variation was spatially structured. Some of our analyses supported the barrier model of resistance to poison frog gene flow (rivers and mountains), which is consistent with a population that is less responsive to contemporary landscape changes. Population sizes were also large for litter frogs, but we did not find an effect of N_e or local abundances on population differentiation.

Second, the focal taxa are representative of amphibian lineages with divergent life histories. Many dendrobatid poison frogs, including *O. pumilio*, are characterized by chemical defences and parental care investment in fewer offspring relative to other amphibian groups (Wells 2007). *Craugastor bransfordii* belongs to the eleutherodactyline clade that comprises about one-third of Neotropical frog diversity and is characterized by lack

© 2014 John Wiley & Sons Ltd

of a free-living tadpole stage (Heinicke *et al.* 2007). The two focal species exhibit differences in reproductive phenology (Donnelly 1989c, 1999), and some evidence suggests that poison frogs (*O. pumilio*) live longer (up to 2–3 years; M. Donnelly *pers obs*, Richards-Zawacki *et al.* 2012) than litter frogs (*C. bransfordii*), which are typically not observed more than a year after first capture in mark–recapture studies (S. Whitfield, personal communication). Divergent life history strategies, particularly longer generation times coupled with spatially autocorrelated population sizes of poison frogs, could contribute to the different responses to landscape change observed between species. Field studies have shown that amphibians, reptiles, mammals and birds often exhibit species-specific abundance and occupancy responses to habitat alterations (Daily *et al.* 2001, 2003; Kurz *et al.* 2014), which underscores the importance of comparative multi-species studies in landscape genetics.

Third, poison frogs are known to use pastures, whereas litter frogs do not (Kurz *et al.* 2014). Therefore, pastures are likely semi-permeable to poison frog gene flow but may represent substantial barriers to litter frogs. Poison frogs often use remnant trees in pastures as small habitat patches (Robinson *et al.* 2013), which could extend populations from the forest boundaries allowing for greater genetic continuity among fragments. However, single trees or small clusters of trees are not represented by our land-cover data at 30 m resolution. Therefore, missing information on variation in habitat quality among pasture areas could contribute to the lack of a consistent landscape signal for poison frogs. Remnant native vegetation and other features, such as live fences and hedgerows, are known to modify habitat quality of agricultural land uses for multiple taxa (Felton *et al.* 2010; Fischer *et al.* 2010). Future studies should make use of field data and high-resolution imagery to investigate the importance of microhabitat variation to landscape resistances.

Field studies suggest that palmito plantations provide greater resistance to poison frog movement than pastures (Kurz *et al.* 2014; Nowakowski *et al.* In Press). However, palmito and similar land uses represent a smaller proportion of the landscape than pastures and are typically more dynamic in space and time. In contrast, survey data show that pastures and associated trees are mostly uninhabited by litter frogs and therefore probably create large gaps in population distributions on the landscape (Kurz *et al.* 2014).

Evaluating time lags in population responses

Contrary to our expectations, we did not find evidence of a time lag between landscape state and observed population structure. For litter frogs, model support was greatest for the contemporary landscape and effect sizes increased from 1986 to 2011 (Fig 4). Effect sizes for poison frogs were similar between 2001 and 2011 landscapes and lowest for the 1986 landscape. Lack of a substantial lag time is in agreement with recent empirical (Zellmer & Knowles 2009) and simulation (Landguth *et al.* 2010) studies that found contemporary landscape effects on populations were not only detectable, but stronger than historical landscape conditions when using hypervariable markers like microsatellites. Our results provide further evidence that microevolutionary changes can often occur rapidly over ecological timescales, particularly in response to anthropogenic disturbances (Schoener 2011).

Maintaining connectivity in Neotropical landscapes

General agreement among survey data, field experiments and population-genetic analyses indicates that pastures represent significant dispersal barriers for litter frog populations. Pastures are widespread landscape features in Sarapiquí that are characterized by a thick ground cover of non-native grasses and scattered remnant trees. Maximum daytime temperatures in pastures can exceed those in forests by as much as 10°C, and remnant vegetation provides limited refugia from microclimatic conditions (Robinson *et al.* 2013). Although much of the study area remains forested, most forest occurs as small fragments or as linear riparian zones. Typical distances between forest patches range from 100 to 400 m, which may represent substantial expanses of open habitat to cross for small frogs (Nowakowski *et al.* 2013). In this study, we observed significant differentiation of litter frog populations in forest sites separated by as little as 540 m (i.e. F_{ST} values were significant). Because pastures generally support low diversities and low abundances of amphibians (Kurz *et al.* 2014), this land use may create strong population discontinuities for other species in the area and elsewhere in Mesoamerica.

There are ongoing conservation measures within the study region that could provide mechanisms for increasing connectivity of amphibian populations; these include the management of protected areas and biological corridors (Sánchez-Azofeifa *et al.* 2003; Fagan *et al.* 2013), as well as environmental service payments (ESPs; Morse *et al.* 2009). The ESP programmes active in Sarapiquí provide incentives to landowners for conversion of other land uses to native forest regrowth, native species plantations and exotic timber plantations. These programmes have likely contributed to the maintenance of tree cover in the region since 1996 (Fagan *et al.* 2013).

Currently, provision of ESPs is prioritized towards areas within biological corridors, properties adjacent to

© 2014 John Wiley & Sons Ltd

protected areas and land-owners with low income (Morse *et al.* 2009). An additional criterion could be added for prioritization of ESPs to specifically target pastures for conversion to secondary regrowth and native plantations. Increased conversion of pastures to forest could reduce average geographic distances among forest patches and minimize the amount of pasture that occurs between forested habitats, thereby reducing effective distances. In addition, incentives could be used to increase retention of remnant trees and allow for limited tree recruitment in pastures that would provide conservation benefits in these anthropogenically influenced landscapes.

Conclusions

Tropical species are underrepresented in the landscape genetics literature (Storfer *et al.* 2010; Emel & Storfer 2012), despite high levels of diversity and disproportionate numbers of threatened species (Vié *et al.* 2009). We present a comparative study of two dominant Neotropical amphibians that identifies similarities between species in the scale and level of population structure, but divergent responses to contemporary landscape change. We link dispersal costs measured with survey data and experiments to landscape-scale patterns of genetic structure through resistance models. Our results support continuities between local process and landscape patterns for one species and a potential overriding effect of population size on differentiation for the other. We conclude that when possible, the use of empirically derived resistance surfaces will allow for clearer interpretation of processes underlying landscape connectivity compared to the use of expert opinion and model-fitting methods. This study and previous work suggest that pastures can represent inimical habitat and impede gene flow for some forest species (Felton *et al.* 2010; Nowakowski *et al.* 2013). Programmes that incentivize conversion of pastures to secondary forest and tree plantations should improve habitat connectivity by simultaneously increasing tree cover and decreasing extent of pastures on the landscape.

Acknowledgments

We thank M. Veiman for help in the field and laboratory and members of the DeWoody laboratory for valuable feedback and assistance. We are grateful to S. Spear, A. Storfer and J. Watling for comments that improved the analyses and quality of the manuscript, to the Costa Rican government (MINAE-SINAC) for permits and to The Organization for Tropical Studies for logistical support. AJN was supported by Florida International University Dissertation Evidence Acquisition and Dissertation Year Fellowships, as well as by grants from OTS, Tinker Foundation, ASIH, NCHS, and Idea Wild. All experimental protocols received IACUC approval (#s 09-007, 10-023, & 12-003). This is contribution number 295 to the programme in Tropical Biology at Florida International University.

References

Amos W, Hoffman JI, Frodsham A, Zhang L, Best S, Hill VS (2007) Automated binning of Microsatellite alleles: problems and solutions. *Molecular Ecology Notes*, **7**, 10–14.

Aronson R, Heck K, Valentine J (2001) Measuring predation with tethering experiments. *Marine Ecology Progress Series*, **214**, 311–312.

Baguette M, Blanchet S, Legrand D, Stevens VM, Turlure C (2012) Individual dispersal, landscape connectivity, and ecological networks. *Biological Reviews*, **88**, 310–326.

Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*, **32**, 818–830.

Barnosky AD, Hadly EA, Bascompte J *et al.* (2012) Approaching a state-shift in Earth's biosphere. *Nature*, **486**, 52–58.

Borcard D, Legendre P (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling*, **153**, 51–68.

Busch JD, Waser PM, DeWoody JA (2009) The influence of density and sex on patterns of fine-scale genetic structure. *Evolution*, **63**, 2302–2314.

Butterfield RP (1994) The regional context: land colonization and conservation in Sarapiquí. In: *La Selva: ecology and natural history of a neotropical rainforest* (eds McDade L. A., Bawa K. S., Hespenheide H. A. & Hartshorn G. S.), pp. 299–306. University of Chicago Press, Chicago, IL.

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. *Journal of Heredity*, **99**, 616–623.

Catenazzi A, Lehr E, Vredenburg VT (2013) Thermal physiology, disease, and amphibian Declines on the eastern slopes of the Andes. *Conservation Biology*, **28**, 509–517.

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution*, **24**, 621–631.

Consentino BJ, Schooley RL, Phillips CA (2011) Connectivity of agroecosystems: dispersal costs can vary among crops. *Landscape Ecology*, **26**, 371–379.

Daily GC, Ehrlich PR, Sánchez-Azofeifa GA (2001) Countryside biogeography: use of human-dominated habitats by the avifauna of southern Costa Rica. *Ecological Applications*, **11**, 1–13.

Daily GC, Ceballos G, Pacheco J, Suzán G, Sánchez-Azofeifa A (2003) Countryside biogeography of neotropical mammals: conservation opportunities in agricultural landscapes of Costa Rica. *Conservation Biology*, **17**, 1814–1826.

DeClerck FAJ, Chazdon R, Holl KD *et al.* (2010) Biodiversity conservation in human-modified landscapes of Mesoamerica: past, present and future. *Biological Conservation*, **143**, 2301–2313.

Deutsch CA, Tewksbury JJ, Huey RB *et al.* (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, **105**, 6668–6672.

Donnelly MA (1989a) Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. *Ecological Monographs*, **59**, 207–221.

© 2014 John Wiley & Sons Ltd

Donnelly M (1989b) Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. *Oecologia*, **81**, 212–218.

Donnelly M (1989c) Reproductive phonology and age structure of *Dendrobates pumilio* in Northeastern Costa Rica. *Journal of Herpetology*, **23**, 362–367.

Donnelly M (1999) Reproductive phonology of *Eleutherodactylus bransfordii* in Northeastern Costa Rica. *Journal of Herpetology*, **33**, 624–631.

Driscoll DA, Banks SC, Barton PS, Lindenmayer DB, Smith AL (2013) Conceptual domain of the matrix in fragmented landscapes. *Trends in Ecology and Evolution*, **28**, 605–613.

Dyer RJ, Nason JD, Garrick RC (2010) Landscape modelling of gene flow: improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology*, **19**, 3746–3759.

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Elmer KR, Dávila JA, Lougheed SC (2007) Applying new inter-individual approaches to assess fine-scale population genetic diversity in a neotropical frog, *Eleutherodactylus ockendeni*. *Heredity*, **99**, 506–615.

Emel SL, Storfer A (2012) A decade of amphibian population genetic studies: synthesis and recommendations. *Conservation Genetics*, **13**, 1685–1689.

Excoffier L, Lischer HEL (2010) ARLEQUIN suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Fagan ME, DeFries RS, Sesnie SE *et al.* (2013) Land cover dynamics following a deforestation ban in northern Costa Rica. *Environmental Research Letters*, **8**, 034017.

Felton A, Knight E, Wood J, Zammit C, Lindenmayer D (2010) A meta-analysis of fauna and flora species richness and abundance in plantations and pasture lands. *Biological Conservation*, **143**, 545–554.

Fischer J, Stott J, Law BS (2010) The disproportionate value of scattered trees. *Biological Conservation*, **143**, 1564–1567.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge.

Funk WC, Blouin MS, Corn PS *et al.* (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology*, **14**, 483–496.

Goldberg CS, Waits LP (2010) Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. *Molecular Ecology*, **19**, 3650–3663.

Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, **22**(7), 1–19.

Hammerschlag N, Heithaus M, Serafy J (2010) Influence of predation risk and food supply on nocturnal fish foraging distributions along a mangrove-seagrass ecotone. *Marine Ecology Progress Series*, **414**, 223–235.

Hansen MC, Potapov PV, Moore R *et al.* (2013) High-resolution global maps of 21st-century forest cover change. *Science*, **342**, 850–853.

Hauswaldt JS, Ludewig AK, Hagemann S, Prohl H, Vences M (2009) Ten microsatellite loci for the strawberry poison frog (*Oophaga* pumilio). *Conservation Genetics*, **10**, 1935–1937.

Hobbs RJ, Higgs E, Harris JA (2009) Novel ecosystems: implications for conservation and restoration. *Trends in Ecology and Evolution*, **24**, 599–605.

Heinicke MP, Duellman WE, Hedges SB (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences*, **104**, 10092–10097.

Hubisz M, Falush D, Stephens M, Pritchard J (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**, 1322–1332.

Huey RB, Deutsch CA, Tewksbury JJ *et al.* (2009) Why tropical forest lizards are vulnerable to climate warming. *Proceedings of Royal Society B:Biological Sciences*, **276**, 1939–1948.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, **23**, 1801–1806.

Jenkins DG, Carey M, Czerniewska J *et al.* (2010) A meta-analysis of isolation by distance: relic or reference standard for landscape genetics? *Ecography*, **33**, 315–320.

Jost L (2008) G_{ST} and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015–4026.

Knowlton JL, Graham CH (2010) Using behavioral landscape ecology to predict species' responses to land-use and climate change. *Biological Conservation*, **143**, 1342–1354.

Koen EL, Bowman J, Walpole AA (2012) The effect of cost surface parameterization on landscape resistance estimates. *Molecular Ecology Resources*, **12**, 686–696.

Kurz DJ, Nowakowski AJ, Tingley MW, Donnelly MA, Wilcove DS (2014) Forest-land use complementarity modifies community structure of a tropical herpetofauna. *Biological Conservation*, **170**, 246–255.

Lampert KP, Rand AS, Mueller UG, Ryan MJ (2003) Fine-scale genetic pattern and evidence for sex-biased dispersal in the túngara frog, *Physalaemus pustulosus*. *Molecular Ecology*, **12**, 3325–3334.

Landguth EL, Cushman SA, Schwartz MK, McKelvey KS, Murphy M, Luikart G (2010) Quantifying the lag time to detect barriers in landscape genetics. *Molecular Ecology*, **19**, 4179–4191.

Laurance WF, Useche DC, Rendeiro J *et al.* (2012) Averting biodiversity collapse in tropical forest protected areas. *Nature*, **489**, 290–294.

Legendre P, Fortin MJ (2010) Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetics data. *Molecular Ecology Resources*, **10**, 831–844.

Legendre P, Legendre L (2012) *Numerical Ecology*, 3rd edn. Elsevier, Quebec, Canada.

Legendre P, Lapointe FJ, Casgrain P (1994) Modeling brain evolution from behavior: a permutational regression approach. *Evolution*, **48**, 1487–1499.

Lowe WH, Allendorf FW (2010) What can genetics tell us about population connectivity? *Molecular Ecology*, **19**, 3038–3051.

Manel S, Holderegger R (2013) Ten years of landscape genetics. *Trends in Ecology and Evolution*, **28**, 614–621.

von May R, Jacobs JM, Santa-Cruz R, Valdivia J, Huaman JM, Donnelly MA (2010) Amphibian community structure as a function of forest type in Amazonian Peru. *Journal of Tropical Ecology*, **26**, 509–519.

© 2014 John Wiley & Sons Ltd

McRae BH, Beier P (2007) Circuit theory predicts gene flow in plant and animal populations. *Proceedings of the National Academy of Sciences*, **104**, 19885–19890.

McRae BH, Shah VB (2009) *Circuitscape User's Guide*. The University of California, Santa Barbara. Available at: http://www.circuitscape.org.

McRae BH, Dickson BG, Keitt TH, Shah VB (2008) Using circuit theory to model connectivity in ecology, evolution, and conservation. *Ecology*, **89**, 2712–2724.

Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources*, **11**, 5–18.

Moilanen A, Nieminen M (2002) Simple connectivity measures in spatial ecology. *Ecology*, **83**, 1131–1145.

Morse W, Schedlbauer J, Sesnie S *et al.* (2009) Consequences of environmental service payments for forest retention and recruitment in a Costa Rican Biological Corridor. *Ecology and Society*, **14**, 1–20.

Nowakowski AJ, Otero Jiménez B, Allen M, Diaz-Escobar M, Donnelly MA (2013) Landscape resistance to movement of the poison frog, *Oophaga pumilio*, in the lowlands of northeastern Costa Rica. *Animal Conservation*, **16**, 188–197.

Nowakowski AJ, Willoughby JR, DeWoody JA, Donnelly MA (2014) Polymorphicmicrosatellite loci for a Neotropical leaf-litter frog (*Craugastor bransfordii*) characterized through Illumina sequencing. *Conservation Genetics Resources*, **6**, 697–698.

Nowakowski AJ, Veiman-Echeverria M, Kurz D, Donnelly MA (In Press) Evaluating connectivity for tropical amphibians using empirically derived resistance surfaces. *Ecological Applications*, doi: 10.1890/14-0833.1 (in press).

Oksanen J, Blanchet FG, Kindt R *et al.* (2012) Package 'vegan.' http://cran.r-project.org/web/packages/vegan/vegan.pdf

Pearman PB, Garner TWJ (2005) Susceptibility of Italian agile frog populations to an emerging strain of *Ranavirus* parallels population genetic diversity. *Ecology Letters*, **8**, 401–408.

Peterman WE, Connette GM, Semlitsch RD, Eggert LS (2014) Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. *Molecular Ecology*, **23**, 2402–2413.

Pflüger FJ, Balkenhol N (2014) A plea for simultaneously considering matrix quality and local environmental conditions when analysing landscape impacts on effective dispersal. *Molecular Ecology*, **23**, 2146–2156.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multi-locus genotype data. *Genetics*, **155**, 945–959.

Pritchard JK, Wen X, Falush D (2010) Documentation for *structure* software: Version 2.3.

R Core Team, 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-projectorg/.

Rayfield B, Fortin M, Fall A (2010) The sensitivity of least-cost habitat graphs to relative cost surface values. *Landscape Ecology*, **25**, 519–532.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Richardson JL (2012) Divergent landscape effects on population connectivity in two co-occurring amphibian species. *Molecular Ecology*, **21**, 4437–4451.

Richards-Zawacki CL, Wang IJ, Summers K (2012) Mate choice and the genetic basis for colour variation in a polymorphic dart frog: inferences from a wild pedigree. *Molecular Ecology*, **21**, 3879–3892.

Rittenhouse TAG, Semlitsch RD (2006) Grasslands as movement barriers for a forest associated salamander: migration behavior of adult and juvenile salamanders at a distinct habitat edge. *Biological Conservation*, **131**, 14–22.

Rittenhouse TAG, Harper EB, Rehard LR, Semlitsch RD (2008) The role of microhabitats in the desiccation and survival of anurans in recently harvested oak-hickory forest. *Copeia*, **4**, 807–814.

Robinson D, Warmsley A, Nowakowski AJ, Reider KE, Donnelly MA (2013) The value of remnant trees in pastures for a neotropical poison frog. *Journal of Tropical Ecology*, **29**, 345–352.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Rothermel BB, Luhring TM (2005) Burrow availability and desiccation risk of mole salamanders (*Ambystoma talpoideum*) in harvested versus unharvested forest stands. *Journal of Herpetology*, **39**, 619–626.

Rothermel BB, Semlitsch RD (2006) Consequences of forest fragmentation for juvenile survival in spotted (*Ambystoma maculatum*) and marbled (*Ambystoma opacum*) salamanders. *Canadian Journal of Zoology*, **84**, 797–807.

Sambrook J, Russell DW (2001) *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.

Sánchez-Azofeifa GA, Daily GC, Pfaff ASP, Busch C (2003) Integrity and isolation of Costa Rica's national parks and biological reserves: examining the dynamics of land-cover change. *Biological Conservation*, **109**, 123–135.

Sanford RL Jr, Paaby P, Luvall JC, Phillips E (1994) Climate, geomorphology, and aquatic systems. In: *La Selva: ecology and natural history of a neotropical rainforest* (eds McDade L. A., Bawa K. S., Hespenheide H. A. & Hartshorn G. S.). The University of Chicago Press, Chicago, IL.

Saporito RA, Donnelly MA, Norton R, Garraffo HM, Spande TF, Daly JW (2007) Oribatid mites as a major dietary source for alkaloids in poison frogs. *Proceedings of the National Academy of Sciences*, **104**, 8885–8890.

Savage JM (2002) *The amphibians and reptiles of Costa Rica: A herpetofauna between two continents, two seas*. The University of Chicago Press, Chicago, IL.

Schoener TW (2011) The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science*, **331**, 426–429.

Sesnie SE, Gessler PE, Finegan B, Thessler S (2008) Integrating Landsat TM and SRTM-DEM derived variables with decision trees for habitat classification and change detection in complex neotropical environments. *Remote Sensing of Environment*, **112**, 2145–2159.

Smith AM, Green DM (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, **28**, 10–128.

Smith MJ, Forbes GJ, Betts MG (2013) Landscape configuration influences gap-crossing decisions of northern flying squirrel (*Glaucomys sabrinus*). *Biological Conservation*, **168**, 176–183.

Spear SF, Balkenhol N, Fortin MJ, Mcrae BH, Scribner K (2010) Use of resistance surfaces for landscape genetic studies: considerations for parameterizations and analysis. *Molecular Ecology*, **19**, 3576–3591.

© 2014 John Wiley & Sons Ltd

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences*, **101**, 15261–15264.

Stevens VM, Verkenne C, Andewoestijne SV, Wesselingh RA, Baguette M (2006) Gene flow and functional connectivity in the natterjack toad. *Molecular Ecology*, **15**, 2333–2344.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? *Molecular Ecology*, **19**, 3496–3514.

Stuart SN, Chanson JS, Cox NA *et al.* (2004) *Science*, **306**, 1783–1786.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.

Vié JC, Hilton-Taylor C, Stuart SN (eds) (2009) *Wildlife in a Changing World– An Analysis of the 2008 IUCN Red List of Threatened Species*. IUCN, Gland, Switzerland.

Wang IJ, Summers K (2009) Highly polymorphic microsatellite markers for the highly Polymorphic strawberry poison-dart frog and some of its congeners. *Conservation Genetics*, **10**, 2033–2036.

Wang IJ, Summers K (2010) Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Molecular Ecology*, **19**, 447–458.

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, **8**, 753–756.

Wells KD (2007) *The Ecology and Behavior of Amphibians*. The University of Chicago Press, Chicago, IL.

Whitfield SM, Bell KE, Philippi T *et al.* (2007) Amphibian and reptile declines over 35 years at La Selva, Costa Rica. *Proceedings of the National Academy of Science*, **104**, 8352–8356.

Whitlock MC (2011) G'_{ST} and D do not replace F_{ST}. *Molecular Ecology*, **20**, 1083–1091.

Zamudio KR, Wieczorek AM (2007) Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. *Molecular Ecology*, **16**, 257–274.

Zeller KA, McGarigal K, Whiteley AR (2012) Estimating landscape resistance to movement: a review. *Landscape Ecology*, **27**, 777–797.

Zellmer AJ, Knowles LL (2009) Disentangling the effects of historic vs. contemporary landscape structure on population genetic divergence. *Molecular Ecology*, **18**, 3593–3602.

Zuur AF, Ieno EN, Walker NJ, Saveliev AZ, Smith GM (2009) *Mixed effects models and extensions in ecology with R*. Springer, New York.

A.J.N. designed the study, collected field data, conducted analyses and drafted the manuscript. JRW contributed to laboratory analyses. M.E.F. developed landcover data sets. M.A.D. and J.A.D. directed the study and contributed to analyses. All authors contributed to the writing of the final manuscript.

Data accessibility

Genetic, geographic, and resistance distances used in MRDM analyses; site-specific variables used in RDA analyses; and STRUCTURE input files are archived on DRYAD: doi:10.5061/dryad.gq08c.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Empirically-derived cost values used to develop resistance surfaces.

Table S2 Final sample sizes, observed heterozygosities, and allelic richness at each site.

Table S3 Pairwise genetic distance matrices.

© 2014 John Wiley & Sons Ltd