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ASSESSING THE EFFECTS OF GENETIC DIVERGENCE AND MORPHOLOGY ON *ANOLIS* LIZARD MATING

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Abstract. The brown anole (*Anolis sagrei*) is a widespread neotropical lizard found on many islands in the West Indies as well as the coast of Central America. Across their range, brown anole populations show extensive ecomorphological trait variation and substantial genetic divergence. It is unclear, however, whether this genetic and morphological divergence is indicative of reproductive isolation between populations. We investigated variation in mating behavior across populations by analyzing 4 h of video for each of 234 captive male-female pairs encompassing all 36 possible pairings from six sampled islands. For each pair of individuals, we tested for an association between the occurrence of mating, morphological traits, and genetic relatedness of their populations. We found no support for the hypotheses of ecological divergence, nonecological divergence, or both ecological and nonecological divergence driving premating reproductive isolation in *A. sagrei*. We did find that males with relatively short heads tend to mate more quickly and hypothesize potential explanations that warrant future investigation.

Key words: mating; behavior; reproductive isolation; speciation; evolution; *Anolis*

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INTRODUCTION

The iguanid lizard genus *Anolis* has been extensively studied because of its hyperdiversity (Losos and Thorpe, 2004; Pincheira-Donoso et al., 2013; Uetz and Stylianou, 2018) and exceptional repeated convergent evolution (Williams, 1972). However, despite the volume of anole literature, intrinsic mechanisms of speciation and reproductive isolation in anoles remain largely unknown (Losos, 2009; Losos and Schneider, 2009). These avenues of study are of particular interest given that *Anolis* is

so species-rich, and many speciation events must therefore have occurred to produce today's diversity (Losos and Thorpe, 2004).

Anoles rely on visual signals to recognize conspecifics. One of the most conspicuous of these signals is the dewlap, an expandable flap of throat skin that is often extended in tandem with species-specific head-bobbing patterns. These species recognition signals may dissuade potential matings with heterospecifics; their effectiveness is indicated by the paucity of observations of interspecific matings and the rarity of interspecific hybridization (Losos, 2009, but see Jezkova et al., 2013).

Premating isolation—barriers to mating between individuals—can take a variety of forms (reviewed in Butlin, 2011). One such form is ethological, which entails behavioral differences that preclude mating between individuals from different populations. One index of such isolation is mating latency (i.e., time between introduction of female to male and copulation; Matute, 2014; Arthur and Dyer, 2015; Giesen et al., 2017). This measure of isolation can be extended to include whether individuals mate at all. However, caution must be exercised in the interpretation of variation in the occurrence and latency of mating, as other traits unrelated to divergence can promote or hinder copulation. In other taxa, these include success in intrasexual combat (Parker, 2009) and the presence of rivals during development (Taylor et al., 2013).

Not only is the study of premating isolation between populations relatively new ground in *Anolis*, it is also generally rare in the squamate literature. Two studies have found a negative correlation between genetic distance and premating isolation. In sea snakes (genus *Laticauda*), the probability of courtship decreased with genetic distance; additionally, males from a smaller species were more likely to court females from a larger one than vice versa (Shine et al., 2002). In western skinks (*Plestiodon skiltonianus*), in which the clades studied were morphologically and genetically divergent, the probability of mating was negatively related to genetic distance. Moreover, morphologically similar individuals from divergent clades were more likely to mate than morphologically dissimilar ones (Richmond and Jockusch, 2007).

Two genetic mechanisms have been proposed to explain the evolution of reproductive isolation: genome-wide divergence and ecological speciation. The former, which is the classic view of the evolution of reproductive isolation (e.g., Dobzhansky, 1937; Coyne and Orr, 1989) posits that isolated populations' genetic divergence over time can, by chance, lead to genetic changes that cause reproductive isolation; thus, more divergent populations are more likely to be reproductively isolated. Coyne and Orr (1989) first showed a positive correlation between genetic divergence and reproductive isolation by examining the literature on *Drosophila*. Ecological speciation is an alternative, nonexclusive theory to explain differential mating behavior with a genetic component. Specifically, this theory suggests that as populations adapt to different conditions, some of the adaptive changes will have the incidental effect of increasing reproductive isolation (Rundle and Nosil, 2005).

We investigated premating isolation in the brown anole (*Anolis sagrei*), which is widely distributed across the West Indies, including populations throughout the Bahamas and on several of the Cayman Islands, as well as elsewhere. This broad range of isolated island populations presents ideal candidates for incipient speciation. Previous analyses have revealed substantial genetic divergence among brown anole populations (Lieb et al., 1983; Kolbe et al., 2004; Marnocha et al.,
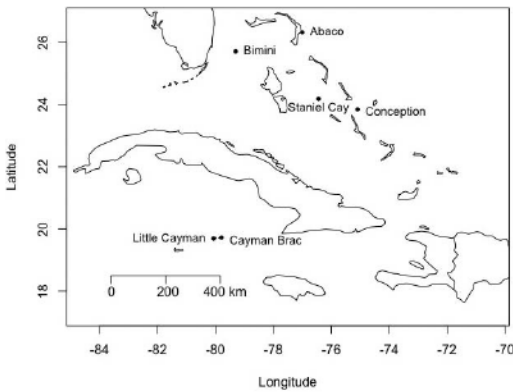
Figure 1. The six sites from which *Anolis sagrei* were collected. Figure created using the package maps (Becker et al., 2018) in R (R Core Team, 2018).

2011; Reynolds et al., 2019). If these populations are differentiated in traits important for conspecific recognition, then premating isolation may have evolved between pairs of brown anole populations. Given that populations of *A. sagrei* have diverged genetically, we can assess the extent of morphological divergence and test the relative importance of morphology and genetic distance in driving the evolution of premating isolation.

To investigate whether genetic or morphological variation is associated with brown anole premating isolation, we collected *A. sagrei* from its native range at six sites across the West Indies (Fig. 1). We brought these anoles to a breeding facility and established a replicated, full diallel cross—all 36 possible pairwise crosses of males and females from these six populations (including intrapopulation pairings and counting both possible pairings of males and females in each between-population cross). We tested for the presence of premating isolation, genetic divergence, and morphological divergence. We then assessed the predictive power of morphology and genetic divergence in estimating premating isolation.

METHODS

Lizard collection and mating behavior

Adult *A. sagrei* were collected in the summer of 2016 from the Bahamian islands of Abaco, Bimini, Conception, and Staniel Cay and from Cayman Brac and Little Cayman in the Cayman Islands. Animals were collected from coppice forest—a low, closed-canopy forest habitat type found throughout the West Indies—on each island. The lizards were kept in a facility described in de Meyer et al. (2019) and reared according to the protocol therein. Animals were kept in 15-L enclosures, provided water twice daily, and fed crickets dusted with a multivitamin supplement three times weekly. Female *A. sagrei* can store sperm from previous matings and lay unfertilized eggs in the absence of fertilization, so females were kept isolated from the males until < 1% of eggs were fertile.

At that point, males were introduced to a cage containing soil, wooden dowels for perching, plastic leaves, and a yogurt cup with a hole cut in it filled with vermiculite, in which females preferentially lay their eggs (de Meyer et al., 2019). After males had been allowed to acclimate to their cages for more than 1 mo, one female was added to each cage, and a GoPro camera facing the cage began filming. This occurred in the mid-morning, during the peak activity time for the species (Baeckens et al., 2016). We observed video of each trial for the first 4 h or until mating occurred (whichever happened first). Mating, defined as cloacal alignment, occurs when the male is on the female's dorsum and curves his tail underneath hers, toward her ventrum. Videos were shot for 24 cages at a time, and filming was performed 10 separate times as new breeding groups were established. Six videos from the first taping were immediately discarded

because of poor camera angle, resulting in a data set with 234 replicates. The plastic leaves were no longer placed in cages after the first three filming sessions because they obscured portions of the video frame.

For some trials ($n = 50$), we were unable to determine whether mating had occurred. If the male and female disappeared behind the cup together, were obscured by the ground or leaves, or were otherwise not visible, they might have mated during that time. Because brown anole matings last at least 1 min (Tokarz, 1999), we concluded that mating did not occur in periods of nonvisibility shorter than 1 min.

For other trials ($n = 8$), a period of nonvisibility greater than 1 min occurred, but mating definitively occurred later in the video. Male *A. sagrei* exhibit a refractory period in their mating habits, in which they do not mate again immediately after mating (Tokarz, 1988). We concluded it would be highly unlikely that these eight trials had two matings, and thus we logged their latency to mate as the time at which mating was observed.

Genetic divergence

The island populations we sampled have previously been shown to be reciprocally monophyletic (van de Schoot, 2016). To estimate population-level genetic divergence, we used sequences of the mitochondrial gene NADH dehydrogenase subunit 2 (ND2) in five individuals from each of the six populations from van de Schoot (2016). We calculated uncorrected p-distances, or the proportion of bases that differ, between each of the 15 interisland pairings using the software program MEGA (Kumar et al., 2018).

To assess whether geographically distant populations have significantly greater ND2 divergence than geographically close ones, we tested for a correlation between p-distance and geographic distance for the 15 pairings using the Pearson product-moment correlation ("cor.test" function in R). To further test this hypothesis, we also used a Mantel test from the package vegan (Oksanen et al., 2019), which is often used to compare genomic and geographic variation between sites in population genetics; although it has been criticized, it remains robust if properly applied (Diniz-Filho et al., 2013). The classic interpretation of a significant Mantel test comparing geographic and genomic divergence is that isolation by distance (IBD) is occurring, although problems exist with this assumption (Diniz-Filho et al., 2013). Under IBD, even if populations are geographically connected, genetic distances may accrue such that physically distant populations become genetically isolated (Wright, 1943).

Morphological traits

The majority of individuals in the study were ultimately euthanized and preserved in 95% ethanol and were therefore available for x-ray imaging. Specimens were scanned with the INSPEX 20i digital x-ray system in the Museum of Comparative Zoology's Digital Imaging Facility. Heads and limbs were taped to the surface of the x-ray plate to control for foreshortening effects; if a limb was not orthogonal to the x-rays and in the same plane as the scale bar, the captured image can misrepresent relative sizes between individuals. X-ray images were traced in ImageJ (Schneider et al., 2012).

We measured a suite of morphological characters from these x-rays. Given the importance of body size in previous studies of squamate premating isolation, we used snout–vent length (SVL) as a proxy for body size, and measured it from the tip of the snout to the first caudal vertebra (Fig. 2A).
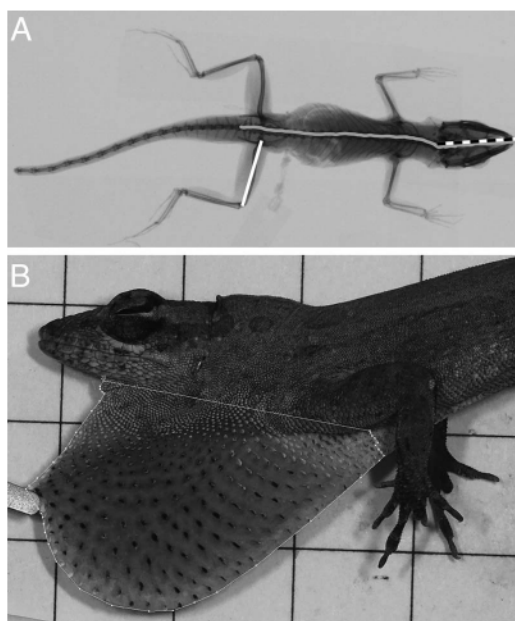
Figure 2. Morphological measurements. (A) X-ray measurements. Snout–vent length is the solid gray line, head length is the dashed black and white line, and femur length is the solid white line. (B) Sample dewlap and its tracing, following the protocol of Ingram et al. (2016).

Because male head size can be sexually selected in reptiles (Olsson and Madsen, 1998), this measurement was included, as was female head size for comparative purposes. Heads were measured longitudinally from the tip of the snout to the juncture between the skull and first vertebra (Fig. 2A). Given the importance of limb size to ecomorphological divergence (Losos, 2009), femur length was included as a proxy for overall limb length (Fig. 2A).

Because SVLs were traced digitally with a freehand line, a mean of three measurements of each individual's SVL was used for analysis. These three SVL measurements were highly repeatable: the mean difference between the largest and the smallest mea-surement of each SVL was 0.28% of the mean SVL for that lizard, and the maximum difference between the largest and the smallest SVL was 1.8% of the mean SVL for that individual. Because of foreshortening effects, the longest femur in any individual was determined to be less foreshortened, and its length was used for analysis.

We photographed male dewlaps for a subset of each population with a tripod-mounted Olympus EM-5mkII digital camera with a 60-mm macro lens. To determine surface area, dewlaps were traced from these photographs with the ImageJ (Schneider et al., 2012) plugin ObjectJ (Vischer and Nastase, 2009) according to the protocol of Ingram et al. (2016) (Fig. 2B).

Not all traits could be measured for all individuals; the number of measurements of each trait, categorized by population, is in Supplementary Table 1. To examine sexual dimorphism, we used one-way analyses of variance (ANOVAs) to test for statistically significant differences between males and females in SVL, absolute head length, and absolute femur length. We also used one-way analyses of covariance (ANCOVAs) to test for differences in head length and femur length when controlling for SVL. All AN-OVAs and ANCOVAs in this study were conducted with the "Anova" function from the R package "car" (Fox and Weisberg, 2019).

We were also interested in examining morphological differences among populations for males and females separately. To examine trait variance among populations, we used ANOVAs (for SVL) and ANCOVAs (for heads, femurs, and dewlaps, controlling for SVL). For traits that significantly differed among populations, we used Tukey's honest significant difference test to conduct pairwise population-level comparisons and examine which populations significantly differ in the traits compared.

Predicting mating occurrence from traits and genetic divergence

We were interested in assessing whether any pairings of populations were significantly more or less likely to mate than any others. We tested this in two ways. First, at a broad scale, we used the chi-square test to examine whether any of the 36 pairings of populations showed a significantly different prevalence of mating during the trials. To investigate preferential mating more closely, a Fisher's exact test was used to compare the mating success rate for each population–sex combination between inter- and intra-island pairings. Because 12 tests were conducted, one for each population–sex combination (e.g., comparing whether Abaco males were more likely to mate with Abaco females than non-Abaco females), we used a sequential Bonferroni correction to control for multiple comparisons.

Beyond testing specific pairings of populations of origin as predictors of latency to mate, we compared generalized linear models (GLMs) predicting the occurrence of mating in 4 h by Akaike's information criterion (AIC) (Akaike, 1974). Because this response variable is binary, the GLMs are binomial and the regression is logistic. We used both the genetic distance between those populations and individual morphological characteristics as potential predictors of mating presence/absence in our models. For the latter, we omitted dewlaps because of the low number measured and considered body size–corrected head and femur length. This correction was conducted separately for each trait within each sex by calculating residuals from a regression against SVL. We visually inspected these residuals and found them to be approximately normal. We narrowed our data set to only contain trials for which male and female head, femur, and SVL were measured, so as not to skew model selection by data set size ($n = 134$, with 49 matings). With this subset, we wrote 57 models containing various combinations of traits, relevant trait interactions, and p-distances (Supplementary Table 2).

Because logistic regression predicts the occurrence of mating during the duration of the videos, it discards information about the exact latency to mate. To investigate predictors of specific mating latency, we used a linear model to predict the time of mating in the trials in which mating occurred, using the best predictor(s) of the presence or absence of mating from the lowest-AIC GLM. We also applied the best predictor(s) in three more logistic regressions to the full data set, with a response variable of the presence of mating in the first hour, the first 2 h, and the first 3 h, respectively, to approximate at which timepoint into the videos any observed trends occurred.

RESULTS

Lizards in the experiment performed behaviors they exhibit in nature. When courtship occurred, males were observed extending their dewlaps and push-upping in view of the female—typical anole behaviors (Losos, 2009). Female receptiveness to this courtship varied, as did male reactions in turn. In some instances, males and females appeared equally willing to initiate mating, with females allowing male approach following this signaling. When females did not appear receptive to mating, sometimes males initiated it nonetheless through pinning the females by biting their neck, whereas in other cases, males ceased courtship attempts.

Of the 184 pairings in which mating conclusively did or did not occur during video recording, 67 pairs mated (36.4%). The mean latency to mate among these 67 replicates was 99.88 min, with the minimum latency being 5.42 min. Supplementary Table
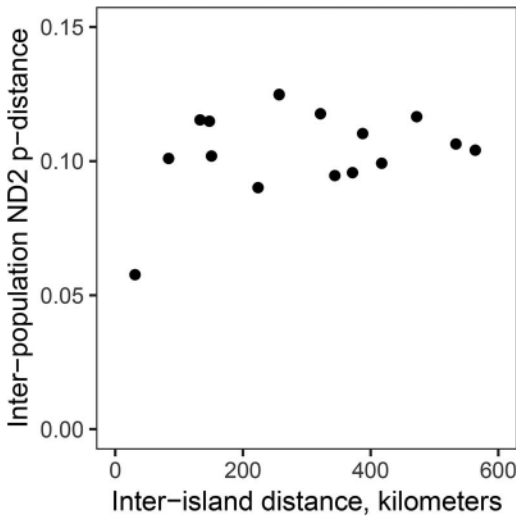
Figure 3. Scatterplot depicting the pairwise geographic distance and uncorrected ND2 *p*-distance for each of the 15 interpopulation pairings.

3 displays the mean mating latency, frequency of mating, and number of replicates of each of the 36 interisland pairings.

We found substantial genetic divergence between brown anole populations, but no relationship between ND2 *p*-distance and geographic distance (Pearson product–moment correlation $P = 0.240$, Mantel test $P = 0.082$; Fig. 3).

With regard to sexual dimorphism, males had longer SVLs, heads, and femurs than females (one-way ANOVAs, $P < 0.0001$ for each), and when controlling for SVL, males had significantly larger heads (one-way ANCOVA, difference in intercepts, $P = 0.011$) and longer femurs (one-way ANCOVA, difference in intercepts, $P < 0.0001$) than females. We also found a series of significant morphological differences between populations within sexes (Fig. 4).

We found no evidence for differences in mating incidence among the 36 pairings of sexes and populations ($\chi^2 = 33.039$, df $= 35$, $P = 0.563$). Moreover, mating was not more likely between individuals of the same population than individuals from different populations (uncorrected *P*-values $0.018 \leq P \leq 1.00$, none of which are significant under sequential Bonferroni-corrected Fisher's exact tests). Figure 5 shows the relationship between ND2 divergence and latency to mate. Of the pairings that mated, genetic distance and time to mating were not correlated when using mean values for each of the 36 pairwise population comparisons (Pearson product–moment correlation, $P = 0.155$) (Supplementary Table 3). This correlation was calculated from 30 of 36 population pairings, however, because the remaining six had no pairs that mated (Supplementary Table 3).

Of the 57 linear models using combinations of individual morphological traits, relevant trait interactions, and population-level ND2 *p*-distances (Supplementary Table 2), the model with the lowest AIC, and thus the best fit, incorporated only body size–corrected male head size. This model estimated the coefficient of body size–corrected male head size at -1.255 ($P = 0.003$; AIC $= 170.13$); that is, males with small heads relative to body size were more likely to mate in the videos than those with larger heads relative to body size.

We considered five additional models because their AIC was less than two greater than that of the best model (Burnham and Anderson, 2002). These models are listed in Table 1. In all, body size–corrected male head size has a negative coefficient and is significant at the $P = 0.05$ level, whereas none of the other predictors are significant at that level.

As mentioned above, these GLMs are based on the subset of trials for which all morphological traits were quantified, so as not to influence model selection by data set size. However, applying the lowest AIC model to the full data set leads to a similarly significant estimate of the coefficient of body
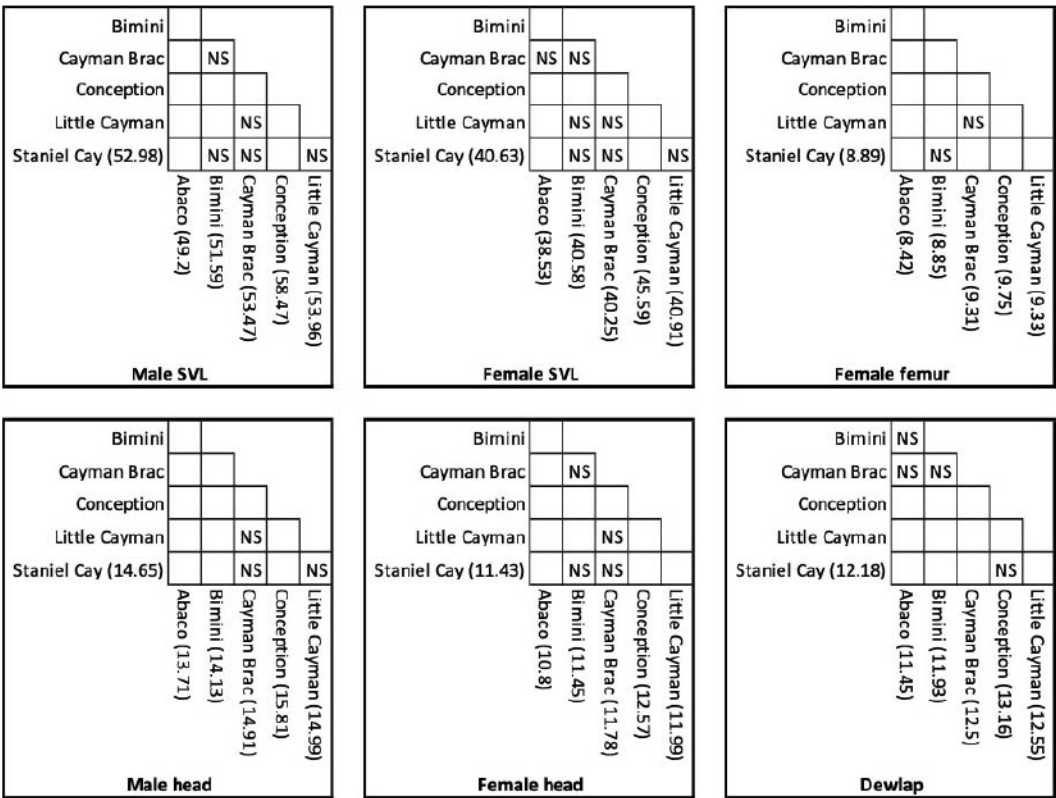
Figure 4. Results of post hoc Tukey's honest significant difference tests of differences in traits between populations after ANOVAs (male SVL; female SVL) or ANCOVAs with SVL of the sex as a covariate (female femur; male head; female head; dewlap). NS indicates a nonsignificant Tukey's honest significant difference test between populations. Mean values of each trait for each population are in parentheses (mm, except for the dewlap, which is cm^2). Male femur is not depicted because the ANCOVA was not significant (difference in intercepts, $P = 0.0596$).

size–corrected male head size, -1.127 ($P = 0.002$), again implying males that are smaller-headed relative to body size are likelier to mate. Figure 6 visually explores the relationship between male SVL, absolute male head size, and the probability of mating in 4 h.

Of the pairs that mated, head size was not a significant predictor of latency ($P = 0.228$). Additionally, male head length residuals do not significantly predict the presence of mating in the first hour or first 2 h ($P = 0.598$ and $P = 0.113$, respectively), but they

do predict the presence of mating in the first 3 h ($P = 0.002$, coefficient estimate $= -1.139$).

We used the statistical computing language R (R Core Team, 2018) for all analyses, unless otherwise stated above, and generated graphs with the R package "ggplot2" (Wickham, 2016). All data collected are available on Dryad (https://doi.org/10.5061/dryad.j9kd51c8f).

DISCUSSION

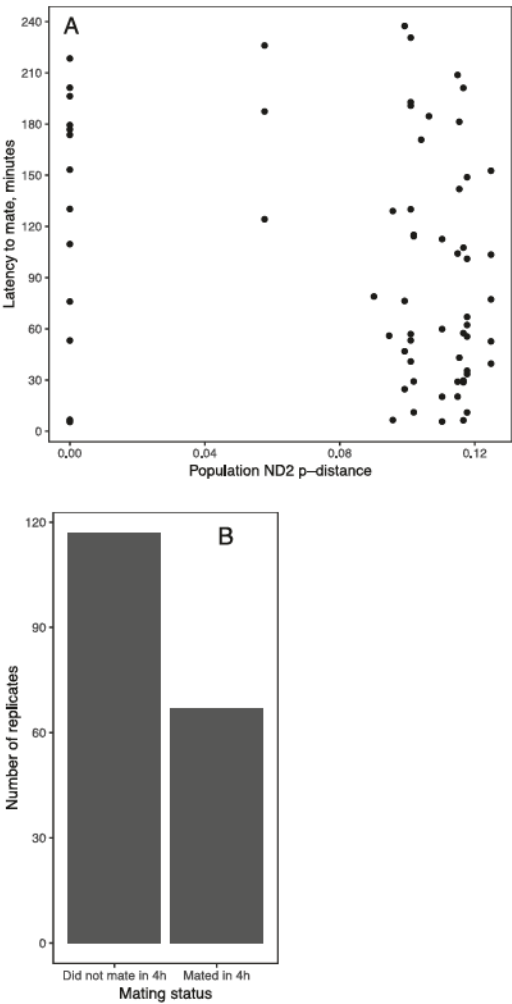This study aimed to estimate predictors of mating occurrence and latency between

Figure 5. (A) The relationship between genetic distance and mating latency for each replicate that showed mating. ND2 *p*-distance and mating latency showed a negative correlation (Pearson product–moment correlation, $P = 0.0214$). (B) Mating success. Number of replicates that mated and did not mate in 4 h.

TABLE 1. BEST-FITTING MODELS, ACCORDING TO AKAIKE INFORMATION CRITERION. ALL MODELS WITH AN AIC NO GREATER THAN THE BEST-FITTING MODEL'S AIC PLUS TWO ARE SHOWN. "HEAD" AND "FEMUR" ARE CORRECTED FOR BODY SIZE, AND "DIVERGENCE" IS MEASURED AS POPULATION-LEVEL ND2 *P*-DISTANCE.

Model Predictor(s)	AIC
Male head	170.13
Male head + female head	170.88
Male SVL + female SVL + male head + female head	171.21
Male SVL + male head	171.98
Male head + male femur	172.11
Male head + divergence	172.11

[1]AIC, Akaike information criterion; ND2, NADH dehydrogenase subunit 2; SVL, snout–vent length.

whether genetic divergence negatively correlates with mating occurrence, as first shown experimentally by Coyne and Orr (1989). Such a correlation would imply that genetic divergence may be leading to reproductive isolation in our system. Second, we investigated whether divergence in ecomorphological traits leads to a lower rate of mating occurrence, implying the possibility that adaptation in different environments could incidentally cause isolation (Rundle and Nosil, 2005). Although we did not find evidence of either process in our study populations, we unexpectedly found that relatively small-headed males had a higher probability of mating.

We observed a wide range of mating outcomes during the behavioral trials. At times, mating was initiated quickly, the shortest elapsed time being 5 min 25 sec. In 117 of the pairings (63.6%), however, mating never occurred during the window of observation. Of the 67 (36.4%) pairs that exhibited mating behavior during a trial, the mean latency to mate was 1 h 39 min. No population exhibited a higher probability of mating with individuals of the other sex from that population than with individuals from other populations, and none of the 36

individuals from divergent populations of *A. sagrei* in the West Indies. We quantified the extent of genetic and morphological divergence and asked whether either was a predictor of mating behavior to test two nonexclusive hypotheses. First, we tested
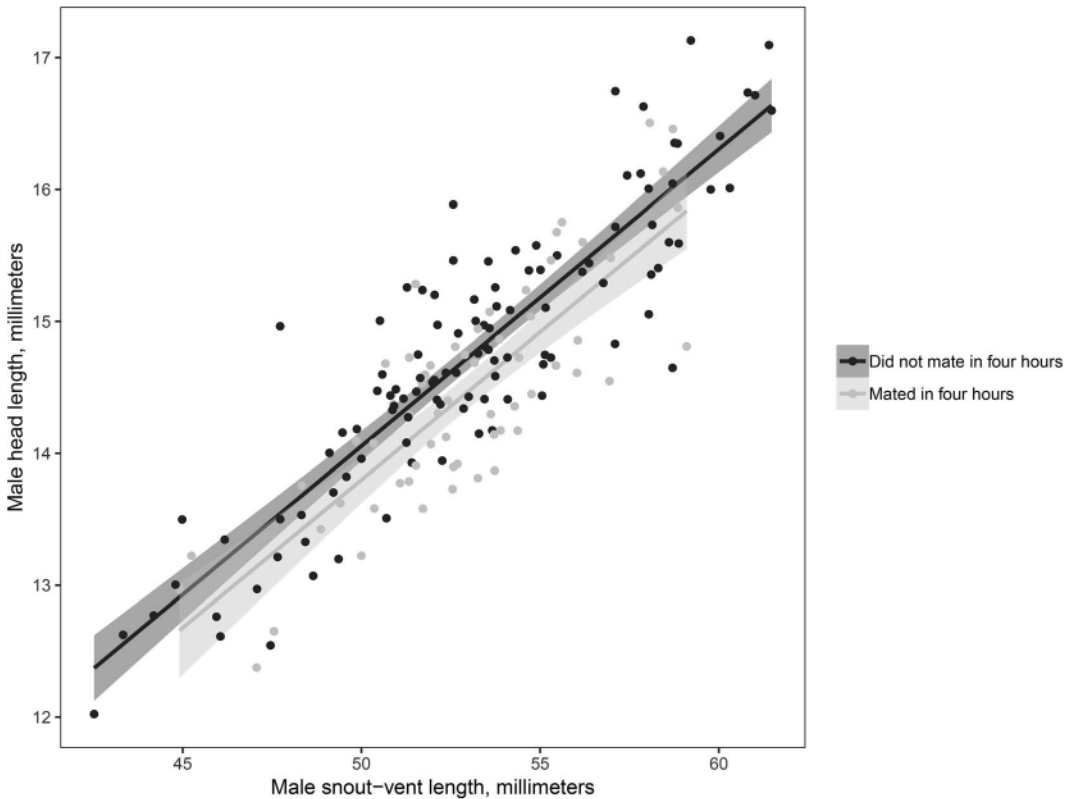
Figure 6. The relationship between SVL and head length for the males in the experiment. Black dots indicate males that did not mate, and gray dots indicate males that did mate. Trendlines for each of these categories and 95% confidence intervals are shown.

population pairings mated significantly more or less than expected by the chi-square test.

We observed genetic (Fig. 3) and morphological (Fig. 4) variation among the populations we studied, but this variation is not a good predictor of differences in the occurrence of mating in laboratory conditions. We used both genetics and morphology as predictors of mating presence–absence and compared the AIC of 57 models predicting mating presence/absence from the skeletal morphological traits measured, trait interactions, and genetic distances (Supplementary Table 2). We found no support for a relationship between premating isolation and genetic distance, because only one of the six best models included a genetic distance term, which was not significant ($P = 0.895$). We also found no evidence of a relationship between morphology and premating isolation, because we saw no association between divergence in ecomorphological traits and the rate of mating occurrence in any of the six best models. These results thus provide no support for the hypothesis that ecological speciation drives the evolution of behavioral reproductive isolation.

We also saw no evidence of IBD among *A. sagrei*, contrary to previous work by Reynolds et al. (2019). However, in addition to that paper's more extensive sampling (95 locations), the authors include brown anole

populations from across several landmasses, including 53 sites on Cuba. Although IBD may occur between islands because of a higher probability of dispersal events between closer islands—a phenomenon we test for here—the terrestrial connectivity of intra-landmass populations may lead to stronger IBD than we see between islands. Alternatively, given the fairly low but not significant P-value of one of our tests for IBD (Mantel test $P = 0.082$), we acknowledge this may be a false negative.

Regardless, were we to have seen a negative correlation between genetic distance and mating probability, observing IBD would allow us to speculate on the role of geographic dispersal as a driver of premating isolation. However, because we saw no premating isolation, we can make no such inference from our tests for IBD, no matter their result.

Our finding that premating isolation was not occurring among these populations is, nonetheless, relevant to broader questions pertaining to the strong colonization potential of *A. sagrei* (Kolbe et al., 2004, 2017). Kolbe et al. (2004) showed that introductions of different brown anole populations into Florida led to interbreeding and greater genetic diversity than any of the source populations alone. Increased genetic diversity may improve an invasive population's ability to adapt and, in turn, may have contributed to the successful invasion of *A. sagrei* in the southeastern U.S.A. Our findings support the idea that genetically distinct *A. sagrei* from throughout the species range can mate when brought into contact, leading to introduced populations with elevated genetic diversity and, perhaps, populations primed for adaptation to newly invaded habitats. More work is needed to study the extent of this phenomenon in nature, but our study provides further evidence of its potential.

Additionally, some genetically heterogeneous populations arise not from multiple recent colonization events, as detailed in Kolbe et al. (2004), but from secondary invasions into a species' own native range, known as "cryptic back-introduction" (Guo, 2005). Kolbe et al. (2017) show cryptic back-introduction of *A. sagrei* onto one of the islands in the present study, Cayman Brac, from the nearby island of Grand Cayman, and the authors also documented interbreeding between the invasive and native *A. sagrei* on that island (Kolbe et al., 2017). This study's result of no premating isolation suggests interbreeding may occur in other cases of back-introduction in the species. Further study of this phenomenon in *A. sagrei*, as well as the extent to which it has occurred thus far, should allow us to better understand the biology of *A. sagrei*, as well as the nature of back-introduction.

Of the six best models predicting mating probability in our study, all included a term incorporating relative male head size, and the lowest-AIC model included relative male head size as its only predictor. Relative male head size was significant ($0.002 < P < 0.003$) in all six models and had a negative coefficient for all. This result is somewhat counterintuitive, as data on male head size in anoles has suggested that males with larger heads bite more strongly and are socially dominant (Lailvaux et al., 2004; reviewed in Losos, 2009). Moreover, males and females in denser populations of *A. sagrei*, which likely entail increased intraspecific competition, have larger heads (Wegener et al., 2019). Head size in anoles also is strongly positively correlated with dewlap size (Ingram et al., 2016), affecting another aspect of courtship (Losos, 2009). For all these reasons, the results reported here run counter to our expectations.

We do not know the reason for the unexpected relationship we find between

male head size and mating probability. One hypothesis might attribute this result to females preferring to mate with smaller headed males whose weaker bites are less likely to result in injuries during copulation. For example, female Dalmatian wall lizards (*Podarcis melisellensis*) prefer the scent of males with a weaker bite, even though those males are less successful in intrasexual conflict (Huyghe et al., 2012). Given the no-choice mating design and the 15 L size of the cages (de Meyer et al., 2019), females had a relatively limited ability to exercise mate choice in this study, calling this explanation into question.

This phenomenon could be also be mechanical: we observed males using their mouths to hold females by the napes of their necks while mating, and perhaps smaller relative head size allows them to do so more efficiently. Alternatively, Sih et al. (2004) argued that animal behaviors and behavioral correlates persist within individuals across contexts ("behavioral syndromes"). There may be a behavioral correlate of head size not assessed in the present study that leads to faster mating. These suggestions are, as yet, speculative, and further studies of this pattern are needed.

While not significant when corrected for multiple comparisons, one relationship between population of origin and latency to mate did appear. The Fisher's exact test comparing the rate of mating of males from Conception with Conception females to the rate of mating of Conception males with non-Conception females was significant without correction for multiple tests ($P = 0.018$). Of the six Conception males paired with Conception females, four mated (67%), but of the 18 paired with females of other populations, only two mated (11%). Given that Conception males have significantly larger SVLs than all other populations

(Fig. 3), body size might be driving this pattern, although further study is needed.

The present study was limited by several factors. Females may have had a limited ability to avoid copulation with their paired male, as discussed above. Whereas males acclimated to their cages before the start of the trial, filming began as females were introduced, which may have affected the latter's behavior in a way unrelated to behavior in nature. We observed only 4 h of footage for each trial, which, although longer than the 45-min observation time used by Richmond and Jockusch (2007), was briefer than the 8-h trials of Shine et al. (2002). Finally, although we had replicates of all 36 pairwise population pairings, more replicated pairings would have strengthened our analyses, especially because six of these 36 had no replicates that mated (Supplementary Table 3).

Despite these drawbacks, the lizards did exhibit realistic anole behavior. Whether our negative findings are correct or are laboratory artifacts requires further study. Future studies should use larger cages and allow longer periods for both males and females to become acclimated to the cages. Moreover, longer observation periods, as seen in Shine et al. (2002), would allow for the collection of further data.

ACKNOWLEDGMENTS

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