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Brief Communication

The genetic architecture of dewlap pattern in Hispaniola anoles (*Anolis distichus*)

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Abstract

Color and pattern are often critical to survival and fitness, but we know little about their genetic architecture and heritability in groups like reptiles. We investigated the genetic architecture for the pattern of the dewlap—an extensible throat fan important for communication—in anole lizards. We studied the Hispaniolan bark anole (*Anolis distichus*)—a species that exhibits impressive intraspecific dewlap polymorphism across its range—by conducting multigenerational experimental crosses with 2 populations, one with a solid pale yellow dewlap and another with an orange dewlap surrounded by a yellow margin. Upon rejecting the hypothesis that the extent of the orange pattern is a quantitative trait resulting from many loci of minor effect, we used a maximum likelihood model-fitting framework to show that it is better explained as a simple Mendelian trait, with the solid yellow morph being dominant over the blush orange. The relatively simple genetic architecture underlying this important trait helps explain the complex distribution of dewlap color variation across the range of *A. distichus* and suggests that changes in dewlap color and pattern may evolve rapidly in response to natural selection.

Keywords: pattern, genetic architecture, heritability, Mendelian, *Anolis*

Introduction

Patterns like the bold stripes of coral snakes, countershading on sharks, and the contrasting colors of a peacock's feathers are fundamental for aposematism, crypsis, and mate attraction (Thayer, 1918). Given the potentially major fitness consequences of pattern variation and the relative ease with which they are quantified, studies on the genetic basis of color and pattern have also profoundly impacted the field of evolutionary biology (Hoekstra, 2006). The classic work on the peppered moth (*Biston betularia*), for example, illustrated that wild populations can evolve dramatic new phenotypes in a short period of time when strong selection acts on one or a few loci of large effect (Bowater, 1914; Ford, 1937; Hof et al., 2011, 2016). Studies across many other animal groups further support the idea that distinct pattern morphs resulting from differences in melanin production are often due to classic Mendelian inheritance involving one or a few loci of large effect (Eizirik et al., 2003; Papa et al., 2008; Rosenblum et al., 2004; Steiner et al., 2009; Steiner et al., 2007). Although understanding the basic genetic architecture and heritability underlying pattern is key to understanding how these traits are likely to evolve, few studies have investigated these questions for nonmelanic pattern variation.

While nonmelanistic patterns resulting from other types of pigments, like carotenoids and pteridines, are widespread in fish, reptiles, and birds, we know little about their genetic architecture or inheritance (Kronforst et al., 2012; Olsson et al., 2013). Do these nonmelanic polymorphisms in pattern—which we define as variation in the distribution of pigment across an organism's body—also result from a few genes of major effect, or are they quantitative traits resulting from many loci of small effect? Squamates, a group that includes over 12,000 species of snakes, lizards, and amphisbaenians, exhibit a variety of colors and patterns, from the vibrant color-shifting panther chameleon to the boldly striped coral snake. Even though nonmelanistic patterns are common and important for squamate natural history, few studies have looked at the genetic architecture of patterns. Instead, the few existing studies have focused on the pigments responsible for the production of nonmelanistic colors (de Gazda et al., 2020; de Mello et al., 2021; Rankin et al., 2016; Teasdale et al., 2013). Here, using crosses from captive populations, we test the hypothesis that the genetic architecture of a pattern formed by nonmelanic colors in a unique and colorful Caribbean lizard is Mendelian.

To test this hypothesis, and further our understanding of the genetic architecture of patterns in squamates, we focus on a Caribbean lizard, the Hispaniolan bark anole (*Anolis distichus*). Anoles are a diverse lizard group with over 400 species that are broadly distributed across the neotropics (Losos & Ricklefs, 2009). Anoles use extendable throat fans, or dewlaps, often with bright colors and distinct patterns for species recognition, defending territories, attracting mates, and discouraging predators (Jenssen, 1977; Leal & Rodríguez-robles, 1997). There is extensive variation in both dewlap color and pattern across anole species; some

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species have solid-colored dewlaps, while others have complicated patterns that include blotches, spots, and stripes (Schwartz, 1980). These differences in dewlap color and pattern are thought to be key for species recognition and play a role in reproductive isolation (Leal & Fleishman, 2004; Ng & Glor, 2011; Ng et al., 2017). However, some species exhibit impressive intraspecific variation (Case, 1990; Leal & Fleishman, 2004), such as the Hispaniola bark anoles (Schwartz, 1971). In the South-Central part of Hispaniola, two populations of *A. distichus* with distinct dewlap patterns that have been recognized as distinct subspecies come in contact and experience gene flow along a narrow contact zone (Schwartz & Henderson, 1991). Dewlaps from populations found in the drier (xeric) southern environments (*A. d. ravitergum*) are entirely pale yellow (solid pattern) (Figure 1A), while dewlaps from the wetter (mesic) northern environments (*A. d. ignigularis*) are typically orange with thin yellow margins (blush pattern) (Figure 1A; Schwartz & Henderson, 1991). Where these two populations come into contact, individuals with intermediate phenotypes are identified based on the presence of dewlaps with varying sizes of orange surrounded by yellow margins (Glor & Laport, 2012; Schwartz, 1971). To understand how their patterns will evolve in the face of continued hybridization, it is necessary to know the genetic architecture and heritability patterns. Previous work within *A. distichus* has shown that differences in dewlap pattern are heritable, independent of diet-based carotenoid availability, not due to differences in UV reflectance, and consistent with autosomal inheritance (Ng, Kelly et al., 2013). Furthermore, the existence of animals with intermediate phenotypes in the field suggests ongoing hybridization and, thus, the possibility of breeding these phenotypically distinct populations to test hypotheses regarding the genetic architecture of patterns. This makes *A. distichus* a valuable model for determining the genetic architecture of a nonmelanic pattern via a breeding experiment.

We studied the genetic architecture of the dewlap pattern in *A. distichus* by using experimental crosses to address two questions. First, is the dewlap pattern in *A. distichus* a Mendelian trait (resulting from one locus of large effect) or an additive quantitative trait (multiple loci, each with a small effect)? Second, if the trait is Mendelian, is it dominant, codominant, or additive? To answer these questions, we used samples from two phenotypically distinct parental populations and set up crosses to produce F1s, F2s, and both backcrosses. Based on prior work examining the heritability of patterns in squamates (Bechtel & Whitecar, 1983; King, 2003; Zweifel, 1981), we predicted that the quantitative hypothesis would be rejected and that the dewlap pattern is instead a Mendelian trait. Determining the genetic architecture and heritability of the dewlap pattern will help us better understand how this unique polymorphism evolved and how the dewlap pattern might change in response to strong selection pressures as parental populations continue to hybridize.

Materials and methods

We quantified the dewlap pattern by measuring the extent of the orange area in each dewlap. To assess whether the dewlap pattern is a Mendelian or quantitative trait, we generated mean-variance graphs for the proportion of orange area from wild-caught male parents and captive-bred offspring. To answer whether the extent of the orange area is determined by a dominant allele, we used a graph of means and variance as well as likelihood-ratio tests.

Housing and husbandry

Adult lizards from the two parental populations were collected along the Rio Baní in the southern Dominican Republic. The southern population consisted of individuals with largely or completely pale, yellow dewlaps found in dry forests that have been recognized as *A. d. ravitergum*. The northern population consisted of individuals with a distinct orange blush at the base of their otherwise pale, yellow dewlaps found in more mesic environments that have been recognized as the subspecies *A. d. ignigularis*. The two populations are genetically and phenotypically distinct but meet along a hybrid zone where some gene flow occurs (MacGuigan et al., 2017; Ng et al., 2013; Ng et al., 2016).

After transportation to the United States, lizards were housed in an Association for Assessment and Accreditation of Laboratory Animal Care certified animal care facilities under Institutional Animal Care and Use Committee-approved protocols at the University of Rochester and the University of Kansas. Husbandry was modified from Sanger et al. (2008) with lizard enclosures divided into breeding groups made up of one male and two females. All breeding groups were fed 3/5″ *Acheta* crickets ad libitum three times a week and misted with water at least twice daily. During the nonbreeding season, room temperature was maintained at 28.3 °C with a light cycle of 10 hr light/14 hr dark. During the breeding season (April–November), room temperature

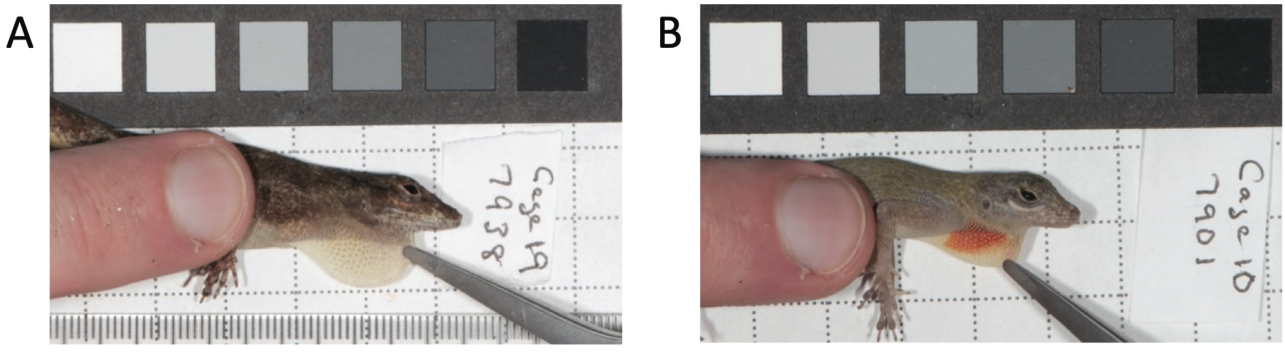

Figure 1. (A) Image of solid yellow dewlap, *Anolis distichus ravitergum*, from the parental population. (B) Image of blush orange dewlap, *Anolis distichus ignigularis,* from the parental population.

was maintained at 29.4 °C and the light cycle was 14 hr light/10 hr darkness.

We provided each breeding group with a location for egg-laying during the breeding season. The egg-laying sites were made from 32-ounce yogurt containers with 1-inch diameter holes for lizard entry cut into the side and lid and half filled with damp vermiculite (2:1 ratio of vermiculite:DI water). We checked for eggs every two weeks and transferred all calcified eggs into their own Solo deli cups (about 11.5 cm diameter) that were 3/4 filled with moist vermiculite. The deli cups had 1 mm prepunched holes near the top for ventilation and were placed next to a small fan to further facilitate airflow. Eggs were incubated at 29.4 °C and checked daily for new hatchlings. Eggs hatched approximately one month after being laid, after which point the hatchlings were toe-clipped for individual identification and transferred to acrylic enclosures. Except for feeding them smaller pinhead crickets, hatchlings were kept under the same conditions as adults.

We first conducted experimental crosses involving the wild-caught representatives of the two parental populations differing in dewlap color and pattern. For convenience, we will refer to the southern population representing *A. d. ravitergum* with mostly solid yellow dewlaps as "P1" or "solid," and the northern population representing *A. d. ignigularis* with a basal blush of orange pattern as "P2" or "blush." The F1 individuals resulting from the initial crosses were then crossed with other F1s to produce F2s or lab-reared individuals representing one of the two pure parental populations to produce backcross progeny (BC) (Figure 2A and B).

Quantification of dewlap pattern

Dewlaps were photographed by placing live adult males on their left side over white paper printed with a 1 cm^2 grid. Dewlaps were then extended parallel to the grid paper by grasping the hyoid with forceps and gently pulling before taking high-resolution digital photographs with a Nikon D90 digital SLR. A Macbeth ColorChecker (X-rite mini) and ruler were included in each photograph for brightness and size standardization, respectively.

We standardized and quantified patterns across all images in ImageJ using a three-step procedure (Schneider et al., 2012). First, we used the white standards on the ColorChecker card to standardize for brightness with the "chart white balance" macro (Vander Haeghen & Naeyaert, 2006). Second, we used the ruler in each picture to standardize the size. Third, we used the polygon tool to select the dewlap, avoiding the forceps, and converted the nondewlap portion of the image to a solid white background. We then saved processed dewlap images in TIFF format to prevent the white background from being included in dewlap area quantification.

To quantify the extent of orange while accounting for differences in dewlap size, we calculated the percent orange area of each dewlap. First, we used the "auto threshold" feature within ImageJ, which identified a threshold value for each image that best-distinguished orange from yellow regions of the dewlap. Then, we averaged the auto-threshold values across all samples, which we then applied as a global threshold to all images to separate the orange from yellow pixels. Next, we used the micaToolbox (Troscianko & Stevens, 2015) to calculate the orange area for each dewlap based on the global threshold value. The orange area for each dewlap was then divided by the total dewlap area (not including the forceps) to get the proportion of the orange dewlap area. Finally, we normalized the percentage data for downstream analyses using a standard arcsine square root transformation (Supplemental Table S1).

Is the dewlap pattern a quantitative or a Mendelian trait?

To test whether the inheritance of dewlap pattern is consistent with the expectations for a quantitative trait, we first calculated the mean and variance of percent orange area for each generation. The expected distribution of means and variances across generations are well characterized for quantitative traits (Lynch & Walsh, 1998): parental populations fixed for distinct pattern alleles are expected to have large differences in mean phenotypes and low variance within populations; first-generation hybrids resulting from crossing the two parental populations (F1s) are

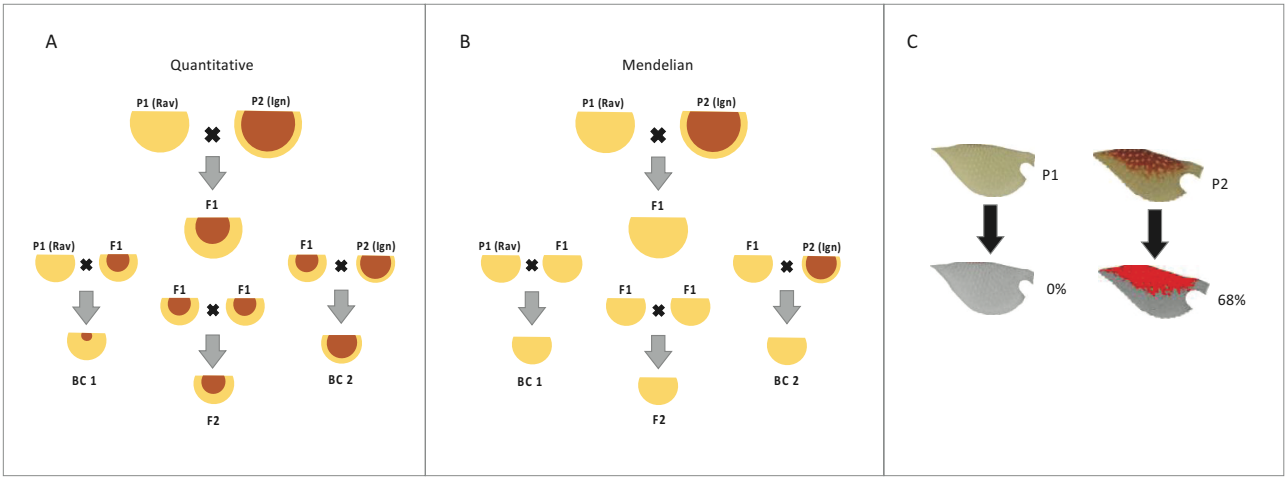

Figure 2. (A) Hypothetical husbandry crossing scheme with the expected dewlap phenotype for each generation under a quantitative hypothesis; (B) hypothetical husbandry crossing scheme with the expected dewlap phenotype for each generation under a Mendelian hypothesis; (C) example of two dewlaps before and after pattern quantification.

expected to have intermediate mean phenotypes and low variance; second-generation F2 hybrids resulting from crossing F1 individuals are expected to have intermediate mean phenotypes and high variance due to recombination of parental alleles; and backcross individuals resulting from crossing an F1 hybrid to one of the parental populations are expected to have phenotypes and variances that are intermediate between F2s and each parental phenotype. When the expected means and variances of quantitative traits are graphed, they produce a classic triangular pattern (Figure 3A). We predicted that if the percent of orange is inherited as a Mendelian trait, the observed means and variances would deviate from this expected triangular pattern. Specifically, the F1, F2, and BC generations would be biased toward one parent.

Because our low sample size in the F2 and backcross generations could potentially bias the variance, we also graphed the mean proportion orange area of each generation to the proportion of recessive alleles. We then did a one-way ANOVA to test if the means of any generations were significantly different and used a Bartlett test to check for differences in variance between groups. For this analysis, quantitative traits are expected to produce a linear pattern (Lynch & Walsh, 1998; Figure 3C). We predicted that if the percent of orange is inherited as a Mendelian trait, the observed means would be the same, except for the recessive parent mean, making a horizontal line with a peak at one end (Figure 3D).

Is dewlap pattern in *A. distichus* determined by a dominant allele?

Given that our data did not match the expectations of a quantitative trait (see *Results* section), we tested whether the dewlap pattern is determined by a dominant inheritance pattern using likelihood-ratio tests. To do so, we created five models that varied in their type of inheritance (simple, codominant, or additive), number of loci, and whether parents belong to separate populations or to a single population with ongoing gene flow (Table 1). We then calculated the maximum likelihood of each model and determined which model best fits the data. To avoid potential biases of small sample size, only data from the parental populations and F1 generation were used. The models we implemented were set up as follows.

Model 1: One locus, two alleles, simple dominance, fixed parents

Because solid dewlaps are more common than blush dewlaps in progeny from a solid × blush cross, we first hypothesized that the solid morph would be completely dominant over the blush phenotype. We classified individuals with less than 25% orange dewlap area as "solid" and those with above 25% orange dewlap area as "blush" (Figure 2C). This model assumed that the two wild populations from which parents were collected were fixed (i.e., homozygous) for either allele associated with the pattern.

Model 2: One locus, two alleles, simple dominance, parents under Hardy–Weinberg equilibrium

We also tested for the possibility that the parental populations are not fixed for either allele, given the evidence for gene flow between solid and blush morphs (Ng et al., 2016). Therefore, we assume in Model 2 that the parental phenotypes belong to a single population under Hardy–Weinberg equilibrium. We used the phenotypes of the parental populations to calculate the expected allele frequency, which we then used to estimate the expected genotype frequency of F1s.

Model 3: One locus, two alleles, codominance, fixed parents

Given that the blush pattern found in hybrids is similar to the pattern found in the blush-bearing parental population, but smaller in area, we hypothesized that the area of the orange blush could be determined by a locus with codominant alleles. The other assumptions of this model are similar to model 1; however, to test the hypothesis of codominance, we included three categories by classifying individuals possessing 5%–25% orange area as heterozygotes with both blush

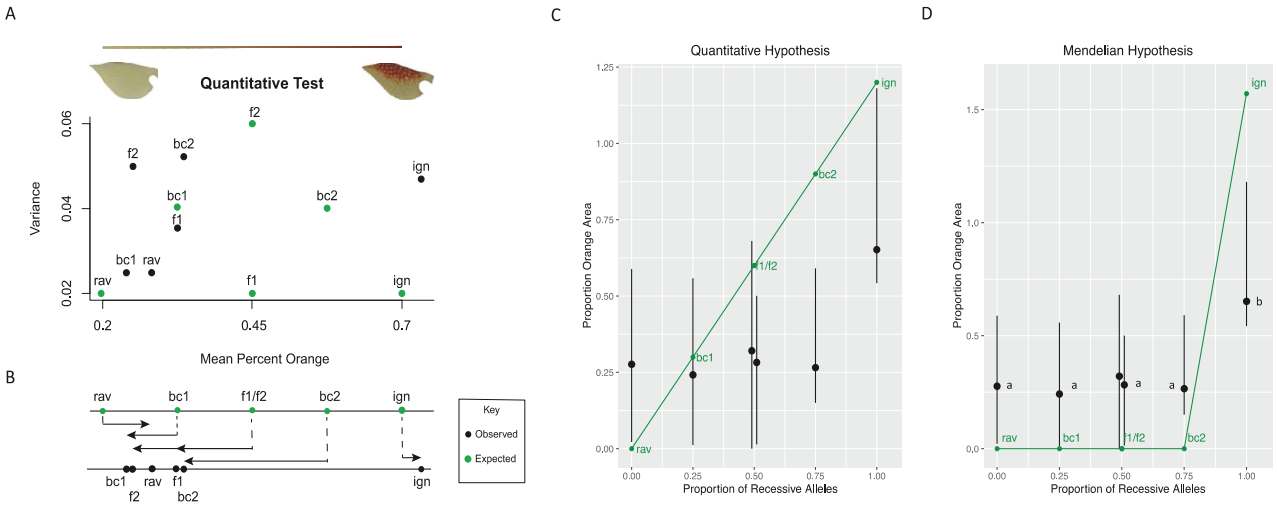

Figure 3. (A) Graph of observed and expected means vs. variance for each generation under the quantitative hypothesis; (B) the horizontal deviation of each generation from expected means; (C) graph of transformed (arcsine square root) observed means and expected means vs. proportion of recessive alleles under a quantitative hypothesis; (D) graph of transformed (arcsine square root) observed means and expected means vs. proportion of recessive alleles under a Mendelian hypothesis (letters designate groups that are significantly different, one-way ANOVA, $p < 0.01$).

Table 1. Number of progeny with expected and observed phenotype under each model's assumptions and results of maximum likelihood test for each model.

	Model 1		Model 2		Model 3			Model 4			Model 5				
Type	Simple dom, fixed parents		Simple dom, parents HWE		Codominance, fixed parents			Codominance, parents HWE			2 Loci, additive, fixed parents				
Phenotype	Yellow	Orange	Yellow	Orange	Yellow	Yellow/ Orange	Orange	Yellow	Yellow/ Orange	Orange	0% Orange	15% orange	30% orange	45% orange	60% orange
Expected	16	0	11	5	0	16	0	0	11	5	0	0	16	0	0
Observed	15	1	15	1	1	14	1	1	14	1	1	12	3	0	0
Probability	1	0	0.007	0.284	0	1	0	0.284	0.009	0	0	0	1	1	1
Likelihood	0		0.002		0			0			0				

and solid alleles, individuals with less than 5% orange area as homozygous for the partially dominant solid yellow alleles, and individuals with more than 25% orange area as homozygous for the recessive blush orange alleles.

Model 4: One locus, two alleles, codominance, parents HWE

This model tests the same hypothesis as model 3, but with the assumption that parents are under HWE rather than fixed for one allele or the other. We took the same approach to calculate expected genotypes as described in model 2.

Model 5: Two loci, two alleles, additive, fixed parents.

In model 5, we tested the hypothesis of an additive genetic architecture where recessive orange alleles at each of two separate loci add 15% orange area. Consequently, we classified individuals into five possible phenotypes, with 0%–15% orange area resulting from homozygosity of the dominant yellow alleles at both loci, 15% and 30% orange area resulting from one of the two loci being heterozygous while the other was homozygous dominant, 30%–45% orange area resulting from possession of two recessive alleles total across both loci, 45%–60% orange area resulting from three recessive alleles, and 60% or more orange area resulting from all four alleles being recessive.

Results and discussion

Our results support the hypothesis that the proportion of orange in the dewlaps of *A. distichus* is largely Mendelian—determined by a single locus of large effect with complete dominance. We rejected the alternative hypothesis of it being a quantitative trait because all specimens—including F1s, F2s, and BCs—have less orange in their dewlaps than expected (Figure 3A and B). In our first graphical analysis comparing means and variances in the proportion of orange on dewlaps across generations, all progeny are shifted toward P1, the population with solid yellow dewlaps (Figure 3A). This deviation from the expected triangular pattern of a quantitative trait suggests the dominance of the P1 solid yellow pattern over the P2 blush orange pattern. The variances of the second-generation (BC1 and F2) progeny are also lower than expected for a quantitative trait, further supporting the hypothesis of one or few loci. In our second graphical analysis comparing means to the proportion of recessive alleles, we also recovered deviation from the expectation of a quantitative trait (Figure 3C and D); instead of a linear pattern, all genotypes with a potential dominant allele have similar means at about 0.25 proportion orange, while the homozygous recessive genotype, 1.2 proportion orange (transformed), is significantly different from the other groups (one-way ANOVA, $p < 0.01$). Similar graphical deviations in a study that investigated color morphs of cichlid fish similarly interpreted the shifts of F1, F2, and back cross generations towards a parent as evidence for the dominance of one allele over the other (Magalhaes & Seehausen, 2010).

Another possible explanation for the patterns we observed in our graphical analyses is sex chromosome linkage. However, testing this hypothesis is challenging because we could not phenotype females, which do not possess dewlaps in this species. While some prior studies have used testosterone-treated females to overcome this issue (Cox et al., 2015; Rankin et al., 2016), we instead relied on reciprocal crosses. The fact that dewlap patterns in each of our two reciprocal hybrid crosses did not differ strongly suggests that dominance at an autosomal locus is a more likely explanation of the pattern than sex linkage.

After using our graphical analyses to reject a quantitative model, we tested if a model involving complete dominance fits our data better than models involving codominance or additive inheritance. We rejected three models involving parental populations being fixed for one of two alternative alleles (models 1, 3, and 5) because we recovered one individual from a first-generation hybrid cross that possessed the blush phenotype (Table 1). Since such progeny are impossible with fixed parental populations, these models all produced likelihood scores of zero. We also rejected the codominant model (model 4) because we recovered progeny with solid yellow dewlaps from hybrid crosses, which would not be expected under codominance. However, model 2 involving simple dominance with ongoing gene flow between the parental populations was recovered as the best fit to our data. We also calculated the likelihoods for each model after excluding the single F1 progeny with a blush phenotype, which recovered model 1 as the best fit. Since the only difference in model 1 and model 2 was relative to the presence or absence of HWE in the parental populations, these analyses further supported our conclusion that most of the variance in dewlap color pattern is due to a single dominant locus.

We further confirmed our graphical results by testing an additive quantitative model (model 5). Like the other models, we excluded the F2 and BC generations because the relatively small sizes of these generations could impact phenotype means and variances. Under this model, all F1 progeny would be expected to have the same intermediate pattern, but 1

individual was solid yellow, 12 were blush orange, and only 3 had an intermediate blush phenotype (~30% orange area), resulting in a likelihood of 0 and rejection of the quantitative model (Table 1).

These results are also in agreement with a previous study examining the development of dewlap pattern in response to carotenoid intake in *A. d. ignigularius*, the blush morph in this study, and *Anolis d. properus*, a second *A. distichus* population found in the eastern part of Hispaniola with a solid cream-colored dewlap (Ng et al., 2013). As in our study, the authors found evidence that a dominant Mendelian locus is responsible for the area of the orange spot in hybrids between *A. d. ignigularis* and *A. d. properus*. However, unlike our findings, the blush orange pattern in *A. d. ignigularius* was dominant over the cream-colored dewlaps of *A. d. properus*. We propose three hypotheses to explain these results. First, we hypothesize that the extent of the orange area in *Anolis distichus* could be determined by a single locus, which regulates the synthesis of orange-producing pigments. Therefore, we would predict that the differences in dominance between orange and yellow (or cream) phenotypes are the consequence of multiple segregating alleles for the same locus across the population. Second, we hypothesize that the cream/yellow colors from *A. d. properus* and *A. d. ravitergum*, respectively, are determined by distinct cellular processes. Therefore, we would predict that the cream color is a consequence of a lack of xanthophores or melanophores, whereas the yellow color would be determined by a thin layer of xanthophores distributed across the dewlap of *Anolis distichus*. In this scenario, the differences in "dominance" seen in *A. d. properus* would instead be due to a presence or absence of orange-pigment-producing xanthophores between *A. d. ignigularis* and *A. d. ravitergum*. This would imply convergent evolution of the yellow/cream dewlap in the xeric areas of Hispaniola. Lastly, as the eastern *A. d. ignigularis* are also geographically close to the red-dewlap *A. distichus dominicensis*, we hypothesize that the allele responsible for the orange blush in *A. d. ignigularis* at the eastern and western part of the range, close to *A. d. ravitergum* and *A. d. properus*, respectively, could be determined by distinct loci, which themselves have distinct dominance dynamics with the loci or alleles responsible for the yellow or cream colors of the *A. d. properus* and *A. d. ravitergum* populations. This hypothesis, like the second hypothesis, also invokes one or more events of convergent selection for color across mesic and xeric environments within *Anolis distichus*. Given that thirteen differentially expressed color and pattern genes, as well as two potential orange-pigment-producing pathways, have been identified in *A. distichus* and that loci responsible for differences in color within *A. distichus* is likely neo-sex linked (de Mello et al., 2021), the *Anolis distichus* species complex is emerging as an ideal system for studying the dynamics between strong directional selection and gene flow in establishing geographically separated phenotypically distinct populations across a continuous range.

Furthermore, understanding the genetic architecture of dewlap patterns can help us better predict how dewlap patterns might change in response to strong selection pressures. For example, this polymorphism likely evolved by divergent selection across a heterogeneous landscape with differing climates. This correlation between dewlap patterns and environmental conditions (Fitch & Hillis, 1984; Leal & Fleishman, 2002, 2004; Ng et al., 2013) suggests that a changing climate could shift dewlap patterns. Since our results point toward solid patterns being dominant over the blush pattern in *A. distichus*, shifts to a drier climate could lead to directional selection towards the solid dewlap pattern as blush morphs are less fit. Not only would this impact parental populations, but the solid dewlaps would likely increase in frequency in hybrid populations as well. This in turn could impact reproductive isolation barriers and cause more back-crossing between the hybrids and *A. d. ravitergum* population. On the other hand, a shift toward wetter environments could lead to detrimental outcomes for individuals with solid dewlaps until the recessive but more fit blush pattern increases in frequency. Like other Mendelian-based color and pattern phenotypes, dewlap pattern in *A. distichus* could evolve quickly in the face of strong selection.

In conclusion, the results of both our graphical analyses and our maximum likelihood tests show support for the hypothesis that the dewlap pattern in *A. distichus* is Mendelian with complete dominance of the solid pattern over the blush. This lays the foundation for future research to examine how similar dewlap patterns have evolved and how they may continue to change in response to strong selective pressures.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Data is provided in the supplemental table and code for the ANOVA analysis, and graphs are available on dryad. https://doi.org/10.5061/dryad.qv9s4mwnm

Author contributions

Conceptualization: R.E.G. and A.J.G. Methodology: A.B. and A.J.G. Investigation: A.B., P.M., and A.J.G. Visualization: A.B. Supervision: R.E.G. and P.M. Writing—original draft: A.B. Writing—review & editing: A.B., P.M., R.E.G., and A.J.G.

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