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Quantitative traits and mode of speciation in Martinique anoles

ROGER S. THORPE,* YANN SURGET-GROBA*† and HELENA JOHANSSON*‡
School of Biological Sciences, Bangor University, Bangor, LL57 2UW, UK,

Abstract

We investigate extensive quantitative trait variation (dewlap hue, colour pattern, dorsum hue, body proportions and scalation) in the Martinique anole across eight transects representing nascent parapatric ecological speciation, nascent allopatric speciation and allopatric divergence without sufficient genetic structure to suggest speciation. Quantitative trait divergence can be extremely large between adjacent sets of populations, but with one exception that this is associated with difference in habitat rather than past allopatry. Nascent ecological speciation shows the greatest level of quantitative trait divergence across all character sets including those implicated in natural, as well as sexual selection. The sole example of nascent allopatric speciation is associated with fairly strong quantitative trait divergence among most character sets, but not the set most implicated in natural (rather than sexual) selection. The role of sexual selection in ecological speciation is discussed, both in terms of female choice with assortative mating and male–male competition with condition-dependant sexual signals.

Keywords: allopatric speciation, natural selection, parapatric ecological speciation, quantitative traits, sexual selection

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Introduction

The interest in speciation, as a focal issue in evolution, continues unabated (Rundle & Nosil 2005; Nosil *et al.* 2009; van Doorn *et al.* 2009; Schluter 2009; Pinho & Hey 2010; Rieseberg & Blackman 2010; Sobel *et al.* 2010; Thorpe *et al.* 2010; Maan & Seehausen 2011; Nosil & Schluter 2011; Elias *et al.* 2012; Nosil 2012; Nosil & Feder 2012; Ostevik *et al.* 2012). However, there has been relatively little work on how divergence in sets of quantitative traits may differ among modes of speciation. We investigate this question using small, insectivorous tree lizards of the genus *Anolis*. *Anolis* lizards are among the most speciose land vertebrates (Losos 2009). They are frequently used in evolutionary and ecological studies (Losos 2009), have many quantitative traits and are particularly suitable for studies of speciation and population evolution.

Martinique, in the Lesser Antilles, is currently a single mountainous island, but in the geological past, it was several separate precursor islands. These have only recently coalesced into a single island (Thorpe & Stenson 2003; Thorpe *et al.* 2008, 2010). The solitary Martinique anole, *Anolis roquet* complex, appears to have lived on these precursors for a considerable time (up to 8 mybp) prior to their coalescence and has phylogeographic lineages that closely match the geological time and distribution of these precursors (Thorpe *et al.* 2010). Moreover, the Martinique anole, similar to other Lesser Antillean anoles, shows marked quantitative trait divergence in relation to habitat differences (coastal xeric vs. montane rainforest) that are largely independent of the phylogeographic lineages (Malhotra & Thorpe 1991, 2000; Thorpe 2002a; Thorpe & Stenson 2003; Thorpe

Correspondence: Roger S. Thorpe, Fax: +44 (0) 1248 370731; E-mail: r.s.thorpe@bangor.ac.uk
† Current address: Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan Province, 666303, China
‡ Current address: Department of Biosciences, University of Helsinki, PO Box 65, Helsinki, FI-00014, Finland

et al. 2004). This species complex therefore provides an opportunity to investigate both divergence among parapatric habitats because of current selection regimes and divergence in allopatry.

A recent study (Thorpe *et al.* 2010) investigated models of speciation in this system by assessing the genetic isolation between populations of this complex from different habitats (xeric vs. montane rainforest) and the four precursor islands with ancient lineages (northwest, central, south-west and south) (Fig. 1). A series of intensively sampled, replicated, transects were taken across ecotones, to test for ecological speciation, and across precursor island lineage secondary contact zones to test for conventional interisland geographic speciation (Fig. 1). Geological information and mtDNA were used to estimate phylogenetic lineage and historical factors (Thorpe & Stenson 2003; Thorpe *et al.* 2008, 2010), hypervariable microsatellite markers were used to assess population structure and genetic isolation, and multivariate analysis of numerous climate variables and quantitative traits gave additional information. This study showed that the four lineages from precursor islands were not genetically isolated separate species as predicted by the allopatric speciation model, but generally showed evidence of strong nDNA introgression. In contrast, there was greater genetic isolation between adjacent populations from distinct habitats (xeric vs. rainforest) than between putative allospecies of 6–8 my divergence (Thorpe *et al.* 2010), suggesting nascent parapatric ecological speciation. The one exception to this trend is transect I (Fig. 1), which provides a single example of nascent allopatric speciation for comparison. Hence, partial genetic isolation conformed to a model of nascent parapatric ecological speciation across the ecotone of transects III and IV, while it conformed to a model of nascent allopatric speciation model only at the lineage contact zone of transect I (Fig. 2).

In this ecosystem, the pronounced habitat differences, and hence different selection regimes, occur within mountainous islands between parapatric populations from adjacent habitats, such as xeric western coastal habitats and montane rainforest (Thorpe *et al.* 2005), rather than among allopatric populations on different islands. Field experiments on Lesser Antillean anoles have shown strong directional selection and significant selection intensity on anoles from adjacent habitats within islands (Thorpe *et al.* 2005). Speciation involving divergent adaptation to these habitats, without any extrinsic barriers to gene flow, can be regarded as ecological speciation. Ecological speciation is not mutually

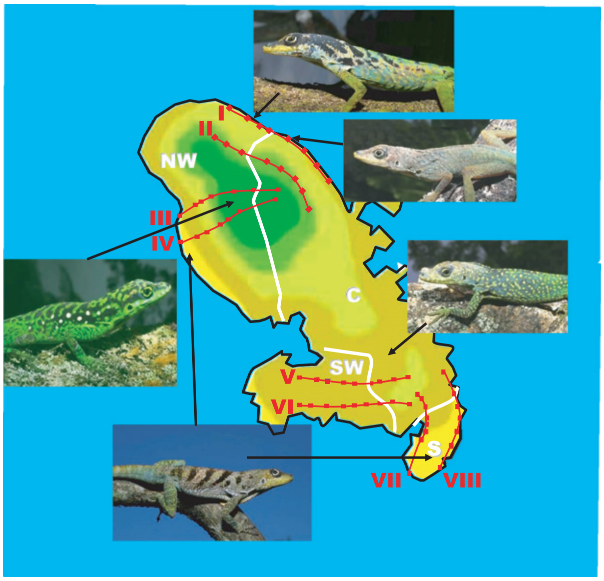

Fig. 1 Martinique transects (I–VIII red lines). The sites (red squares) are numbered (1–7/9) in sequence from the position of the transect label. The approximate precipitation (yellow low, dark green high) and lineages (not geology) (north-west, south-west, south and central in white) are given. The images (clockwise from the top) indicate the north-east coastal type, the eastern littoral ecotype, the widespread mesic/transitional type, the xeric ecotype and the montane ecotype. Apart from transect I, these are distributed according to habitat irrespective of lineage. Examples of dewlap reflectance spectra from these anoles can be seen in Thorpe *et al.* (2008).

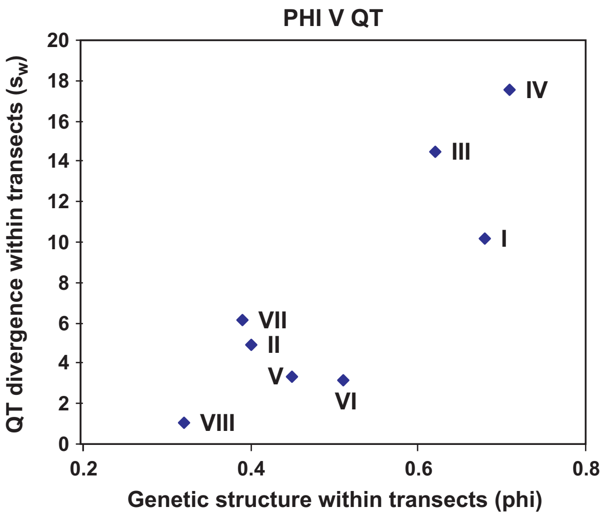

Fig. 2 Scatter diagram of QT divergence of lineages (I–II, VI–VIII) and ecotypes (III–VI) against the extent of their (lineages/ecotypes) genetic isolation. The horizontal axis represents phi as a measure of goodness of fit to the allopatric speciation model (I–II, VI–VIII) or parapatric ecological speciation model (III–IV) using microsatellite variation to estimate genetic isolation (0, no isolation; 1, complete isolation) as in Thorpe *et al.* (2010). The vertical axis represents the extent of divergence in quantitative traits between lineages or ecotypes using contrast ANOVA and measured in units of within-site standard deviations.

exclusive to allopatric speciation (Rundle & Nosil 2005), as allopatric populations may be subject to divergent selection regimes. While there may inevitably be small-scale indirect effects of selection among allopatric island populations, there is no evidence of strong selection regime differences among similar habitats on different islands in this system that are comparable to the strong selection differences among different habitats on the same island. However, there is an effective extrinsic barrier to gene flow between allopatric populations on different islands, allowing genetic drift, as well as mutation and indirect effects of natural selection (Hoskin *et al.* 2005). While recognizing that ecological and allopatric speciations are not mutually exclusive, in this study, we abbreviate nascent (incomplete) parapatric ecological speciation to 'ecological speciation' and nascent allopatric speciation to 'allopatric speciation'.

Thorpe *et al.*'s (2010) study of the Martinique anole employed a wide range of quantitative traits. They showed that in transects with substantial climatic variation, where the model of ecological speciation predicted genetic isolation between habitat types, there was extreme quantitative trait divergence between population from different habitats, and the strongest association between quantitative trait and climate variation. That is, nascent ecospecies show extreme differences in their quantitative traits. Moreover, in the single transect I supporting allopatric speciation, there was also quite marked quantitative trait divergence between nascent allospecies. Nevertheless, Thorpe *et al.* (2010) treated all quantitative traits together. Moreover, they did not investigate directly the relationship between genetic isolation and quantitative traits.

Here we treat sets of quantitative traits separately and relate quantitative trait divergence to genetic isolation (nDNA population structure). We attempt to gain an empirical insight into the extent and nature of quantitative trait variation in relation to allopatric and ecological speciation using five sets of quantitative traits, dewlap hue, colour pattern, dorsum hue, body proportions and scalation. These sets of traits and individual traits are independently compared with the extent of genetic structure, lineages and climate. We consider various explanations for the role of sexual and natural selection in ecological speciation, including female choice and assortative mating and male–male competition and condition-dependant sexual signals (van Doorn *et al.* 2009).

Materials and methods

The lineages represent the allopatric populations from the precursor islands. To compare between lineages for the allopatric speciation model, transects were taken across the north-west–central lineage contact (I coastal, II transitional/montane forest), the south-west–central lineage contact (V, VI) and the south–central lineage contact (VII, VIII). Also, the sites within the NW lineage along transects III and IV were used to compare xeric and montane ecotypes (parapatric ecological speciation model) without the influence of lineage (Fig. 1). The number of sites per transect (I–VIII) was 8, 8, 7, 7, 9, 9, 8 and 7, respectively, but the seventh site was subsequently excluded for the final habitat comparisons in transects III–IV so that these comparisons were within a single lineage. Sample size per site was 48 individuals for genetic analyses and 10 adult males for quantitative trait analyses. We used the genetic structure along each transect from Thorpe *et al.* (2010), which was obtained from the variation in nine neutral nuclear microsatellite loci subjected to Bayesian clustering executed in Structure 2.1 (Pritchard *et al.* 2000). Thorpe *et al.*'s (2010) estimate of cluster frequency at each site (Fig. 3) gave a pattern of variation along a transect that may represent a good fit (phi) to ecological speciation (III, IV, goodness of fit 0.62, 0.72, respectively), a good fit to allopatric speciation (I, goodness of fit 0.68) or a weaker fit to allopatric differentiation (II, V–VIII, goodness of fit 0.40, 0,45,0.51,0.39 and 0.32, respectively). For each site of each transect, 48 individuals were assigned to a lineage by PCR–RFLP (Thorpe *et al.* 2010).

An estimate of the environment at each site was obtained from a principal component analysis (PCA) of $\log_e$-transformed 19 climatic variables from Worldclim (http://www.worldclim.org/) across all sites from all transects. PC1 was visualized on a 0–1 scale.

Independent (Table S1, Supporting information) quantitative traits (30) were analysed from scalation (2), body proportions (7), dorsum hue (3), dewlap hue (12) and colour pattern (6). Scalation traits were the number of ventral and dorsal scales along the trunk. Hue of the anterior and posterior dewlap was recorded using reflectance spectrometry, and the spectrum of each was divided into six independent hues (from 330 to 710 nm) following a multiple-group eigenvector procedure (Thorpe 2002a; Thorpe *et al.* 2008), giving 12 traits. Anoles have an all-cone retina with four types of cone, including one in the UV range. This spectrum covers the range of the anoles visual system. Body dimensions were size-adjusted for (ANCOVA against snout–vent length) jaw, head, upper leg, lower leg and dewlap length, head depth and head width. Dorsum hue was recorded as the percentage of red, green and blue on the posterior trunk assessed from standardized images using Adobe Photoshop. Sexually mature male colour pattern traits are dorsal chevron number, chevron intensity, occipital 'A' mark intensity, extent of black dorsal reticulation, white spot size and extent of black cloak as in Thorpe *et al.* (2008).

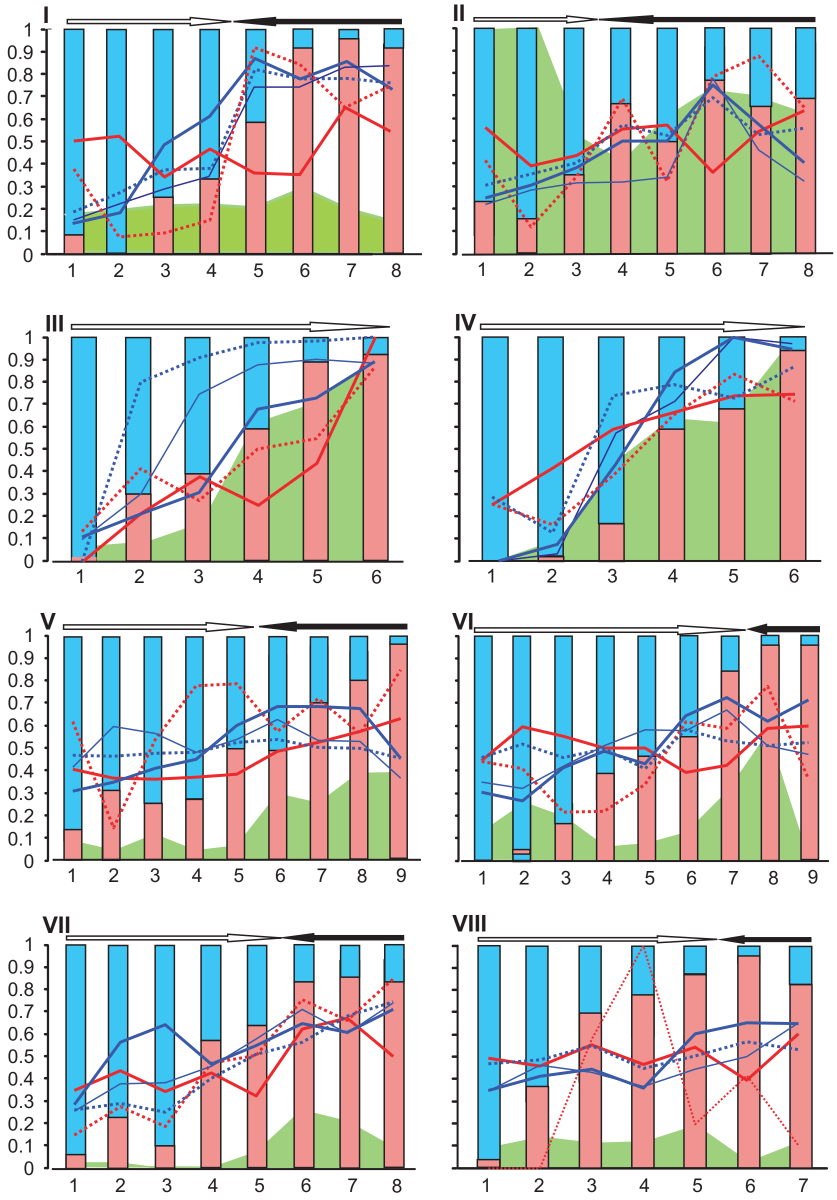

Fig. 3 Genetic structure in relation to variation in quantitative traits. The proportion of two Bayesian clusters (coded pink and blue) showing neutral nuclear genetic structure is plotted along sites ($1-n$) of transects I–VIII. On the same 0–1 scale, the principal component representing climate variation (low xeric, high montane rainforest) is given as a green background. The lineage is indicated by an arrow, unfilled for the peripheral lineages (north-west lineage transects I–IV, south-west lineage transects V–VI, southern lineage transects VII–VIII), with black for the central lineage (with some SW haplotypes in trans VII–VIII). The site mean for the multivariate variation in the quantitative traits is also scaled to unity against the transect with the greatest magnitude for comparative purposes. The magnitude therefore varies among character sets with unity representing 4.10, 5.52, 6.43, 8.9 and 9.9 within-group (site) standard deviations for body proportions (broken red line), scalation (solid red line), dorsum hue (thin blue line), colour pattern (broken blue line) and dewlap hue (thick blue line), respectively.

As well as analysing individual characters, character sets were analysed by multivariate analysis. Discriminant function analysis (DFA) was used for body proportions, scalation, dorsum hue and dewlap hue, while PCA was employed on the normalized colour pattern characters. For comparison, the colour pattern principal component for each transect was scaled so that the pooled within-site standard deviation was unity.

Contrast ANOVA was used to compare sets of sites within a transect for both individual characters and DF1 and PC1 for the multivariate character sets, where sites are the statistical groups, and the mean divergence between contrast sets is expressed in terms of within-site standard deviations (s_w). These contrast sets were based on either lineage (transects I–II, V–VIII) or ecotype sites 1–2 vs. 4–6 on either side of the ecotone (site 3) for transects III–IV. Significance was estimated by sequential Bonferroni correction by column. When a character showed a significant contrast between lineage sets, the effect of climate (PC1 on Worldclim data) was regressed out and the character re-tested.

The extent of quantitative state divergence was compared with the extent of genetic structure at each transect. Goodness of fit of the genetic structure to the allopatric speciation (transects I–II, V–VIII) or ecological speciation model (III–IV) was estimated using phi from Thorpe *et al.* (2010). For the quantitative state divergence, we estimated the overall divergence as the mean difference between contrast sets using contrast ANOVA (as with the individual characters) with the contrast along DF1 (run on body proportions, scalation, dorsum hue and dewlap hue simultaneously) plus the contrast value for PC1 for the colour pattern (both of which are in units within-site standard deviations).

For each transect, we calculated the correlation between site mean PC or DF scores for a set of quantitative traits and population structure (site cluster frequency). The association (correlation) between genetic structure (site frequency of nDNA cluster) and site mean multivariate score for a given character set was estimated across transects (I–VIII) shown in Table 3. The correlation between mtDNA lineage frequency and nDNA cluster frequency (genetic structure) was also computed for each transect.

Results

Extent of quantitative trait divergence

There can be very considerable differentiation in the quantitative traits between spatially adjacent sets of sites (lineages or habitats). The mean differentiation between adjacent sets across the total character set may be up to 17.6 within-site standard deviations (s_w) (Fig. 2, Table 1), up to 8.8 s_w between-character sets (e.g. dewlap hue) (Table 1) and up to 6.2 s_w for individual characters (Table 2). Maximum differentiation between any pair of sites along a transect is of course greater (22.2 and 9.9 s_w, respectively, for total and specific character sets). Overall, the extent of quantitative trait divergence is related to the extent of genetic differentiation between spatially adjacent sets of sites defined by either lineage or habitat type (Fig. 2). The transects tend to fall into two groups, those that have nascent speciation and high QT divergence (trans I, III, IV) and those that have a lower fit to a model of speciation (less genetic structure among sets) and little QT divergence (trans II, V–VIII) (Fig. 2). Overall, ecological speciation (trans III, IV) shows greater QT divergence than allopatric speciation (trans I), irrespective of genetic differentiation (Figs 2 and 3).

Table 1 Mean difference between ANOVA contrast sets of multivariate character sets in units of pooled within-site standard deviation. Body proportions (BP), scalation (SC), dorsum hue (DORH), colour pattern (CP) and dewlap hue (DEWH). Nascent allopatric speciation (AS), allopatric differentiation with levels of genetic structure below partial speciation (AD) and nascent parapatric ecological speciation (ES). Transects (Trans) I–VIII from Fig. 1. Mean allopatric divergence (Mean allo) taken across transects I–II, V–VIII)

Trans	Model	BP	SC	DORH	CP	DEWH	Total
I	AS	2.5	0.1	3.4	4.1	4.6	10.2
II	AD	1.5	0.4	1.1	2.0	2.0	4.9
III	ES	1.5	2.5	4.4	5.2	6.0	14.5
IV	ES	2.1	2.1	5.7	5.2	8.8	17.6
V	AD	0.5	1.0	0.0	0.1	2.0	2.9
VI	AD	0.7	0.6	0.0	0.3	2.0	2.5
VII	AD	1.8	1.2	1.8	2.8	1.5	6.1
VIII	AD	0.4	0.0	1.1	0.1	2.2	0.6
Mean allo		1.2	0.6	1.2	1.6	2.4	4.5
Mean ES		1.8	2.3	5.0	5.2	7.4	16.1

As the extent of divergence in a multivariate character set (Table 1) is going to be, in part, a function of the number of characters, the extent of divergence in individual characters (Table 2) can also be informative. It is evident that some character sets show more differentiation than others. Generally, some dewlap hue characters, colour pattern characters and transects dorsal hue show the greatest differentiation, while body proportions and scalation generally show the least (Tables 1 and 2).

Divergence associated with ecological speciation

For transects III–IV, there is a strong climatic variation along each transect and strong nDNA genetic structure (Figs 2 and 3). The neutral genetic structure is associated with this habitat differentiation rather than with the mtDNA lineages (Thorpe *et al.* 2010). The variation in every QT character set varies along the transect in concert with the genetic structure and climate; this is particularly notable for transect IV. The scaling of the ranges of character set divergence allows comparison between transects in Fig. 3. As well as showing the highest total QT variation, every character set shows its highest, or high, divergence (range) for these transects (Fig. 3). Mean differences between habitat types from the contrast ANOVAS are higher than the average differences between lineages for every character set (Table 1), and this is reflected in the mean differences in some individual characters (Table 2). Correlations between genetic structure and all five character sets are consistently high for the transects representing ecological speciation (Table 3).

Table 2 Significant mean difference between ANOVA contrast sets of individual characters in units of pooled within-site standard deviation. Significance at $P < 0.05$ after sequential Bonferroni correction by column. An asterisk indicated significance after climate is regressed out (trans I–II, V–VIII)

Character	I	II	III	IV	V	VI	VII	VIII
JL	—	—	—	1.1	—	—	—	—
HL	—	—	—	—	—	—	—	—
HD	1.4	1.2	1.2	—	—	—	1.1*	—
HW	1.4	1.2	1.0	1.0	—	—	—	—
UL	—	—	—	—	—	—	—	—
LL	—	—	—	—	—	—	—	—
DL	1.7	—	—	—	—	—	1.2*	—
VS	—	—	0.9	2.1	0	—	1.2*	
DS	—	—	2.3	1.4	1.0*	—		—
R	1.0	—	—	1.3	—	—	1.7*	—
G	2.4	1.1	3.9	4.1	—	—	0.8*	1.1
B	—	0.8	3.6	5.2	—	—	1.1*	0.7*
DCC	—	—	4.9	5.0	—	—	2.4	—
AM	—	—	2.3	1.0	—	—	1.9*	—
CI	—	—	4.4	3.3	—	—	2.6*	—
CLOAK	3.0	3.2	—	—	—	—	—	—
RETIC	3.3	0.8*	3.3	3.0	—	—	1.4	—
SPOT	1.4	0.8*	1.8	3.3	—	—	—	—
AD uv1	0.9	1.1*	1.0	—	—	1.3	—	—
AD2 uv2	0.7	1.7	1.7	2.6	—	1.2	—	—
AD blue	—	1.8	4.5	4.8	1.1*	—	—	—
AD green	0.7	1.6	4.0	6.2	1.1*	—	—	0.8
AD yellow/ orange	0.8	1.3	2.4	2.8	0.7*	0.7*	—	0.8
AD red	2.4	1.1	—	2.2		1.5	0.8*	—
PD uv1	—	—	—	—		—	—	0.9
PD uv2	—	—	—	—		—	—	—
PD blue	—	—	—	1.1		—	—	—
PD green	1.1	—	—	1.5	0.6*	—	—	—
PD yell/ oran	1.4	—	—	1.0		—	—	0.9
PD red	—	—	—	—	0.7*	—	—	—

Divergence associated with past allopatric lineages

In transect I, the north-west lineages occupy sites 1–4, and the central lineage occupies sites 5–8. They show very little introgression of the mtDNA (Thorpe *et al.* 2010), even with large sample sizes at each site (Fig. 3). The neutral nuclear genetic structure shows some gene exchange between these lineages, but a close enough association exists between the genetic structure and the past allopatric lineages to support the model of allopatric speciation (Thorpe *et al.* 2010). Some character sets (body proportions, dorsal hue, dewlap hue and colour pattern, but not scalation) show a quite high range of divergence (but never the highest) (Fig. 3) across this transect associated with genetic structure and lineage. The contrast ANOVAS also show that these character sets (but not scalation) show relatively high mean differences between the two lineages (Table 1), which is also reflected in the mean differences in some individual characters (Table 2). Dewlap hue, colour pattern, dorsum hue and body proportions, but not scalation, are correlated with genetic structure along this transect (Table 3).

The remaining transects (II, V–VIII) across previously allopatric lineages do not show strong genetic structure associated with the allopatric speciation model (Fig. 3) (Thorpe *et al.* 2010). Unlike transect I, there is little QT differentiation associated with allopatry (Figs 2 and 3) in these transects. The extent of divergence (range) is low to modest (except body proportions transect VIII), and character sets generally do not tend to change at the point of lineage transition (Fig. 3) in these transects. The contrast ANOVAS show that the mean difference between lineages across character sets is nonexistent to modest (0–2.8 $s_{\mathrm{w},}$ Table 1), which is also reflected in the generally low or insignificant mean differences in individual characters (Table 2). Moreover, a QT difference among lineages may be due to coincident habitat differ-

Table 3 Correlation between genetic structure on one hand and mtDNA lineage and multivariate character sets on the other

Transect	Model	Lineage	Dewlap hue	Dorsum pattern	Dorsum hue	Body shape	Scalation
I	AS	**0.925**	**0.893**	**0.930**	**0.962**	**0.835**	0.146
II	AD	**0.918**	**0.749**	**0.948**	0.651	**0.872**	0.222
III	ES	0.634	**0.939**	**0.790**	**0.869**	**0.848**	**0.852**
IV	ES	0.467	**0.926**	**0.859**	**0.930**	**0.926**	**0.915**
V	AD	**0.879**	0.566	0.085	0.247	0.392	**0.916**
VI	AD	**0.727**	**0.933**	0.404	**0.689**	0.517	0.084
VII	AD	**0.931**	0.633	**0.942**	**0.932**	**0.803**	**0.752**
VIII	AD	0.496	0.715	0.542	0.491	0.489	0.062

Significance at $P < 0.05$ in bold. Abbreviations as in Table 1.

ences along a transect (e.g. Trans VII, Fig. 3). Apart from transect I, and to some extent transect II, when climate is regressed out, very few individual characters differ among lineages along a transect, and when they do, the difference is almost always small (Table 2). While the correlations between genetic structure and the five character sets are consistently high for those transects representing ecological speciation, these correlations are not consistently high for those transects representing lower levels of allopatric divergence.

Discussion

The extent of divergence in quantitative traits among spatially adjacent parapatric sets of populations within this island can be very large, and many studies of quantitative traits within Lesser Antillean anoles in particular (Thorpe *et al.* 2004) and island lizards in general (Thorpe & Richard 2001; Thorpe 2002b) show that within-island variation can be pronounced. Exact quantitative comparison with within-island quantitative trait variation in other Lesser Antillean anoles is not readily available, particularly at the level of individual characters, but some comparisons can be made. To the north, *Anolis oculatus* (Dominica) and *Anolis marmoratus* (Basse Terre) show about 3–4 within-group standard deviations among quantitative trait character sets (scalation, body proportions) from spatially adjacent sets of populations (Malhotra & Thorpe 1994, 1997). To the south, a spectrometric study of hue (both body and dewlap) of *Anolis trinitatis* from St. Vincent showed over 10 s_w between xerix and montane populations within the same lineage (Thorpe 2002a).

This QT divergence in the Martinique anole appears to be primarily related to variation in climate and habitat rather than the phylogeographic lineages, even though these phylogenetic divisions can be old and reflect separation on ancient precursor islands for a very substantial time (up to 8 mybp). The one exception to this being the QT variation associated with the allopatric speciation along transect I. This general pattern conforms closely to the pattern seen in other Lesser Antillean anoles and within-island variation in lizards in general (Thorpe 2002b; Thorpe *et al.* 2004) where, in spite of deep phylogeographic divisions, and even evidence of past allopatry (Thorpe & Richard 2001), the patterns of QT variation generally relate to factors implicating natural selection for current ecological conditions, rather than historical factors such as divergence in allopatry.

The extent of QT divergence is a good predictor of the extent of genetic structure as measured by the hypervariable nDNA loci (microsatellites), while the correlation between nDNA variation and lineage frequency can be quite low at this level (e.g. $r = 0.63$, 0.47, 0.50 for transects III, IV and VIII, respectively). This could be due to the independent microsatellite loci being linked to the quantitative traits under selection. However, the microsatellite loci are unlinked to one another and are not under selection (Thorpe *et al.* 2010). It may simply be that quantitative traits vary with selection to habitats, and it is selection to habitats (rather than lineage) that primarily determines the extent to which populations interbreed.

Both natural selection and sexual selection can impact these sets of quantitative traits, and the impact is unlikely to be constant among character sets. For example, dewlap hue is likely to be impacted by sexual selection. In Lesser Antillean anoles, only sexually mature males have large dewlaps. These are usually kept hidden except when extended for conspecific communication. The role of the dewlap in male–male competition, in mating behaviour and potentially in female choice has recently been reviewed by Losos (2009). In complex Greater Antillean communities, dewlap hue may be involved with species recognition; in the solitary Martinique anole, it can be considered to be largely involved in sexual selection. Colour pattern is also strongly sexually dimorphic; sexually mature males may have distinct patterns unlike immature males and females. These are likely to be involved in sexual selection, but are visible all the time and vary with habitat type (Fig. 1), so may also be impacted by natural selection. Dorsum hue also varies with habitat in Lesser Antillean anoles (greener in more montane rainforest areas), but the intensity of the hue may vary slightly between the sexes, with the male more intensely coloured (Thorpe 2002a), so this may also be involved in sexual selection. Body proportions vary with habitat type (Malhotra & Thorpe 1997) and are likely to be influenced by factors such as physical habitat structure (Losos 2009) and perhaps dehydration. However, the shape of an anole may impact on sexual selection, particularly during head-bobbing displays and fighting, so this cannot be ruled out. Scale number, on the other hand, does not obviously respond to sexual selection. Rather, scale number in anoles and other squamates is implicated in desiccation resistance (Horton 1972; Soule' & Kerfoot 1972; Lister 1976; Thorpe & Baez 1987; Daltry *et al.* 1998; Malhotra & Thorpe 2000; Alibardi 2003; Thorpe *et al.* 2005; Calsbeek *et al.* 2006) and shows very pronounced geographic variation within individual Lesser Antillean islands, along moisture gradients from coastal dry woodland to montane rainforest (Malhotra & Thorpe 2000; Thorpe *et al.* 2004, 2005). Several studies indicate that anole scalation is genetically controlled (Thorpe *et al.* 2005; Calsbeek *et al.* 2006; Eales *et al.* 2010). These together with evidence from large-scale field experiments on natural selection (Thorpe *et al.* 2005), correlative studies (Thorpe & Malhotra 2000; Thorpe *et al.* 2004), parallel evolution (Thorpe *et al.* 2004) and studies of rapid adaptation of invasive species (Eales *et al.* 2010) suggest that this geographic variation in scale number is primarily caused by natural selection. Martinique anoles, like those on other Lesser Antillean islands, show variation in scale number between xeric and montane rainforest habitats. Consequently, for the sake of discussion, we can loosely ordinate our character systems on a sexual to natural selection gradient as dewlap hue, colour pattern, dorsum hue, body proportions and scalation, with dewlap hue most likely to be influenced by sexual selection and scalation most likely to be influenced by natural selection.

Ecological speciation involves all of these character sets including those like scalation, which are implicated in natural selection. Allopatric speciation on the other hand does not involve the character set (scalation) that is most implicated in natural selection. Moreover, mean divergence of individual scalation characters between lineages is never significant once climate has been regressed out. Allopatric divergence below the level of nascent speciation shows few individual characters that differ between lineages, but where they do differ, they are more likely to come from the sexual selection end of the spectrum (Table 2).

These results suggest that both strong divergent natural selection and sexual selection are necessary for the ecological speciation in this complex (Fig. 2). The hue of the dewlap, an important facet of the sexual selection, differs notably between xeric and montane rainforest habitats (Thorpe *et al.* 2008), as will the light environment in which they operate drive (Endler 1992; Boughman 2002). The combination of sensory drive, female choice and the divergence of mating preferences could lead to speciation in these organisms (Endler 1992; Boughman 2002; Leal & Fleishman 2004; Rundle & Nosil 2005). The requirement for the divergence of mating preferences in models of ecological speciation is frequently problematic (van Doorn *et al.* 2009). In this particular example, the acceptance of the conventional explanation of assortative mating is compromised by the observation that there has been no very robust demonstration of female choice in lizards in general, and anoles in particular, in spite of substantial interest in the area (Losos 2009). Male–male competition is more apparent in lizards in general (Pough *et al.* 2004), including anoles (Jenssen *et al.* 2005). Of course, the habitat differences and sensory drive may just as well impact on the effectiveness of the signals, such as dewlapping, if used in male–male competition. If these habitat differences generated strong divergent selection on dewlap hue, this may, perhaps, facilitate speciation. While a male from one habitat (and his offspring) migrating to the contrasting habitat, may be perceived by males in that habitat as having a poorer dewlap signal, sensory drive will not necessarily result in the migrating male perceiving the local males' signal as better. This would not obviously result in the migrating male loosing the competition, particularly if it escalated to fighting. It may be that as the local males perceive the immigrant's dewlap signal as poorer, he is challenged more frequently. Even so, this intrasexual selection does not lend itself to the simple, direct and intuitive prezygotic mechanism as is afforded by female choice and assortative mating.

Another explanation, not necessarily mutually exclusive to those mentioned earlier, derives from the recent simulation study of the role of condition-dependent sexual signals in ecological speciation (van Doorn *et al.* 2009). That is, an organism under selection will be in better condition (fitter) if it is locally adapted for that specific habitat. If the sexual signals (such as head bobbing, or dewlap hue) are condition dependent, then an organism in the correct habitat (one that it is adapted to by divergent natural selection) will give more effective sexual signals. Simulations indicate that this can lead to ecological speciation without the need for divergence of mating preferences (van Doorn *et al.* 2009). Moreover, this condition-dependent model also overcomes the need for sexual selection by female choice, because the concept should operate as well with male–male competition, where fitter males (those adapted to the correct habitat) have better condition-dependent sexual signals (robust head bobbing, brighter dewlap display and better fighting capacity) and win more territory and mating success. This model does not require the sexually selected trait to differ between types (even though dewlap hue does in this case), but it does require them to be condition dependent. At least some dewlap pigments (e.g. carotenoids) may be condition dependent, as suggested by a series of studies in birds (Saks *et al.* 2003; Peters *et al.* 2008). However, the only study of anoles, which used nutrition as a surrogate for condition, did not demonstrate this (Steffen *et al.* 2010). Hence, explanations based on both intrasexual and intersexual selection are not free from problems.

However, this is a very poorly studied area, and more remains to be done investigating the role of condition (not just nutrition) in a variety of species and habitat types. The energetic head-bobbing and press-up displays associated with dewlapping are intuitively condition dependent, and prolonged fighting is likely to be condition dependent, although this has not been demonstrated. There has been no comparison of head-bobbing and press-up displays among habitat types in these anoles, but a difference is not required by the condition-dependent model. This research suggests that further investigation into condition dependence of the complete range of the various components of anole sexual signals would be valuable.

The strong divergent selection between rainforest and xeric habitat anoles (Thorpe *et al.* 2005), the observation that ecological speciation in this case is not associated solely with sexual selection (as is allopatric speciation), but also requires strong divergent natural selection, and the absence of clearly demonstrated female choice in this complex (Losos 2009) suggest that we should consider a range of explanations, including the condition-dependent model of ecological speciation (van Doorn *et al.* 2009). This model may contribute to the explanation of partial genetic isolation of these habitat forms (Thorpe *et al.* 2010), if condition dependence for components of the sexual signal can be established.

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Data accessibility

Microsatellite, RFLP–PCR, multivariate and quantitative trait data: Dryad entry doi: 10.5061/dryad.c75nn.

Supporting information

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

Fig. S1 Frequency of mtDNA lineages along transects I–VIII.

Table S1 Character and character set independence.