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Contrasting Patterns of Gene Flow for Amazonian Snakes That Actively Forage and Those That Wait in Ambush

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Abstract

Knowledge of genetic structure, geographic distance and environmental heterogeneity can be used to identify environmental features and natural history traits that influence dispersal and gene flow. Foraging mode is a trait that might predict dispersal capacity in snakes, because actively foragers typically have greater movement rates than ambush predators. Here, we test the hypothesis that 2 actively foraging snakes have higher levels of gene flow than 2 ambush predators. We evaluated these 4 co-distributed species of snakes in the Brazilian Amazon. Snakes were sampled along an 880 km transect from the central to the southwest of the Amazon basin, which covered a mosaic of vegetation types and seasonal differences in climate. We analyzed thousands of single nucleotide polymorphisms to compare patterns of neutral gene flow based on isolation by geographic distance (IBD) and environmental resistance (IBR). We show that IBD and IBR were only evident in ambush predators, implying lower levels of dispersal than the active foragers. Therefore, gene flow was high enough in the active foragers analyzed here to prevent any build-up of spatial genotypic structure with respect to geographic distance and environmental heterogeneity.

Subject area: Conservation genetics and biodiversity
Keywords: dispersal, isolation by distance, isolation by resistance, landscape genomics, SNPs

Quantifying the spatial distribution of genetic variation has provided knowledge of the environmental factors influencing gene flow and dispersal (e.g., Stow et al. 2001; Dudaniec et al. 2013). These data have been applied to measure how heterogeneity of the natural environment influences dispersal and gene flow, and also evaluate anthropogenic habitat loss and fragmentation (Banks et al. 2007; Peterman et al. 2014). In more recent years, spatial models have been used to provide more sensitive tests of landscape influences on genetic structure, but these landscape genetic approaches have rarely been applied to tropical rainforest taxa (Ruiz-Lopez et al. 2016). Obtaining information on dispersal via direct observation can be extremely difficult (Lowe and Allendorf 2010), for example, due to species size, their habitat use, or levels of detectability. In this respect, species of tropical rainforests are often typified by low detectability (e.g., Fraga et al. 2014) and as such, there is a paucity of data on patterns of gene flow and dispersal for some taxonomic groups. This is

exemplified by snakes for which most data on gene flow and dispersal are available from temperate biomes (e.g., Lougheed et al. 1999; Blouin-Demers and Weatherhead 2002).

Tropical rainforests are known to contain a spectacular diversity of snakes (Bernarde et al. 2012; Fraga et al. 2013a), exhibiting a vast range of natural history traits, yet there is a lack of data on gene flow and dispersal for this group. These data can be used to compare levels of connectivity for species with different life history traits, thus contributing to our knowledge on the ecology of these species, and the mechanisms underpinning their current distributions. This information can also be valuable for conservation, because it provides data on how habitat loss influences gene flow, and how much intraspecific genetic variation is captured within reserve systems (Stow et al. 2004; Bell and Okamura 2005).

The relationships among genetic structure, geographic distance, and environmental heterogeneity allows the dispersal characteristics and gene flow for particular species to be characterized, and an assessment of the environmental features that shape species distributions (Lowe and Allendorf 2010; Wang and Bradburd 2014). Isolation-by-distance (hereafter IBD) and isolation-by-resistance (hereafter IBR) are the 2 models to which spatial patterns of genetic variability can be fitted (e.g., Wright 1943; McRae 2006; Koen et al. 2012; Marrote et al. 2014). The first is estimated simply by testing for correlations between genetic distance and geographic distance. The latter may be quantified by calculating the probability of an individual dispersing from one location to another after weighting the "resistance" to dispersal in each of the intervening environments. The implication of this resistance-weighting to dispersal between these 2 points is then assessed over a range of possible pathways (Manthey and Moyle 2015; Wang and Bradburd 2014).

Resistance surfaces can be used to detect subtle influences of environmental differences on gene flow that may not be detected using population genetic measures such as genetic diversity, divergence and structure, inbreeding and genetic bottlenecks (Radespiel and Bruford 2014). Landscape influences on gene flow for fauna in tropical forests have been especially hard to evaluate (Radespiel and Bruford 2014). However, the application of analyses based on resistance surfaces has been shown to be an effective means of disentangling anthropogenic and natural influences on tropical forests (Ruiz-Lopez et al. 2016). By optimizing resistance surfaces, researchers are able to better evaluate ecological processes (e.g., levels of dispersal) underlying gene flow and habitat connectivity. Environmental resistance-based models are suitable to compare patterns of gene flow of different species, because they assume that gene flow may be influenced by diverse mechanisms, such as nonrandom migration, dispersal capability, life history, and the geographic features of the study area (Dudaniec et al. 2013). These approaches clearly have useful application within very heterogeneous environments, and with organisms that have low detectability, for which measuring the effect of habitat on connectivity has hitherto been unachievable.

Snakes constitute approximately 6% of vertebrate diversity (Uetz and Hošek 2015) and are known to have key ecological functions, in addition to making numerous contributions to human societies in culture, medicine, and economics (Konar and Monak 2010). Knowledge of their dispersal patterns will enable conservation managers to better predict the consequences of environmental change. However, knowledge of dispersal in snakes can be difficult to obtain because of their typically cryptic life style (Steen 2010). Quantifying levels of connectivity at landscape scales and species–habitat associations may be difficult to achieve in the Amazon rainforests (Fraga et al. 2014). This includes obtaining knowledge of dispersal for particular species through continuous heterogeneous forest, and consequently, whether some habitats facilitate gene flow more so than others. In particular, obtaining sufficient numbers of individuals for analyses of gene flow and the inference of dispersal is challenging. Recently, more powerful analyses based on thousands of single nucleotide polymorphisms (SNPs) have become accessible, and these analyses allow reliable estimates of genetic structure from fewer individuals (e.g., Willing et al. 2012).

Although the Amazon lowlands have an exceptionally high number of snake species (e.g. Bernarde et al. 2012), the mechanisms generating and maintaining snake diversity over landscapes are poorly known. Environmental gradients may influence patterns of snake community assembly at regional scales (Fraga et al. 2011) and dispersal through different habitats at local scales (Fraga et al. 2013b). However, gene flow and genetic structuring of snakes in the Amazon has not been studied. The biodiversity of the region sampled in this study is under pressure by rapid urban growth in the last decades (Filho 1997), road construction (Soares-Filho et al. 2006) and artificial flooding by hydroelectric power plants (Fearnside 2014).

Knowledge of patterns of gene flow and dispersal for organisms sharing particular traits may allow generalizations to be drawn, whereby one could use these traits to predict the consequences of habitat change to dispersal potential and genetic structure. When evaluating the impacts of environmental change on dispersal, detailed species-level knowledge of dispersal is often lacking, and in these cases, species traits have been used to predict dispersal potential, for example, larval mode in marine organisms and wing morphology in birds and insects (Angert et al. 2011). Therefore, characterizing gene flow and dispersal in snake species with contrasting foraging modes might contribute to data that will eventually enable predictions on connectivity of snakes, to be made on the basis of species traits.

In this study, we investigate whether genetic variation can be explained by IBD and/or IBR in order to describe patterns of dispersal for snake species of the Amazon Basin. We test whether gene flow follows IBD and/or IBR patterns for 4 co-distributed snake species, 2 of which are ambush predators (*Bothrops atrox* and *Corallus hortulanus*) and the other 2 are active foragers (*Leptodeira annulata* and *Philodryas georgeboulengeri*). Foraging modes may be associated with dispersal through attributes such as activity patterns (Cooper et al. 2001), habitat use (Fedriani et al. 1999), digestive physiology (Hilton et al. 1999), seasonal patterns of reproduction (Colli et al. 1997) and mortality (Willette et al. 1999). Radio tracking data have shown that some ambush predator snakes have low mobility, for example, *B. atrox* can move up to 150 m in four months (Fraga et al. 2013b), and *Crotalus durissus* can move up to 20 m per day on average (Tozetti et al. 2009). In contrast, active foragers such as *Natrix natrix* can cover 150 m in a single day (Madsen 1984), and females of *Stegonotus cucullatus* can move up to 400 m per day during the breeding season (Brown et al. 2005). Here, we hypothesize that lower levels of dispersal observed in the ambush predators will result in less gene flow at the landscape scale than the two species of snakes that actively forage.

Here, we present a landscape-scale overview where we examine how a combination of geographic distance, environmental heterogeneity, and species behavioral traits influence gene flow. Specifically, we are interested in quantifying patterns of spatial genetic variation driven by IBD and/or IBR, and test whether foraging mode is associated with levels of genetic structure.

Materials and Methods

Target Species

The common lancehead, *B. atrox*, is a nocturnal pitviper (Crotalinae), widely distributed throughout the Amazon Basin. The species is primarily terrestrial, but individuals, especially juveniles, can climb in vegetation up to 2 m from the ground. It is an ambush predator that is found in a variety of habitats (Martins and Oliveira 1999), but a greater density of individuals has been found in areas near streams (Fraga et al. 2013b).

The garden treeboa, *C. hortulanus*, is a nocturnal boid (Boinae), widely distributed in different forested habitats in South America. The species is an ambush predator, arboreal, and it tends to be sedentary, spending long periods in small areas (Henderson 1997). The species shows marked polychromatism, with up to 5 sympatric color morphs (Duarte et al. 2015).

The cat-eyed banded snake *Leptodeira annulata annulata* is a nocturnal dipsadid (Dipsadinae), widely distributed in northern South America. The species is an active forager, which often moves and hunts at ground level, although it may climb onto vegetation to sleep (Martins and Oliveira 1999). The species is a habitat-generalist, and individuals may be found in disturbed areas.

The southern sharpnose snake *P. georgeboulengeri* (Dipsadinae) has the smallest geographic range of the species investigated in this study, being limited to south-southwestern Amazonia (Prudente et al. 2008). Its biology is poorly known, but it shows similar habits to the closely related *Philodryas argentea* (see *Xenoxybelis argentea* in Martins and Oliveira 1999). It is an active, diurnal, arboreal predator.

Study Area and Snake Sampling

We sampled snakes along approximately 880 km from the central (Manaus) to the southwest (Porto Velho) regions of the Amazon lowlands. Environmental heterogeneity along the study area consists of gradients of vegetation cover type and climatic seasonality (Figure 1). In the north of our study area we sampled the Ducke Reserve, which is covered by 100 km^2 of primary nonflooded forest and supports perennial streams. In the central region of our study area (Purus – Madeira interfluve) we sampled primary and old secondary forests which are crossed by a federal highway (BR-319) which was partially abandoned in the 1980s. This region is characterized by forests which are seasonally flooded by overflow from intermittent streams, and patches of arboreal *Campinarana*, which is a forest growing on white sand. In the southern part of our study area (Madeira River, Porto Velho) we sampled primary and old secondary forests, characterized by a drier climate compared to the rest of the study area, with most streams being completely dry in the dry season. The region is mostly covered by primary and old secondary rainforest, but patches of forest seasonally flooded by rivers and open understory forest are also present. Despite snake co-occurrence often being influenced by ecological gradients in the Amazon region (Fraga et al. 2011), all the species studied here are found in the different habitats within the study area.

We sampled snakes from 21 RAPELD modules (Magnusson et al. 2013), each of which is 5 km^2, with an average distance of 40 km between neighboring modules. Each module contains 10 plots, 250 m long and 10 m wide each, following the altitudinal contour lines, and distributed along 2 parallel trails (5 plots per trail), with 1 km between neighboring plots.

We found snakes by actively searching the modules at night, limited by space (plot area) and time (1 h per plot), with 2 observers per plot. We sampled each plot 4 times, at intervals of 2–8 months. We captured snakes by hand or with Pilstrom tongs and maintained them in soft cotton bags until the next day, when they were sacrificed, material removed for genetic analyses, and fixed for deposition in the Herpetological section of the Zoological collections of the Instituto Nacional de Pesquisas da Amazônia (INPA-H, Manaus, Brazil). We collected snakes under permits from IBAMA/SISBIO (Ministry of Environment, Government of Brazil) process numbers 02001.000508/2008–99 and 13777. Detailed information on sample

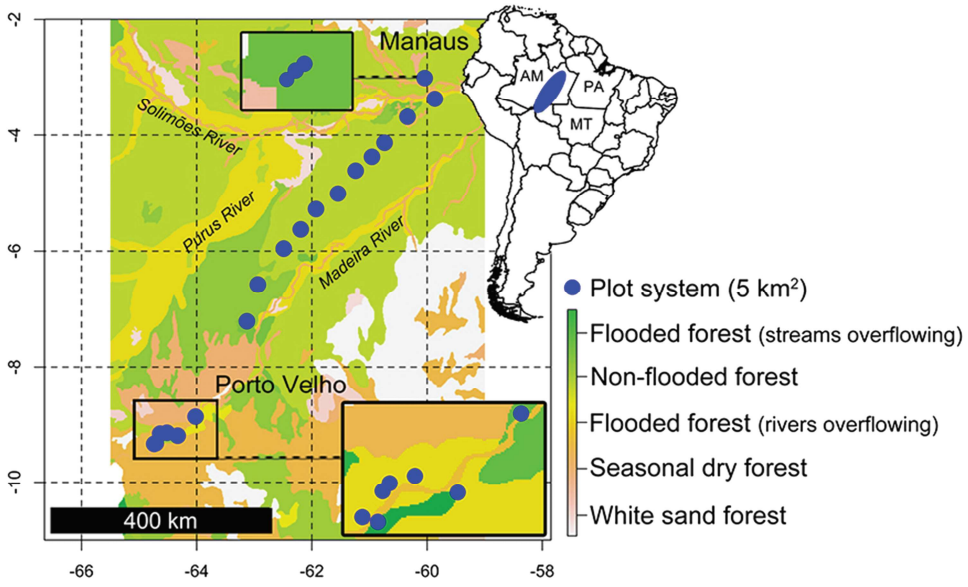

Figure 1. Sampling modules (blue circles) across the Amazon basin, that have been used to contrast patterns of gene flow among 4 snake species. The study area comprises different habitats based on vegetation-cover types and climate seasonality. The rectangles zoom in on areas with modules that are too close together to be distinguished on larger scale. The ellipse on the map of South America shows the study area on a continental scale, and the acronyms are the Brazilian states of Amazonas (AM), Pará (PA), and Mato Grosso (MT).

size per species and localities may be found in the Supplementary Material (Appendix 1).

SNP Data

We extracted and purified DNA from muscle tissue samples using the GenCatch™ Genomic DNA Extraction Kit. We mostly followed the protocols suggested by the manufacturer, but we added an extra hour of incubation for digestion. We checked DNA quality visually using 0.8% agarose gel. We sent subsamples of 0.5 µg of high quality DNA samples to Diversity Arrays Technology Pty. Ltd. (Canberra, Australia), where SNPs were discovered and genotyped using the standard DartSeq™ protocol (Petroli et al. 2012). It is based on a combination of Diversity Arrays (DArT) markers (Jaccoud et al. 2001; Kilian et al. 2012) and next-generation Illumina sequencing (Sansaloni et al. 2011). We present a brief description of the DartSeq™ protocol in the Supplementary Material (Appendix 2).

DArTs genotyped more than 14,000 SNPs for each species. We filtered this dataset by excluding SNPs with unknown scores for genotyping, read depth <10, call rate <70%, and repeatability <90%. Additionally, we excluded monomorphic loci and loci with more than one SNP to reduce statistical bias from linkage disequilibrium.

Patterns of neutral gene flow were evaluated using 2 methods to identify loci with a signal of selection. These were F_{ST} outlier tests based on Bayesian modeling (Bayescan, Foll and Gaggiotti 2008) and the latent factors mixed models (LFMM) approach, which tests for correlations between genetic polymorphisms and environmental variables. The LFMM approach was applied using the LEA package (Frichot and François 2015) in R (R Development Core Team 2015). We set both Bayescan and LEA with false discovery rate of 0.01. However, we did not identify any locus with a strong signal of positive or negative selection and therefore our data set only detected patterns expected under neutrality.

Investigating Genetic Structure

The small sample sizes prevented us from using analyses based on allele frequencies estimated per locality. Instead, we investigated global genetic structure for each species and also carried out individual-based analyses. Using the entire data set for each species, we tested for significant deviation from Hardy–Weinberg Equilibrium (HWE) per locus, to evaluate the null hypothesis of random mating. We also contrasted the values per locus for expected heterozygosity (H_E) and observed heterozygosity (H_O). Limited gene flow and the presence of genetic structure will result in a spatial Wahlund effect (Wahlund 1928). This is because pooling genetic data where genetic structure is present will result in heterozygosity deficit compared to HWE expectation.

Measuring Genetic Differentiation

To measure genetic differentiation between individuals we used pairwise genetic distances based on genotypic relatedness, which is better suited to detecting subtle genetic variation. This is because genotypes are shuffled at each generation and genotypic structure derived from genotypic similarity between individuals can be influenced by short-term processes, such as the spatial distribution of close relatives (Stow et al. 2001). We used the R-package related (Pew et al. 2015), which estimates pairwise relatedness with 7 different indices, and identifies the best index for the dataset by comparing Pearson's correlations between observed and expected relatedness values for each estimator (Pew et al. 2015). The Ritland index (Ritland 1996) was identified as the best estimator of relatedness between individuals for all species in our dataset. We used distance matrices based on the Ritland index to test the effects of IBD and IBR on genetic differentiation, and in PCA's to assess for genetic clustering.

Constructing the Environmental Resistance Surfaces

The study area is characterized by a mosaic of different vegetation types influenced by differences in climatic seasonality over a latitudinal gradient of about 7 degrees. The southern forests of the study area contain more open vegetation and more pronounced climatic seasonality compared to the northern forests of the study area. These, in turn, are denser and more humid throughout most of the year (Supplementary Material, Appendix 3). Therefore, in order to capture environmental heterogeneity in our resistance surface, we selected vegetation-cover type as a discrete variable and seasonality in temperature and rainfall as continuous variables. Each of these variables is likely to drive, or reflect large-scale ecological changes, and consequently we considered these as likely to be associated with any changes in connectivity for the species in question. The environmental variables were obtained in raster format in the public repository Ambdata (Amaral et al. 2013; www.dpi.inpe.br/Ambdata). Ambdata provides environmental data for the entire Amazon basin, and the raster files have a resolution of 1 km. We cropped the raster files to our study area and reduced the resolution to 12 km. This is an appropriate scale, because the scale at which we defined habitats, such as the distribution of different vegetation cover types and variation in climate seasonality along the study area is far greater than 12 km. Likewise, the distance between localities where sampling took place is far greater than 12 km (Supplementary Material, Appendix 1). We modified the raster files using the raster R-package (Hijmans 2015).

Most of the methods used to build resistance surfaces require a priori definition of maximum resistance values to environmental variables. Factors limiting dispersal can be conspicuous, such as low altitudes to mountain goats in northern United States (Shirk et al. 2010). However, here we were interested in investigating factors influencing gene flow across subtle variation in vegetation and climatic seasonality in the Amazon rainforests. Therefore, we weighted resistances using genetic algorithms that combine genotypes, geographic locations, and environmental layers (Peterman 2014). Genetic algorithms (GAs) are used to solve optimization problems and simulate the evolutionary process in natural systems (Scrucca 2013). The optimum weighting of resistance surfaces was appraised by running the evolutionary process simulated by the GA as implemented by the R-package ResistanceGA (Peterman 2014). This approach overcomes the challenge of complex environmental heterogeneity and having no a priori information on the influence of habitat type on dispersal. In addition, the method used in this study allows multiple resistance surfaces (e.g., 3 environmental layers combined) to be optimized (Peterman 2014).

We developed IBR models for each species in an iterative fashion using the GA to weight the resistance imposed by vegetation cover type, seasonality in temperature, and seasonality in rainfall. At each iteration, we then calculated pairwise resistance values between our sampling localities using both circuit theory (Circuitscape, McRae and Beier 2007; McRae et al. 2008; McRae and Shah 2009) and least-cost path (e.g., Driezen et al. 2007; Wang et al. 2009). The ResistanceGA R-package then fits a linear mixed effect model where our observed pairwise genetic distances were the response and pairwise resistance distance was the predictor (Peterman 2014). The best

model for each species was determined using Akaike information criterion (AIC; Peterman 2014). These steps are carried out iteratively, with the individuals belonging to the population with the best AICs carried forward to the next generation in the evolutionary process modeled using the GA. Iterations are continued to a maximum of 1000 iterations and are stopped after 25 generations have passed without improvement to AIC.

To further compare levels of dispersal between ambush predators and active foragers we used the PopGenReport R-package (Gruber and Adamack 2015). We used pairwise cost distance matrices (generated by ResistanceGA) to estimate the probability of 5% of individuals from a sampling module dispersing to a neighboring module, using the average distance between modules (40 km). We predicted that ambush predators would have lower dispersal probabilities than active foragers. Because we can't make any a priori prediction of migration rates for the species studied, we simulated the same dispersal rate for all species, and then tested for differences in dispersal probabilities according to foraging mode using a Kruskal–Wallis test. Dispersal probabilities are given by exp $(x/d0) \times \log(p)$, where exp = exponential function, x = cost distance matrix, $d0$ = dispersal distance, and p = proportion of all individuals in a "population."

Evaluating the Role of Isolation-By-Distance

To assess the statistical coefficients of the linear relationships between the genotypes and geographic distance (IBD) decoupled from cost distance (IBR), we used redundancy analysis (RDA) per species in the Vegan R-package (Oksanen et al. 2016), assuming a full model with IBD and IBR as independent variables, a model with IBD as independent variable, conditioned on IBR, and a third model with IBR conditioned on IBD. The different models were useful to test for the effects of respectively IBD + IBR, only IBD and only IBR on genetic variation among samples. We obtained statistical coefficients from each RDA model using ANOVA.

As an alternative to the RDA analysis, we used PCoA scores (axis 1) from each distance matrix in multiple linear regressions. We assumed the general formula PCoA1-Ritland genetic distance = a + b (PCoA1-geographic distance) + b (PCoA1-cost distance). Plots of the partials from each model are presented in the Supplementary Material (Appendix 8).

To further test for IBD, we carried out an independent test for spatial autocorrelation of genetic similarity and geographic distance. IBD was concluded if the whole correlogram reflected a significantly negative correlation. This was tested using a heterogeneity test, where the null hypothesis was no genotypic divergence associated with geographic distance at $P < 0.01$ (Banks and Peakall 2012). We set geographic distance categories to give approximately even numbers of pairwise comparisons in each distance class. Spatial correlograms were obtained for each species using GenAlEx (Peakall and Smouse 2012).

Results

Global Genetic Structure Based on HWE

Using SNP data pooled across all individuals sampled per species, ambush predators consistently had higher proportions of loci that significantly deviated from HWE, compared to active foragers (Table 1). Additionally, the majority of loci that significantly deviated from HWE showed a homozygote excess, suggesting stronger genetic structure in ambush predators (Supplementary Material, Appendix 4). This result is consistent with active foragers having significantly greater dispersal probabilities (Kruskal–Wallis chi-squared = 60.808, $P < 0.0001$) between neighboring sampling modules (Supplementary Material, Appendix 5). PCA provided no evidence of discrete genetic clusters for any of the species studied here (Supplementary Material, Appendix 6).

Isolation by Geographic Distance and Environmental Resistance

The RDA full models significantly captured 8% ($F_{2,29}$ = 1, 121; P = 0.01) of the variance in genotypes of the lancehead (Figure 2) and 18% ($F_{2,12}$ = 1.378; P = 0.001) of the variance in the treeboa (Figure 3). However, the partial models showed that genetic variation in the lancehead is exclusively associated to the effects of IBD ($F_{2,29}$ = 1.207; P = 0.01), while the genetic variation in the treeboa was affected by both IBD ($F_{2,12}$ = 1.301; P = 0.01) and IBR ($F_{2,12}$ = 1.572; P = 0.006). Forests flooded by streams and overflowing rivers had greater resistance scores, showing that flooded habitats are not optimal routes for dispersal of garden treeboas. We found no significant effects of IBD or IBR on the genetic variation for the active foragers, the cat-eyed banded (Figure 4) and southern sharpnose (Figure 5) snakes ($P > 0.34$ in all analyses). The coefficients from the RDA models are summarized in Table 2.

Although genetic algorithms were used to optimize the resistance surfaces, this does not appear to have influenced the IBR analyses, which were based on an independently generated distance measure. Patterns of resistance for 3 of the 4 species were not significantly associated with genetic distance. This shows that the use of a genetic algorithm to optimize resistance surfaces did not bias our data toward finding a correlation between resistance surfaces and the measure of genetic distance that we used.

The resistance scores based on least-cost path and Circuitscape were at least 86% correlated ($P < 0.001$ in all cases). Therefore, we show only the results from LCP in the main text, but, resistance surfaces based on LCP and CS are given in the Supplementary Material, Appendix 7.

The multiple linear regressions based on PCoA scores returned results that were consistent with the RDA models. Multiple regressions explained 35% (P = 0.001) and 79% ($P > 0.001$) of the relationship for the lancehead and the treeboa, respectively. The influence

Table 1. Summary of genetic structure of snakes from the Amazon based on deviation from HWE

Species	Foraging behavior	N	Filtered loci	HWE ($P \leq 0.05$)	% Deviating from HWE	$H_E > H_O$	% $H_E > H_O$
Lancehead	AP	32	3169	466	14.89	329	10.51
Treeboa	AP	15	7805	898	11.50	853	10.92
Cat-eyed	AF	23	2173	32	1.47	28	1.28
Sharpnose	AF	19	720	2	0.27	1	0.27

The table shows the proportions of loci that significantly deviated from HWE and proportions of loci for which H_E was greater than H_O. Genetic structure was compared between ambush predators (AP) and active foragers (AF).

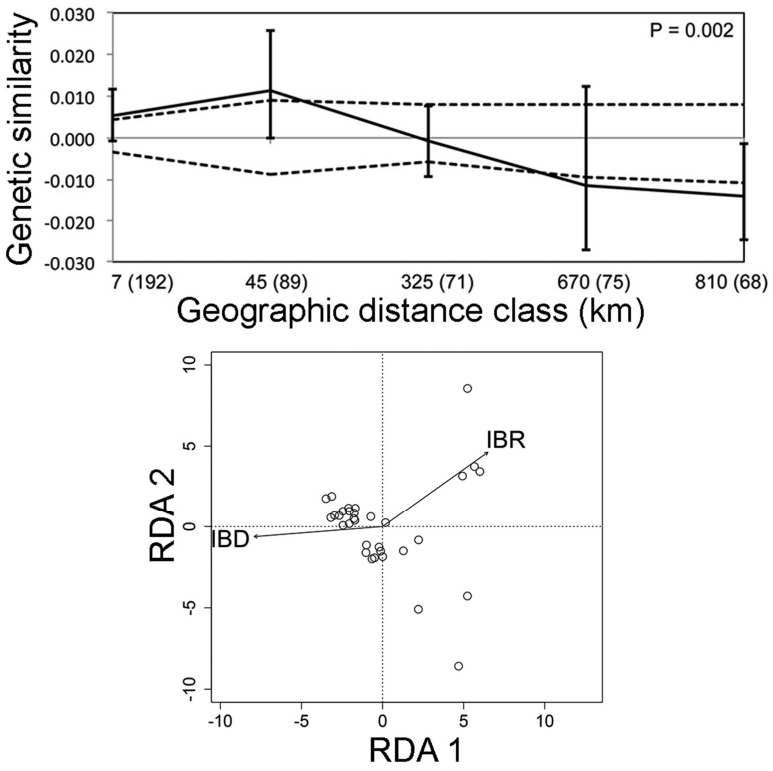

Figure 2. Patterns of gene flow in the common lancehead *Bothrops atrox* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the error bars around mean genotypic similarity per distance class show 95% confidence intervals, the dashed lines show 95% confidence intervals for the null hypothesis of no spatial structure and the values in brackets show numbers of pairwise comparisons per geographic distance class. The biplot shows individuals as open circles and the explanatory variables as vectors (black arrows).

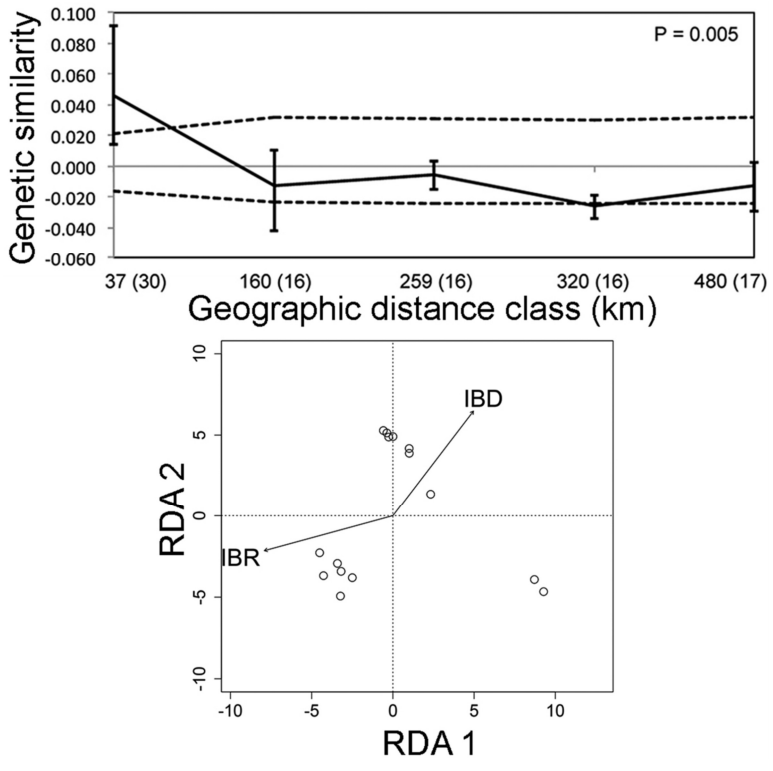

Figure 3. Patterns of gene flow in the garden treeboa *Corallus hortulanus* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the error bars around mean genotypic similarity per distance class show 95% confidence intervals, the dashed lines show 95% confidence intervals for the null hypothesis of no spatial structure and the values in brackets show numbers of pairwise comparisons per geographic distance class. The biplot shows individuals as open circles and the explanatory variables as vectors (black arrows).

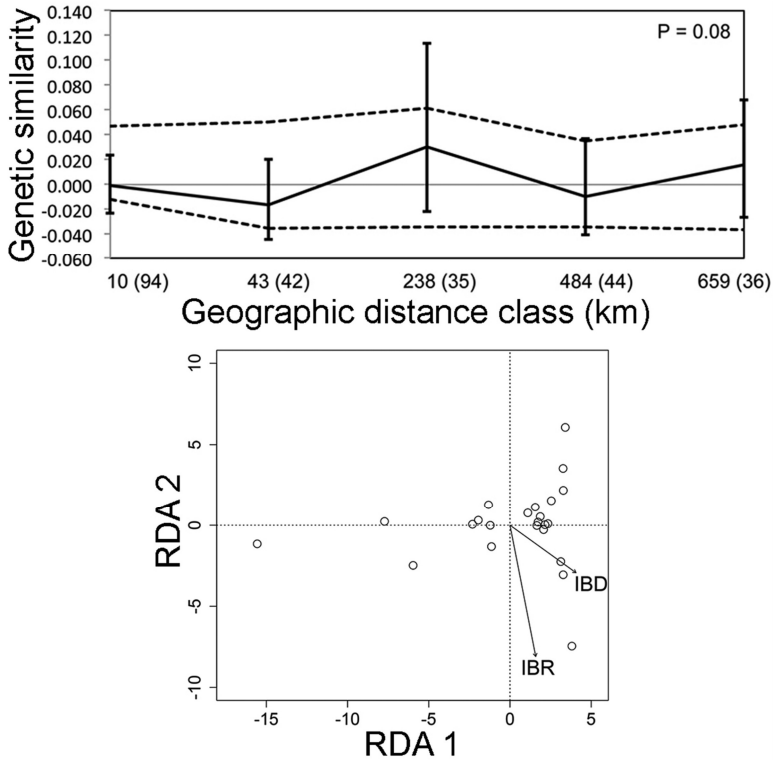

Figure 4. Patterns of gene flow in the cat-eyed banded snake *Leptodeira annulata* annulata based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the error bars around mean genotypic similarity per distance class show 95% confidence intervals, the dashed lines show 95% confidence intervals for the null hypothesis of no spatial structure and the values in brackets show numbers of pairwise comparisons per geographic distance class. The biplot shows individuals as open circles and the explanatory variables as vectors (black arrows).

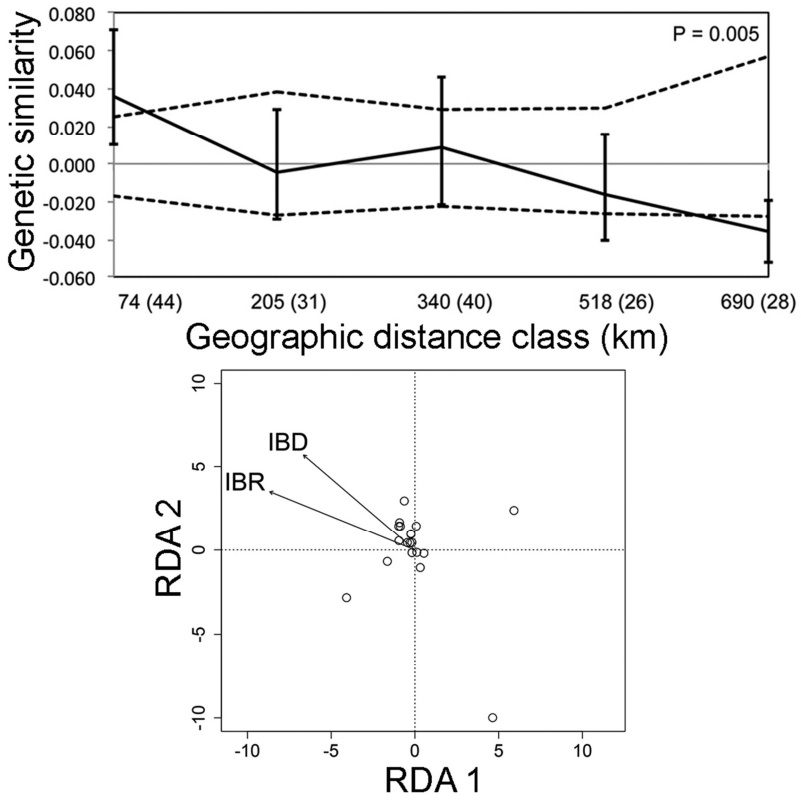

Figure 5. Patterns of gene flow in the southern sharpnose *Philodryas georgeboulengeri* based on spatial autocorrelation in genetic similarity, isolation by geographic distance and isolation by cost distance. In the correlogram, the error bars around mean genotypic similarity per distance class show 95% confidence intervals, the dashed lines show 95% confidence intervals for the null hypothesis of no spatial structure and the values in brackets show numbers of pairwise comparisons per geographic distance class. The biplot shows individuals as open circles and the explanatory variables as vectors (black arrows).

of IBR on genetic distance was restricted to the treeboa ($P < 0.001$). All PCoA ordinations of distance matrices (genetic, geographic, and environmental cost) returned axis 1 representing at least 70% of the variation in the original distances observed. The coefficients from multiple linear regressions are summarized in Table 3, and the partials from each model are plotted in the Supplementary Material, Appendix 8.

The spatial autocorrelations of genetic similarity for classes of geographic distance were generally consistent with the RDA and multiple regression models. They showed a continuous decrease in genetic similarity over geographic distance classes in the ambush predators ($P < 0.01$ in all cases), and no pattern in genetic similarity over geographic distance classes for the cat-eyed banded ($P = 0.01$). However, the southern sharpnose showed significant spatial correlogram ($P = 0.003$), despite this pattern not being captured by the linear models.

Discussion

Our data from 4 snake species co-distributed across ~880 km in the central-southwestern Amazon show that in these species' foraging mode is associated with genetic divergence. The 2 ambush predators showed greater genotypic partitioning with geographic and environmental cost distances than the active foraging species. Furthermore, the higher proportion of loci with homozygote excess in ambush predators may be due to the Wahlund effect, where an apparent homozygote excess results from data being pooled across a genetically subdivided sample (Wahlund 1928). Overall, these data imply higher levels of gene flow and dispersal across the sampled landscape for the active foragers.

Knowledge of gene flow in snakes may be severely limited by the ability to obtain large enough sample sizes in places where detectability is low (Steen 2010; Fraga et al. 2014). Despite lower than typical sizes used in population-genetic studies, the sample sizes obtained here were sufficient to detect differences in patterns of genetic distance among species. The significant association of genotypic distance with geographic or cost distances for the ambush predators matches our prediction that a sedentary lifestyle results in lower levels of gene flow than that measured in active foragers. Ambush hunting has been associated with low vagility and tendency to spend long periods in relatively small areas in both the garden treeboa (Henderson 1997) and the common lancehead (Fraga et al. 2013b). Limited dispersal has also been linked to fitness advantages in particular habitats, such as matching organisms and background colors (Rosenblum and Harmon 2011). It has been unclear whether this could be the case of the species studied here, because both garden treeboa and common lancehead are polychromatic, but different colormorphs are often sympatric at local scales (Fraga et al. 2013a; Duarte et al. 2015). This observation has been thought to indicate high gene flow regardless of geographic distance and environmental heterogeneity (Henderson 1997; Duarte et al. 2015). However, in this study, IBD is present in both species of ambush snakes, and IBR is present in the garden treeboa. Furthermore, it is possible that these species are displaying high levels of phenotypic plasticity. Phenotypic plasticity with respect to color type has been demonstrated for several reptile species (Broadie 1992; King and Lawson 1995; Rosenblum et al. 2004). Interestingly, phenotypic plasticity itself may be under selection (Pigliucci et al. 2006), potentially explaining the lack of IBR that we observed in 3 of the 4 species studied.

The only species for which we found significant effects of IBR on gene flow is the garden treeboa. In this species, nonflooded rainforests are habitats providing genetic connectivity. Effects of IBR on gene flow has been linked to localized adaptation in several organisms, such as grasses (Freeland et al. 2010), fishes (Smith et al. 2005), amphibians (Dudaniec et al. 2012; Peterman et al. 2014), birds (Smith et al. 2005; Manthey and Moyle 2015) and invertebrates

Table 2. Summary of RDA showing the effects of IBD and IBR on genetic distance of snakes from the Amazon

Species	IBD + IBR				Pure IBD			Pure IBR		
	Inertia	CP	*P* Geo	*P* Cost	Inertia	CP	*P*	Inertia	CP	*P*
Lancehead	142	0.07	**0.01**	0.76	74.93	0.03	**0.01**	59.76	0.02	0.76
Treeboa	771.1	0.18	**0.002**	**0.002**	359.9	0.08	**0.03**	377.2	0.09	**0.008**
Cat-eyed	28.41	0.05	0.38	0.97	8.39	0.03	0.46	6.21	0.01	0.99
Sharpnose	13.07	0.11	0.44	0.33	6.42	0.05	0.39	6.85	0.06	0.32

The *P* values were obtained by ANOVA, separately to geographic distance (*P* Geo) and environmental resistance (*P* Cost). The inertia values are equivalent to variance, and the CP values show the constrained proportion of variance on genetic data captured by RDA. Bolded *P* values show significant effects of IBD and/or IBR on genetic distance.

Table 3. Coefficients from multiple linear regressions using PCoA scores representing genetic distance as response variables and PCoA scores representing geographic distance (IBD) and environmental cost (IBR) as predictor variables

Species	Predictor	r^2	Residuals	Standard error	*t*-value	*P*
Lancehead	IBD	0.35	0.004	0.001	3.97	**<0.001**
	IBR		−0.0002	0.0003	−0.77	0.44
Treeboa	IBD	0.79	0.005	0.002	2.64	**0.02**
	IBR		0.08	0.013	6.35	**<0.001**
Cat-eyed	IBD	0.06	5.065	0.03	1.73	0.1
	IBR		−0.01	0.03	−0.35	0.72
Sharpnose	IBD	0.01	−0.11	0.17	−0.64	0.52
	IBR		0.09	1.12	−0.08	0.93

Bolded values are statistically significant.

(Funk et al. 2011). However, because we were not able to identify loci with selection signal in this study, the effect of IBR on gene flow in garden treeboa is more likely explained by nonrandom dispersal across heterogeneous environments (Davis and Stamps 2004; Stevens et al. 2005; Feder and Forbes 2007). Garden treeboas have mainly been found in the understory across the study area (Martins and Oliveira 1999), which is relatively open in the flooded forests in the Amazon, because many plant species cannot survive flooding for long periods. It is possible that the higher exposure to predators and prey in flooded forests might render this habitat less suitable for dispersal by treeboas. We are not, however, concluding that different selective pressures have not been acting on treeboas in these different habitats. It is possible that we did not detect any loci under selection because we did not recover any SNPs within or closely linked to genes under selection.

Gene flow was high enough in active foragers to prevent significant deviation from the HWE from samples pooled across the whole data set, or any evidence of IBR and limited evidence of IBD. These findings suggest that there has been a sufficient level of gene flow to offset genetic differentiation that would otherwise result from the localized accumulation of mutations, and the effects of genetic drift (Wright 1931; Slatkin 1987). Further, our modeling suggests that active foragers more often disperse to neighboring plots, irrespective of the intervening habitat types. While high levels of gene flow imply higher levels of dispersal across different habitats, it is also possible that effective population sizes are of a sufficient size that demographically independent groups of individuals are approximately genetically homogeneous (Guo and Thompson 1992). At present, there are insufficient data to estimate effective population sizes for these species. In future work, our knowledge of how dispersal capacity is driving genetic differentiation could be refined by radio telemetry data and finer-scale genetic analyses based on a higher density of individuals.

The relationship between geographic distance and genetic variation provided instances of high genetic differences between individuals from sites in close proximity to each other, and low genetic differences between individuals from sites further apart. This was especially prevalent in the cat-eyed banded snake (Supplementary Material, Appendix 8c). High variation in individual-based estimates of genetic distance is a characteristic expected with high dispersal (Goudet et al. 2002) and thus consistent with our finding of general high levels of gene flow for all species.

Regardless of foraging mode, in each of the species studied here, gene flow is large enough that the error bars around mean estimates of genotypic similarity mostly overlap across the distance categories assessed in the spatial-autocorrelation analysis. The sample sizes for each distance category are above those that have generated significantly different inter-class differences of the same measure of genotypic similarity in spatial autocorrelation analysis of other species (e.g., brush tail possums; Stow et al. 2006). Therefore, the high variance is most likely a consequence of high levels of gene flow across the geographic extent of sampling, and not a sampling bias. This finding is consistent with studies of other snakes, where gene flow has been shown to occur at high levels, at distances greater than 1000 km (Lawson and King 1996), and even among samples collected from islands isolated from the mainland by up to 108 km (Bittner and King 2003). However, temperate snakes that use communal hibernacula to overwinter may show genetic structure at finer scales, with IBD evident at distances of 15–50 km (Lougheed et al. 1999; Blouin-Demers and Weatherhead 2002).

Despite the differences in levels of gene flow between ambush predators and active foragers, each of the species has distributions that extend to habitats not represented within our study area. Assessing the influence of IBD and IBR at larger scales (e.g., Amazon basin) would sample biogeographically different regions, which would incorporate greater geographic isolation and environmental heterogeneity. Further assessment at larger scales may reveal genetic structuring for the active foragers, and potentially, identify habitats that provide resistance to gene flow. Additionally, by sampling larger areas in Amazonia researchers are able to distinguish ecological factors driving gene flow from historical factors, such as vicariance caused by the rivers acting as barriers to dispersal (e.g., Ribas et al. 2012). We also emphasize that although we found a consistent pattern of greater gene flow in active foragers, data from additional species are required to suggest that this constitutes a general pattern.

Tropical rainforests have provided an exceptionally challenging environment to collect basic information on dispersal for snakes, as with many other organisms, because of low detectability and the effort required to collect enough individuals. We have shown that obtaining measures of gene flow and inferring patterns of dispersal might now be accessible using new technologies that allow thousands of SNPs to be sequenced de novo in nonmodel organisms (Sansaloni et al. 2011; Peterson et al. 2012), in conjunction with new spatial modeling techniques (Peterman 2014).

Supplementary Material

Supplementary data are found at *Journal of Heredity* online.

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Data Availability

In accordance with the Journal of Heredity data archiving policy, we have submitted all the data and R scripts to Dryad (http://datadryad.org/) doi:10.5061/dryad.mq4p7.

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