Divergence in coloration and ecological speciation in the *Anolis marmoratus* species complex

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Abstract

Adaptive divergence in coloration is expected to produce reproductive isolation in
species that use colourful signals in mate choice and species recognition. Indeed, many
adaptive radiations are characterized by differentiation in colourful signals, suggesting
that divergent selection acting on coloration may be an important component of specia-
tion. Populations in the *Anolis marmoratus* species complex from the Caribbean island
of Guadeloupe display striking divergence in the colour and pattern of adult males
that occurs over small geographic distances, suggesting strong divergent selection.
Here we test the hypothesis that divergence in coloration results in reduced gene flow
among populations. We quantify variation in adult male coloration across a habitat
gradient between mesic and xeric habitats, use a multilocus coalescent approach to
infer historical demographic parameters of divergence, and examine gene flow and
population structure using microsatellite variation. We find that colour variation
evolved without geographic isolation and in the face of gene flow, consistent with
strong divergent selection and that both ecological and sexual selection are implicated.
However, we find no significant differentiation at microsatellite loci across popula-
tions, suggesting little reproductive isolation and high levels of contemporary gene
exchange. Strong divergent selection on loci affecting coloration probably maintains
clinal phenotypic variation despite high gene flow at neutral loci, supporting the
notion of a porous genome in which adaptive portions of the genome remain fixed
whereas neutral portions are homogenized by gene flow and recombination. We
discuss the impact of these findings for studies of colour evolution and ecological
speciation.

Keywords: adaptation, ecological genetics, population genetics – empirical, reptiles

Received 8 March 2012; revision received 6 February 2013; accepted 12 February 2013

Introduction

Recent theoretical and empirical support for models of
ecological speciation and divergence with gene flow
have sparked renewed interest in the role that divergent

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natural selection and local adaptation plays in the evo-
lution of reproductive isolation (Rice & Hostert 1993;
Dieckmann & Doebeli 1999; Gavrilets 2004; Nosil 2008,
2012). Many studies have demonstrated that local adap-
tation in response to divergent selection across ecologi-
cal gradients can generate phenotypic diversity in spite
of high gene flow (Smith *et al.* 1997; Schneider *et al.*
1999; Freedman *et al.* 2010; Kirschel *et al.* 2011) and that

the role of clinal adaptive variation in ecological speciation is potentially strong (Endler 1977; Rundle & Nosil 2005; Schluter 2009; Nosil 2012). Speciation is facilitated when characters subject to divergent selection also affect reproductive compatibility—so-called magic traits (Gavrilets 2004). In species that use colourful signals in mating interactions, divergent selection on coloration may result in reproductive isolation (Seehausen *et al.* 2008). Here we test the hypothesis that divergent selection on coloration across an ecotone between mesic and xeric habitats decreases gene flow and promotes speciation in the Guadeloupean anole, *Anolis marmoratus*.

Ecological speciation is likely an important mechanism of diversification in the adaptive radiation of Caribbean *Anolis* lizards (Ogden & Thorpe 2002; Losos & Thorpe 2004; Thorpe *et al.* 2010; reviewed in Losos 2009). In this adaptive radiation ecological types, termed 'ecomorphs', are divergent primarily in body size and limb dimensions (Williams 1972; Beutell & Losos 1999). However, intraspecific variation, which may represent the initial phase of speciation, occurs primarily in adult male colour and pattern (Underwood & Williams 1959; Lazell 1972; Williams & Rand 1977; Thorpe *et al.* 2004) rather than in characters associated with ecomorph divergence (Knox *et al.* 2001). Geographically divergent body colour may result from drift in small populations, but is more often adaptive, resulting from ecological selection for background matching and crypsis (Rosenblum 2006), intra- or intersexual selection (Stuart-Fox & Ord 2004; Stuart-Fox & Moussalli 2008), or both. Divergence in coloration may lead to speciation because the traits under selection potentially have a direct link to reproductive isolation in taxa, such as anoles, that use colourful signals in species recognition (Losos 1985, 2009; Ng & Glor 2011).

Geographic variation in male head and body coloration is extreme in *A. marmoratus* from the islands of the Guadeloupean archipelago in the Lesser Antilles. *Anolis marmoratus* comprises a polytypic species complex, with 12 subspecies described based primarily on the colour and pattern of adult males (Lazell 1972). There are six parapatrically distributed subspecies on the two main islands, five on Basse Terre and two on Grande Terre, one of which is shared between islands (Fig. 1). In addition there are six allopatric populations on offshore islands that have variously been treated as subspecies or species (Lazell 1972; Schwartz & Henderson 1991; Schneider *et al.* 2001). Mitochondrial DNA variation among subspecies may be substantial, suggesting that populations may represent several stages along the speciation spectrum (Schneider *et al.* 2001). On the main island of Grande Terre, two subspecies are present, *A. m. speciosus* and *A. m. inornatus*. While these two subspecies are very similar both in mtDNA (Schneider *et al.*

2001) and in morphological dimensions (Knox *et al.* 2001), they differ dramatically in adult male colour and pattern (Fig. 1). *Anolis m. speciosus* has a green body, a blue wash on the head with distinctive blue eyering and, often, a blue wash on the tail (Lazell 1972). In contrast, *A. m. inornatus* has a dull, yellow-green body, a brown-green head, a yellow or cream-coloured eyering and, often, a blue wash on the tail. Both subspecies have a similarly coloured yellow-orange dewlap, which is an extendable throat fan used in *Anolis* communication (Williams & Rand 1977; Jenssen 1978; Losos 2009). The females and juveniles are drably coloured and show less distinct geographic variation (Lazell 1972).

The southwest of Grande Terre (Grands Fonds) is a hilly region composed of limestone laid onto a volcanic basement (Feuillet *et al.* 2002). The Grands Fonds is covered with broadleaf vegetation and transitions into the open, dry lowlands in the north and east of the island. The transition from mesic to xeric habitat is sharp and occurs on a scale of two to four kilometers. Clinal variation in lizard coloration is associated with habitat and occurs on a similar scale. The *speciosus* phenotype is found in the mesic Grands Fonds, whereas *inornatus* occupies the dry, open lowlands.

The pattern of green lizards in moist, forested habitat and dull-coloured (ranging from dull green to grey or brown) animals in open, dry habitat mirrors the general pattern found among species of *Anolis* and is thought to reflect adaptation for crypsis (Thorpe & Malhotra 1996; reviewed in Macedonia 2001; Losos 2009). However, variation in male coloration may also play a role in sexual selection. Blue coloration is typically conspicuous and involved extensively in lizard intraspecific communication (Cooper & Burns 1987; Zamudio & Sinervo 2000; Macedonia 2001; Robertson & Rosenblum 2009, 2010). Thus, the blue coloration of the head in *A. m. speciosus* is more likely associated with intra- or intersexual selection than with crypsis. Williams & Rand (1977) proposed that on single-species islands in the Lesser Antilles, such as Grande Terre, body and head colour are the most salient visual cue for social communication or population recognition. Although the role of conspicuous colours in *Anolis* lizards remains unresolved, it is reasonable to hypothesize that both ecological and sexual selection may shape patterns of geographic colour variation and represent important components of the speciation process in anoles.

Here we address three principal questions. First, is variation in adult male head and body colour on Grande Terre consistent with hypotheses of ecological and sexual selection? Ecological selection for crypsis suggests that the reflectance of the body and head should fall within the reflectance of background vegetation and leaf litter,
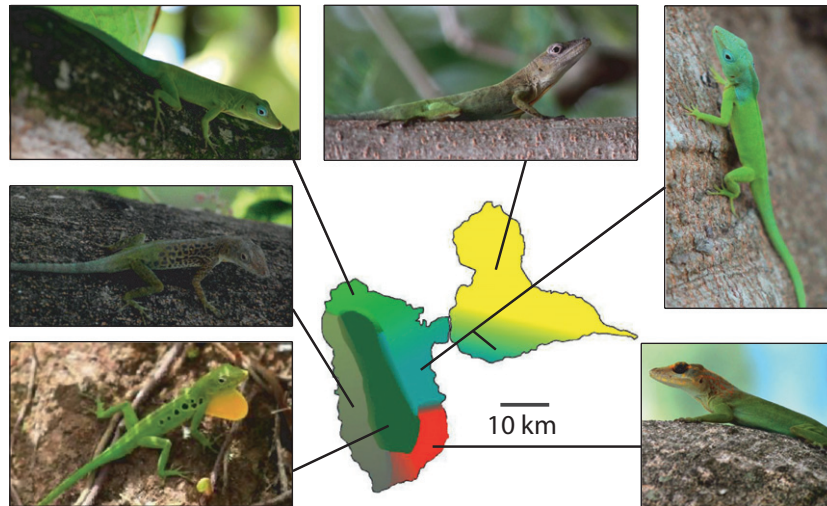
Fig. 1 Adult males of the six named forms on the two main islands of the Guadeloupean Archipelago: Basse Terre (left) and Grande Terre (right). The named forms are, clockwise from bottom left, *Anolis marmoratus alliaceus* (green with black spots); *A. m. girafus* (grey mottled); *A. m. setosus* (green with blue eyering and conical scales); *A. m. inornatus* (brown head and dull green-brown body, sometimes blue tail); *A. m. speciosus* (green with blue head and tail); and, *A. m. marmoratus* (red marbled head and blue tail). Areas of intergradation between forms are depicted as graduated shades of color. Females are generally cryptically coloured and show less distinct geographic variation (Lazell 1972). Photographs of *A. m. alliaceus* and *A. m. speciosus* by Pierre Legreneur and used with permission. All other photographs by André Schneider.

while sexual selection is expected to favour conspicuous colour elements that stand out from the background. Second, is there a history of isolation between differentiated populations of *A. marmoratus* on Grande Terre? This question addresses the process of divergence—clinal variation in phenotype may result either from neutral processes of secondary contact and introgression among previously isolated populations, or from divergent selection in primary contact (i.e., parapatric divergence). Third, is the transition across the ecotone in phenotype (head and body colour) associated with decreased gene flow? If divergence in coloration has a direct impact on reproductive isolation then we expect less gene flow per unit geographic distance across the ecotone than within habitats.

To answer these questions, we first analysed phenotypic variation in coloration in relation to the background vegetation in each habitat. Second, we estimated historical demographic parameters for the two subspecies with a multilocus coalescent approach (Wakeley & Hey 1997; Hey & Nielsen 2004) using 10 sequenced anonymous nuclear loci to reconstruct the historical demographic parameters of divergence. Third, we examined population structure and gene flow using microsatellite markers assayed among populations across two transects spanning the ecotone and encompassing the full gamut of phenotypic variation on Grande Terre. Finally, we discuss the impact of our findings for understanding the processes of colour evolution, local adaptation and ecological speciation.

Materials and methods

Sampling and sequencing

We collected tissue samples (tail tips) from adult male lizards from 11 localities (Fig. 2, Table 1) across Grande Terre ($n = 5$–11, mean = 9) and preserved them in 95% ethanol. We sampled populations spanning the habitat and phenotypic transitions on Grande Terre along two transects: a south–north transect (Cocoyer to Port Louis) and a west–east transect from Cocoyer to Desvarieux. The S-N transect encompassed a sharp ecological transition between the mesic habitat in Lebaire and open habitat in Morne-a-L'Eau, which are only 2.4 km apart but are separated by an abrupt escarpment, whereas the W-E transect (Cocoyer to Desvarieux) traversed a shallower ecological transition from mesic to xeric habitats between Fouché and Chateaubrun (Fig. 2).

We extracted genomic DNA from tissue following standard protocols using Qiagen™ DNAEasy kits. We created primers using Primer 3.0 (Rozen & Skaletesky 2000) for 40 haphazardly selected nuclear loci derived from 3072 Sanger sequence reads from a whole-genome library developed by the Broad Institute of MIT and Harvard. Primers were designed to yield sequences 300 –500 base pairs (bp) in length. We screened all 40 primer sets on a test panel of six individuals (two each from Cocoyer, Port Louis and Desvarieux) and retained those that resulted in efficient single-copy gene amplification, as determined by single PCR products that

Fig. 2 Sampling sites on Grande Terre, Guadeloupe. Locality abbreviations as in Table 1. Genetic sampling sites are connected by dashed lines and values are G'_{ST} between adjacent populations estimated from microsatellite loci. Pointe-a-Pitre, Leroux and Beauplan are sites where additional measures of animal reflectance were taken; reflectance measurements were not taken for animals from Port Louis or Les Abymes.
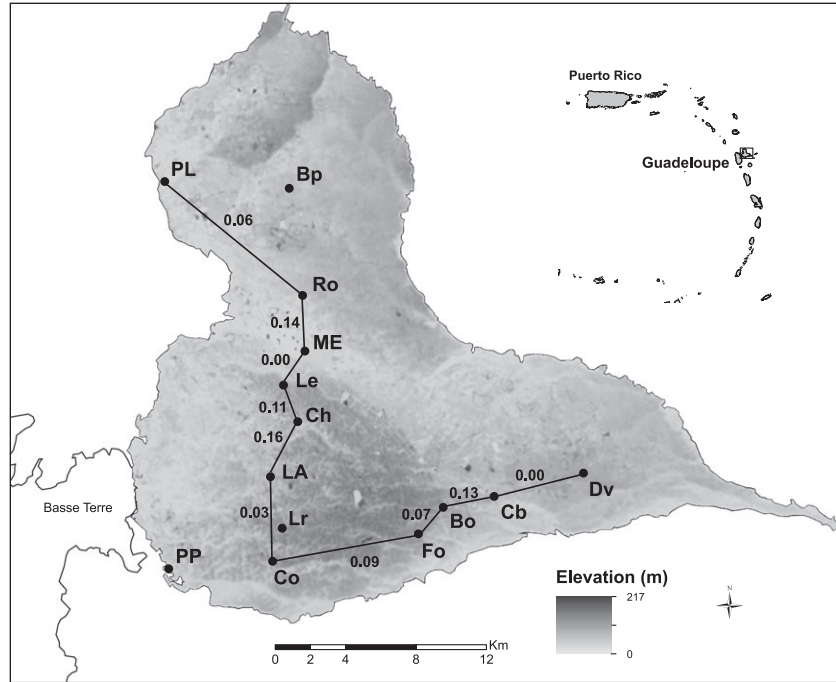
Table 1 Summary of sampling localities on Grande Terre, including coordinates and elevation. Sample sizes denote number of individuals used in microsatellite analyses. DNA sample sizes denote number of individuals used in microsatellite analyses. Phenotypic sample size refers to number of male *Anolis marmoratus* sampled for each body part (head background, eyering, dewlap, lateral body and lateral tail). Phenotypic sample size that only have one value had the same value for all body parts

Locality	Abbreviation	Coordinates	Elevation (m)	DNA sample Size	Phenotypic sample Size
Port Louis	PL	16.42, −61.53	4	5	0
Rougeole	Ro	16.37, −61.46	17	9	5
Morne-a- l'Eau	ME	16.34, −61.46	2	9	4
Lebaire	Le	16.32, −61.47	61	8	11
Chazeau	Ch	16.30, −61.46	31	10	5
Les Abymes	LA	16.27, −61.48	48	9	0
Cocoyer	Co	16.23, −61.48	49	11	5, 0, 5, 5, 5
Fouché	Fo	16.24, −61.40	65	10	9
Bombo	Bo	16.26, −61.38	62	10	8, 9, 9, 9, 9
Chateaubrun	Cb	16.26, −61.36	26	10	5
Desvarieux	Dv	16.27, −61.31	49	5	10
Pointe-à-Pitre	PP	16.23, −61.53	6	0	28, 8, 27, 28, 28
Leroux	Lr	16.25, −61.47	49	0	35, 30, 35, 35, 35
Beauplan	Bp	16.42, −61.46	49	0	6

showed only two alleles per variable site. Of 28 single-copy loci, we retained the 10 loci that most reliably yielded high-quality sequences. We sequenced both strands of each locus using BigDye™ (Applied Biosystems) terminator chemistry on an ABI-3100 sequencer and, for subsequent analysis, deleted regions containing indels due to uncertain alignment and phasing. We used PHASE 2.1.1 (Stephens *et al.* 2001) to reconstruct haplotypes from sequence genotype data and, where phase at a particular polymorphic site was uncertain,

those individuals were excluded from the analysis. We used IMgc (Woerner *et al.* 2007) to identify the largest, non-recombining region of sequence common to the greatest number of individuals.

Phenotypic analysis

We measured coloration of adult male lizards from 12 sites along two transects that spanned the ecotone on Grande Terre (Fig. 2). We characterized phenotypic

variation in colour by measuring reflectance of the dorsal head, eyering, dewlap, dorsal body and lateral tail using an Ocean Optics USB 2000 field-portable spectrometer. Reflectance values were recorded as per cent reflectance relative to a barium sulphate white standard using an Ocean Optics R400 UV-VIS reflectance probe attached to a DT-1000 tungsten halogen/deuterium UV-VIS light source (Ocean Optics, Inc., Dunedin, FL, USA). We also sampled reflectance of vegetation and leaf litter to characterize the visual background.

To visualize variation in colour components along each transect, we used the hue angle estimate from the four-segment classification scheme of Endler (1990). To calculate hue angle, we used a custom python script (spec.py; http://github.com/ngcrawford/Coloration) to interpolate spectral reflectance values to 1 nm, smooth the spectral reflectance curve using a 100 nm hanning window, and calculate brightness, hue and chroma. We used TetraColorSpace (Stoddard & Prum 2008) to visualize lizard and background vegetation colour as an avian predator with a U-type sensitivity might perceive the signal (Endler & Mielke 2005; Stoddard & Prum 2008). While most birds have a V-type retina, both U- and V-type retinas are sensitive to the UV so we chose to use the U-type model in our analysis to include the full range of reflectance from 300 to 700 nm. Also we used information on the visual sensitivity of *A. cristatellus* (Loew *et al.* 2002) to visualize colour components as the lizards might perceive them.

Historical demography and subspecies population structure

Because the geographical context, time of divergence, and migration rate are central to understanding the process of divergence, we used a multilocus coalescent analysis to infer the demographic history of the two subspecies. We used the site variation data from 10 nuclear loci in an Isolation-with-Migration (IM) model as implemented in the program IMa2 (Hey & Nielsen 2007) to simultaneously estimate the time since divergence between subspecies (t), migration rates ($m1$ and $m2$) between subspecies, and the effective population size for the ancestral (qA), and modern ($q1$ and $q2$) populations. We pooled sequences from populations where eyering, head and body coloration were all consistent with subspecies description and excluded intergrade populations.

We ran a large number of preliminary IMa2 analyses with various priors, heating schemes, and number of chains to determine the parameters for our final run. Based on the observation of trend plots and ESS values, we found that MCMC runs with 40 chains and geometric heating with parameters set at -ha 0.96 and -hb 0.90 provided mixing as thorough as any other combination

of heating parameters and number of chains that we explored (from 40 to 120 chains with geometric heating parameters of -ha from 0.96 to 0.99 and –hb from 0.75 to 0.90). We performed six independent replicate MCMC simulation runs for more than 28 million generations, each with 40 chains, geometric heating (-ha 0.96 and –hb 0.90), a different random number starting seed, a burn-in of 200,000 generations and priors of $q = 15$, $m = 100$ and $t = 6$. An infinite sites model was used and inheritance scalar priors were broad (10^{-3} to 10^{-10}). To convert parameter scalars to demographic units, we used a mutation rate per year of 0.5 substitutions per site per lineage per million years based on an estimate of synonymous substitution rate for the *Anolis carolinensis* genome (Janes *et al.* 2011). Generation time was assumed to be 1 year (Andrews 1976).

Microsatellite estimates of gene flow and population structure

To estimate fine-scale population genetic structure and gene flow among all pairs of populations, we screened seven microsatellite loci designed specifically for *A. marmoratus*. All loci were scored independently by two observers and cross-checked for accuracy (see Appendix S1, Supporting information).

We checked for violations of Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP on the Web (v. 4.0.10; Raymond & Rousset 1995; Rousset 2008). We used GENALEX (Peakall & Smouse 2006) and custom code publicly available at http://github.com/ngcrawford/pypgen to calculate the following summary statistics: P, the proportion of polymorphic loci; H_S, Nei's unbiased estimate of heterozygosity (Nei 1987); and $a_{private}$, the number of alleles unique to a single population. To estimate the relative genetic differentiation among populations, and determine whether patterns of allele frequencies were associated with ecotones, geographic distance or both, we calculated Jost's D (Jost 2008) and G'_{ST} (Hedrick 2005) for all pairwise comparisons among populations using the web-based software SMOGD (Crawford 2010). We used an exact G-test for pairwise population differentiation (Goudet *et al.* 1996), as implemented in GENEPOP.

We also used the Bayesian clustering method in STRUCTURE 2.1 (Pritchard *et al.* 2000) to identify the number of populations that best explains patterns of allelic variation. Because of the shared recent ancestry and potential for mixed ancestry across populations, we employed the admixture model and the correlated alleles model with 100,000 generations as burn-in and run lengths of over two million generations for all values of K between 1 and 11. We used the log likelihood ($-\ln L$) to determine the best estimate of K.

Results

Phenotypic variation across Grande Terre

Patterns in dorsal and head colour display clinal variation across Grande Terre and are broadly concordant with ecological gradients (Fig. 3). Between Lebaire (Le) and Morne-a-L'eau (ME) eyering colour in the majority of animals shifts from blue to yellow, body colour shifts from bright green to dull yellow-green and head colour shifts from blue to brown. These populations are separated by only 2.4 km, and the phenotypic shift is coincident with the sharp ecological transition between the mesic closed habitat in the southwest (Le) and the scrubby open habitat found in the north (ME). Similarly, along the west to east transect, the ecological transition from mesic forest to open scrub occurs between Bombo (Bo) and Chateaubrun (Cb), over a distance of 2.9 km.
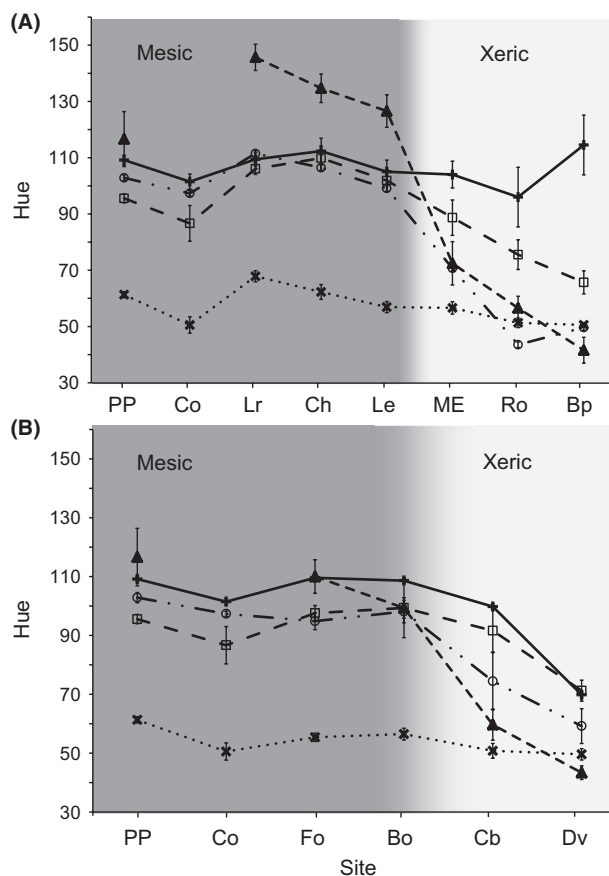
Fig. 3 Average hue angle ± 1 SE for head, eyering, dewlap, lateral body and tail reflectance measured along (A) south to north and (B) west to east transects across an ecological gradient on Grande Terre, Guadeloupe. ▲ = eyering; + = lateral tail; ○ = dorsolateral head (temporal region); □ = dorsolateral body (shoulder region); and × = center of the dewlap. The shaded background of the figures represents the approximate transition from mesic to xeric habitat. Locality abbreviations as in Table 1.

Patterns in eyering, body and head colour follow a similar transition from the majority of individuals being blue, green and blue or green respectively, in the west, to yellow, dull yellow-green and brown in the east.

Plots of colour components in colour space reveal that *A. m. speciosus* males in the southwest of Grande Terre are generally conspicuous, with even the green body coloration standing out from background (Figs 4 and S1, Supporting information). In contrast, *A. m. inornatus* male colour components fall within, or much closer to, background reflectance (except for the dewlap and tail) making them generally cryptic. Dorsal coloration of female lizards overlaps with background reflectance consistent with female crypsis.

Coalescent-based inference of divergence parameters from sequenced nuclear loci

Sequences from the 10 nuclear loci ranged in size from 108 to 323 bp, and the complete data set comprises 2128 bp containing 56 variable sites (Table 2). All sequences mapped to non-repetitive, non-coding portions of the *A. carolinensis* genome (AnoCar 2.0) supporting the assumption that sequence polymorphism is selectively neutral.

The six independent MCMC runs of IMa2 converged on similar estimates of the model parameters, and ESS values for all parameters in each run were above 50, which, together with the trend plots, demonstrated sufficient mixing of the chains in each of the MCMC runs. We used the Lmode option in IMa2 to read in the genealogies from the output files of all six MCMC runs and summarize the results from a total of 1,731,863 genealogies (Table 3 and Fig. S2, Supporting information). We visualized the results using the output file from the Lmode run as input for IMfig (Fig. 5). Time since divergence (t) was estimated at *c.* 650 000 years ago, but with broad confidence intervals (351 000 – 14.5 million years ago). Gene flow among populations was asymmetrical with high gene flow from *A. m. speciosus* into *A. m. inornatus* ($2Nm$ = 21.36; 95% CI = 7.87–423.2) and lower, but still substantial, gene flow from *A. m. inornatus* into *A. m. speciosus* ($2Nm$ = 2.64; 95% CI = 0.37–11.62). The estimate of ancestral effective population size (qA) is *c.* 370,000 individuals (95% CI = 126,247–1,079,800) while recent effective population size is estimated at *c.* 136,000 individuals (95% CI = 32,731–8.97 million) for *A. m. speciosus*, and *c.* 1.14 million individuals (95% CI = 565,773–9.00 million) in *A. m. inornatus* (Fig. 5; Table 3).

Microsatellite genetic diversity and population structure

We analysed seven microsatellite loci in 11 populations of *A. marmoratus* from Grande Terre. In seven popula-
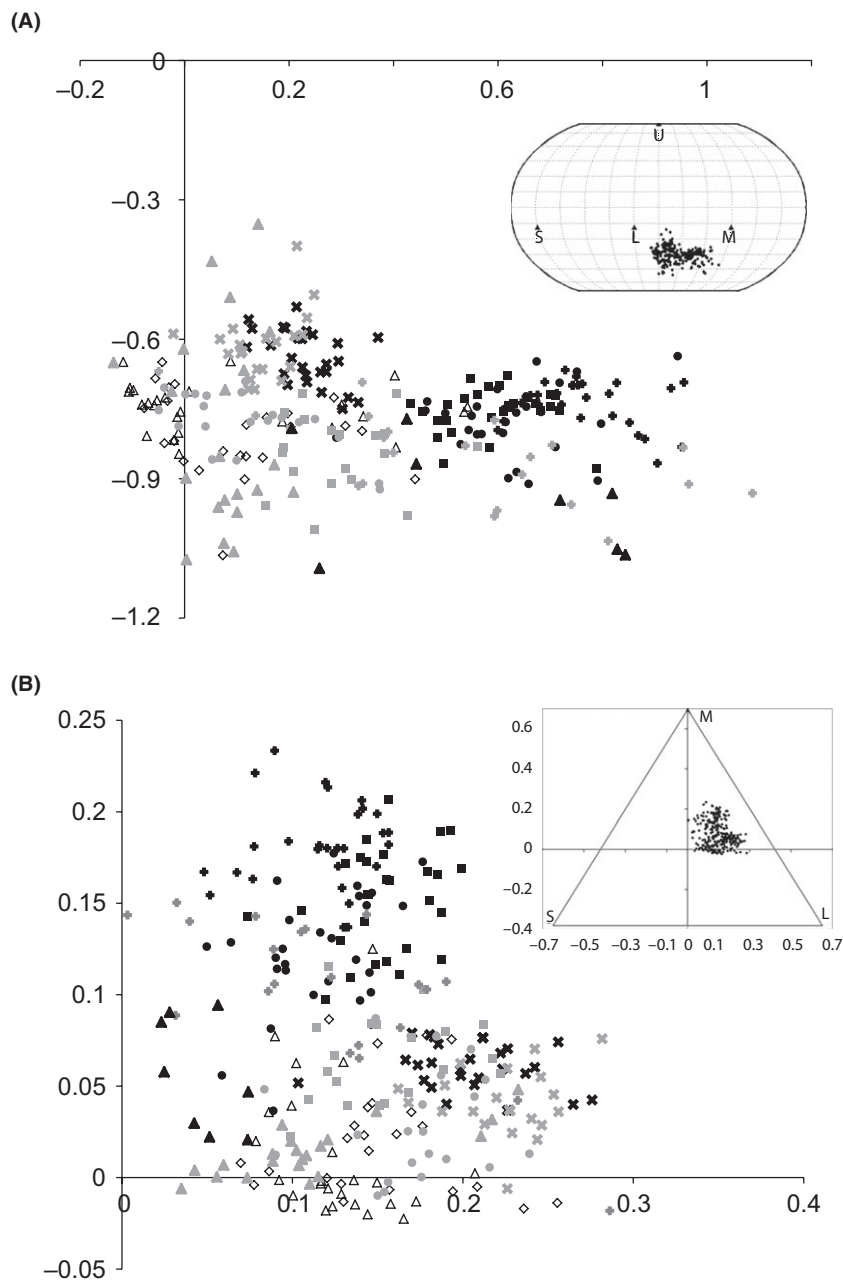
(A)

(B)

Fig. 4 (A) Detail of Robinson projection (Endler *et al.* 2005; Stoddard & Prum 2008) of lizard and visual background colour components showing hue longitude on the *x*-axis and hue latitude on the *y*-axis. Inset shows position of all points in Robinson projection of complete tetrahedral colour space △ = reflectance of visual background (vegetation and leaf litter). ◊ = dorsolateral reflectance of females. Other symbols are reflectance from adult males. Grey symbols represent *A. m. inornatus*, black symbols represent *A. m. speciosus*: ▲ = eyering; + = lateral tail; ● = dorsolateral head (temporal region); ■ = dorsolateral body (shoulder region); × = centre of the dewlap. (B) Plots of lizard colour components and visual background projected on the S, M, L face of the colour space tetrahedron (looking down along the UV axis). The scale and orientation of the S, M, L colour space on the *x–y* plot is shown in the inset. Symbols are the same as above.

tions, all microsatellite loci examined were polymorphic ($P = 1.0$), while the proportion of polymorphic loci remained high in the remaining four populations ($P = 0.86$; Table 4). The average number of genotypes analysed per population was 8.7 and ranged from 5 to 11. Similarly, estimates of unbiased heterozygosity (H_S) were consistently high across populations, and ranged from 0.566 (Port Louis) to 0.731 (Fouché). The average number of alleles unique to each population ($a_{private}$) was 1.7, and ranged from 0 (Les Abymes) to 5 (Fouché). In

Table 2 Characteristics of sequenced nuclear loci for IMa2 analysis. Size reflects number of base pairs analysed. Mutation rate (μ) per year is the per-locus mutation rate used to convert IM parameter scalars to demographic parameters. Mutation rate per site per million years per lineage of 5×10^{-7} is taken from the analysis of Janes *et al.* (2011). Generation time is assumed to be 1 year. The geometric mean of the per-locus mutation rates is 1.01×10^{-4}. The position in the *Anolis carolinensis* genome reflects high-scoring BLAST alignments of locus sequence reads to the AnoCar2.0 genome build. None of the loci mapped to exon regions.

Locus	Size (bp)	Variable sites	n speciosus, n inornatus	μ per year	Position in *Anolis carolinensis* genome
1FA3	137	5	28, 18	0.000069	chr3:109,228,783-109,228,909
1FA4	286	9	26, 16	0.000148	chr2:73,603,309-73,603,389
1FA5	153	2	26, 18	0.000077	chrUn_GL343268:21,075-21,208
1FA7	108	7	28, 16	0.000054	chr1:45,032,731-45,032,808
1FB1	169	7	24, 8	0.000085	chr4:99,942,237-99,942,407
1FB3	202	8	24, 12	0.000101	chr6:24,722,277-24,722,488
1FC8	323	10	24, 10	0.000162	chrUn_GL343584:350,635-350,954
10FB3	201	4	28, 16	0.000101	chrUn_GL343289:1,546,328-1,546,516
10FB8	320	2	30, 14	0.000160	chr5:103,583,017-103,583,215
10FB12	229	2	26, 10	0.000115	chr1:16,986,237-16,986,465

most populations, however, a_{private} was consistently low (≤ 2), except in Bombo and Fouché ($a_{\text{private}} = 3$ and 5, respectively).

Microsatellite variation shows low levels of genetic differentiation among populations. Multilocus estimates of G'_{ST} ranged from 0 to 0.182 and D ranged from 0 to 0.171 (Table 5). G'_{ST} values between populations in the west–east transect (Cocoyer to Desvarieux) were similar to estimates between populations in the south–north transect (Cocoyer to Port Louis). Only two multilocus estimates of population differentiation were significant after Bonferroni correction. These were Bombo-Chateaubrun ($G'_{\text{ST}} = 0.145$; Exact G-test $P = 0.0009$) and Bombo-Lebaire ($G'_{\text{ST}} = 0.118$; Exact G-test $P = 0.0007$). Exact G-tests for allele frequency differences among pairs of populations for each locus revealed that a single locus (12RF3) appeared to be driving the pattern; after Bonferroni correction for multiple comparisons, only comparisons for that locus and for the two population comparisons above were significant ($P < 0.02$).

Overall, there was no pattern of isolation by distance (IBD) (Fig. 6). The sharp phenotypic transition between *A. m. speciosus* and *A. m. inornatus* that occurs between Lebaire and Morne-a-L'eau is not accompanied by increased G'_{ST} values. However, the phenotypic transition between Bombo and Chateaubrun is associated with increased Gst although, as noted above, the pattern may be caused by a single locus. Overall, G'_{ST} values across the ecotone are not higher than values within habitats when standardized for geographic distance (Fig. 6). Pairwise estimates of gene flow (Nm) derived from G'_{ST} are all substantially greater than one suggesting that gene flow between adjacent populations is high enough to homogenize neutral genetic variation.

The pattern of low genetic differentiation among populations was further supported by the STRUCTURE analysis (Fig. 7). A model with two populations ($K = 2$) had the highest likelihood (lnL = −2132) of the 11 models examined ($K = 1$–11). The likelihood of a model with two populations was significantly better than the likelihood of a model with one population (lnL = −2145; $D = 25.8$; chi-square test, d.f. = 1; $P \ll 0.001$), and likelihoods for all other values of K were substantially worse (lnL −2209 to −2620). However, an examination of the probability of assignment to either of the two clusters for $K = 2$ shows no distinguishable pattern (Fig. 7). Most individuals were classified as belonging to a single population with high probability. Three individuals from Chazeau, one from Les Abymes, and one from Port Louis were assigned to a second genetic cluster with fairly high probability. However, these five individuals span the breadth of the S-N transect and do not form a coherent cluster in space. With the exception of these five individuals, all others are assigned with high probability to a single nearly homogeneous population.

Discussion

Local adaptation in multiple colour elements of *A. marmoratus*

The results from our phenotypic analysis are concordant with the hypothesis that ecological and sexual selection mediate colour evolution in *A. marmoratus*. As expected, patterns in dorsal and head colour vary clinally across the island, and phenotypic transitions coincide with the mesic–xeric ecotone (Fig. 3). Colour space plots reveal that the head and body coloration of

Table 3 Summary of the six independent IMa2 runs showing estimates of demographic parameters for the divergence histories for *Anolis m. speciosus* and *A. m. inornatus*. The values for the peak (HiPt), as well as the Low (95%Lo) and High (95%Hi) points of the 95% confidence interval are given

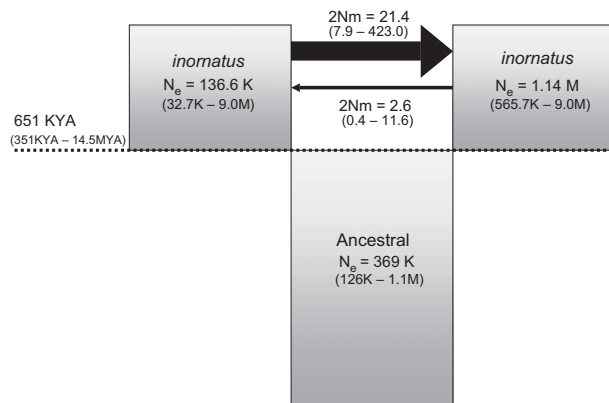| Measure	N_e (ancestral)	N_e (*inornatus*)	N_e (*speciosus*)	2Nm (*inornatus* → *speciosus*)	2Nm (*speciosus* → *inornatus*)	Time since divergence (years)
HiPt	369,389	1,136,534	135,598	2.64	21.36	650,872
95%Lo	126,247	565,773	32,731	0.37	7.87	351,621
95%Hi	1,079,800	9,002,494	8,965,087	11.62	423.20	14,476,309

Fig. 5 Divergence histories for the two subspecies on Grande Terre. Effective population size (N_e with bounds of 95% highest posterior density) for *Anolis m. speciosus*, *A. m. inornatus* and the ancestral population are shown in the shaded boxes (boxes not drawn to scale). Time is on the vertical axis, with recent time at the top, and the estimated divergence time (with 95% HPD) is shown. Gene flow is indicated by arrows indicating the direction of gene movement, and values for 2Nm (with 95% HPD) are shown.

Table 4 Summary statistics of genetic diversity for seven microsatellite loci used in this study. *N*, total number of individual genotypes; *P*, proportion of polymorphic loci; $a_{private}$, number of alleles unique to a single population; H_s, Nei's unbiased heterozygosity per locus with the standard error (SE) of that estimate shown in parentheses

Population	*N*	*P*	$a_{private}$	H_S (±1 SE)
Bombo	10	1.00	3	0.624 (0.236)
Chateaubrun	10	1.00	2	0.651 (0.257)
Chazeau	10	1.00	2	0.675 (0.246)
Cocoyer	11	1.00	1	0.654 (0.222)
Desvarieux	5	0.86	1	0.599 (0.273)
Fouché	10	1.00	5	0.731 (0.156)
Lebaire	8	0.86	1	0.618 (0.278)
Les Abymes	9	1.00	0	0.639 (0.204)
Morne-a-L'eau	9	0.86	2	0.650 (0.284)
Port-Louis	5	0.86	1	0.566 (0.288)
Rougeole	9	1.00	1	0.628 (0.245)

A. m. inornatus overlap with background reflectance (Fig. 4) consistent with crypsis due to background matching (e.g., Hoekstra *et al.* 2004; Endler *et al.* 2005; Rosenblum 2006). In contrast, plots of colour components reveal that *A. m. speciosus* is conspicuous in its environment, as individuals show little overlap with background reflectance in colour space (Fig. 4). Even the green body coloration stands out from the background in this subspecies. Sexual selection may favour general conspicuousness when predation risk from visual predators is low (Reznick & Endler 1982; Kemp *et al.* 2009), and we suspect that the predation by avian predators may be driving this pattern. Our results strongly suggest that divergent selection for crypsis (in open habitats) and conspicuity (in closed habitats) are mediating evolution in multiple colourful elements in *A. marmoratus*.

The history of divergence among subspecies

The observed colour variation among populations may result either from secondary contact and introgression or from primary divergence, without geographic isolation, due to divergent selection (Endler 1977). Our analysis of historical demography strongly supports the hypothesis that divergent natural selection across the ecological gradient generated clinal patterns of phenotypic divergence in this complex (classic parapatric divergence of Mayr 1947). Furthermore, the analysis of nuclear sequence variation in an Isolation-with-Migration (IM) model found a strong signal of migration between subspecies and large effective population sizes throughout their history, supporting a model of divergence with gene flow (Fig. 5).

Our results are consistent with *in situ* diversification of *A. marmoratus* on Grande Terre. It is not surprising that the alternative model of geographic isolation followed by secondary contact is not strongly supported. Grande Terre is a geologically young island, having formed through the deposition of limestone platforms during the Pleistocene, building east and northward from the Grands Fonds in the southwest of the island,

Table 5 Pairwise comparisons of G'_{ST} (bottom left) and Jost's D (top right) estimated from seven microsatellite loci among all populations. Populations are ordered North to South, West to East

Population	PL	Ro	ME	Le	Ch	LA	Co	Fo	Bo	Cb	Dv
PL	–	0.049	0.091	0.000	0.007	0.161	0.017	0.000	0.035	0.105	0.006
Ro	0.063	–	0.029	0.030	0.163	0.099	0.010	0.101	0.025	0.108	0.000
ME	0.140	0.182*	–	0.038	0.032	0.171	0.024	0.021	0.013	0.025	0.025
Le	0.000	0.062	0.111*	–	0.061	0.077	0.003	0.049	0.035	0.024	0.001
Ch	0.112	0.169*	0.085	0.120	–	0.124	0.010	0.018	0.128	0.098	0.002
LA	0.164	0.124	0.163	0.101	0.149*	–	0.038	0.065	0.056	0.066	0.038
Co	0.025	0.042	0.050	0.018	0.070	0.064	–	0.044	0.050	0.013	0.001
Fo	0.088	0.142	0.056	0.095	0.067	0.123	0.064	–	0.092	0.017	0.001
Bo	0.072	0.113	0.071	0.118***	0.167**	0.123*	0.089*	0.149*	–	0.099	0.004
Cb	0.128	0.138*	0.065*	0.077	0.098	0.098*	0.035	0.063	0.145***	–	0.000
Dv	0.000	0.051	0.053	0.000	0.043	0.056	0.005	0.038	0.030	0.000	–

Multilocus G'_{ST} *P*-values for genotypic differentiation resulting from an exact *G*-test (Goudet *et al.* 1996) as implemented in GENEPOP are denoted as follows: *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. Population abbreviations are as in Table 1.
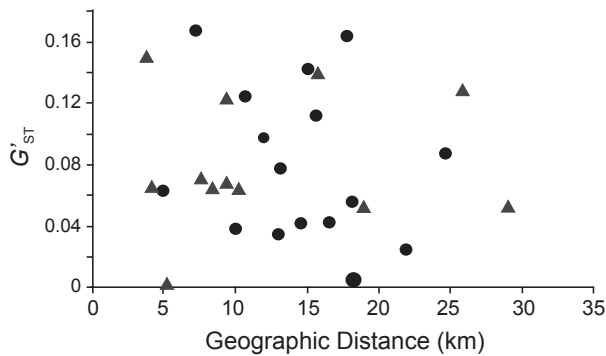

Fig. 6 G'_{ST} plotted against geographic distance within (triangles) and between (circles) habitats. No pattern of isolation by distance is evident (Mantel test $P = 0.5$) nor is G'_{ST} among populations between habitats higher than G'_{ST} within habitats over similar geographic distances.
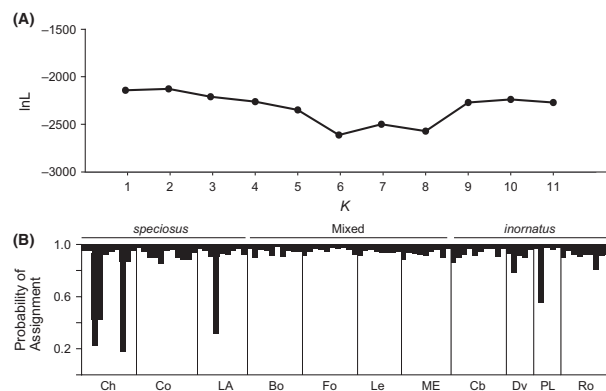

Fig. 7 Structure analysis. (A) lnL for $K = 1$–11 populations. (B) Probability of assignment of individuals to either of two populations.

and there is limited evidence for periods of geographic separation between regions of the island (Feuillet *et al.* 2002; Komorowski *et al.* 2005). The time since divergence, estimated at about 650 KYA, suggests that the populations on Grande Terre diverged well after the formation of the island. Finally, there is no sign of a population bottleneck or founder event, and the ancestral population size for these subspecies is inferred to be large, with N_e estimated at 369,000 individuals. This is not surprising given that anoles are good dispersers and that population densities, especially in the Lesser Antilles, can be extraordinarily high; more than 32,000 individuals per hectare have been estimated for *A. trinitatis* from St. Vincent (Hite *et al.* 2008). Thus, all indications are that phenotypic divergence among subspecies on Grande Terre occurred due to divergent selection in primary contact.

Phenotypic variation is maintained despite near panmixia at neutral loci

Our finding that *A. m. inornatus* is cryptic in its environment, while *A. m. speciosus* is conspicuous suggests a strong role for divergent selection and, consequently, the potential for genomic divergence among subspecies. Indeed, previous research in anoles and other lizards has demonstrated that neutral genetic structure can be stronger across ecotones than within the same habitats (Ogden & Thorpe 2002; Rosenblum 2006; Thorpe *et al.* 2010). However, we find little indication of reduced gene flow among phenotypically differentiated populations of *A. marmoratus* on Grande Terre suggesting that gene flow and recombination are high enough to homogenize genomic variation except at loci that have a strong effect on fitness.

Our finding of high gene flow among populations is somewhat surprising given that phenotypic variation among populations is mainly in the coloration of adult males, a character that may impact reproductive isolation in highly visual animals in which mating success is related to social signalling. A possible explanation for the lack of genetic structure is that divergence among populations may be so recent that genetic variation at marker loci is not at migration—drift equilibrium (Wright 1943). At equilibrium, we expect to see a pattern of IBD if dispersal is limited relative to the geographic scale of analysis (Slatkin 1993). Our genetic data are not consistent with a pattern of IBD (Fig. 6), but given the temporal depth of divergence of the *A. marmoratus* subspecies on Grande Terre (~650 KYA corresponding to an equivalent number of generations), we expect populations to be at migration—drift equilibrium. The lack of genetic structure, therefore, probably reflects rates of gene flow and recombination that are high enough to homogenize allele frequencies at neutral loci, while frequencies at loci affecting coloration have diverged.

Anole coloration displays little phenotypic plasticity (Steffen *et al.* 2010) and therefore phenotypic variation maintained along ecological gradients in the face of high gene flow probably reflects divergence at a small number of loci under selection (e.g., Mullen & Hoekstra 2008; Freedman *et al.* 2010; Rosenblum & Harmon 2011). These loci remain differentiated between phenotypically divergent populations despite high levels of gene flow across the genome. Although colour variation loci are not driving the evolution of reproductive isolation (e.g., Turner *et al.* 2005), they may be viewed as 'adaptation islands', as they underlie the evolution of a strong phenotypic cline in the face of high gene flow at loci that are selectively neutral. This view of a porous genome (Feder 1998; Gavrilets & Vose 2005) suggests that strong selection can overcome gene flow to fix adaptive loci while gene flow and recombination homogenize variation across loci that are selectively neutral.

Implications for divergence with gene flow, ecological speciation and adaptive radiation

Because of the strong phenotype-habitat correlation observed across *Anolis*, we might expect an important role for ecological speciation in the adaptive radiation of anoles. Some species of *Anolis* show evidence for stages in the process, but we do not yet have clear examples of complete ecological speciation (i.e., largely or fully reproductively isolated species). For example, *A. roquet* from Martinique shows patterns consistent with ecological speciation in that levels of gene flow across an ecological transition are lower than among previously isolated populations that currently occupy the same habitat (Ogden & Thorpe 2002; Thorpe *et al.* 2010). However, reproductive isolation is not sufficient to allow differentiated species to coexist, a necessary step in speciation within islands. Similarly, while *A. m. inornatus* and *A. m. speciosus* clearly represent a case of divergence with gene flow, likely due to divergent selection on multiple traits, we see no sign of reproductive isolation. Thus, taxa from geographically complex islands in the Lesser Antilles appear to represent cases in which the processes of population divergence are revealed, but where speciation has stalled at different stages. What, then, inhibits ecological speciation on small islands?

In models of ecological speciation, reduction in gene flow between divergent phenotypes is an important step in transforming ecotypic variation into reproductively isolated species (Kirkpatrick & Ravigné 2002; Nosil 2008; Hendry 2009). The evolution of reproductive isolation in the face of gene flow is difficult because recombination randomizes associations between genes under divergent selection and those that affect reproductive isolation (Felsenstein 1981). However, this selection/recombination antagonism disappears when the loci under selection pleiotropically affect reproductive isolation (Rice & Hostert 1993; Gavrilets 2004; Mallet *et al.* 2009; Servedio *et al.* 2011).

Are there traits in *Anolis* that are under divergent selection and that may pleiotropically result in reproductive isolation—so-called magic traits (Gavrilets 2004)? There is strong evidence that dewlap coloration is important in species recognition in *Anolis* (Rand & Williams 1970; Losos 1985; Nicholson *et al.* 2007; Ord & Stamps 2009; reviewed in Losos 2009) and sympatric species nearly always differ in dewlap colour. It has been suggested that tight dependence on dewlap coloration combined with species-specific display patterns may explain the near absence of hybridization among sympatric species (Losos 2009). If adaptive divergence in dewlap coloration occurred among populations (e.g., local adaptation for effective signalling in different light environments), reproductive isolation may follow as a consequence. There is some evidence that divergence among populations in dewlap coloration is related to decreased gene flow (Ng & Glor 2011), but there is little evidence that dewlap coloration is a direct target of divergent selection prior to speciation (Nicholson *et al.* 2007). Williams & Rand (1977) hypothesized that head and body coloration also may be important components of the mate recognition system but we find no evidence to support such a link. Finally, an analysis of multiple quantitative traits associated with reduced gene flow across an ecotone in *A. roquet* identified

several characters that covary across the ecotone (Thorpe *et al.* 2012). However, it does not seem likely that those characters, which may be under divergent selection (e.g., number and size of scales), have a direct impact on reproductive isolation. Thus, there is no evidence to date for magic traits in anoles that may be both the target of divergent selection and also affect reproductive compatibility.

Sexual selection is likely an important cause of phenotypic evolution in anoles (reviewed in Losos 2009), and variation in female preference associated with habitat could provide a mechanism for speciation via sexual selection by female choice (Kirkpatrick & Ravigné 2002; Seehausen *et al.* 2008). However, the prevailing view is that there is no evidence for female preference in *Anolis*, or any other lizard for that matter (Stamps 1983; Andrews 1985; Tokarz 1995; Lailvaux & Irschick 2006), and the lack of assortative mating suggested by our data is consistent with that view. Rather, sexual selection in *Anolis* appears to take the form of male–male competition for territory, and divergent male coloration may evolve in the context of intrasexual interaction without concomitant evolution of assortative mating. In the absence of geographic isolation, therefore, divergent sexual selection may not have a large impact on the initial stages of adaptive radiation of anoles, although it may still play a role in reinforcement.

Our findings suggest that loci controlling head and body coloration are not involved in reproductive compatibility. For reproductive isolation to evolve without direct selection on loci that affect reproductive isolation, linkage disequilibrium between reproductive compatibility loci and loci under divergent selection must evolve (Felsenstein 1981; Rice & Hostert 1993; Kirkpatrick & Ravigné 2002; Gavrilets 2003, 2004). The evolution of linkage disequilibrium among disparate loci is unlikely given the high rates of gene flow and large effective sizes that we estimated for divergent populations on Grande Terre. Therefore, some degree of geographic isolation or IBD may be necessary for the evolution of linkage disequilibrium and speciation in *Anolis*.

Losos & Schluter (2000) identified an evolutionary species–area relationship for *Anolis* whereby speciation occurs only on islands above a threshold size. This pattern may be general; Kisel & Barraclough (2010) surveyed a wide variety of taxa on oceanic islands and found a similar evolutionary species–area relationship. Both sets of authors suggested that gene flow inhibited speciation on islands below a threshold size. Our findings are consistent with that hypothesis, but we point out that, at least in *Anolis* and probably other taxa as well, there appears to be a gradation of differentiation with island size—small, topographically simple islands contain single, monotypic species while intermediate-sized islands often show minor geographic variation within a single species, and similar-sized volcanic islands, with substantially more topographic complexity, harbour polytypic species, sometimes with extreme geographic variation (Losos & Schluter 2000). Notably, this pattern is reflected within the *A. marmoratus* species complex, with six subspecies on the volcanic island of Basse Terre and two on the similarly sized, but more topographically simple, island of Grande Terre. Such continuity of pattern suggests continuity of process and highlights the fact that the homogenizing effects of gene flow may be overcome by strong divergent selection to produce substantial phenotypic variation over small spatial scales. With sufficient isolation from gene flow, linkage disequilibrium between loci under divergent selection and those controlling assortative mating could evolve. Without geographic isolation, the balance between gene flow and selection will determine where along the speciation spectrum population divergence comes to rest.

Conclusions

Male *Anolis marmoratus* on the island of Grande Terre show striking phenotypic differentiation in multiple colourful elements. Our analysis of coloration in relation to habitat suggests that sexual selection for conspicuousness is primarily responsible for the striking blue and bright green coloration of males in the mesic southwest of the island, whereas in the open habitat of the north and east, ecological selection for crypsis has resulted in the drab coloration of males. Our multilocus coalescent and microsatellite analyses support a model of divergence with gene flow and high contemporary rates of gene exchange. Thus, while phenotypic divergence in adult male coloration is striking, we find no indication that reproductive divergence has occurred as a consequence. This suggests that the anoles on Grande Terre represent an early stage along the speciation continuum and that assortative mating is not complete. High gene flow among populations may inhibit further progress toward speciation, particularly if the loci affecting fitness in the two habitats are not the same as loci affecting traits involved in reproductive isolation; thus, these populations are likely at a stable endpoint that reflects a balance between divergent selection and gene flow. This migration–selection antagonism may explain the evolutionary species–area relationship noted by Losos & Schluter (2000) and also explain the fact that ecological speciation appears to have repeatedly stalled at different stages on topographically complex islands in the Lesser Antilles (Losos 2009; Thorpe *et al.* 2010).

Our findings are consistent with strong divergent natural and sexual selection acting on coloration among populations. Similar selection may be playing out in other portions of the *A. marmoratus* species complex but we do not yet know whether stronger selection is correlated with reduced gene flow as predicted by models of ecological speciation. The *A. marmoratus* species complex provides a rare opportunity to examine the interaction of gene flow, geographic isolation, and ecological and sexual selection on population divergence and speciation across a wide portion of the speciation spectrum. Analyses of population divergence across the steeper ecological gradients on the older volcanic island of Basse Terre, and analyses of populations isolated on small offshore islands, will allow additional tests of ecological speciation and identify the factors that determine the outcome of population divergence in response to ecological and sexual selection.

Acknowledgements

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Data accessibility

DNA sequences (200 bp or longer): GenBank accessions JQ064574–JQ064941. Final DNA sequences for IMa2, spectral reflectance data, and microsatellite data: Dryad doi:10.5061/dryad.15570.

Supporting information

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

Fig. S1 (A) Detail of Robinson projection (Endler et al. 2005; Stoddard & Prum 2008) of lizard and visual background color components showing hue longitude on the x-axis and hue latitude on the y-axis calculated using the cone sensitivities of Anolis cristatellus (Loew et al. 2002). Inset shows position of all points in Robinson projection of complete tetrahedral color space. Δ = reflectance of visual background (vegetation and leaf-litter). ◊ = dorsolateral reflectance of females. Other symbols are reflectance from adult males. Grey symbols represent A. m. inornatus, black symbols represent A. m. speciosus: ▲ = eye ring; + = lateral tail; ● = dorsolateral head (temporal region); ■ = dorsolateral body (shoulder region); × = center of the dewlap. (B) Plots of lizard color components and visual background projected on the S, M, L face of the color space tetrahedron (looking down along the UV axis). The scale and orientation of the S, M, L color space on the x-y plot is shown in the inset. Symbols are the same as above.

Fig. S2 Posterior probability distributions for the demographic parameters of Anolis marmoratus estimated from 1,731,863 genealogies generated by six independent MCMC runs of IMa2 (see text for model parameters).

Appendix S1 Microsatellite testing and selection.