

# The roles of allopatric divergence and natural selection in quantitative trait variation across a secondary contact zone in the lizard *Anolis roquet*

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## Abstract

Populations of the Caribbean lizard, *Anolis roquet*, are thought to have experienced long periods of allopatry before recent secondary contact. To elucidate the effects of past allopatry on population divergence in *A. roquet*, we surveyed parallel transects across a secondary contact zone in northeastern Martinique. We used diagnostic molecular mitochondrial DNA markers to test fine-scale association of mitochondrial DNA lineage and geological region, multivariate statistical techniques to explore quantitative trait pattern, and cline fitting techniques to model trait variation across the zone of secondary contact. We found that lineages were strongly associated with geological regions along both transects, but quantitative trait patterns were remarkably different. Patterns of morphological and mitochondrial DNA variation were consistent with a strong barrier to gene flow on the coast, whereas there were no indications of barriers to gene flow in the transitional forest. Hence, the coastal populations behaved as would be predicted by an allopatric model of divergence in this complex, while those in the transitional forest did not, despite the close proximity of the transects and their shared geological history. Patterns of geographical variation in this species complex, together with environmental data, suggest that on balance, selection regimes on either side of the secondary contact zone in the transitional forest may be more convergent, while those either side of the secondary contact zone on the coast are more divergent. Hence, the evolutionary consequences of allopatry may be strongly influenced by local natural selection regimes.

*Keywords:* allopatry, *Anolis roquet*, cline, natural selection

Received 8 April 2008; revision received 25 September 2008; accepted 1 October 2008

## Introduction

Hybrid zones are areas where distinct genotypes or phenotypes meet and produce hybrids (Barton & Hewitt 1985), and as such they are relevant to studies of speciation (Harrison 1991; Nurnberger *et al.* 1995; Jiggins & Mallet 2000). Hybrid zones arise from either primary or secondary contact (Barton & Hewitt 1985). In the latter case, differences built up during a period of geographical isolation are effectively put to a test of compatibility (Fitzpatrick & Shaffer 2004). On initial contact, steep and congruent clines in multiple characters may form. With time, and in the absence of strong selection, these clines can

disappear due to the homogenising effects of dispersal and recombination (Endler 1977; Barton & Hewitt 1985), and previously isolated populations may merge (Sequeira *et al.* 2005). Alternatively, the centre and width of some clines may displace and flatten (respectively) in response to varying selection pressures on different traits (Parsons *et al.* 1993; Nurnberger *et al.* 1995; Brumfield *et al.* 2001; Takami & Suzuki 2005). Finally, multiple character clines may remain steep and congruent in response to strong selection against hybrids (Dasmahapatra *et al.* 2002; Phillips *et al.* 2004), offering the possibility of continued population divergence.

Estimating the relative contribution of two types of selection, endogenous (intrinsic) and exogenous (extrinsic), is a fundamental problem in hybrid zone study (Kruuk *et al.* 1999). Endogenous selection leads to the formation of

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tension zones, which are independent of environment and predominantly maintained by reduced fitness in hybrids due to an incompatible mix of genomes (Barton & Hewitt 1985; Nurnberger *et al.* 1995; Phillips *et al.* 2004). Exogenous selection results in hybrid zones where different types may be favoured on either side of an environmental gradient, and it is the environmental gradient that ultimately determines the position of the cline (Nurnberger *et al.* 1995). In patchy environments, exogenous selection can lead to the formation of mosaic hybrid zones. In these zones, character traits show abrupt reversals and transitions in concordance with habitat distribution (Ross & Harrison 2002; Vines *et al.* 2003; Fitzpatrick & Shaffer 2004). Endogenous and exogenous selection can act together (Szymura & Barton 1986; Szymura & Barton 1991; Sites *et al.* 1995) and distinguishing the effects of one from the other can be difficult or impossible (Kruuk *et al.* 1999; Marshall & Sites 2001). The relative strengths of endogenous and exogenous selection pressures can also vary in different areas of a zone of secondary contact (Szymura & Barton 1991; Hairston *et al.* 1992; Vines *et al.* 2003) and clines can move, either as a response to change in environment (Dasmahapatra *et al.* 2002; Leache & Cole 2007), due to competition (Rohwer *et al.* 2001) or into areas of low population density (Barton & Hewitt 1985). The fate of clines in secondary contact zones is ultimately concerned with fundamental theories of speciation and species concepts. Case studies from hybrid zones can offer insight into the effects of allopatry and the importance of other forces driving population divergence upon secondary contact.

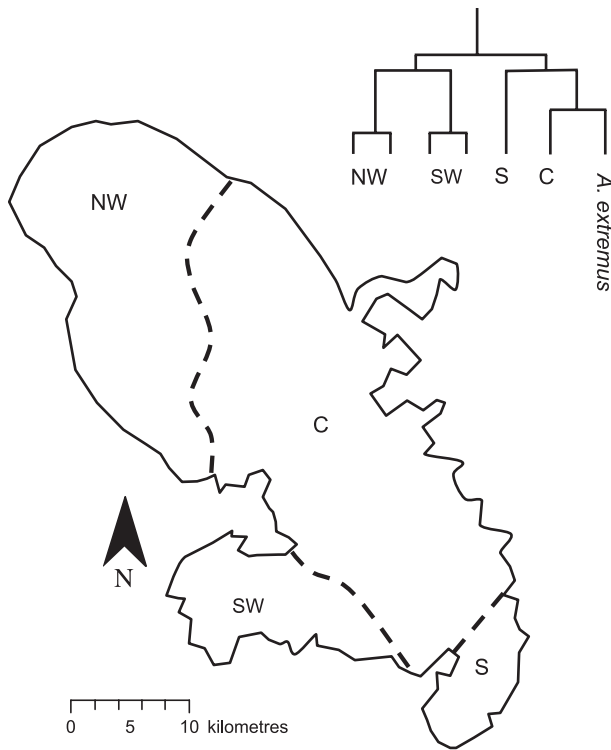
Adaptive radiations, such as the *Anolis* radiation in the Caribbean, are particularly useful for the empirical study of the processes involved in speciation. The c. 150 species of anoles in the Caribbean demonstrate high levels of in-situ speciation from as few as two colonisation events (Jackman *et al.* 1999). The islands involved in this adaptive radiation are grouped into two sets based on island size and geology: the Greater Antilles, which include the large islands of Cuba, Hispaniola, and Puerto Rico, and the Lesser Antilles, a chain of small islands extending from Anguilla, south towards Venezuela. The anole communities on the two sets of islands share some fundamental similarities. Specifically, there is strong evidence in both areas of natural selection acting on quantitative traits. Selection has led to both intraspecific within-island adaptation to habitat in the Lesser Antilles (Thorpe & Malhotra 1996; Malhotra & Thorpe 2000) and divergent habitat specialisation among species in the Greater Antilles (Losos 2004; Calsbeek *et al.* 2006). However, the Greater Antillean islands support multispecies anole communities with up to 55 endemic species on a single island (Losos *et al.* 2003), whereas the Lesser Antilles have only one or two native species on each island (Thorpe *et al.* 2004). In the Lesser Antilles, both the biogeographical pattern of species distribution and phylo-

genetic analyses of molecular variation suggest that anole colonisation and subsequent speciation occurred progressively from island to island (Thorpe & Stenson 2003; Thorpe *et al.* 2004, 2008), suggesting that allopatry is important for speciation in this particular set of islands.

Because the Lesser Antilles are a chain of discrete islands, their respective species of anole are geographically isolated from each other. Therefore, reproductive isolation in this group cannot usually be tested in natural conditions. However, the island of Martinique is unusual due to its geological history: it appears that it was formed into a single island from five separate precursor islands, when two precursors from the older arc (Caravelle, and St Anne peninsulas, formed during the Eocene and early Miocene) and two precursors from the younger arc (Trois-Ilets peninsula and the northwest, formed during the Miocene and Pleistocene) were joined by the uplifting of the central area between them, possibly as recently as 1.5 million years ago, or less. (Andrieuff *et al.* 1976; Bouysse *et al.* 1983; Maury *et al.* 1990; Sigurdsson & Carey 1991; Thorpe *et al.* 2008). This has allowed organisms that evolved in allopatry to come into secondary contact, offering an exceptional opportunity in which to test the strength of allopatric divergence and its role in speciation, while also evaluating the importance of selective forces driving differentiation.

Martinique is inhabited by a single species of anole, *Anolis roquet*, which is an endemic, arboreal, iguanid lizard. A recent phylogenetic analysis of a 1139-bp mitochondrial DNA (mtDNA) (cytochrome *b*) fragment revealed four main monophyletic lineages (Fig. 1) (Thorpe & Stenson 2003). Across the island, these main lineages were found to be very closely associated with the geological regions described above. The age of the lineages directly corresponds to the emergence of the most recent precursor islands and regions, and it is thought that young precursor islands were colonised as they emerged (Thorpe & Stenson 2003). When volcanic activity and orogeny eventually connected the precursor islands to form present-day Martinique, lizard lineages came into secondary contact (Thorpe *et al.* 2004). Three major contact zones were identified on Martinique, where divergence between the different lineages has been estimated at between 6 and 8 million years, probably followed by less than 1.5 million years of secondary contact (Thorpe *et al.* 2008).

The mountainous younger arc Lesser Antillean islands, such as Martinique, have pronounced environmental zonation, with specific vegetation types (Beard 1948). Rainforest covers the montane interiors, and is replaced by transitional forest as altitude decreases towards the coast. Coastal habitat varies, with xeric woodland in the rain shadow of the Caribbean coast, and littoral woodland on the exposed central Atlantic coast. Furthermore, rainfall and habitat change on the southern and northern tips of the islands. As a result, in Martinique the Atlantic littoral



**Fig. 1** Main phylogenetic relationships and lineage boundaries of *Anolis roquet* on Martinique (Thorpe & Stenson 2003; Thorpe *et al.* 2008). *Anolis extremus* on Barbados is a sister clade to the central *A. roquet* lineage (Thorpe & Stenson 2003).

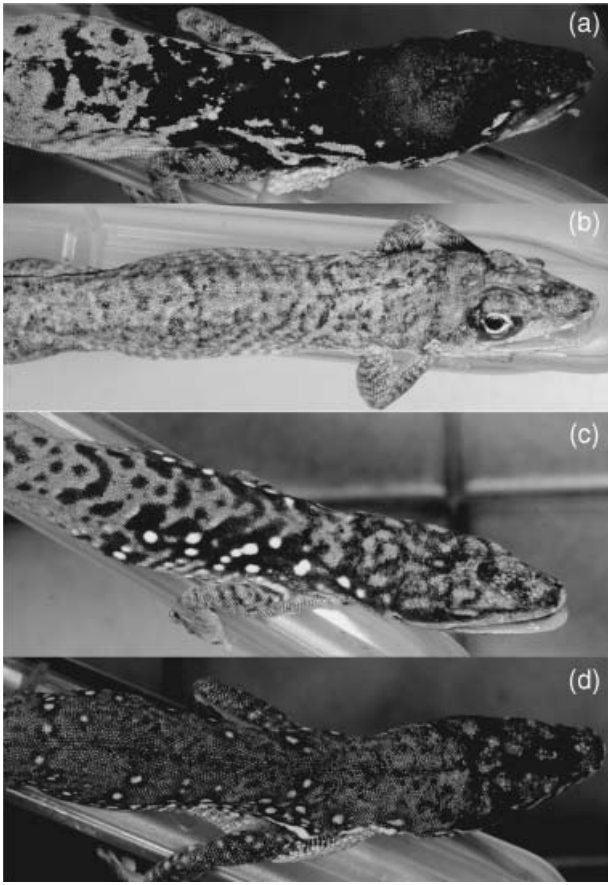
woodland is replaced by xeric woodland to the south and mesic forest to the north.

The Martinique anole, like the anoles on the other Lesser Antillean islands with environmental zonation, shows marked geographical variation in hue and pattern, as well as scalation, body dimensions and size (Lazell 1972; Thorpe & Malhotra 1996; Thorpe & Stenson 2003; Thorpe *et al.* 2004, 2008). There may be specific exceptions, but overall, the geographical variation in quantitative traits (QT) of anoles on Martinique, and the other Lesser Antillean islands, is associated with this environmental zonation rather than phylogeographical lineages. This is interpreted as natural selection for current conditions and is supported by common garden experiments indicating genetic control rather than plasticity (Thorpe *et al.* 2005), large-scale field experiments on natural selection determining the intensity and targets of selection (Malhotra & Thorpe 1991; Thorpe *et al.* 2005), correlations between environmental and QT patterns (taking into account phylogeny and other factors), and parallel patterns of adaptation (Thorpe 2005). The latter is particularly notable in the Martinique anole where populations from different lineages experiencing similar environmental conditions have very similar appearance due to strong convergent selection (Thorpe 2005). An example

of this is found in montane forms which, irrespective of lineage, are an intense saturated green hue with black and non-ultraviolet (UV) white markings. Indeed, Ogden & Thorpe (2002) and Thorpe *et al.* (2008) show that where northwestern and central lineages meet in montane rainforest after prolonged allopatry, convergent selection renders them identical (in patterns of QTs) and there is no indication of reduction in gene flow across the secondary contact zone as estimated by neutral nuclear markers. In sharp contrast, divergent selection between adjacent habitats results in marked difference in QTs and a notable reduction in gene flow estimated by the same nuclear markers (Ogden & Thorpe 2002; Thorpe *et al.* 2008). Despite this general pattern, Thorpe & Stenson (2003) identified two areas where, at least superficially, quantitative trait variation seems to correlate to lineage, one in southern Martinique (Ste Anne peninsula) and the other on the north Atlantic coast.

This study examines one of the regions that may represent an exception to the general pattern. In northeastern Martinique, two of the most divergent mtDNA lineages meet (central and northwestern; 7.9% uncorrected divergence). Where these lineages meet on the coast, the Atlantic littoral woodland (Beard 1948) gives way to wetter and more seasonal climatic conditions to the north. However, inland at the transitional forest, conditions are more consistent at any given altitude, and in the montane rainforest, there is thought to be strong convergent selection for identical habitats either side of the secondary contact between these two lineages. Along the coastal strip of littoral woodland, anoles from the central lineage (C) have a brown, dull, uniform dorsum with a low UV reflective dewlap, whereas anoles from the northwestern (NW) lineage are green with black markings and a bright dewlap in the yellow/orange part of the spectrum and higher UV reflection (Fig. 2). Colour and pattern appear correlated with lineage, and 'lineage forms' are easily distinguished on the coast over the space of a few kilometres. The change in dewlap hue along the Atlantic coast of Martinique (Thorpe & Stenson 2003) may be in response to habitat change, as *A. trinitatis* on the Atlantic coast of St Vincent also has a highly UV reflective dewlap (Thorpe 2002). However, in the transitional forest, a short distance towards the interior of the island, lizards from both lineages are superficially indistinguishable (Fig. 2).

With two parallel transects, one on the coast and one in the transitional forest, we explore the transition between mtDNA lineages and relate these to quantitative traits known to be under selection (colour, body dimensions, markings and scale counts, Malhotra & Thorpe 1991; Ogden & Thorpe 2002; Thorpe *et al.* 2005). We also measure climatic and habitat data along the transects in order to give an insight into the nature of selection (convergent or divergent) influencing populations on either side of the



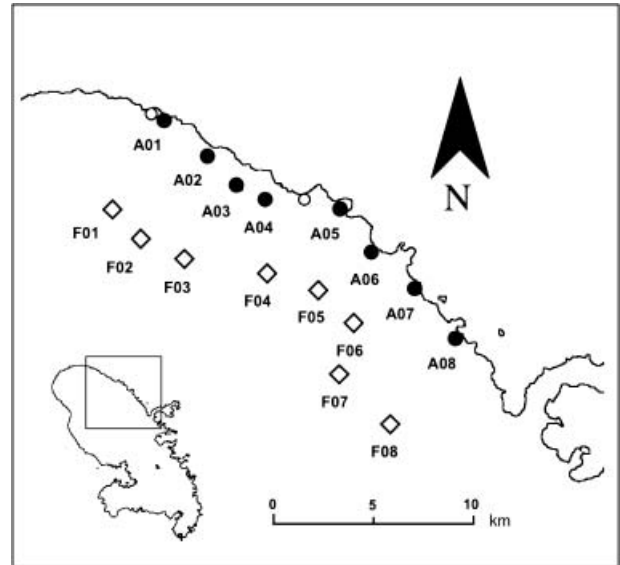
**Fig. 2** Marking patterns in *Anolis roquet* from northeastern Martinique. a) Northern coastal form (northwestern lineage) with black head, black cloak and light spots on a green background. b) Southern coastal form (central lineage). Black markings and light spots are entirely absent and lizards are brown. c)–d) Typical lizards from transitional forest habitat (both lineages); black markings and light spots are present to varying degrees, background colour is green.

contact zone. The estimation of the position of mtDNA and quantitative traits cline centres, and their respective widths, allows us to gain insight into the formation and maintenance of the secondary contact zones (Leache & Cole 2007). The influence of allopatry and natural selection on quantitative traits variation also is discussed.

## Methods

### Sampling

Eight localities along the coast and eight localities in transitional forest habitat were sampled along two linear parallel transects approximately 15 km in length (Fig. 3). The average distance between transect was approximately 6 km. Transects were designed to traverse the boundary between NW and C lineages of *Anolis roquet* as described



**Fig. 3** Map showing the position of the transects. The townships of Basse-Pointe and Lorrain are included for geographical reference, and are represented by unfilled circles. Inset shows the island of Martinique; study area is boxed.

by Thorpe & Stenson (2003). Forty-eight lizards from each locality were hand caught and sampled for DNA (autotomised tail tips). Tail tips were stored in tubes containing absolute ethanol for subsequent genetic analysis.

### Diagnostic polymerase chain reaction–restriction fragment length polymorphism assignment

We designed a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) assay to quickly and confidently assign individuals to their mitochondrial clade without the need to sequence them. To do this, we aligned all the cytochrome *b* sequences published by Thorpe & Stenson (2003) and examined them for fixed differences between clades that corresponded to restriction sites. The restriction enzyme *SspI* was found to cut the cytochrome *b* fragment at position 160 in the NW clade, but not in the C clade, and was subsequently used to distinguish the two clades. A total of 765 DNA samples were extracted from collected tail tips using QIAGEN DNeasy Blood & Tissue Kit (QIAGEN). A 1063-bp fragment of the cytochrome *b* gene was amplified using the primers MtA-S (5′-ATCTCAGCATGATGAAACTTCG-3′) and MtF-S (5′-TTTGGTTTACAAGACCAATG-3′) in 10- $\mu$ L reactions using 5 ng of template DNA, 3 mM MgCl<sub>2</sub>, 0.1 mM of each nucleotide, 0.4  $\mu$ M of each primer, 0.5 U of *Taq* DNA polymerase (Promega), and 10 $\times$  buffer (50 mM KCL; 10 mM Tris-HCl, pH 9.0). PCRs were performed using a profile of denaturation of 5 min at 95 °C, followed by 34 cycles (20 s for 95 °C, 30 s at 48 °C, and 45 s at 72 °C) with a

final extension period of 5 min at 72 °C. After amplification, a mixture of 0.2 µL *SspI* (New England Biolabs), 2 µL buffer 2 (NEB) and 7.8 µL H<sub>2</sub>O was added and the samples were digested for 3 h at 37 °C. The reaction was stopped by denaturation of the enzyme at 85 °C for 15 min. Products were visualised on 1.5% agarose gels stained with ethidium bromide under UV illumination, and scored from photographs.

#### *Quantitative trait variation*

A subset of 10 adult males from each locality were subject to quantitative trait analysis of the following 20 characters from four character sets: (i) body dimensions: snout to vent length (SVL) jaw length (JL), head length (HL), head depth (HD), head width (HW), upper hindleg length (ULL), lower hindleg length (LLL), dewlap length (DL); (ii) scale counts: postmental (PSC), supra-orbital semicircle (SSC), dorsal (DSC) and ventral (VSC); (iii) markings: number of light patches on head (LPH), number of light patches on anterior body (LPA), number of light patches on posterior body (LPB), percentage of black hood covering head (HEAD), percentage of black cloak covering anterior body (CLOAK). Before recording body measurements and marking patterns, lizards were photographed in standardised light conditions with a Canon EOS 350D fitted with a 100-mm Canon macro lens and a Macro Twin Lite MT-24EX flash. Body dimension measurements were taken in millimetres using electronic digital callipers (Linear Tools), accurate to two decimal points. Photographs were used to confirm scoring of marking patterns, to perform the ventral and dorsal scale counts and were also manipulated in Photoshop (Adobe Systems) to extract the fourth character set; a measure of hue based on the relative proportion of green, red and blue pixels within a standardised area on the dorsal trunk, just behind the front legs. Earlier spectrophotometric analysis of populations in this area (Thorpe & Stenson 2003) indicated that this was an acceptable procedure for dealing with the hue variation of this part of the body when using large samples.

Quantitative traits were analysed independently for each transect. Unless stated, variables were normally distributed. Body dimensions were adjusted against snout-vent length by analysis of covariance (ANCOVA). For each transect, five categorical markings data and two scale counts (SSC and PSC) that had distributions that violate the assumptions of canonical variate analysis (CVA) were entered into a principal components analysis (PCA). The seven components did not violate these assumptions and were inputted into subsequent CVAs as in Duntzman (1989), Zhao *et al.* (1998), Macedonia (2001), Weisrock *et al.* (2005) and Stein & Uy (2007). Hence, we inputted the seven principal components, adjusted body dimensions, two raw scale count variables (DSC and VSC) and two hue variables

(red and green) into a canonical variate analysis using *SPSS* version 14, to study the variation along the transects.

#### *Climate data*

The climate in the northeast Martinique is characterised by moist trade winds that come in from the Atlantic Ocean and hit the slopes of the Pitons du Carbet and Mount Pelée a few kilometres inland. Climate changes rapidly with increasing elevation towards the interior of the island with increased precipitation and lower temperatures in the mountains. Conditions also change with latitude towards the northern and southern extremities of the island. A large body of evidence (see above) indicates that Lesser Antillean anoles adapt by natural selection to this environmental zonation. To elucidate how environmental conditions, and hence selection regimes, varied along the transects, we carried out a PCA (normalised data) on altitude [Institut Géographique National, Carte de Randonnée 4502 MT and 4503 MT (French national 1:25 000 resolution maps)], habitat type (Lassere 1979), and three climatic variables, (annual mean temperature, annual precipitation and precipitation seasonality data) from [www.worldclim.com](http://www.worldclim.com). Habitat types were given a nominal code for the analysis. For both transects, the first and second principal components were correlated to the spatial position of the localities along the transect.

#### *Cline fitting*

We fitted tanH clines to mtDNA frequency and quantitative trait data using the Fit 1D cline in the program Analyse 1.3 (Barton & Baird 1999: [www.biology.ed.ac.uk/research/institutes/evolution/software/Mac/Analyse/Version1.3.html](http://www.biology.ed.ac.uk/research/institutes/evolution/software/Mac/Analyse/Version1.3.html)). The program fits tanH curves to cline data using four variables: cline width, cline centre, and  $P_{\min}-P_{\max}$  (where  $P_{\min}$  and  $P_{\max}$  denote minimum and maximum gene frequencies in the tail end of a cline), using a Metropolis algorithm. Normally distributed phenotypic data can be fitted to clines either as single characters or from composite variables (e.g. PCA or CVA scores) where  $P_{\min}$  and  $P_{\max}$  are the maximum and minimum character scores in the tails of the cline (Bridle *et al.* 2001; Brumfield *et al.* 2001; Dasmahapatra *et al.* 2002; Takami & Suzuki 2005; Leache & Cole 2007).

Model parameters for mtDNA and quantitative trait clines were estimated independently for both transects, allowing centre and width to vary, fixing  $P_{\min}$  and  $P_{\max}$  at 0 and 1, respectively, over 2000 iterations along a best-fit axis. CV 1 locality means from the quantitative trait CVA were transformed to a 0–1 scale before cline fitting, and mtDNA data were represented as 0–1 haplotype frequencies at each locality (Takami & Suzuki 2005). Support values were generated using the support values option in Analyse 1.3,

where parameter values within two likelihood units were generated, equivalent to 95% confidence limits. Significant coincidence and concordance between mtDNA and quantitative traits was attained if centre and width values from one cline could be found within the support limits of the other cline, corresponding to 95% confidence limits (Takami & Suzuki 2005).

## Results

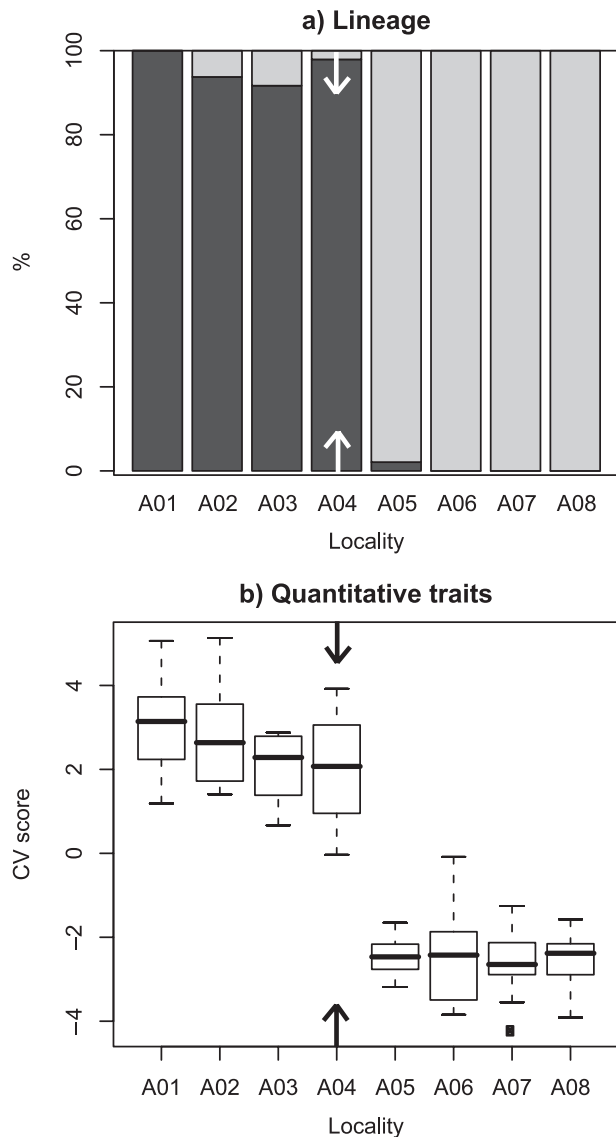
Group means and variances for each trait and locality can be found in Tables S1 and S2, Supporting information; correlations between characters for each transect can be found in Tables S3 and S4, Supporting information.

### Coastal transect

On the coastal transect, the haplotype frequency change shown by the mtDNA PCR-RFLPs was a sharp step; at each extreme locality there was 100% of NW or C haplotype, a further three localities contained only C haplotypes, and in the remaining four localities more than 90% of individuals were of the common haplotype (Fig. 4a). The changeover in quantitative traits mirrored that of the mtDNA, and the total change between extreme localities amounted to 5.5 pooled within-groups standard deviations (Fig. 4b). There was no overlap of CV scores between the two sets of localities (A01–A04, and A05–A08), and there was a strong and significant correlation between quantitative trait locality means and lineage ( $r = 0.99$ ,  $P < 0.01$ ,  $N = 8$ ). Lizards from either lineage were thereby clearly separated by their appearance: lizards belonging to the northwestern lineage were green with black markings and light-patches, this contrasted with lizards belonging to the central lineage which were brown, without black markings or light-patches, and with relatively wider heads (Tables S5 and S6, Supporting information). The bioclimatic PC2 indicated a significant linear change in likely selection regime along transect A ( $r = 0.92$ ,  $P < 0.001$ ,  $N = 8$ ) (Table S7, Supporting information), where the south is less seasonal and habitat is dominated by xerophilous vegetation, although PC1 did not change along the transect ( $r = 0.17$ ,  $P > 0.68$ ,  $N = 8$ ). There was also a strong significant correlation between QT pattern and bioclimatic PC2 variation ( $r = -0.84$ ,  $P < 0.01$ ,  $N = 8$ ). Furthermore, both mtDNA and QT clines were narrow with sharp transitions between locality A04 and A05, coinciding with a geological boundary (Fig. 6a). Width and centres coincided and concurred (Table 1).

### Transitional forest transect

In the transitional forest transect, mtDNA haplotype frequency change was gradual, with pure NW and C



**Fig. 4** Lineage distribution and quantitative trait pattern along the coastal transect. (a) Lineage distribution. Dark grey represents northwestern lineage, and pale grey represent central lineage. (b) CV scores for quantitative traits. Haplotype and quantitative trait pattern are similar with sharp, stepped transitions at the geographical boundary (boundary is indicated by arrows).

lineage at the extreme localities (Fig. 5a). The changeover occurred over four localities in the northern part of the transect, and the three southernmost localities were pure C lineage. The quantitative traits showed some variation across the transect; there was a minor gradient between the first and last four localities with 2.2 pooled within-group standard deviations of change between extreme localities (Fig. 5b). In contrast to the coastal transect, QT scores overlapped extensively so no distinct sets could be recognised. However, there was a significant correlation between QT and lineage ( $r = 0.79$ ,  $P < 0.02$ ,  $N = 8$ ). Here,

	Width (m)	Centre position (m)	Log likelihood
Coastal transect			
Mitochondrial DNA	3016 (2549–3587)	6715 (6344–7155)	-16.304
QT	2990 (2057–4044)	6450 (6193–6767)	-0.37
Transitional forest transect			
Mitochondrial DNA	4000 (3171–4893)	5656 (5248–6052)	-2.779
QT	13122 (8868–20518)	9834 (8444–11210)	-12.627

**Table 1** Cline widths and centre positions (metres from transect starts) are given for both transects, with best log likelihood for the estimations. Support limits are in parenthesis

extreme northern localities were populated with bright green lizards with black markings, while greenness and markings became less prominent further south and head depths increased (Table S8 and S9, Supporting information). Neither bioclimatic PC1 or PC2 (Table S10, Supporting information) changed significantly along transect F (PC1  $r = -0.43$ ,  $P = 0.29$ ,  $N = 8$ ; PC2  $r = 0.63$ ,  $P = 0.09$ ,  $N = 8$ ) and we found no significant correlation between QT pattern and bioclimate (PC1  $r = -0.33$ ,  $P = 0.43$ ,  $N = 8$ ; PC2  $r = 0.63$ ,  $P = 0.10$ ,  $N = 8$ ).

Quantitative trait and mtDNA clines had very different centre and widths (Table 1). The QT cline was nearly twice as wide as the mtDNA cline (Fig. 6b). The centre of the mtDNA cline was located between locality F02 and F03, and therefore coincided with the geological boundary, whereas the centre of the QT cline was displaced south.

## Discussion

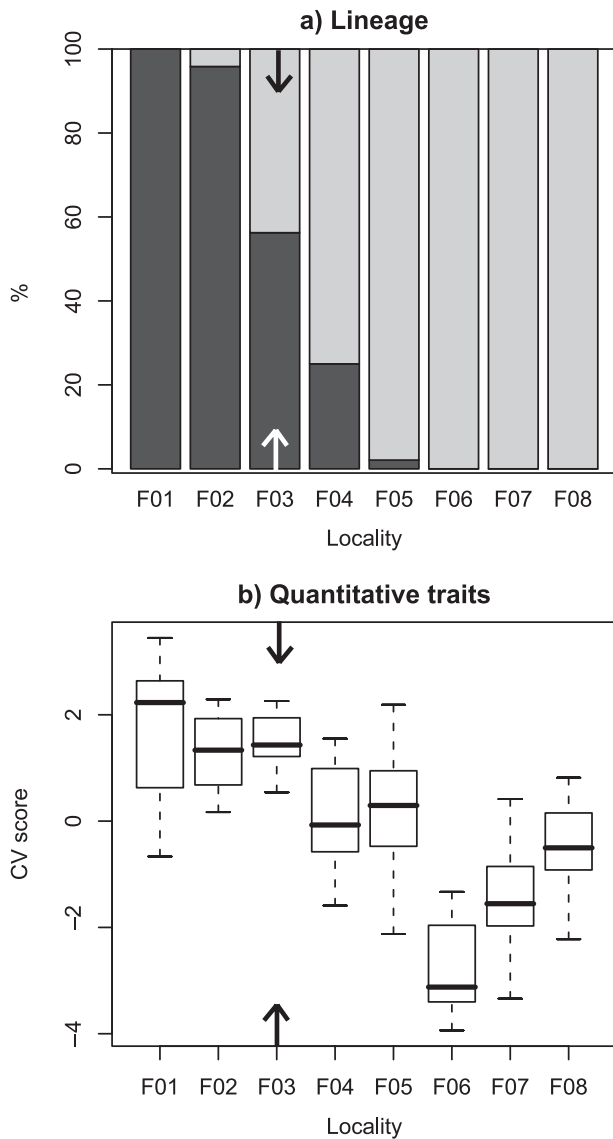
Geological events corresponding with phylogenetic data show evidence of secondary contact of distinct *Anolis roquet* mtDNA lineages on Martinique (Thorpe & Stenson 2003; Thorpe *et al.* 2008). Here we confirmed this relationship on a finer scale and with larger sample numbers; mtDNA transition examined in both transects (identified by the mtDNA cline centre) occurred at the geological boundary where the substrate composition changes from calc-alkali to andesite volcanic rock (Andreieff *et al.* 1976; Maury *et al.* 1990; Sigurdsson & Carey 1991). Broadly speaking, the northwestern precursor island is likely to have joined the central precursor island relatively recently (1–1.5 million years ago), after their respective anole populations had spent an extended period (6–8 million years ago) in allopatry (Thorpe *et al.* 2008). However, more recent volcanic activity of Mount Pelée (500 000–5000 BP) (Traineau *et al.* 1989) may have caused episodes of local extinction and recolonisation. As the transect in the transitional forest was between Mount Pelée and the coastal transect, it is most unlikely that this volcanism could have impacted the coastal region without simultaneously impacting the adjacent transitional forest region, so the timing of secondary contact should be the same in both transects.

Nevertheless, there were notable differences between the two transects despite their proximity and similar history. The wide, flat shape of the QT cline in the transitional forest transect was characteristic of a neutral cline (Barton & Hewitt 1985), suggesting that barriers to gene flow were either absent or very weak in this area. In contrast, the QT cline along the coastal transect showed steepness typical of a cline that was either recently formed, or is maintained by selection (Barton & Hewitt 1985). We can estimate the dispersal rate from the QT clines using the equation  $T = 0.35(w/L)^2$  where  $T$  is the number of generations necessary to create a cline  $w$  metres wide with a rate of  $L$  metres of gene flow per generation (Endler 1977). Assuming identical timing of secondary contact in the two transects (see above), the dispersal distance was 4.5 times larger in the transitional forest transect than in the coastal transect. This result strongly suggests restriction of gene flow in the coastal transect.

Endogenous or exogenous selection, or a combination of both, can maintain clines in secondary contact zones long after initial contact (Dasmahapatra *et al.* 2002; Phillips *et al.* 2004). Environmental selection has been shown to be important in anoles and can restrict gene flow (Ogden & Thorpe 2002). Habitat variation (in the shape of a north–south gradient) and QT pattern were correlated on the coastal transect. But compared to the very steep ecotonal transitions in bioclimate and habitat between coast and mountain that drive ecological selection on QTs in *A. roquet* (Ogden & Thorpe 2002; Thorpe *et al.* 2008), the difference in bioclimate and habitat between extreme ends of the coastal transect was relatively small, and the change along the transect was a gentle, linear gradient. Hence, exogenous selection may be a contributing factor, but on its own, it is unlikely to explain the sharp QT transition observed here.

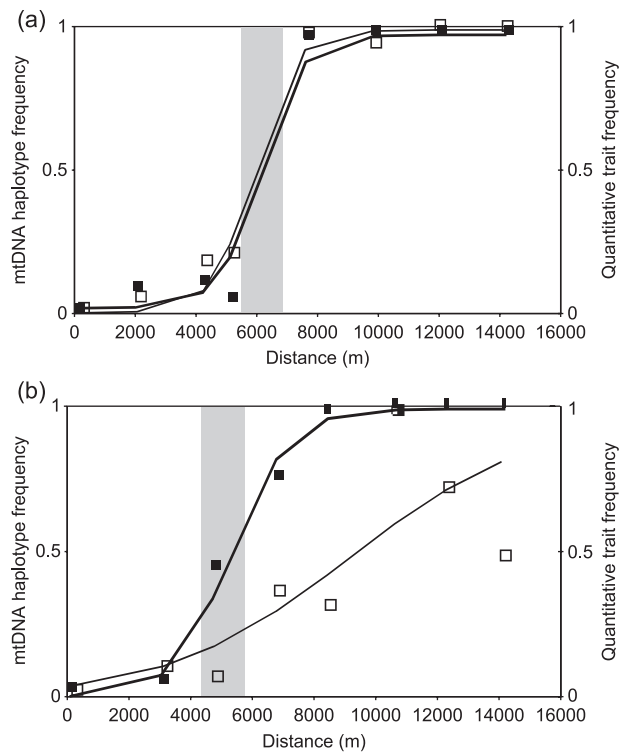
Endogenous selection in secondary contact zones is evidenced by coincidence and concurrence of multiple character clines independent of environmental variation (Brumfield *et al.* 2001; Phillips *et al.* 2004), and occasionally by phenotypic anomalies (Brumfield *et al.* 2001). We found a strong correlation between lineage and QT patterns on the coast, and this finding was also corroborated by the coincidence and concurrence of mtDNA and QT cline





**Fig. 5** Lineage distribution and quantitative trait pattern along the transitional forest transect. (a) Lineage distribution. Dark grey represents northwestern lineage, and pale grey represent central lineage. (b) CV scores for quantitative traits. Lineage and quantitative trait variation do not show similar patterns; lineage transition is gradual but coincides with geological boundary (indicated by arrows). Quantitative trait pattern show variation that does not appear to coincide with lineage transition or geological boundary.

centres and widths. Furthermore, at the point where the lineages meet on the coast (locality A05) unusual pigmentation anomalies occur on the head and neck: blotches of white, or skin lacking scales and pigmentation (personal observation). Anomalies of this type were previously described for this region (and this region only) by Lazell (1972); in secondary contact, genetic differences that evolve in allopatry can lead to genic incompatibilities, where



**Fig. 6** Mitochondrial DNA and quantitative traits clines. Closed squares and thick line represent mtDNA. Open squares and thin line represent quantitative traits. Geological boundary (see discussion) indicated with grey shading. Distance was measured from locality A01 on the coastal transect, and F01 on the transitional forest transect, and projected distance between localities was used. Mitochondrial DNA and quantitative traits clines concur and coincide on the coast (a) but not on the transitional forest transect (b).

alleles tend to function better in the population they are sourced from, but can nevertheless be functional in a different genetic background (Coyne & Orr 2004). This process can occur with or without natural selection; however, when driven by natural selection, the process is likely to occur more rapidly (Coyne & Orr 2004).

On this relatively fine spatial scale, the QTs on the coast were bimodal which could suggest assortative mating (Jiggins & Mallet 2000). Although it may occur in lizards (Bleay & Sinervo 2007), it is not readily demonstrated and has yet to be unambiguously shown to occur in anoles (Tokarz 1995). Nevertheless, several experimental studies of male dewlap colouration and display behaviour (dewlapping and head bobbing) suggest the possibility of female choice (Crews 1975; Sigmund 1983; Fleishman 1992; Tokarz 1995). Moreover, adaptation to habitat may include changes in dewlap hue to increase visibility/detectability in specific light conditions, which may influence anole interactions (Leal & Fleishman 2002; Leal & Fleishman 2004). Hence, assortative mating as a contributor to the QT pattern merits further investigation.



Results for the coastal transect thus conformed to the expectations of the allopatric speciation model, where differences built up in allopatry are maintained after secondary contact. Both endogenous and exogenous selection may contribute to maintaining the QT cline, and our results are consistent with a strong barrier to gene flow. In contrast, we did not find evidence consistent with barriers to gene flow on the transitional forest transect. Patterns of lineage and QT variation were weakly correlated, but this was not supported by a more detailed analysis of their clines which had different centres and widths. Hence, the transitional forest transect, like the montane rainforest transects (Ogden & Thorpe 2002; Thorpe *et al.* 2008) suggest that the populations do not behave consistently with an allopatric model of speciation. There is overwhelming evidence of the importance of natural selection in shaping population divergence in Lesser Antillean anoles (see references above) and the extent of convergent vs. divergent selection along these two transects may contribute to explain the difference between them. Strong convergent selection in the transitional forest habitat may have eradicated the effects of allopatric divergence, as occurs where these two forms meet in the mountains (Ogden & Thorpe 2002; Thorpe *et al.* 2008). Conversely, the absence of strong convergent selection, and the presence of some divergent selection either side of the secondary contact on the coast, may allow the perpetuation of differences built up in allopatry.

### Acknowledgements

This work was funded by BBSRC grant BB/C500544/1 to R.S.T., EC award MEIF-CT-2005-009981 to Y.S.G. and R.S.T., and NERC studentship NER/S/A/2004/12449 to H.J. (supervised by R.S.T.). We wish to thank the DIREN (France) for permissions to carry out this work on Martinique. Jennifer L. Gow and Owen F. Rowe assisted with collection in the field and, the manuscript greatly benefited by comments from Alison Davis, Owen F. Rowe, Alexander D. Rowe and three anonymous reviewers.

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This paper is part of a long-term study on divergence and speciation in the *Anolis roquet* complex, performed by researchers from the Molecular Ecology and Evolution of Reptile Unit at Bangor University, U.K. The paper forms part of Helena Johansson's research for a PhD at this institution. Yann Surget-Groba is interested in the molecular ecology and evolution of lizards, with particular reference to the evolution of viviparity. Roger S. Thorpe is interested in natural selection and adaptation, population genetics, molecular phylogeography and speciation of island lizards.

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### Supporting Information

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

**Table S1** Coastal transect locality population means ( $\bar{X}$ ) and variances ( $\sigma^2$ ) for all quantitative trait characters. Adjusted values for body dimensions are given. See text for key to abbreviations

**Table S2** Transitional transect locality population means ( $\bar{X}$ ) and variances ( $\sigma^2$ ) for all quantitative traits characters. Adjusted values for body dimensions are given. See text for key to abbreviations

**Table S3** Pearson correlations for each character set of quantitative traits for the coastal transect. Upper triangular half shows scale count and marking pattern correlations, lower triangular half shows raw body dimension correlations. See text for key to abbreviations. \*Significant at the 0.05 level. \*\*Significant at the 0.01 level (two-tailed test)

**Table S4** Pearson correlations for each character set of quantitative traits for the transitional forest transect. Upper triangular half shows scale count and marking pattern correlations, lower triangular half shows raw body dimension correlations. See text for key to abbreviations. \*Significant at the 0.05 level. \*\*Significant at the 0.01 level (two-tailed test)

**Table S5** Principal component matrix for markings and scale count data on the coastal transect

**Table S6** The first (un-standardised) canonical function coefficient for the coastal transect

**Table S7** Principal component matrix for bioclimatic data on the coastal transect

**Table S8** Principal component matrix for markings and scale count data on the transitional forest transect

**Table S9** The first (un-standardised) canonical function coefficient for the transitional forest transect

**Table S10** Principal component matrix for bioclimatic data on the transitional forest transect

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