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Population differentiation and nuclear gene flow in the Dominican anole (*Anolis oculatus*)

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Abstract

Allele frequency data from nuclear microsatellite loci were used to investigate patterns of nuclear gene flow and population structure in the morphologically variable Dominican anole (*Anolis oculatus*). All six loci used proved to be highly polymorphic, with an average of 18.8 alleles per locus. Test for Hardy–Weinberg equilibrium revealed small numbers of heterozygote deficiencies at single loci in single populations and consistent patterns of increasingly significant heterozygote deficiency in global tests across populations and loci. No significant relationship between F_{ST} and patristic distances estimated from mitochondrial DNA sequences was detected and estimates of F_{IS} were significantly higher in females than in males, indicating that gene flow may be sex-biased and mediated mainly by male migration. A highly significant correlation between linearized F_{ST} and $\log_e$ (geographical distance) indicates that geographical proximity is a significant factor in the genetic structure of *A. oculatus* populations. However, levels of gene flow between morphologically differentiated parapatric populations are frequently seen to be relatively high. This supports the hypothesis of natural selection being the driving force behind the development and maintenance of morphological variation and shows that adaptive differentiation may be maintained despite the homogenizing influence of gene flow. Generally, the morphologically variable populations of *A. oculatus* are seen to be poor candidates for *in situ* speciation, but an exceptional case on the west coast of Dominica indicates that isolation resulting from vicariant events may lead to rapid differentiation at both mitochondrial and nuclear loci. This provides a possible mechanism for anole speciation on other Caribbean islands.

Keywords: adaptive differentiation, microsatellite loci, natural selection

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Introduction

The Greater Antillean islands (Jamaica, Puerto Rico, Hispaniola and Cuba) support up to 40 or more species of anole (Sauria: Iguanidae), with as many as 10 species occurring sympatrically (Losos & de Querioz 1997). A phylogeny based on analysis of mitochondrial DNA (mtDNA) sequence data (Jackman *et al*. 1999) indicates that in the Greater Antilles a majority of the observed diversity is the result of within-island speciation events (Losos & Schluter 2000). In contrast, the islands of the Lesser Antilles support only one or two species each and, on all but one of the islands where two species occur, the two species are not sister taxa. This indicates that within the Lesser Antilles the occurrence of two species on a single island is the result of independent colonization events as opposed to *in situ* speciation (Losos & Schluter 2000).

Although it has been suggested that the depauperate anole communities of the Lesser Antilles represent the early stages in the evolution of the more complex communities seen on the larger Greater Antillean islands (Williams 1972), this is no longer believed to be the case (Losos & de Querioz 1997). The islands of the Lesser Antilles are now thought by some (Losos 1996; Losos & Schluter 2000) to be below the threshold area required to permit within-island speciation events. Nevertheless, some of the islands of the Lesser Antilles, such as Guadeloupe, Dominica and Martinique, exhibit pronounced geological and environmental heterogeneity and the single species found on these islands tend to display considerable geographical intraspecific variation in morphology (Lazell 1972). Furthermore, the presence of two species of anole occurring sympatrically on some small,

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environmentally homogeneous islands (e.g. Barbuda and St Vincent), indicates that these islands are capable of supporting more than one species. Therefore, studies of the patterns and causes of morphological variation and adaptation may elucidate the processes by which this highly specious group has diversified.

The mountainous topology of the island of Dominica results in highly variable environmental conditions across the island. The eastern, Atlantic, coast is exposed to the salt-laden, prevailing wind and the mountainous centre of the island receives extremely high levels of precipitation throughout the year. The leeward, Caribbean coast receives a lower, more seasonally variable rainfall, with evaporation exceeding precipitation for up to 5 months of the year. Not surprisingly, this variation in environmental conditions dictates the distribution of habitats on the island. The eastern Atlantic coast is fringed with a narrow strip of littoral woodland, while the interior of the island is shrouded in rainforest. On the western Caribbean coast thorny xerophytic vegetation abounds and many species are deciduous, shedding their leaves during the dry season.

The single, endemic anole species present on Dominica, *Anolis oculatus*, displays pronounced variation in morphology, which has been shown to consist of patterns of smooth clines, for example, an east–west cline in tail depth and altitudinal clines in scale size and body size (Malhotra & Thorpe 1997a,b). Furthermore, the patterns observed in different characters are incongruent with each other and much of the observed variation is highly correlated with environmental variables, such as vegetation type and rainfall (Malhotra & Thorpe 1997b). These findings are consistent with ecogenetic adaptation to local variations in environmental conditions. On the west coast of the island there is a notable exception to the general patterns of gentle clines and incongruence between characters (Malhotra & Thorpe 1994, 2000; Thorpe & Malhotra 1996). Despite apparent homogeneity in environmental conditions across this region, congruent changes in many characters result in a transition from a 'northern' form to a 'southern' form which occurs over just a few kilometres (see Fig. 1). Historical influences on the patterns of variation were investigated using mitochondrial cytochrome *b* gene sequences (Thorpe & Malhotra 1996). Although the distributions of mtDNA lineages are generally inconsistent with the patterns of morphological variation, the sharp morphological transition zone found on the west coast again provides an exception, as it marks a change between two mtDNA lineages (Malhotra & Thorpe 2000). In this study, microsatellite loci are employed to investigate levels of subdivision and nuclear gene flow between parapatric populations of *A. oculatus*. In order to assess bias in the levels of gene flow mediated by males and females, the patterns revealed are compared with the distributions of morphological forms and mtDNA haplotype lineages.

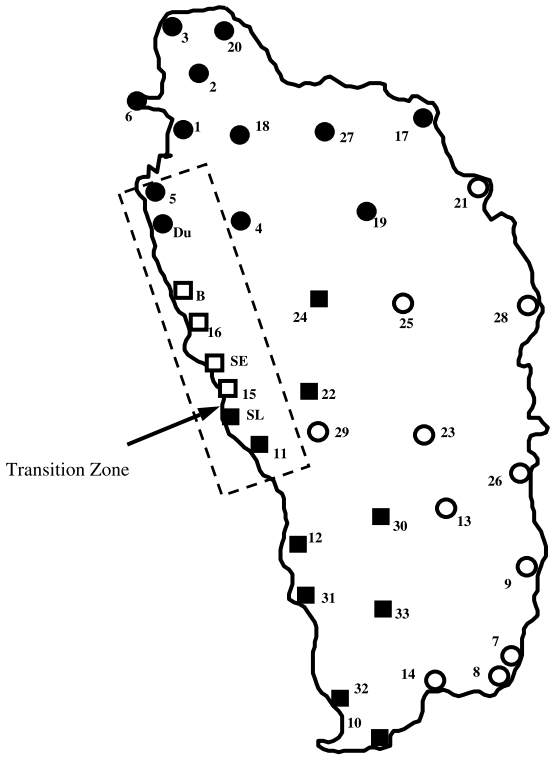

Fig. 1 Sampling sites on the island of Dominica. Site numbers 1–33 correspond with those used in Malhotra & Thorpe (1997a,b). Four additional sites (Du, B, SE and SL) spanning the transition zone on the west coast were also sampled. The geographical ranges of the four mitochondrial haplotype lineages (Malhotra & Thorpe, 2000) are indicated by the use of four symbols (□, ■, ○, ●) and the position of the sharp transition in morphology on the west coast is marked with an arrow. The transect shown in Fig. 3 is enclosed in the box of dashed lines.

Methods

Sampling, DNA extraction and genotyping

Autotomized tail tips were collected from 20 or 30 individuals at each of the sites described in Malhotra & Thorpe (1991a) and four additional sites from the west coast were also sampled (Fig. 1). With four of the original sites, these form a more detailed transect spanning the Caribbean coastal region associated with the dramatic shifts in morphology and mitochondrial haplotype lineages (Malhotra & Thorpe 2000). Tissue collections were obtained on three separate trips, spread over a 12-month period. In order to test for temporal variation in allele frequencies over this timescale, one site (16: Batali Estate) was sampled on all three trips, with care being taken on the second and third visits not to include individuals with regenerated tails. Tail tips were placed into 80% ethanol immediately and refrigerated upon return to the laboratory. The animals were released, otherwise unharmed, at the point of capture.

Whole tissue DNA extractions were prepared using a protocol adapted from Palumbi *et al*. (1991) and all individuals were genotyped for four microsatellite loci (AoGT2, AoGT9, Ao7;73 and Ao10;13) developed from an *Anolis oculatus* genomic library (Stenson *et al*. 2000). Individuals from the eight populations forming the west coast transect were also genotyped for two further loci (AoSA18 and AoBA36). After amplification, aliquots (between 1 and 4 μL) were separated electrophoretically through 6% denaturing polyacrylamide gels and the products were transferred to nylon membranes by Southern blotting. Visualization of the products was achieved by autoradiography after hybridization with complimentary simple motif oligonucleotides 5′-end labelled with ^{32}P or ^{33}P. Accurate and consistent identification of alleles was obtained by including a small number of heterozygote individuals in all batches of polymerase chain reaction. These were mixed to provide an allelic ladder that was then included on all gels.

Intra-population analyses

Tests for within population departures from Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium are necessary to establish panmixia within populations and independence of genetic distances estimated from individual loci, respectively. Therefore, GENEPOP version 3 (Raymond & Rousset 1995) was used to determine allele frequencies, observed heterozygosity and expected heterozygosity and to test the significance of departures from HWE and linkage expectations using a Markov Chain procedure (Guo & Thompson 1992). Parameters for the Markov Chain were set as default; 1000 dememorization steps and 100 batches (20 in global tests for HWE) of 1000 iterations. The Markov chain procedure may fail to detect cases of linkage disequilibrium where the number of alleles is large and/or the sample sizes are small and, under these conditions, the expectation maximization algorithm (Slatkin & Excoffier 1996) provides a more sensitive test for linkage between loci. Therefore, ARLEQUIN version 1.1 (Schneider *et al*. 1997) was used to test for departures from linkage expectations within two subsets of data: 10 of the original 33 populations, each represented by 30 individuals, and the eight transect populations, for which all individuals were genotyped for all six loci.

The highly structured geographical distributions of mtDNA haplotype lineages, with high levels of diversity even within single locations (Malhotra & Thorpe 2000), in the absence of contemporary or recent barriers to dispersal suggest that females are highly philopatric and that gene flow may be male-biased. For 17 sampled populations, the sexes of the individuals had been recorded. To test for differences in small-scale substructuring within collecting sites, males and females were grouped separately and values of F_{IS} were calculated for each sex in each population using FSTAT version 1.2 (Goudet 1995). Values for males and females were then compared using a Mann–Whitney test, with an alternative hypothesis of higher values being observed in females.

Inter-population analyses

Three measures of differentiation between the three collections from Batali were considered. Variation in allelic distributions between the three collections was assessed using a contingency table test as implemented in GENEPOP version 3 (Raymond & Roussett 1995), with a null hypothesis of identical allelic distributions in all populations. Pairwise estimates of F_{ST} and R_{ST} between the collections were then produced using ARLEQUIN version 1.1 (Schneider *et al*. 1997), with the probability of nondifferentiation (F_{ST} or R_{ST} not > 0) being estimated over 10 000 randomizations.

Pairwise estimates of F_{ST} and R_{ST} among the 33 original populations from Malhotra & Thorpe (1991a) were also estimated using ARLEQUIN version 1.1 (Schneider *et al*. 1997) and the probability of F_{ST} or R_{ST} being not > 0 was estimated over 10 000 randomizations. Isolation by distance was tested by regression of pairwise genetic distances, linearized by $D/(1 - D)$ where D is the genetic distance, against $\log_e$ (geographical distance), using the ISOLDE routine in the GENEPOP program, with 10 000 randomizations. Substituting a matrix of patristic distances (Malhotra & Thorpe 2000) for the geographical distance matrix and repeating the test investigated the relationship between genetic distances estimated from maternally and biparentally inherited markers. Partial matrix correlation tests (Thorpe *et al*. 1994), also over 10 000 randomizations, were carried out to determine whether any relationship between the genetic distances persisted after removal of the influence of geographical proximity.

Detection of patterns in the genetic structuring of populations, or departures there from, may be problematic when the number of populations is large and/or there is no *a priori* prediction of hierarchical substructure. To provide a graphical summary of the data, neighbouring sites were linked to form a network consisting the smallest, most equilateral, nonoverlapping triangles possible. For each of the population pairs linked in this way, the residuals from the regression of $F_{ST}/(1 - F_{ST})$ against $\log_e$ (geographical distance) were plotted at the mid-point between them. Interpolation and two-dimensional contouring, using a distance-weighted least-squares algorithm, was performed using UNIRAS UNIMAP (European Software Contractors A/S).

For the eight transect populations analysed using six loci, pairwise estimates of F_{ST} were obtained using ARLEQUIN, as described above. Similarly, isolation by distance was tested using GENEPOP as described above. The residual

Table 1 The numbers of alleles (N_A), expected heterozygosity (H_E) and observed heterozygosity (H_O) for each locus in each population. Averages across populations and across loci are also shown. Significant heterozygosity deficiencies, after Bonferroni correction, are highlighted in bold typeface

	AoGT2			AoGT9			Ao7;73			Ao10;13			AoSA18			AoBA36			Mean of four loci			Mean of six loci		
Pop.	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O
1	7	0.792	0.800	8	0.767	0.850	8	0.783	0.750	11	0.841	0.850							8.50	0.796	0.813			
2	9	0.822	0.700	6	0.738	0.700	10	0.855	0.700	11	0.812	0.750							9.00	0.807	0.713			
3	8	0.800	0.650	9	0.863	0.800	**9**	**0.696**	**0.400**	9	0.773	0.600							**8.75**	**0.783**	**0.613**			
4	11	0.854	0.833	11	0.769	0.733	11	0.883	0.867	13	0.846	0.767							11.50	0.838	0.800			
5	7	0.749	0.750	8	0.795	0.700	9	0.832	0.950	11	0.764	0.737	9	0.630	0.450	8	0.792	0.778	8.75	0.785	0.784	8.67	0.760	0.727
6	9	0.857	0.733	9	0.826	0.767	8	0.838	0.767	9	0.785	0.667							8.75	0.827	0.733			
7	7	0.721	0.567	10	0.819	0.800	8	0.728	0.567	9	0.800	0.633							**8.50**	**0.767**	**0.642**			
8	6	0.664	0.684	7	0.799	0.842	9	0.861	0.789	7	0.780	0.632							7.25	0.776	0.737			
9	6	0.749	0.750	11	0.838	0.750	9	0.833	0.850	8	0.814	0.737							8.50	0.809	0.772			
10	8	0.746	0.667	8	0.842	0.900	9	0.633	0.600	8	0.806	0.621							8.25	0.757	0.697			
11	8	0.729	0.633	11	0.894	0.800	9	0.845	0.933	13	0.875	0.793	8	0.685	0.481	7	0.797	0.769	10.25	0.836	0.790	9.33	0.804	0.735
12	10	0.864	0.889	11	0.876	0.778	7	0.738	0.778	6	0.775	0.667							8.50	0.813	0.778			
13	10	0.781	0.850	11	0.886	0.900	10	0.846	0.850	10	0.783	0.700							10.25	0.824	0.825			
14	7	0.740	0.700	8	0.799	0.650	7	0.786	0.700	**8**	**0.850**	**0.450**							**7.50**	**0.794**	**0.625**			
15	5	0.665	0.650	10	0.862	0.900	9	0.659	0.600	7	0.651	0.500	10	0.884	0.944	6	0.823	0.750	7.75	0.709	0.663	7.83	0.757	0.724
16	9	0.699	0.667	9	0.762	0.767	12	0.773	0.833	12	0.854	0.800	**11**	**0.764**	**0.653**	9	0.843	0.862	9.75	0.772	0.767	9.83	0.783	0.763
17	9	0.802	0.733	11	0.858	0.833	11	0.840	0.933	12	0.898	0.767							10.75	0.850	0.817			
18	8	0.785	0.850	8	0.717	0.700	8	0.854	0.900	9	0.810	0.800							8.25	0.792	0.813			
19	10	0.815	0.733	11	0.888	0.900	12	0.831	0.733	14	0.815	0.767							11.75	0.837	0.783			
20	11	0.809	0.850	10	0.873	0.850	9	0.860	0.900	**14**	**0.909**	**0.650**							11.00	0.863	0.813			
21	9	0.794	0.800	12	0.840	0.700	9	0.835	0.900	15	0.932	1.000							11.25	0.850	0.850			
22	8	0.764	0.800	11	0.890	0.900	12	0.892	0.850	12	0.881	0.800							10.75	0.857	0.838			
23	8	0.720	0.767	12	0.895	0.900	13	0.865	0.933	13	0.894	0.828							11.50	0.844	0.857			
24	7	0.756	0.750	13	0.854	0.900	9	0.847	0.750	11	0.865	0.850							10.00	0.831	0.813			
25	8	0.783	0.600	10	0.849	0.750	12	0.854	0.900	12	0.853	0.950							10.50	0.835	0.800			
26	9	0.741	0.800	15	0.921	0.867	12	0.856	0.733	16	0.888	0.862							**13.00**	**0.852**	**0.816**			
27	9	0.792	0.800	9	0.882	0.800	9	0.845	0.750	13	0.890	0.900							10.00	0.852	0.813			
28	8	0.772	0.700	10	0.871	0.900	11	0.888	0.750	11	0.860	0.900							10.00	0.848	0.813			
29	7	0.721	0.750	11	0.797	0.850	11	0.894	0.850	**13**	**0.895**	**0.650**							10.50	0.827	0.775			
30	6	0.769	0.700	12	0.878	0.900	7	0.723	0.800	13	0.905	0.950							9.50	0.819	0.838			
31	9	0.864	0.850	12	0.859	1.000	7	0.756	0.850	9	0.733	0.700							9.25	0.803	0.850			
32	8	0.758	0.750	11	0.844	0.900	8	0.801	0.900	8	0.812	0.900							8.75	0.804	0.863			
33	10	0.767	0.800	9	0.814	0.800	9	0.813	0.650	9	0.832	0.700							9.25	0.807	0.738			
B	**8**	**0.640**	**0.450**	11	0.863	0.800	9	0.864	0.850	13	0.718	0.700	8	0.781	0.700	10	0.854	0.800				**9.83**	**0.787**	**0.717**
Du	8	0.854	0.900	8	0.851	0.900	9	0.794	0.800	10	0.714	0.550	7	0.664	0.600	6	0.672	0.550				8.00	0.758	0.717
SE	4	0.632	0.500	10	0.830	0.800	10	0.767	0.650	12	0.886	0.950	8	0.717	0.800	10	0.876	0.850				9.00	0.785	0.758
SL	8	0.737	0.900	**10**	**0.869**	**0.900**	9	0.846	0.750	10	0.831	0.900	8	0.540	0.550	8	0.844	0.900				8.83	0.778	0.817
Mean	**8**	**0.765**	**0.738**	10.08	0.840	0.824	**9.46**	**0.814**	**0.786**	**10.84**	**0.828**	**0.756**	**8.63**	**0.696**	**0.637**	8	0.813	0.782	**9.62**	**0.814**	**0.777**	**8.92**	**0.774**	**0.743**

values from this regression were plotted, at the mid-points between the relevant populations, along an axis scaled to represent the geographical distances between sample sites. Multi-locus population genetic data are multivariate in nature (Guinand 1996), so a univariate statistic such as F_{ST} may be insufficient to adequately describe the variation between populations. PCA-GEN version 1.2 (Goudet 1999) was used to carry out Principal Components Analysis based on the genotypic data obtained for individuals from the transect populations. The statistical significance associated with each axis was calculated over 10 000 randomizations. This multivariate ordination technique is frequently used with genetic data (e.g. Barker *et al*. 1986; Cavalli-Sforza *et al*. 1993).

Results

Intra-population analyses

All six loci proved to be highly polymorphic (Table 1) with between 13 and 25 alleles being observed over all populations at each locus (mean = 18.8). With few exceptions, all of the possible allele sizes within the observed size range were detected. None of the observed alleles differed in size from other alleles by less than the two base pairs predicted by the microsatellite motif. The most polymorphic loci were the two compound loci (AoGT9 and Ao10; 13). The smallest number of alleles were observed at the two loci (AoSA18 and AoBA36) used only for the eight transect populations. These two loci remained the least polymorphic when compared with the number of alleles observed at the other loci in the eight transect populations.

Tests for departures from HWE detected small numbers of heterozygote deficiencies ($P < 0.05$) for single loci in single populations, but most were not significant after Bonferroni correction. In global tests, pooling across loci and/or populations revealed increasing numbers of departures from HWE, with an increasing proportion of the heterozygote deficiencies remaining significant after Bonferroni correction. In all three data sets, the heterozygote deficiency was highly significant ($P < 0.0089$) across all populations and loci. Although a small number of departures from linkage expectations were detected in single populations, very few remained significant after Bonferroni correction and no cases of significant linkage between loci were detected across all populations ($P > 0.11$). For the 17 populations for which sexes had been recorded, the estimates of F_{IS} for females (mean = 0.0631) were significantly higher ($P = 0.0219$) than the values for males (mean = 0.0042).

Inter-population analyses

Among the three collections from Batali, no significant differentiation was detected across all samples and loci ($P = 0.3423$). Furthermore, no single locus detected differentiation between any pair of samples ($P > 0.07$) or across all samples ($P > 0.05$). Estimates of F_{ST} and R_{ST} between pairs of samples were all very low (maximum $F_{ST} = 0.00352$, maximum $R_{ST} = 0.01600$) and were not significantly greater than 0 (F_{ST} $P = 0.26177$; R_{ST} $P = 0.07589$).

Pairwise estimates of F_{ST} among the 33 populations varied between −0.00148 and 0.11138, with only 41 (7.8%) of the comparisons not being significantly greater than zero. In contrast, 304 (57.6%) of the R_{ST} estimates, which varied between −0.02085 and 0.17957, were not significantly greater than zero. This is presumed to be the result of the higher variance associated with estimation of R_{ST} (Gaggiotti *et al*. 1999). Both genetic distances were highly correlated to geographical distance ($P < 0.0001$), but due to the apparent nondifferentiation detected between many population pairs using R_{ST}, this measure of population structure was excluded from further consideration. A significant correlation between F_{ST} and patristic distance was also detected ($P < 0.0001$). However, with the common influence of geographical proximity regressed out, the relationship between the two distances was no longer significant ($P = 0.287$).

The contoured plot of residual F_{ST} estimates (Fig. 2) shows that, over large parts of the island, levels of gene flow are relatively high, even between populations with pronounced variation in morphology and between populations belonging to different mitochondrial haplotype lineages. However, a region is highlighted on the west coast of the island where a relatively large apparent barrier to gene flow occurs in the same region of the coastline associated with the transition in morphology and mtDNA. Pairwise estimates of F_{ST} between the transect populations varied between 0.00122 and 0.09306. Of the 28 comparisons, only two were not significantly greater than 0 ($P > 0.05$) and a highly significant relationship between F_{ST} and geographical distance was detected ($P < 0.0001$). The result of plotting residual F_{ST} estimates for consecutive population pairs along the mid-point between the populations is shown in Fig. 3. Higher estimates of F_{ST} and higher levels of significance are seen to be associated with changes between mitochondrial lineages, at both the northern and southern ends of the distribution of the central Caribbean lineage. Principal components analysis revealed only one axis to be significant at the 5% level ($P = 0.0020$). A second axis was marginally significant ($P = 0.0649$) and a two-dimensional plot was generated using these two axes (Fig. 4). Three population clusters can be seen, corresponding to the three different mitochondrial lineages found in this area. In relation to the horizontal axis, the only one significant at the 5% level, the populations to the south of the transition zone can be seen to be more genetically distinct from central populations than are the populations to the north.

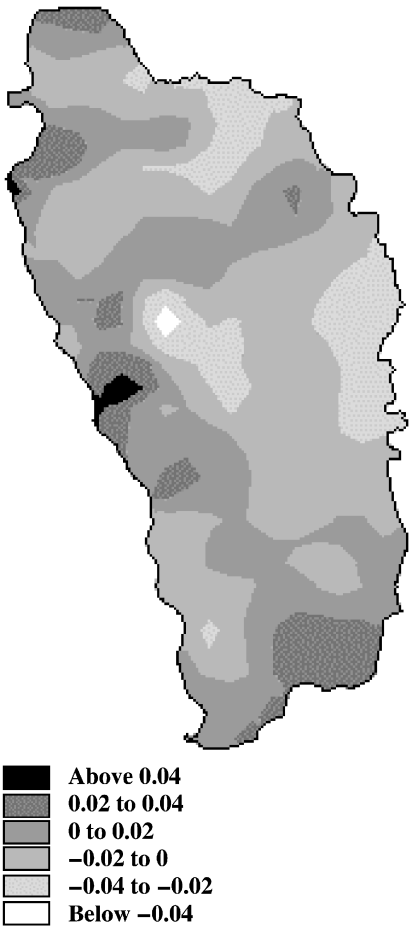

Fig. 2 Contoured plot of residual F_{ST} estimates. Collection sites were joined to form a network and residual values from the regression of F_{ST} estimates against $\log_e$ (geographical distance) were plotted at the mid-points between the relevant populations (see text for further details).

Discussion

Population structure and gene flow in the Dominican anole

Levels of within-population variation were consistently high both among loci and among populations. Loss of genetic variability has been used to detect demographic bottlenecks in threatened or endangered species and/or populations (Taylor *et al*. 1994; Gibbs *et al*. 1998) and, where bottlenecks have occurred, apparent cases of linkage disequilibrium may be detected between independently segregating loci (Slatkin 1994). Although a small number of significant cases of disequilibrium were detected, these were not clustered either by locus-pair or population and, over all populations, no significant departures from linkage expectations were observed. Therefore, the six loci can be accepted as independently segregating units, providing independent estimates of population differentiation and it would seem that severe and/or recent bottlenecks have not been a significant feature of the demographic history of *Anolis oculatus*. This is consistent with the high levels of nucleotide diversity observed in cytochrome *b* haplotypes, both within and among sites (Malhotra & Thorpe 2000). Dominica has experienced numerous highly destructive volcanic eruptions during the last 50 000 years (Sigurdsson & Carey 1981). The absence of any apparent influence of such a turbulent history on the genetic record of either mitochondrial or nuclear markers indicates that events perceived by humans as catastrophic may be of little significance in terms of the demographic history of anoles (Malhotra & Thorpe 2000). Population bottlenecks following volcanic events may have been of short duration and/or may not have been severe, with high population densities allowing relatively large numbers of animals to survive in small enclaves.

In all three data sets there is a consistent pattern of increasing within-population heterozygote deficiency in global tests across both loci and populations. However, departures from HWE within individual populations were small and infrequently statistically significant, so estimates of genetic distances between populations should not be biased. Small deviations from HWE are frequently observed in microsatellite data and are thought to be the result of one or a combination of factors (Goodman 1998). Selection acting on a microsatellite locus (or a closely linked locus) can result in excess homozygosity, as can nonrandom mating. The presence of null alleles can also result in apparent departures from HWE, as heterozygotes carrying a single null allele are erroneously identified as homozygous for their single amplifying allele (Pemberton *et al*. 1995). The presence of null alleles is only clearly indicated when null allele homozygotes (or heterozygous individuals carrying two different alleles from a series of null alleles) are detected. Amongst all of the individuals screened (900 in total), only 20 individuals persistently failed to produce amplification products for one locus when other loci amplified readily. Although these were from widely distributed populations, only three of the six loci were involved — eight cases involved locus AoSA18, while loci Ao10;13 and AoBA36 were each involved in six cases — so these three loci may be affected by nonamplifying alleles. However, as these occurrences of nonamplification and the observed departures from HWE occur in different populations, the frequencies of null alleles are probably relatively low and evenly spread. Where null alleles are of limited occurrence, overall population structure analysis is not expected to be biased (Goodman 1998), especially if these alleles are not restricted to one or a small number of localized populations.

Significant departures from HWE were observed at loci other than those suspected of including null alleles and,

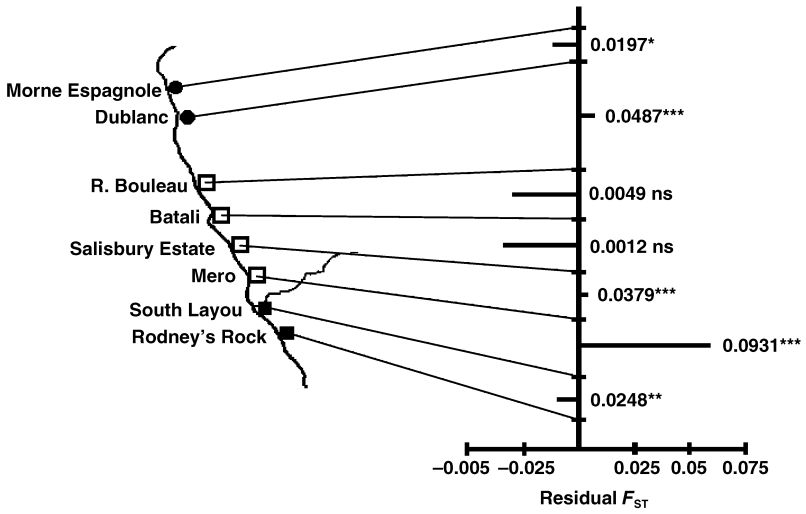

Fig. 3 Residual values of regression of F_{ST} against $\log_e$ (geographical distance) between transect populations. The original estimates of F_{ST} and their levels of significance are indicated to the right of each bar (***$P < 0.0001$; **$P < 0.01$; *$P < 0.05$; ns, $P > 0.05$). The geographical distributions of the three mitochondrial haplotype lineages in the area are indicated by different symbols as in Fig. 1.

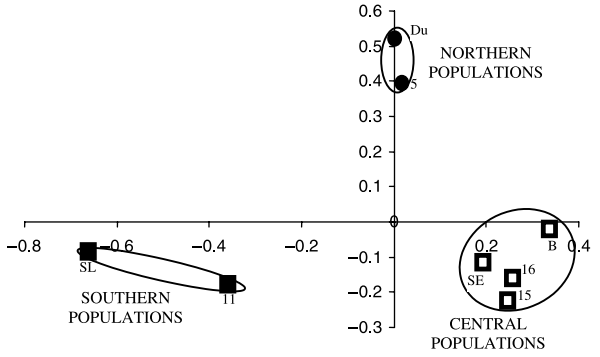

Fig. 4 Principal Components Analysis of allele frequencies in transect populations. The horizontal axis is statistically significant ($P = 0.0020$), while the vertical axis is only marginally significant ($P = 0.0649$). The statistical significance of the axes was calculated over 10 000 randomizations. Key to site identities: 5 = Morne Espagnole; Du = Dublanc; B = R. Bouleau; 16 = Batali; SE = Salisbury Estate; 15 = Mero; SL = South Layou; 11 = Rodney's Rock.

over all loci, highly significant departures from HWE were identified. It is therefore apparent that the observed pattern of heterozygote deficiencies may be the result of population substructure within the sampling areas (Wahlund Effect; Hartl & Clark 1989). Where gene flow is mediated through sex-biased dispersal, genetic distances estimated from bi-parentally inherited nuclear markers are expected to be poorly correlated with genetic distances estimated from maternally inherited mtDNA (e.g. Rassmann *et al*. 1997). Although a significant correlation between F_{ST} and patristic distance was detected, once the effect of geographical proximity was removed there was no significant relationship between the two matrices. Given that the strong phylogeographic structure detected in mtDNA haplotypes (Malhotra & Thorpe 2000), is not apparent in the analysis of microsatellite allele frequencies, gene flow would appear to be dominated by male migration. The significantly higher F_{IS} detected in females, compared with males, indicates that they are subject to a degree of fine-scale substructure within sampling sites that is less significant in males and further indicates that males may be the major vectors of gene flow. A pattern of male-biased gene flow is also consistent with observations of anole behaviour. Male anoles are highly territorial and defend their non-overlapping territories vigorously. Although females are also territorial, the areas they defend are roughly half the size of male territories (Stamps 1973) and, at high population densities, female territories may overlap (Schoener & Schoener 1982).

Pairwise F_{ST} estimates among the 33 populations and among the eight transect populations were highly correlated with geographical distance, indicating that isolation-by-distance is significant in the structuring of *A. oculatus* populations. However, the two-dimensional contoured plot of residual F_{ST} estimates indicates that high levels of gene flow may occur, even between populations of different ecomorphs. Patterns of gene flow are generally unrelated to patterns of morphological variation or distribution of mitochondrial haplotype lineages. This indicates that morphological adaptations to variations in local conditions are maintained despite the exchange of genetic material between neighbouring populations. Previous analyses and experimentation have indicated natural selection as the most likely causative agent in generating and maintaining the observed patterns of morphological variation in *A. oculatus* (Malhotra & Thorpe 1991b; 1997a,b). The high level of gene flow detected between morphologically differentiated populations lends further support for the role of natural selection. Local adaptations may be maintained despite

high levels of gene flow when selection pressure is high (Endler 1977) and has been demonstrated in Trinidadian guppy populations (*Poecilia reticulata*), exposed to differences in predation pressure (Endler 1995; Magurran 1998). Adaptive differences between parapatric anole populations can apparently be maintained despite the homogenizing influence of gene flow.

An exception to the general patterns of high gene flow and incongruence between mitochondrial and nuclear marker systems is seen on the west coast. An apparent restriction in gene flow is seen to coincide with the transition between mtDNA lineages at the southern end of the geographical range of the Central Caribbean clade. The apparent restriction in gene flow implies that the populations on either side of the transition zone display differences in genetic composition greater than would be predicted from the geographical distance between them. The analyses from which these conclusions have been derived are based on variance in allele frequencies (Weir & Cockerham 1984), which could result from past vicariance, during which differences in allele frequencies developed, followed by subsequent secondary contact. Pairwise differences in mtDNA sequence data between the central Caribbean and northwestern lineages suggest that the lineages originally diverged 4.46 ± 0.08 million years ago (Malhotra & Thorpe 2000). It is impossible to categorically differentiate between contemporary barriers to gene flow, leading to continuing divergence and secondary contact after divergence in allopatry, leading to eventual homogenization. However, at the northern extremity of the Central Caribbean haplotype lineage, where the differentiation in mtDNA is equally deep, the apparent differentiation in microsatellite allele frequencies is considerably less pronounced. Both ends of the geographical distribution of the Central Caribbean lineage are delineated by relatively recent lava flows (i.e. within the last 50 000 years; see Fig. 5 of Malhotra & Thorpe 2000), so it is likely that populations in both areas have been in contact, allowing the opportunity for nuclear introgression, over similar timescales. Therefore, the genetic differentiation between the west coast populations spanning the transition zone may be, at least in part, due to contemporary restrictions in gene flow.

Adaptive differentiation and speciation in the Lesser Antilles

A further implication of the high levels of gene flow between differentiated populations is that, in general, the highly polymorphic species on single-species islands in the Lesser Antilles would appear to be poor candidates for *in situ* speciation. Although population differentiation is considered as a preliminary step in many widely accepted models of speciation (Foster *et al*. 1998), the highly differentiated anole populations on environmentally heterogeneous Lesser Antillean islands may not be destined for eventual isolation. Similarly, highly differentiated populations of Trinidadian guppies appear to be prevented from following independent evolutionary trajectories due to relatively high levels of gene flow, largely mediated by sneak mating by migrating males (Magurran & Seghers 1994; Magurran 1996, 1998). In this case, the observations were consistent with theoretical predictions that, under conditions of incipient sympatric or parapatric speciation, females will act to favour premating isolation, while males will act to prevent such isolation (Parker & Partridge 1998). Although the data presented here do not indicate mate choice preferences in females, it is clear that populations of *A. oculatus* may be prevented from following independent evolutionary trajectories by high levels of male-mediated gene flow.

The general pattern observed in *A. oculatus* gives little indication of a process that may result in the high levels of *in situ* speciation that have been shown to have occurred in Greater Antillean anole radiations (Jackman *et al*. 1999; Losos & Schluter 2000). However, the exceptional case on the west coast of Dominica shows that, where populations become isolated due to vicariant events, differentiation at nuclear and mitochondrial loci may occur readily. If such differentiation extends to behavioural and visual cues important in courtship (Case 1990; Giannasi 1997), upon secondary contact the differentiated populations may fail to hybridize freely due to prezygotic behavioural reproductive isolation. Where maladapted hybrids with reduced fecundity result from heterotypic mating, complete reproductive isolation will tend to evolve through reinforcement (Rubinoff & Rubinoff 1971). Resource or niche partitioning may then occur as interspecific competition acts to elaborate on small existing differences in morphology and/or behaviour. This may provide a mechanism for the evolution of the more complex anole communities of the Greater Antilles, where the larger area of the islands (Losos & Schluter 2000) and timescale may have provided opportunities to diverge in allopatry after vicariant events such as partial submergence.

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Anita Malhotra and Roger Thorpe have been studying the evolution of anoles of the Lesser Antilles since 1988, using studies of within-species morphological divergence, natural selection field experiments, molecular markers and, more recently, spectroradiometry of colour. Andrew Stenson joined them as a PhD student to extend the use of molecular markers, particularly microsatellites, in order to study the radiation of the *bimaculatus* group of anoles in the northern Lesser Antilles. This paper is a result of his PhD work. As well as work on divergence in island lizards, the group has an active research programme on the evolution of venomous snakes.
