ORIGINAL ARTICLE

Male color polymorphism in populations of reef geckos (*Sphaerodactylus notatus*) reduces the utility of visual signals in sex recognition

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Abstract

Sexually dichromatic signals can identify the sex of conspecific, facilitating decisions to initiate agonistic or courtship displays at the onset of social interactions. The utility of sex-specific signals, however, may be reduced when polymorphism evolves and these signals become variable. To explore the impact of polymorphism in the utility of sex-specific signals, we studied Floridian and Bahamian populations of the reef gecko, *Sphaerodactylus notatus*, which vary in the presence of polymorphic males (i.e., populations with monomorphic males that are sexually dichromatic from females; polymorphic with dichromatic and female-like males; polymorphic with dichromatic and intermediate males). We staged encounters between same-sex and different-sex pairs on opposite sides of transparent barriers to measure response to visual signals, quantifying agonistic and courtship behavior. For geckos from the monomorphic population, opposite-sex pairs consistently initiated courtship displays, and same-sex pairs initiated agonistic displays. In contrast, both polymorphic populations lacked consistent sex-specific response to visual signal, and the population with dichromatic and female-like males showed similar responses in both same-sex and opposite-sex pairings, failing to distinguish sex even when interacting with dichromatic males. When individuals from this polymorphic population were tested with the transparent barrier removed to allow olfactory signaling, we found clear courtship and agonistic responses between opposite-sex and same-sex pairs, respectively. Our study suggests that loss of signal reliability when a trait becomes polymorphic can lead to reduction of signal utility, even when the signal remains present in some individuals and still conveys the sex of conspecifics.

Significance

Our study examines partial loss of sex-specific signal in a divergent mainland population of the reef gecko (*Sphaerodactylus notatus*). By staging encounters between same-sex and opposite-sex individuals from three populations that varied in the presence of male polymorphism, we found that clear sex-specific visual signals were associated with visual discrimination between sexes, while unreliable sex-specific visual signal was associated with greater reliance on olfactory sex discrimination. Because the evolution of additional male color morphs (i.e., female-like males) is likely derived in this clade, our results suggest a switch from visual to olfactory signals in sex recognition in the polymorphic population. This presents an example of color polymorphism leading to a reduction in signal utility, and divergence of these traits in an isolated population suggests a possible mechanism for development of reproductive barriers and a potential contributing factor to diversity of the *Sphaerodactylus* clade.

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Introduction

Mate recognition signals are integral at the start of behavioral interactions, as they allow individuals to quickly assess and categorize conspecifics. For instance, sex-specific signals enable the discrimination of potential mates from competitors, facilitating the decision to initiate either

courtship or agonistic displays (reviewed by Darwin 1871; Huxley 1938; Andersson 1994). Variation in individual expression of these sex-specific traits may confer advantages to males with exaggerated traits that advertise status or increase attention from potential mates and competitors (Peek 1972; Jenssen 1977; Borgia 1979; Andersson 1982; Newbolt et al. 2017; Romano et al. 2017; reviewed by West-Eberhard 1983). However, sex-specific signals may increase the visibility of signalers to predators (Endler 1992; Stuart-Fox et al. 2003; Moore and Martin 2018) and/or to dominant sexual competitors (Stuart-Fox et al. 2021). As a result, selection may act to reduce signal conspicuousness when predation is high (Blows 2002; Moore and Martin 2018) or intrasexual competition is very costly (Lee and Bass 2006). The balance between different types of selection may therefore favor the evolution of different mating strategies among males, resulting in variation in sex-specific signals within populations (e.g., Sinervo and Lively 1996; Hurtado-Gonzales et al. 2011; Mank 2022). Variable systems provide unique opportunities to study mate recognition and, importantly, how variation in sex-specific signals leads to the loss of their utility in social interactions.

Sexual dimorphism, a difference in appearance between sexes, is widespread among animals. In particular, sex-associated differences in color and pattern (i.e., sexual dichromatism) can serve as a visual signal for mate recognition or of individual status (Andersson 1994; Dufva and Allander 1995; Grindstaff et al. 2012; Assis et al. 2021). Sexual dichromatism is particularly well-studied in birds, fish, and lizards, but it exists in a diversity of animal taxa (Hamilton 1961; Saetre and Slagsvold 1992; Kohda and Hori 1993; Burns 1998; Badyaev and Hill 2003; Stuart-Fox et al. 2021). Research on the evolution of sexual dichromatism has focused primarily on broad-scale, comparative studies that uncover the factors that influence the gains and losses of sexual dichromatism (Burns 1998; Pfennig 2012; Luro and Hauber 2021), and field studies that quantify sexual selection for specific phenotype within variable or polymorphic species (Robinson and Gifford 2021; Stuart-Fox et al. 2021). In birds, congeneric diversity is correlated with presence of sexual dichromatism in Anatidae and Parulidae (Pfennig 2012), and both the presence of congeners and migratory behavior are correlated with presence of sexual dichromatism in Thrushes (Luro and Hauber 2021). Presumably, species that lose sexual dichromatism do so because these conspicuous signals become more costly than the benefit they convey (Kirkpatrick 1987; Blows 2002).

Sexual dichromatism exists in some taxa in a polymorphic state, characterized by the presence of more than two adult morphs within a single population (i.e., color polymorphic species). Such polymorphisms are found in birds (Pryke et al. 2007; Calhim et al. 2014), fish (Hurtado-Gonzales

et al. 2010; Ahi et al. 2020), and insects (Fincke 2015), as well as lizards (Stuart-Fox et al. 2021). In one common alternative mating strategy, one sex mimics the other in order to avoid aggression from dominant competitors and/or to attempt sneak copulations (Rohwer 1978; Saetre and Slagsvold 1996). Within fish, for instance, female mimicry is frequently found among smaller males until full adult size is attained (Taborsky 1998) or as a distinct sneaker strategy to avoid intrasexual competition (Hurtado-Gonzales et al. 2010). In birds, immature males delay the development of adult male plumage to reduce intrasexual aggression (Chen et al. 2019).

Lizards have evolved polymorphism within many diverse clades including geckos, lacertids, agamas, phrynosomatids, and liolaemids, and many of these examples include female mimicry among male morphs (Stuart-Fox et al. 2021). In lizards, polymorphisms have been linked to differences in aggression, immune response, sperm production, and mating behavior (Sinervo and Lively 1996; Vercken and Clobert 2008; Yewers et al. 2016; Friesen et al. 2020), indicating that the color morphs represent alternative mating or reproductive strategies (Sinervo 2001; Galeotti et al. 2013). Within geckos, male polymorphism in which one morph resembles females while another is more brightly colored has been described in *Cnemaspis* day geckos (Kabir et al. 2019, 2021), *Sphaerodactylus* dwarf geckos (Regalado 2015), and possibly *Gonatodes* dwarf geckos (Rivero-Blanco 2012). Geckos use olfactory and visual signals to identify potential mates, and the relative importance of each signal modality varies among taxonomic groups. *Cnemaspis* day geckos, for instance, use both visual and olfactory signals, but olfactory signals seem to dominate in mate recognition (Kabir et al. 2019). Within *Sphaerodactylus* dwarf geckos, visual signals are used to identify sex from a distance, while olfactory signals are used for identification at close range (Regalado 2012).

Because of the presence of polymorphic males within populations, geckos present an excellent clade for studying the consequences of male polymorphism to signal utility. In species that rely on visual signals for mate recognition, the loss of reliable sex-specific signals could impact both intersexual and intrasexual communication (Stuart-Fox et al. 2021). As evolving signals lose reliability, the behavior of receivers should change in response to the loss of sex-specific courtship or aggression response. Within *Sphaerodactylus* geckos, the Lost Recognition Cue (LRC) model was proposed to describe the adoption of an equivocal response from receivers in species where visual signals evolve variability or become lost among males (Regalado 2015). The LRC model predicts that the loss of sexual dichromatism in a previously sexually dichromatic taxon will result in receivers failing to distinguish sexes until alternative signals can be assessed. In the case of the partial loss of sexual

dichromatism (e.g., male polymorphism with dichromatic morph and female-like morph), the loss of signal reliability is predicted to result in reduced utility of visual signals in sex identification of ambiguously patterned conspecifics and increased reliance on olfactory signals for confirmation of sex. Multiple species of *Sphaerodactylus* possess coloration and courtship traits that fit expectations of the LRC model, highlighting this clade as an excellent focus for the study of how variation in visual signals within populations can affect mate recognition.

Sphaerodactylus geckos present opportunities for such a study because of the multiple gains and losses of sexual dichromatism and the presence of male polymorphism within the clade (Regalado 2003, 2012, 2015). In sexually dichromatic *Sphaerodactylus* species, individuals visually identify potential mates and competitors, modifying their courtship behavior according to sex (Regalado 2003). Furthermore, studies comparing behavioral interactions between individuals from monochromatic and sexually dichromatic *Sphaerodactylus* species showed variation in behavior, with individuals from the sexually dichromatic species being more likely to court a possible mate upon contact, while

individuals from the monochromatic species immediately initiate ambivalent agonistic displays (Regalado 2015). In addition to visual sex recognition, *Sphaerodactylus* geckos also respond to chemical signals (Regalado 2012), suggesting two sensory modes for sex identification.

Here, we focus on the reef gecko, *S. notatus*, a species native to South Florida, The Bahamas, and Cuba, because distinct subspecies vary in presence of male polymorphism (Fig. 1). The subspecies in Cuba and the Bahamas (*S. n. atactus*, *peltastes*, and *amaurus*) are strongly sexually dichromatic, with females (and immatures) having striped heads, and monomorphic dichromatic males losing the stripes when reaching maturity and instead becoming speckled (this population is henceforth referred to as monomorphic with dichromatic males, or MD) (Fig. 1). In contrast, the subspecies in South Florida (*S. n. notatus*) is polymorphic with variation between the mainland and the Florida Keys. Geckos found on the mainland feature dichromatic adult males having the typical speckled heads while others retain the immature coloration that is similar to striped females (this population is henceforth referred to as polymorphic with dichromatic and female-like males, or PDF)
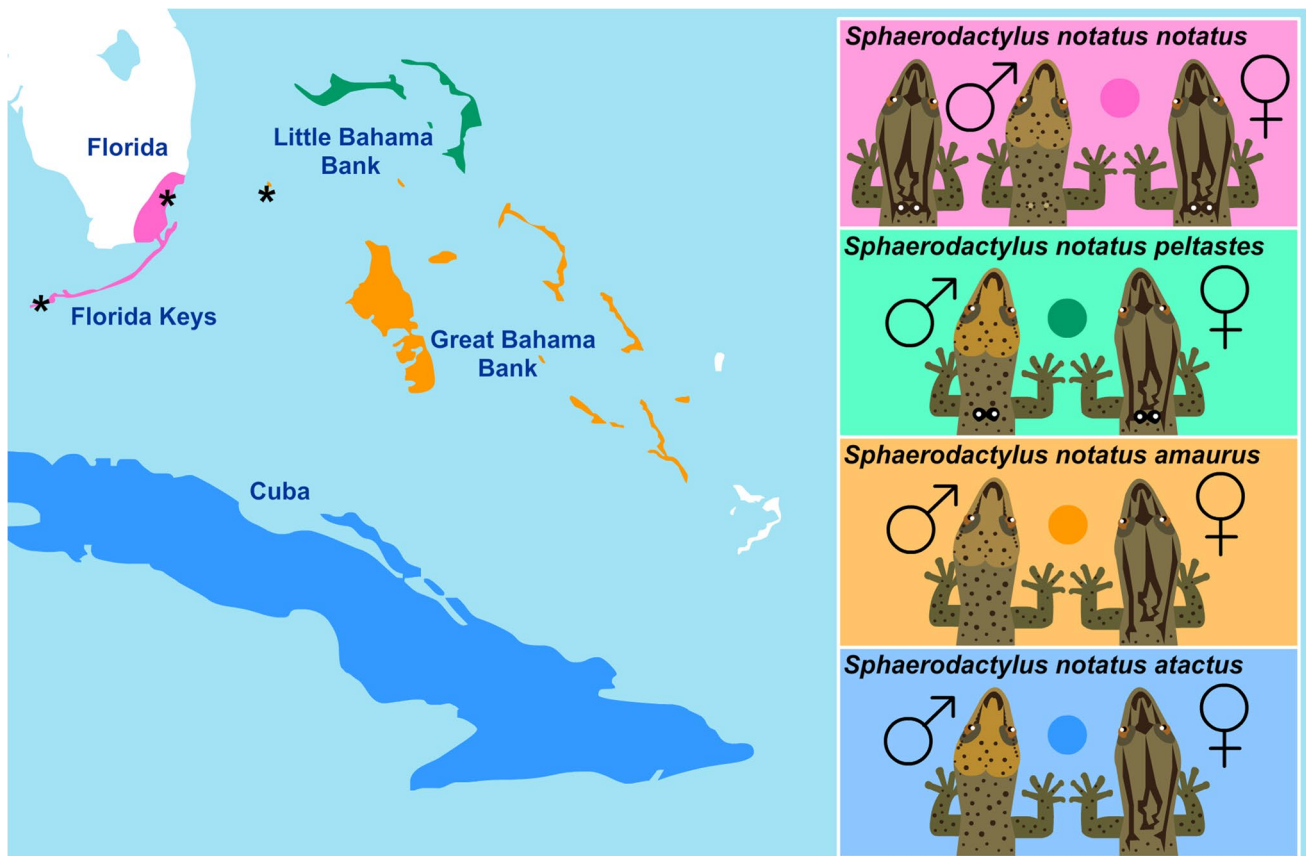

Fig. 1 The range of four subspecies of *Sphaerodactylus notatus* showing the variation in the presence of male color polymorphism and extent of sexual dichromatism for each population. Collection sites for behavioral trials are marked with asterisks

(Fig. 1). Geckos in the Florida Keys, in contrast, have the typical dichromatic morph and a less common morph that is intermediate in appearance to typical males and females (this population is henceforth referred to as polymorphic with dichromatic and intermediate males, or PDI). The sister species to *S. notatus*, *S. altavelensis* (Pyron et al. 2013), is fully sexually dichromatic, suggesting that male polymorphism found in the Florida Keys and mainland Florida is likely the derived state.

We compared decisions to initiate courtship or agonistic behavior in three populations of reef geckos to test how male color polymorphism has influenced sex recognition. We predicted that the variation in presence of polymorphism among populations would be associated with the ability to effectively discern sexes using only visual signals. The MD population and, to a lesser degree, the PDI population have visual signals that can still reliably indicate sex. In contrast, the evolution of female-like males in the PDF population could result in the reduction or loss of the reliability of sex-specific signals. Furthermore, we expected two potential outcomes for how the PDF population would respond. The LRC model predicts that individuals from sexually dichromatic populations will consistently use visual signals to recognize the sex of potential partners, while individuals from the PDF populations will respond differently depending on whether the encountered male is a dichromatic male morph or a female-like morph (Regalado 2015). That is, sex recognition using visual signals is only predicted to occur when the partner is a dichromatic male in the PDF population. Alternatively, variability in sex-specific signals may result in reduced receiver response to the signal and subsequent reliance on a different signal and/or signal modality despite some males still displaying the typical, male-specific visual signal. Variation in presence of polymorphism across populations provides us with an opportunity to distinguish between these two hypotheses. In short, our study aims to identify whether derived variability in visual signal results in continued receiver use of this signal when present, or, alternatively, if receivers no longer respond to signal despite its continued presence in some individuals.

Methods

Study system

Sphaerodactylus notatus is a *ca.* 5-cm-long (snout-vent length or SVL) dwarf gecko with populations that vary in the presence of male polymorphism (Schwartz 1965). Based on preliminary observations of mainland Florida *S. n. notatus* populations, we found that many males never achieve typical male coloration and instead retain the striped pattern (Fig. 1). That is, despite the presence of an escutcheon

(an iridescent patch of scales near the cloaca that positively identifies males), many males retain female color patterns and visually appear to be females. Literature descriptions are mainly based on Florida Keys specimens, where adult-sized males occasionally retain some female head striping in an intermediate morph (Schwartz 1965). In contrast to Florida, the *S. n. amaurus* of South Bimini exhibit clear dichromatism with all mature males lacking head striping (i.e., monomorphic males; Fig. 1).

Collection of geckos

We collected reef geckos from natural habitats during the breeding season (mid-February to early May 2019 (Regalado 2006)). For our initial trials with clear barriers, we collected 20 female and 18 male *S. n. amaurus* from South Bimini, 14 female and 15 male *S. n. notatus* from Key West in the Florida Keys, and 12 female and 16 male *S. n. notatus* from Fairchild Tropical Botanical Garden in mainland Florida (Fig. 1). An additional 12 females and 12 males were collected from mainland Florida for no-barrier trials. Sampling locations were chosen to sample natural variation in male color polymorphism and by density of the gecko population. For the pattern analysis, we used additional photographs taken of *S. notatus* that were captured but excluded from behavioral trials due to incompletely regrown tails, resulting in totals of 20 males and 23 females from South Bimini, 17 males and 17 females from Key West, and 28 males and 20 females from Fairchild Tropical Botanical Garden.

We captured geckos by cupping with a hand and coaxing them into plastic vials for safe transport. Geckos were often located under shelter such as rocks, logs, palm fronds, and other debris. Alternatively, geckos were often located by walking slowly through leaf litter while watching for movement of active geckos. We transported geckos back to a field laboratory and housed them in individual 355-mL plastic containers with 20 ventilation holes (1 mm each). All terraria were kept in approximately the same ambient temperature as their diurnal environment within the breeding season (24–27 °C) with a natural photoperiod. Geckos were provided with fresh water in the cap of a vial (refilled as needed), and native soil with leaf litter for shelter. Fruit flies or other microinvertebrates were provided every 48 h, with the disappearance of these invertebrates confirming feeding. All encounter trials were performed during the breeding season within 2 weeks of capture, and near field sites except for the Florida Keys population which was briefly transported to Miami for trials and returned to the collection site following conclusion of the trials. All transport from field sites to field lab occurred within a 12-h period. At the conclusion of our experiments, we returned geckos to their individual capture locations using photo references to ensure correct release placement.

For each gecko, we took a dorsal photograph and recorded sex. We determined sex by shining a light on the ventral side of the gecko to reveal presence of a male-indicating escutcheon. Because adult females and immatures do not possess an escutcheon, we used a lower mass limit for inclusion of females that was 10% greater than that of the smallest sexually mature male to eliminate the possibility of misidentification of sex. Due to size variation between populations, this resulted in a lower mass limit of 0.24 g for Bimini and 0.30 g for the Florida Keys and mainland Florida populations. We recorded mass using a portable scale. Mass was chosen for size evaluation due to the difficulty of measuring SVL in the field, but SVL was later measured using photographic analysis software.

Pattern analysis

We performed pattern analysis using the PAT-GEOM (Chan et al. 2019) plugin with ImageJ (Schneider et al. 2012) to quantify the extent of male color polymorphism within populations and to categorize males based on morph phenotype. We first outlined patterns by manual selection using high-resolution photographs of dorsal views of each gecko. The region used for analysis was the tip of the nose to the beginning of the neck. The Directionality of Shape analysis was used to compare aspect ratios of pattern markings (see Fig. 2). This analysis fits ellipses to patterns and then calculates the major axis divided by minor axis. To perform this analysis, we selected all dark markings within the analysis region. Females typically have striped heads, and the lines on their heads have high aspect ratio values because they have a greater generalized length relative to width, meaning the markings are longer than they are wide. Males typically have spotted or speckled heads, and their more circular markings have low aspect ratio values because length and width are similar. By differentiating between these two types of markings, this analysis was able to quantitatively plot the variation in patterns of males and females in each population and clearly differentiate the two male morphs in the mainland Florida *S. n. notatus* population.
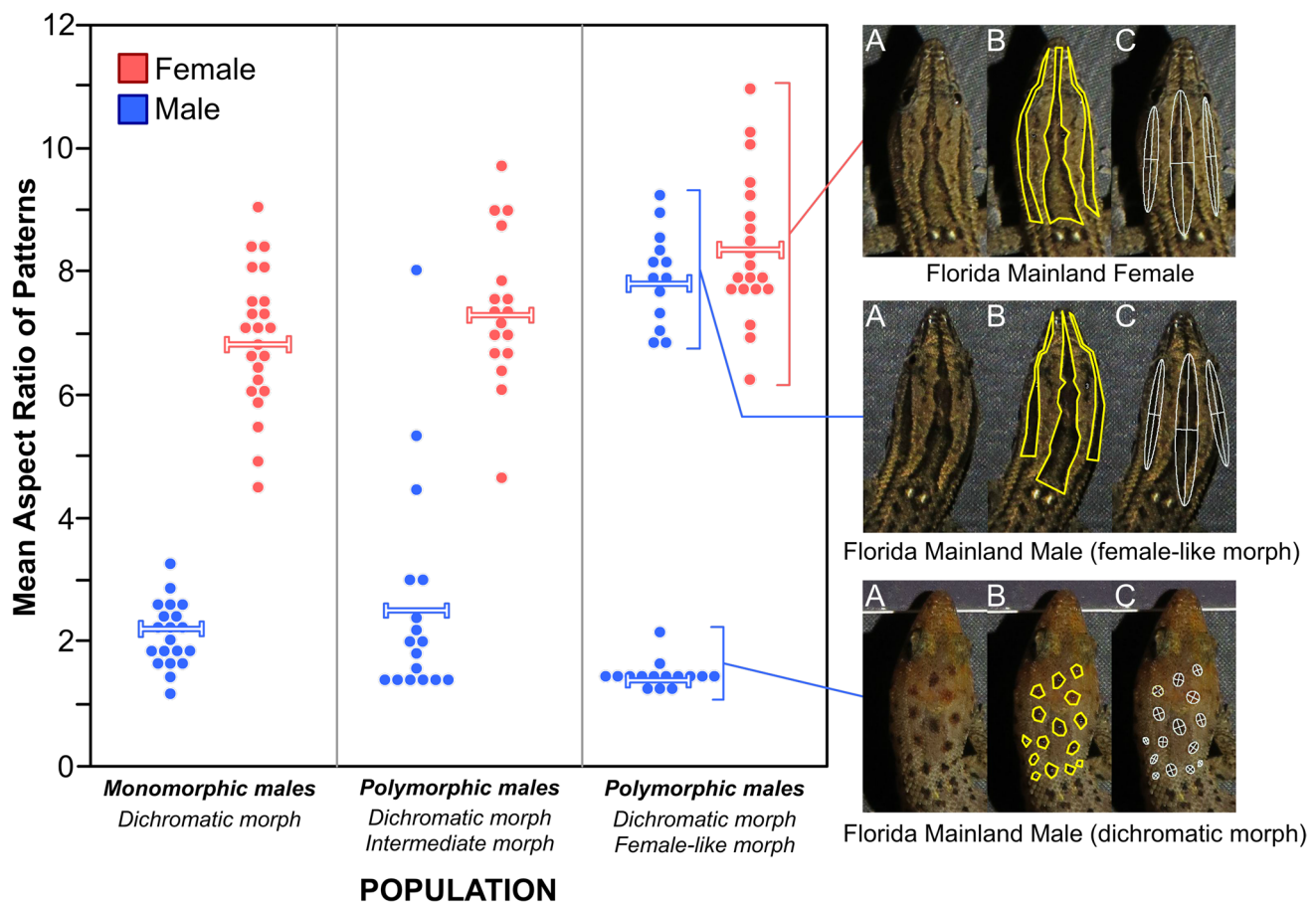

Fig. 2 Scatterplots showing each individual gecko's mean aspect ratio of patterns. White bars indicate mean for each cluster. At right, images demonstrate methods used for analysis with "A" images showing original photo, "B" images showing pattern selection, and "C" images showing the Directionality of Shape calculation of aspect ratio. Each set of images represents a distinct cluster within the PDF population of the Florida mainland

Introduction experiments

We placed the geckos in an encounter chamber for behavioral trials. Geckos were paired for encounter trials based on sex and mass such that geckos differed in size by ≤ 0.05 g. Initial use of geckos in either same-sex or opposite-sex trials was alternated to ensure equal initial presentation. The chamber was a 20 cm × 10 cm × 10 cm acrylic box with a dividing panel (see Fig. S1). The dividing panel was a clear acrylic partition, initially covered with an opaque panel during lizard acclimation. The lid was hinged for access. All sides were painted a neutral tan color other than the transparent front and top of the chamber. Geckos were provided with a water dish and leaf for shelter during the acclimation period. The leaf was removed during the recorded trial.

To limit behavior associated with stress from capture, geckos were allowed to acclimate between all transport and prior to experiments. Geckos were left to acclimate in a terrarium for a minimum of 24 h prior to encounter trials. They were then allowed a minimum of a 1-h acclimation period when transferred to the encounter chamber prior to removal of the opaque divider and subsequent video recording. Encounters took place in a large opaque box to reduce outside distraction.

After the acclimation period, we carefully opened the encounter chamber to remove the opaque divider, leaving only a transparent barrier between geckos for barrier trials that presented only visual signal and removing it entirely for non-barrier trials that also allowed olfactory cues. We removed shelters to ensure view of the other gecko. The chamber was then closed and the video recording began once the researcher (E.P.) disappeared from view of the chamber. Geckos were allowed an additional 24 h between encounters and were assigned a second encounter (either same-sex or opposite-sex) depending on what the first trial was, and opposite- and same-sex trials were performed in alternating order. All videos were recorded with a Samsung Galaxy S7 mounted on a tripod.

Video analysis

For each trial, we recorded behaviors of each gecko independently, but combined results to yield one final score for each trial as a whole. Therefore, each trial quantified the behavior of the gecko in the left and the right chambers, which was then combined to summarize each encounter/trial. Video analysis was performed blind when possible, but in trials that involved sexually dichromatic males and females, the sex of the pair was visually apparent.

We completed video analysis using the behavior-logging program BORIS (Friard and Gambia 2016) to track the number of courtship and agonistic signals/interactions. Behaviors (see Table 1) were chosen based on published accounts of Sphaerodactylidae behaviors (Leuck et al. 1990; Regalado 2012), as well as initial observations of gecko courtship and mating behavior. Based on preliminary observations, we found that the majority of social interactions occurred within 20 min of introducing individuals. We therefore clipped footage to 20 min, beginning when the observer disappeared from view. We measured the time spent by each individual conducting the behaviors listed in Table 1. Courtship behaviors included time spent displaying head bob by males and time spent displaying look back by females (Table 1). Agonistic behavior for both sexes was measured as the time spent displaying stiff behavior (Table 1). Individuals from both sexes sometimes simultaneously used stiff + headbob, which would be classified as an ambivalent behavior by Regalado (2015). However, the use of this combination of behaviors was determined to be a neutral behavior based on use in same-sex and opposite-sex pairs for the MD population. As such, head bob and stiff behavior was only counted as courtship and agonistic when used as a singular behavior, and the combination of simultaneous stiff + headbob was counted as a neutral behavior and not used in analysis (see Tables S2, S3, and S4 for a summary of raw data for opposite-sex trials, female-female trials, and male-male trials respectively).

Statistical analysis

Each trial represented an independent experimental unit, and the courtship or agonistic response was summarized by

Table 1 List of behaviors measured during video analysis with description of each behavior and how it is used by male (M) and female (F) geckos. Based on behaviors described in Regalado (2012) with additional personal observation by EAP regarding the look back behavior

Behaviors measured in barrier trials		
Behavior	Description	Likely function
Head bob	The gecko raises and lowers its head repetitively with equal rhythm	Courtship (M)
Stiff	The gecko straightens its body and rises above the ground	Aggression (F, M)
Look back	The gecko orients body facing away from the other gecko and rotates head backward toward the gecko while waving tail	Courtship (F)

subtracting time (in seconds) spent in agonistic display from time spent in courtship display for each gecko, then combining the values of the two geckos in each trial. As a result, a net agonistic interaction resulted in a negative score, while a net courtship interaction resulted in a positive score. Raw data for each behavior was log transformed ($\log 10(x + 1)$) to normalize data, which was right skewed. Skewed data resulted because most interactions occurred during a small portion of the total encounter, but a few interactions involved much greater time spent displaying.

Encounters for opposite-sex pairs and same-sex pairs were analyzed separately, and single-sample t-tests were conducted to test if responses differed significantly from zero for each population. A mean of zero indicated either a neutral response or a net neutral response if encounters were split between positive and negative, indicating ambiguity of sex. In contrast, values significantly greater or less than zero would indicate courtship and agonistic behaviors, respectively. To test for the potential effects of male morph on response in the two polymorphic populations, these populations were analyzed using ANCOVA to test for interaction between population and male morph on agonistic or courtship responses.

Results

Presence of visual sexual dichromatism

The results of our pattern analysis indicate significant differences between sexes in the Bimini *S. n. amaurus* population (Fig. 2). In contrast, we found a bimodal distribution of patterns in *S. n. notatus* males from mainland Florida, with some males clustering with females and others clustering separately. Finally, we found that males and females showed a low level of overlap in pattern aspect ratio in the Florida Keys *S. n. notatus* population, indicating the presence of the dichromatic and intermediate male morphs.

Lack of morphological sexual dimorphism

Measuring other possible dimorphic traits was important to ensure that the observed visual differences were the only significant signals for sex identification by geckos. We found no significant difference between male and female geckos for any population in body mass (see Fig. S2) ($P = 0.431$ for Bimini geckos, $P = 0.948$ for Keys geckos, $P = 0.552$ for Florida mainland geckos), head width (see Fig. S3) ($P = 0.185$ for Bimini geckos, $P = 0.264$ for Keys geckos, $P = 0.761$ for Florida mainland geckos), or SVL (see Fig. S4) ($P = 0.690$ for Bimini geckos, $P = 0.380$ for Keys geckos, $P = 0.905$ for Florida mainland geckos). Escutcheons, when present, may be used to positively identify sex, but these

pores are located near the cloaca and are not visible without suspending the gecko. The lack of sexual size dimorphism in our measurements supports that the only visible indication of sexual differences are visual color patterns, allowing us to rule out other obvious signals of visual mate recognition for our encounter trials. For full details of statistical tests performed, see Table S5.

Difference in same-sex vs opposite-sex response varies by population

To identify the effect of polymorphic male visual signals, we characterized each trial by measuring time spent in courtship or agonistic display by geckos. The agonistic display time was subtracted from the courtship display time to yield a summary score ranging from negative (for agonistic) to positive (for courtship).

We found that for the opposite-sex trials (Fig. 3), geckos from Bimini (MD) displayed significant courtship responses ($P < 0.001$), geckos from the Florida Keys (PDI) displayed a non-significant trend for courtship ($P = 0.072$), and geckos from the Florida mainland (PDF) displayed no significant courtship response ($P = 0.598$). When geckos from the Florida mainland PDF population were tested in trials allowing for olfactory communication, significant courtship response was found ($P = 0.029$).

For the same-sex trials, patterns of significance followed those of the opposite-sex trials. Bimini (MD) and Florida Keys (PDI) females (Fig. 4) displayed significant agonistic responses ($P = 0.001$, $P = 0.043$) while those from the Florida mainland (PDF) were insignificant ($P = 0.374$). Bimini (MD) and Florida Keys (PDI) males (Fig. 5) displayed significant agonistic responses ($P = 0.001$, $P = 0.013$), while those from the Florida mainland (PDF) were not significantly different from zero ($P = 0.200$). However, when geckos from the Florida mainland PDF population were tested in trials allowing for olfactory communication, we found significant agonistic response for both female same-sex trials ($P = 0.030$) and male same-sex trials ($P = 0.021$).

The Florida mainland PDF and Florida Keys PDI populations were analyzed using ANCOVA to test for interaction between morph and behavioral responses (see Figs. 3 and 5). For these populations, no significance was found with respect to effect of morph on response ($P = 0.215$). For full details of statistical tests performed, see Table S5.

Discussion

Using a quantitative method that measured the pattern of color patches on an individual's head, we confirmed quantitatively that the *S. n. amaurus* population of Bimini has monomorphic, dichromatic males, while the *S. n. notatus*
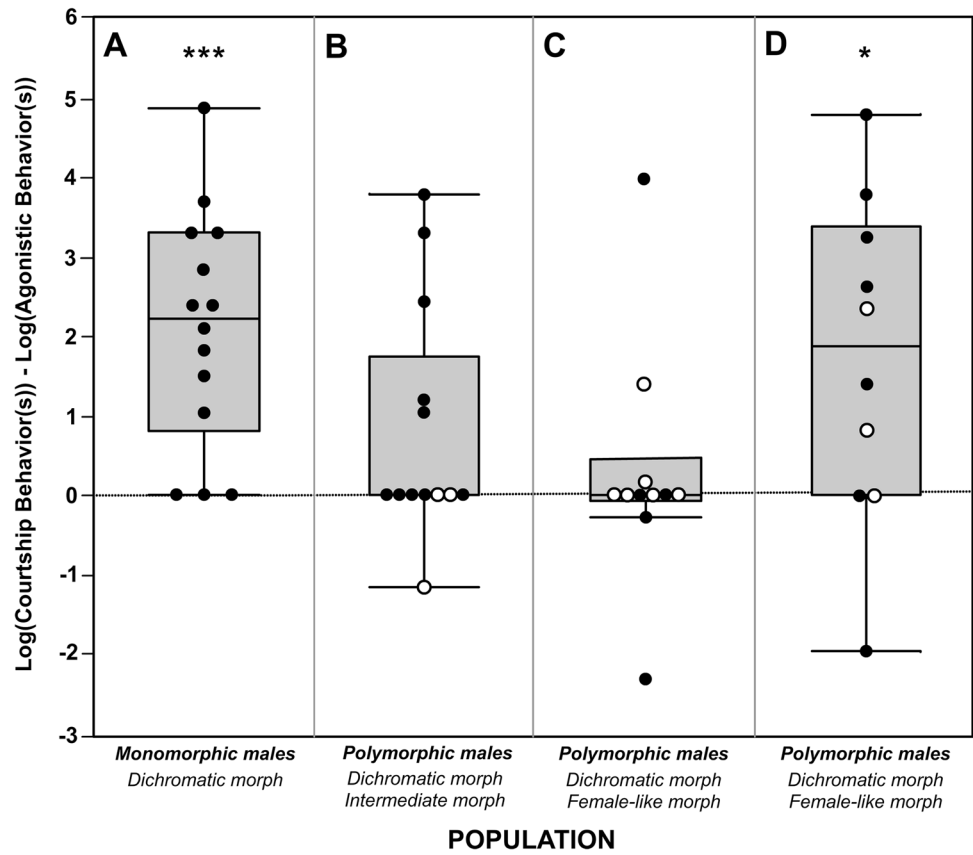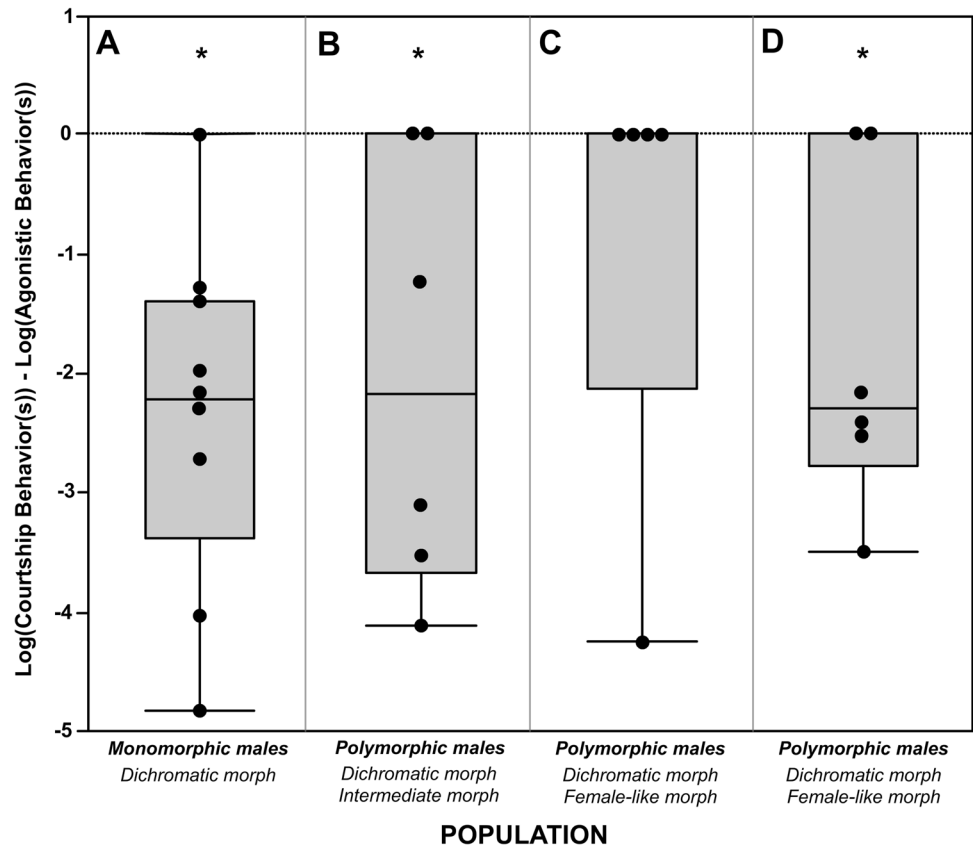
Fig. 3 Box plots of behavioral response for opposite-sex encounters, grouped by population. A–C are experiments with transparent barriers, while D is without transparent barriers. Boxes indicate the inter quartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5*IQR. Data points visible in circles superimposed on each box. The light circles indicate trials with female-like/intermediate morph males, and dark circles indicate trials with dichromatic-morph males. Significance values shown for single-sample *t*-tests for each group indicate single asterisk (*) for $P < 0.05$ and triple asterisk (***) for $P < 0.001$

Fig. 4 Box plots of behavioral response for female-female encounters, grouped by population. A–C are experiments with transparent barriers, while D is without transparent barriers. Boxes indicate the inter quartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5*IQR. Data points visible in circles superimposed on each box. Significance values shown for single-sample *t*-tests for each group indicate single asterisk (*) for $P < 0.05$

Fig. 5 Box plots of behavioral response for male-male encounters, grouped by population. A–C are experiments with transparent barriers, while D is without transparent barriers. Boxes indicate the inter quartile range (IQR), with the central line depicting the median and the whiskers extending to 1.5*IQR. Data points visible in circles superimposed on each box. The light circles indicate trials with two female-like/intermediate morph males, gray circles indicate trials with one female-like/intermediate morph male and one dichromatic-morph male, and dark circles indicate trials with two dichromatic-morph males. Significance values shown for single-sample t-tests for each group indicate single asterisk (*) for $P < 0.05$ and double asterisk (**) for $P < 0.01$
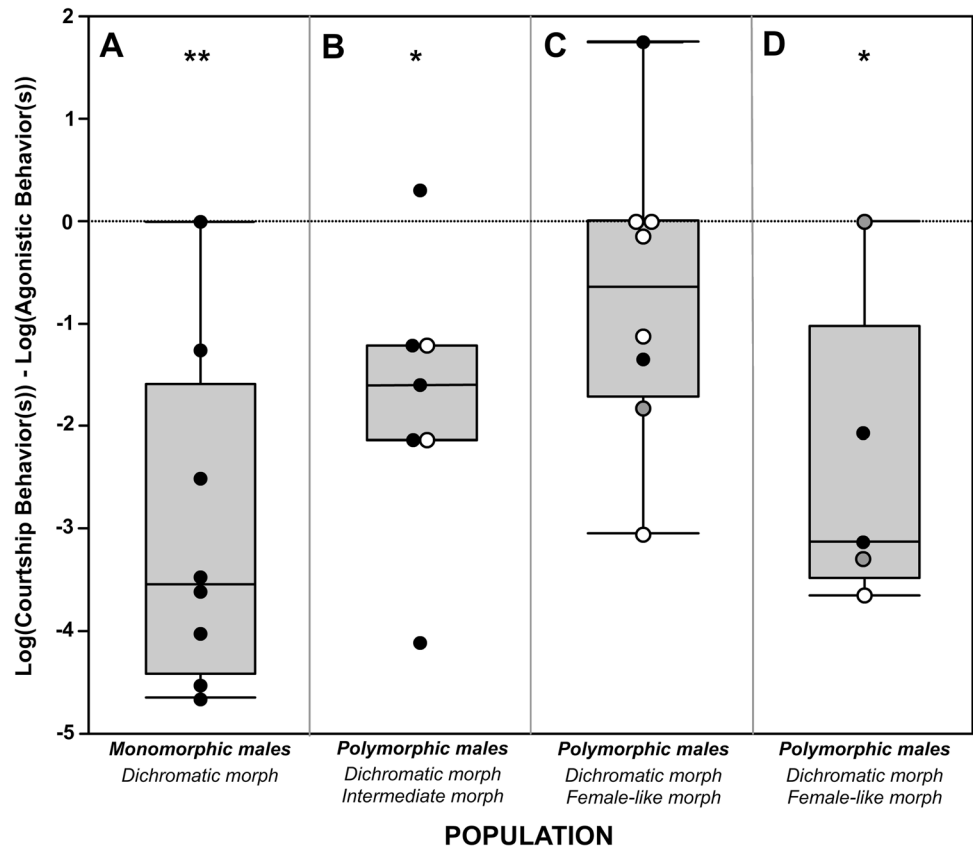
populations of the Florida Keys was polymorphic with a small number of intermediate male morphs, and mainland Florida was polymorphic with a bimodal distribution of dichromatic and female-like males. Importantly, the male polymorphism in the mainland *S. n. notatus* population was due to a subset of adult males resembling females, bearing similarity to systems in which female mimicry or status signaling is used (Lyon and Montgomerie 1986; Slagsvold and Saetre 1991; 1992; Taborsky 1998; Vergara and Fargallo 2007). With this observed variation in male polymorphism across populations, we found that geckos from the male monomorphic *S. n. amaurus* population of Bimini showed discrimination in initiating courtship responses in opposite-sex trials or agonistic responses in same-sex trials. The Florida Keys population with intermediate males showed a non-significant trend for opposite-sex trials and significance for same-sex trials. In contrast, geckos from the Florida mainland *S. n. notatus* population with female-like males did not consistently initiate courtship or agonistic response when presented with only visual signals of opposite or same-sex partners. However, when *S. n. notatus* individuals of the Florida mainland were allowed to interact with access to olfactory signals, they showed discrimination between sexes (with significant results for both opposite and same-sex trials). That is, when individuals from the PDF population were able to assess olfactory signals, they initiated

courtship or agonistic displays according to the sex of their partner, indicating greater reliance on olfactory signals in lieu of visual signals. Overall, results from our experiments suggest that the evolution of male color polymorphism can reduce the reliability of sex-specific signals and thus the utility of visual signals, favoring a switch to different signaling modalities.

In the presence of male color polymorphism within populations, individuals may still use the visual signal for assessing potential mates and rivals when available, or completely rely on alternative signals since sex-specific visual signals are no longer reliable. We found evidence for the latter in polymorphic mainland Florida populations, as neither males nor females modified their behavior in the presence of the dichromatic morph or the female-like male morph. Interestingly, in the Florida Keys population with the intermediate male morph, we found a trend where males and females were more likely to respond with courtship in opposite sex, and with agonistic behavior in same-sex pairings. That is, the consistency of sex-specific courtship and agonistic responses of geckos from the Florida Keys were intermediate to those of the monomorphic Greater Bahama Bank and the polymorphic mainland Florida populations. The less distinct sex-specific response of Florida Key geckos may be due to either the ambiguity of the intermediate color patterns of the second morph or their relatively low frequency within this

population. Overall, geckos in the polymorphic populations simply did not use or rely less on visual signals despite some males still having the male-specific coloration. The results from the Florida trials contrast with many other polymorphic systems in which the visual signal of dichromatic males is still assessed in the presence of female-like males (Sinervo and Lively 1996; Taborsky 1998; Hurtado-Gonzales 2011). However, parallels have been found in other geckos. Similar results were observed in *Cnemaspis* gecko with polymorphic males (Kabir et al. 2019). For *Cnemaspis mysoriensis* geckos, neither sex showed a difference in response to only visual cues when encountering white-gular males, yellow-gular males, or females; however, females did alter their response based on chemical signals, while males altered their response only in the presence of visual and olfactory signals (Kabir et al. 2019). Multimodal signals therefore seem to convey non-redundant information for *C. mysoriensis* males. One possible reason for the apparent differences between *S. notatus* and *C. mysoriensis* is our use of multiple behavioral indicators of courtship, which may more accurately reflect sex-specific response to specific signals. Indicators in Kabir et al. (2019, 2021) included tongue flicking and movement bouts, but these are not courtship-specific signals. In contrast, several signals of *S. notatus* (e.g., look back and head bobs) are used only in courtship, giving a more accurate indication of female and male interest. In addition, the female-morph male of *C. mysoriensis* is not entirely identical to female geckos due to sex-specific eyelid coloration. Because of this, polymorphism in this species may serve primarily for reliable male-male communication. In contrast, a separate study on the sexually dichromatic but not polymorphic *C. littoralis* found that neither males nor females responded to visual signals alone (Kabir et al. 2021), with color signals only used in concert with olfactory signals. These results are inconsistent with what we found in the strongly sexually dichromatic *S. notatus* population, where visual signals alone can be used to determine the sex of a conspecific. *Sphaerodactylus notatus* presents a study system that more readily enables analysis of female and male interest. In our study, we avoided limitations of ambiguous signals that indicate general interest by measuring courtship-specific signals which allowed us to more accurately identify female and male interest as that of courtship or of aggression. Although the dichromatic male morph signal in mainland Florida seems to be unimportant in sex recognition, the color monomorphic population showed clear use of visual signals.

The cause for the evolution of male color polymorphism in *S. notatus* remains to be determined. Female mimicry is hypothesized as a strategy to reduce intrasexual aggression and is supported in bird and fish species (Slagsvold and Saetre 1991, 1992; Sinervo and Lively 1996; Taborsky 1998; Vergara and Fargallo 2007), but this hypothesis

has also been challenged by multiple studies showing clear discrimination between females and female-mimic males (Flood 1984; Hill 1989; Procter-Gray 1991; Muehter et al. 1997). In our study, we likewise failed to find support for this hypothesis, finding that males that resembled females were not treated differently than the typical male morph. An alternative hypothesis, the Status Signaling Hypothesis (Lyon and Montgomerie 1986), proposes that immature coloration serves as a reliable signal of subordinate status. In the case of *Cnemaspis* geckos, the Status Signaling Hypothesis most accurately matches findings of Kabir et al. (2019), but in the polymorphic *S. n. notatus*, the potential advantage of female mimicry is unclear. When limited to visual signal, no discrimination was found between sexes or male morphs. Finally, the loss of the dichromatic male signal in *S. n. notatus* may be influenced by natural selection against conspicuous signal rather than sexual/social selection for alternative strategies. As the only mainland population of the species, *S. n. notatus* may face unique selective pressures that have impacted sexual and natural selection for sexual dichromatism. Within guppies, male coloration varies between streams and is correlated to predation pressures (Endler 1984). Male conspicuousness within *S. n. notatus* may be similarly affected by predation pressures on the mainland due to possible increased predator diversity.

Previous work on the courtship of *Sphaerodactylus* geckos compared interactions between different species and found differences in the use of species-specific signals (Regalado 2012, 2015). For instance, courtship displays differ between species with regard to incorporation of threatening elements like stiff posturing. These differences may be correlated with the utility of visual sexual signals within species (Regalado 2015), suggesting that divergence in communication systems may be a factor that drives species diversification in this genus. Patterns of signal use within *S. notatus* do not follow the expectations of the LRC model (Regalado 2015), which predicts that geckos with ambiguous signals would receive ambivalent responses, while dichromatic males would be approached with either courtship (by females) or threat (by males). Regalado's findings suggest that ambivalent displays would be expected toward female and female-like morphs of male *S. notatus*, but our findings are inconsistent with this prediction. All of our populations of *Sphaerodactylus notatus* often incorporated an ambiguous headbob + stiff display in both courtship and agonistic interactions while using the individual behaviors independently for clear courtship or agonistic intent. This cannot be explained by use of the combined behavior as an introductory display prior to sex determination, because geckos would often switch from head bob displays to headbob + stiff displays and back again. Even *S. notatus* females use a combination of stiff posturing and head bobbing intermittently

during agonistic display, which cannot be interpreted as ambivalent approach because females do not head bob during courtship. It is unclear what the function of this combined behavior might be, but we hypothesize that *S. notatus* uses it as a neutral signaling behavior to attract attention. Sexual dichromatism with monomorphic males is an ancestral state for this species based on its presence in closely related taxa (Pyron et al. 2013), but the use of this ambiguous courtship-threat display in *S. notatus* may be inherited from further ancestral relatives. Continued research and documentation of courtship behavior for additional *Sphaerodactylus* species may shed light on the origin of this behavior. Further comparison between studies is somewhat limited because Regalado (2012, 2015) did not use transparent barriers, and thus allowed geckos to use both olfactory and visual signals. However, our results do mirror Regalado's (2015) descriptions of greater use of olfactory signal in confirmatory mate recognition for monochromatic or polymorphic species. The polymorphic *S. n. notatus* population demonstrated reliance on olfactory signals for confirmatory sex recognition, as evidenced by discrimination of sex by geckos exposed to olfactory signals.

Questions remain on how the evolution of male color polymorphism could affect mating interactions among subspecies upon secondary contact and mediate the evolution of premating reproductive barriers. The divergence of visual signal in *S. n. notatus* and resulting loss of signal utility indicates the capacity for change among isolated populations that could explain the speciose clade of *Sphaerodactylus*. That is, loss of reliable male signal can cause divergence in receiver response, providing a mechanism for rapid divergence among populations that could then lead to behavioral reproductive barriers and speciation. Inter-population encounters, though unlikely given the distribution of *S. notatus*, may be common in other *Sphaerodactylus* species and could shed light on how variations in sexual dichromatism affect reproductive isolation in this clade. Future research will hopefully expand on this knowledge to better explain the link between signal divergence and speciation.

Declarations

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