Herpetologica, 73(4), 2017, 323–330
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Responses of Galápagos Lava Lizards (*Microlophus bivittatus*) to Manipulation of Female Nuptial Coloration on Lizard Robots

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Abstract: Females of some lizard species exhibit conspicuous coloration during the breeding cycle ("nuptial coloration") that elicits male courtship. We conducted two field experiments with San Cristóbal Lava Lizards (*Microlophus bivittatus*) in the Galápagos Islands to determine how the presence and extent of nuptial coloration on lizard robots affected responses of adult males and females. Robots programmed to perform conspecific bobbing displays had a morphological appearance that mimicked (1) a conspecific female without nuptial coloration (non-red control stimulus); (2) a conspecific female with nuptial coloration (normal red stimulus); or (3) a female with an extent of nuptial coloration beyond the range of conspecific variation (super-normal red stimulus). In Experiment 1, subjects witnessed two stimuli in sequence, being presented first with the side of a robot that exhibited conspecific nuptial coloration or with the opposite side of the same robot that lacked nuptial coloration. Results showed no effect of subject sex or stimulus order, but subjects exhibited more display and shorter display latencies in response to the normal red stimulus than to the non-red stimulus. In Experiment 2, new subjects were shown either non-red, normal red, or super-normal red stimulus. In contrast to Experiment 1, results of this experiment revealed sex differences in the amount of display elicited from subjects. Among the findings, males exhibited less display to the super-normal stimulus than to the non-red and normal red stimuli whereas the quantity of display elicited from females by the super-normal stimulus was similar to that evoked by the other two stimuli. We discuss our results in the context of prior studies and offer suggestions for future research.

Key words: Communication; Bobbing displays; Galápagos Islands; Sexual selection; Tropiduridae; Visual signals

Biologists have long been intrigued with the diversity of conspicuous color patterns among animal species and between the sexes. With the exception of aposematic coloration, colorful ornamentation is thought to evolve via sexual selection through mate choice, same-sex competition, or both (Darwin 1871; Andersson 1994; Jones and Ratterman 2009; McCullough et al. 2016). Among reptiles, aposematism is exceedingly rare in lizards (e.g., *Heloderma suspectum*); thus, conspicuous coloration in lizards must arise almost exclusively through sexual selection (see Cooper and Greenberg 1992 and Olsson et al. 2013 for reviews). As with motion displays, lizard color displays can function to attract the opposite sex as well as to intimidate same-sex rivals (see Carpenter and Ferguson 1977 and citations immediately above).

Numerous lizard taxa exhibit seasonal breeding (nuptial) color ornamentation in which red, orange, or yellow coloration (or a combination) covers the neck and adjacent locations (in males, Cooper and Vitt 1988; Martín and Forsman 1999; Sreekar et al. 2011; Batabyal and Thaker 2017; in females, Cooper and Greenberg 1992; Cooper and McGuire 1993; Hagar 2001; Weiss 2002; Chan et al. 2009; Salica and Malloy 2009; Stuart-Fox and Goode 2014). In those species that exhibit female nuptial coloration, color saturation typically increases rapidly at first and then diminishes slowly during each reproductive cycle. The function of nuptial coloration appears to be conditional in such cases, conveying female receptivity to male courtship early in color expression but indicating gravidity when fully expressed (Cooper and Greenberg 1992; Hagar 2001; Chan et al. 2009; Stuart-Fox and Goode 2014). Although males will court females of any reproductive state, the presence of female nuptial coloration strongly stimulates courtship in males (Cooper and Greenberg 1992).

During courtship, males approach females while regularly performing stereotyped bobbing movements variously characterized as headbob displays, pushup displays, shudders, or other actions, depending on the details of the motion patterns and the author describing them (reviewed in Carpenter and Ferguson 1977). Unreceptive females in many species respond to male courtship with rejection displays, particularly when nuptial coloration is fully expressed (e.g., Carpenter and Ferguson 1977; Cooper and Greenberg 1992; Chan et al. 2009). Females behave aggressively toward same-sex conspecifics as well. Although the structure of female bobbing displays is identical to that of conspecific males, females typically exhibit less elaboration in posturing and contest escalation than is observed between males (Carpenter 1966; Werner 1978).

In the tropical lizard genus *Microlophus*, females exhibit conspicuous orange-red (henceforth red) coloration when reproductively active. During each 25–30 d reproductive cycle, throat coloration of female *M. occipitalis* in Western Ecuador progresses from white (nonreproductive or early in the cycle) to pale red and finally to deep red (Watkins 1997). Following oviposition, nuptial coloration begins fading back to white. In Galápagos Lava Lizards, body location and extent of female red nuptial coloration varies considerably among species. Similar to mainland *Microlophus*, nuptial color expression in female Lava Lizards appears, intensifies, and then fades during each 3–4 wk reproductive cycle (Werner 1978). In contrast to mainland *Microlophus*, however, nuptial coloration in female Galápagos Lava Lizards becomes less vibrant following oviposition but does not disappear (Stebbins et al. 1967).

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Researchers have attempted to understand the functions of lizard nuptial coloration experimentally by using paints (in males, Martín and Forsman 1999; Olsson 2001; Husak et al. 2004; in females, Watkins 1997; Cuadrado 2000; Hagar 2001; Weiss 2002; Baird 2004; Stuart-Fox and Goode 2014; both sexes, reviewed in Cooper and Greenberg 1992). Given that behavioral interactions between live (painted) stimulus individuals and experimental subjects can complicate interpretations of results, stimulus–subject interaction can be eliminated (if desired) through the use of artificial models (Olsson 1993; Cuadrado 1998; McLean et al. 2010). Perhaps the most flexible means to achieve stimulus control in research on animal visual signals is through the use of computer-controlled robots (reviewed in Frohnwieser et al. 2016a). Indeed, Cooper and Greenberg (1992:391) pointed out the need for "reasonably realistic mechanical models" in studying behavioral responses to manipulations of coloration. Since the time of that review, numerous studies have been conducted in which lizard robots have been employed (Gillingham et al. 1995; Martins et al. 2005; Smith and Martins 2006; Kelso and Martins 2008; Ord and Stamps 2008, 2009; Thompson et al. 2008; Partan et al. 2011; Nava et al. 2012; Macedonia et al. 2013, 2015; Clark et al. 2015, 2016; Frohnwieser et al. 2016b). In only one of these studies, however, has display coloration (male dewlap color) been manipulated independently of display motion (Macedonia et al. 2013). Moreover, the effects of female lizard nuptial coloration on subjects' responses have yet to be examined using robotic stimuli.

In this study, we used lizard robots in two field experiments to examine the effects of female nuptial coloration on display behavior in adult male and female *M. bivittatus*. Our goal for Experiment 1 was to determine if subjects would differ in response to two versions of a female conspecific robot. We predicted that a robot with conspecific appearance and nuptial coloration would elicit more display from subjects and shorter latencies to display than would the same robot lacking nuptial coloration. The results of this first experiment led us to conduct a second experiment in which we presented an additional treatment—a super-normal stimulus. The goal of Experiment 2 was to determine if increasing the body coverage area of female nuptial coloration would affect subjects' display responses. Specifically, we predicted that a conspecific female robot with elaborated nuptial coloration ("super-normal stimulus") would evoke more display from subjects than would normal red and non-red robots.

Materials and Methods

Study Species

The nine species of Galápagos Lava Lizards (*Microlophus* spp.) comprise two independent evolutionary radiations: a western radiation containing seven species and an eastern radiation containing two species. The smaller eastern radiation includes *M. bivittatus*, which is endemic to the Galápagos island of San Cristóbal (Benavides et al. 2009). Molecular phylogenetic analyses indicate that *M. bivittatus* shares a common ancestor with *M. occipitalis* on the South America mainland, which arrived on San Cristóbal via the Humboldt Current between 2.1 and 2.8 million years ago (Benavides et al. 2009). Unlike some other species of Lava

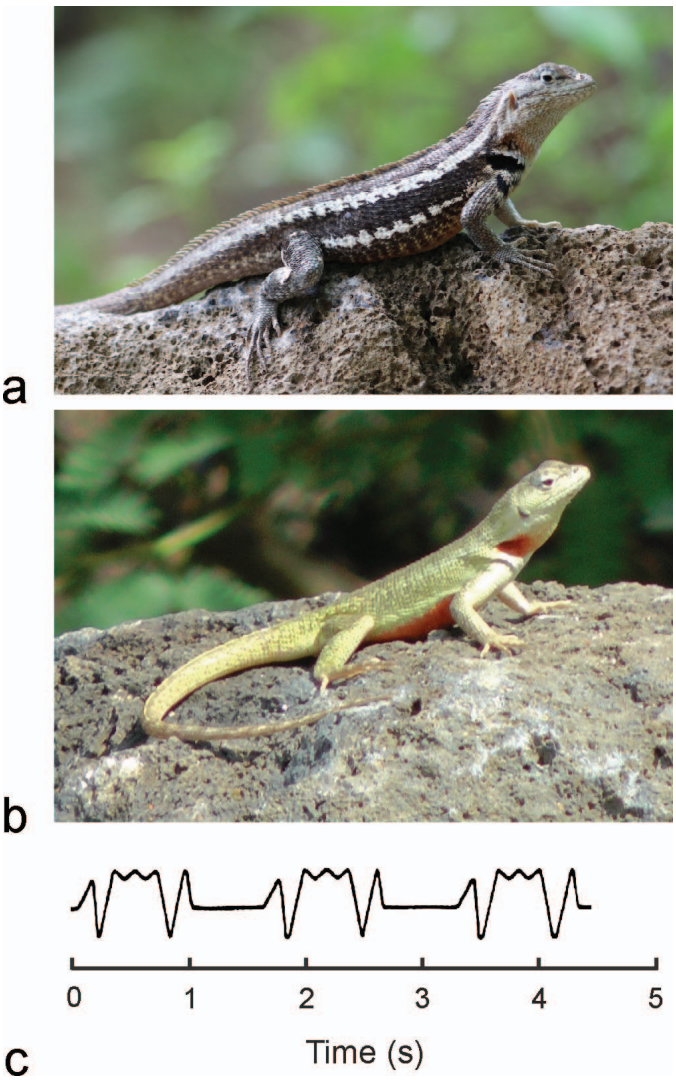

Fig. 1.—Representative *Microlophus bivittatus* adult (a) male and (b) female; (c) *M. bivittatus* display action pattern (DAP) graph showing three consecutive species-specific headbob displays (adapted from Carpenter 1966).

Lizards that might have experienced secondary contact during land bridge exposure at Pleistocene glacial maxima (Clark et al. 2015, 2016), San Cristóbal always has been isolated from other islands in the Galápagos archipelago (Benavides et al. 2009; Ali and Aitchison 2014; Geist et al. 2014). It therefore is improbable that *M. bivittatus* has experienced previous contact with a congener.

Like many lizard species, *M. bivittatus* exhibits male-biased sexual size dimorphism (SSD), measured as male snout–vent length (SVL: mean ± 1 SD) divided by female SVL (male SVL = 71.41 ± 9.78 mm, range = 55–94 mm, n = 161; female SVL = 57.85 ± 5.05 mm, range = 47–70 mm, n = 118; SSD = 1.23; J.W. Rowe and D.L. Clark, personal observations). Males exhibit a color pattern of dark gray-to-black on the dorsum that is interrupted on each side by two longitudinal whitish bands (Fig. 1a). Male coloration sometimes contains pinkish to brownish overtones, particularly on the dorsal crest, and the ventrolateral chest and abdomen are yellowish with orange overtones. By comparison, female *M. bivittatus* are patterned diffusely in muted

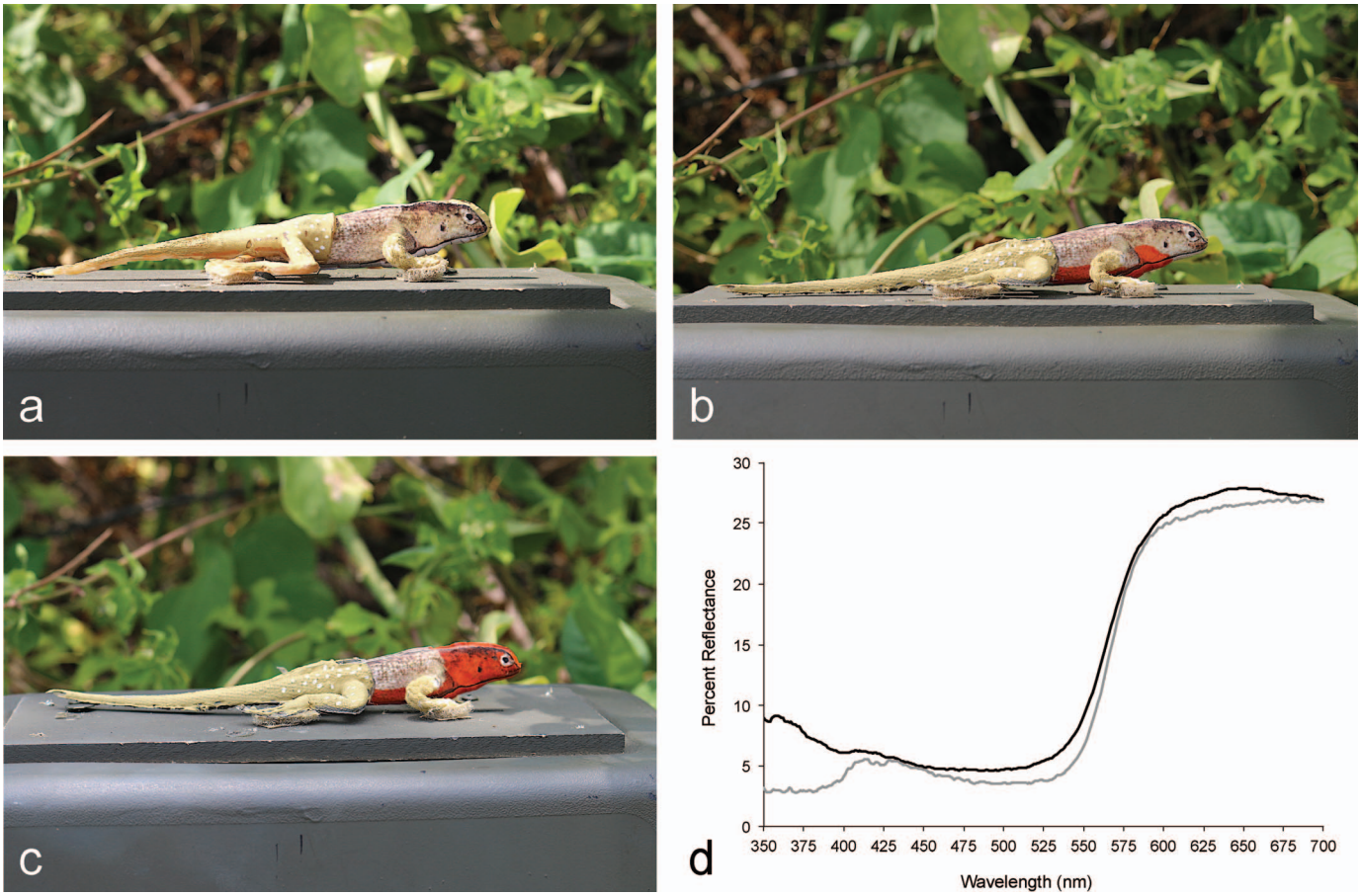

Fig. 2.—Robot models of adult female *Microlophus bivittatus* used to examine responses to visual signals: (a) non-red female, (b) normal red female, and (c) super-normal red female; (d) mean reflectance spectrum of red nuptial coloration on *M. bivittatus* females (black line, $n = 7$) and a reflectance spectrum (gray line) of the red permanent marker ink that was applied to robot skins in (b) and (c).

shades of brown and gray which often are overlaid with a wash of yellow. When gravid, females exhibit chromatic (saturated) red nuptial coloration on the neck and ventrolateral areas of the chest and abdomen (Fig. 1b; also, see Fig. S1 in the Supplemental Materials available online).

Robot Construction and Programming

To create a robot body, lizard shapes (60 ± 2 mm SVL) were hand-carved from wood and secured with eyelets to a pushrod. The pushrod was linked to a servomotor that controlled the robot's vertical bobbing motions. The hindquarters and tail of the robots were made of latex from a Plaster-of-Paris mold of a preserved *Microlophus* specimen. The latex hindquarters were painted to approximate those of an adult female *M. bivittatus* and were glued in place on the robot body.

We created coverings ("skins") for the robot bodies from a photo of a live adult female standing in profile. Using Adobe Photoshop (V 12.04 x32, Adobe Systems, Inc., San Jose, CA), we fit the image to one side of the robot body and then produced a mirror image to fit the opposite side. The skins were inkjet-printed onto photo-quality stretchable adhesive fabric, cut to shape, and adhered to the wooden robot body. We used three variants of adult female *M. bivittatus* skins for our experiments (Fig. 2): a female lacking red sexual coloration (non-red control stimulus), a female exhibiting normal red nuptial coloration (normal red stimulus), and a female in which we extended the red throat coloration to cover the head (super-normal red stimulus, sensu Tinbergen and Perdeck 1951). The super-normal stimulus combined the female nuptial coloration of our study species with that of an allopatric congener, *M. delanonis* (endemic to Española Island), in which fully expressed red nuptial coloration covers the entire head and upper torso (Werner 1978). To produce the normal and super-normal stimuli, red permanent marker ink was applied to the non-red female printed skin (Fig. 2b,c). Although female *M. bivittatus* nuptial coloration has slightly more of a UV-reflective component (~9% at 360 nm) than does our red ink treatment (~3% at 360 nm; Fig. 2d), a difference of 6% in UV reflectivity is not large in the context of our tested hypotheses. Although spectral sensitivities are not known for any *Microlophus* species, long-wavelength sensitivity in most other lizards for which it is known (Loew et al. 2002; Chan et al. 2009; Macedonia et al. 2009) suggests that our subjects may have perceived the red marker coloration on our robotic stimuli in a manner roughly similar to our own perception of it, at least to the extent that this coloration is unlikely to have been viewed as being highly unnatural.

We created a *M. bivittatus* display within an MIDI controller file (Logic Pro v9.1 for Macintosh OS, Apple Inc., Cupertino, CA) following Carpenter's (1966) representative

Display Action Pattern (DAP) graph for this species (Fig. 1c). Our programmed display was assessed carefully for fidelity to Carpenter's DAP graph using the iPad iOS application Video Physics (v3.0, Vernier Software and Technology LLC, Beaverton, OR). The Logic Pro MIDI controller file was exported to an iPod Touch via the Line 6 MIDI Mobilizer so that we could readily present display sequences to subjects in field trials. Each display sequence was 30 s in duration and consisted of 3 consecutive displays lasting a total of 4.25 s followed by a 25.75-s pause. Each 30-s sequence was iterated 12 times over a subject's trial period in Experiment 1 and 16 times in Experiment 2.

Robot Presentation Protocol

We presented our robotic stimuli to adult male and female *M. bivittatus* in the vicinity of Puerto Baquerizo Moreno on San Cristóbal (0°53′45.13′′S, 89°36′24.70′′W; datum = WGS84). Robot presentations were conducted daily from 29 February to 7 March 2016, between 0900 and 1700 h, in temperatures of 26–32°C under clear to partly cloudy skies. We searched for adult-sized lizards by visually scanning lava rock walls and rock outcrops while walking dirt roads and paths. After locating a potential subject, we placed the robot at a standard distance of ~2 m (maximum range ~1–3 m) from the subject in full, broad-side view. Variation in subject–robot distance at the initiation of a trial depended both on the location of the subject with respect to obscuring rocks and plants and features of the terrain on which the robot could be placed in a stable manner. Subjects rarely remained stationary, however, and their distances from the robot varied throughout the duration of a trial. When possible we positioned our tripod-mounted video camera ~2 m behind the robot and in line with the subject so that we could see both the subject and robot in the same video frame. If a subject neither moved out of view nor displayed during the trial setup period (~2–3 min), the trial was initiated by triggering the robot display sequence. We avoided retesting individual lizards in more than one trial and therefore maintained independence of data by sampling new and disparately spaced areas (>100 m) during each round of robot presentations (radiotelemetry data indicate that *M. bivittatus* home ranges are ≤30 m in diameter; J.W. Rowe and D.L. Clark, personal observations).

In Experiment 1, we tested whether subjects differed in response to a female conspecific robot exhibiting or lacking nuptial coloration. For this experiment, one side of the robot body had a non-red (control) conspecific female appearance whereas the other side of the body had red permanent marker added to depict conspecific female nuptial color (Fig. 2a,b). We used a repeated measures (stimulus-switch, Clark et al. 2015) design that allowed us to test each subject with both stimuli, potentially reducing variability in responses to a given stimulus. Subjects were presented with 6 min of display from one side of the robot, which then was turned 180 degrees to show its opposite side, followed by a further 6 min of display. Turning the robot was carried out quickly but carefully with the goal of minimizing disturbance to the subject. We counterbalanced presentation order by alternating which side of the robot the subjects saw first in each new trial.

Upon completion of Experiment 1, we conducted an additional experiment that included a super-normal stimulus as a treatment (Fig. 2c). We chose not to use a repeated measures design in this experiment for several reasons. First, testing each subject with three stimuli would have required use of at least two different robots within the same trial, which would have been challenging to achieve. Moreover, it seemed unlikely to us that we could maintain a subject's attention throughout a trial in which three stimuli were presented sequentially. Alternatively, had we chosen to test each subject with one of three alternative stimulus pairings (i.e., non-red vs. normal red, normal red vs. super-normal red, and non-red vs. super-normal red), we would have had to conduct substantially more trials to obtain an adequate sample size for each treatment. Taken together, these deterrents favored an experimental design in which we presented a single stimulus type to three different groups of subjects. Thus, in Experiment 2, subjects received a single, 8-min trial with one of the three robot stimuli (non-red, normal red, or super-normal red). Images and videos of subjects responding to our robots are provided in the Supplementary Materials.

Statistical Analysis

We used two measures to quantify responses of adult male and female subjects: (1) the summed durations of a subject's bobbing displays during a trial expressed as a proportion of trial duration; and (2) the latency of a subject to engage in bobbing display during a trial, also expressed as a proportion of trial duration. In the present study, *M. bivittatus* subjects performed two types of bobbing displays: the species-specific display (Fig. 1c) and a two-bob "up-down-up-down" display that is present in other lava lizard species. The brief (~0.3 s) two-bob display usually occurred immediately prior to movement (often toward the robot) but also occasionally preceded species-specific displays. Because male and female subjects performed both types of bobbing display in response to our robotic stimuli, we included both display types in our calculations of display duration totals and display latencies. We did not observe bobbing displays with a motion structure unique to male courtship, either in response to our robots or in serendipitous recordings of courting pairs made during the course of the study.

As subjects occasionally moved out of the video camera field of view during trials (and thus could not be scored during these periods), trial durations sometimes were shorter than stimulus presentation periods. Likewise, some subjects continued to display in response to a robot after the robot had completed its final series of bobs in a presentation. For such subjects, we continued to record these displays until subjects had not displayed for 15 s following their last display. In these instances, our calculations were adjusted to account for the longer trial durations. Converting the sum of display durations and display latency to the proportions of trial length standardized these measures across subjects (Clark et al. 2015, 2016). Shapiro-Wilk tests affirmed that the distributions of our two response variables (whether partitioned by sex or with sexes combined) met the assumptions of normality following a log+1 transformation.

In Experiment 1, our repeated measures analyses of variance (ANOVA) tested the null hypothesis that the sum of display durations and display latency during a trial did not differ between the control (non-red) and experimental (normal red) treatment. In this experiment, the within-subjects factor was treatment and the between-subjects

factors were sex and presentation order. For Experiment 2, we used two-way ANOVAs with treatment and sex as fixed factors to test the null hypothesis that neither the sum of display durations nor display latency in a trial differed among the non-red, normal red, and super-normal red treatments. When results of these ANOVAs were significant, we conducted post hoc pairwise comparisons with Fisher LSD tests, which produce acceptable Type I error rates in comparisons involving $\leq$4 treatments (Keselman et al. 1979). All statistical tests were conducted with SPSS (v21.0, IBM Inc., Armonk, NY).

Results

Experiment 1: Repeated Measures Trials

Paired-stimulus presentations, in which one side of the robot exhibited normal red nuptial coloration and the other side lacked this coloration, were conducted with 24 subjects (9 males and 15 females). In these trials, 23 subjects performed bobbing displays in response to at least one of the two stimuli and 19 subjects responded to both stimuli. Only one subject did not respond to either stimulus and was discarded from the statistical analysis. Results of a repeated measures ANOVA on display duration revealed a main effect of treatment ($F_{1,19} = 12.72$, $P < 0.005$): subjects displayed more in response to the normal red side of the robot than to its non-red side (Fig. 3a). By contrast, neither sex ($F_{1,19} = 0.36$, $P > 0.05$) nor presentation order ($F_{1,19} = 0.31$, $P > 0.05$) had an effect on display duration. Interactions with treatment of sex ($F_{1,19} = 1.16$, $P > 0.05$) and presentation order ($F_{1,19} = 0.18$, $P > 0.05$) were absent. Similarly, a repeated measures ANOVA on display latency revealed a main effect of treatment ($F_{1,19} = 29.75$, $P < 0.0001$): subjects took longer to begin displaying to the non-red side of the robot than to its red side (Fig. 3b). Again, neither sex ($F_{1,19} = 0.10$, $P > 0.05$) nor presentation order ($F_{1,19} = 0.18$, $P > 0.05$) had an effect on display latency, and interactions with treatment of sex ($F_{1,19} = 0.10$, $P > 0.05$) and presentation order ($F_{1,19} = 0.18$, $P > 0.05$) were absent.

Experiment 2: Single-Stimulus Trials

Single-stimulus robot presentations were made to a total of 85 subjects (43 males and 42 females) in which 34 males and 33 females responded with at least one bobbing display. Nonresponders (9 males and 9 females) were discarded from the analysis. Results of a two-way ANOVA on display duration revealed main effects both for treatment ($F_{2,61} = 5.09$, $P < 0.01$) and sex ($F_{1,61} = 15.00$, $P < 0.001$); the interaction term was nonsignificant ($F_{2,61} = 2.69$, $P > 0.05$). Post hoc pairwise comparisons showed that male subjects displayed more to non-red ($P < 0.05$) and normal red ($P < 0.01$) robots than to the super-normal red robot (Fig. 4a). Although males displayed more to the normal red than to the non-red stimulus, the differences were not significant (Fig. 4a). In contrast to males, female subjects displayed more to the normal red robot than to the non-red robot ($P < 0.05$); no other comparisons were significant (Fig. 4b). Results of a two-way ANOVA on display latency showed a main effect of treatment ($F_{2,61} = 5.82$, $P < 0.01$), but neither sex ($F_{1,61} = 1.41$, $P > 0.05$) nor the interaction term ($F_{2,61} = 0.85$, $P > 0.05$) were significant. Data from both sexes therefore were combined in a one-way ANOVA, where results showed that response latency differed among treatments ($F_{2,64} = 5.65$, $P < 0.01$). Post hoc pairwise comparisons revealed that subjects exhibited shorter response latencies to the normal red robot than to the non-red robot ($P < 0.001$); no other comparisons were significant (Fig. 4c).

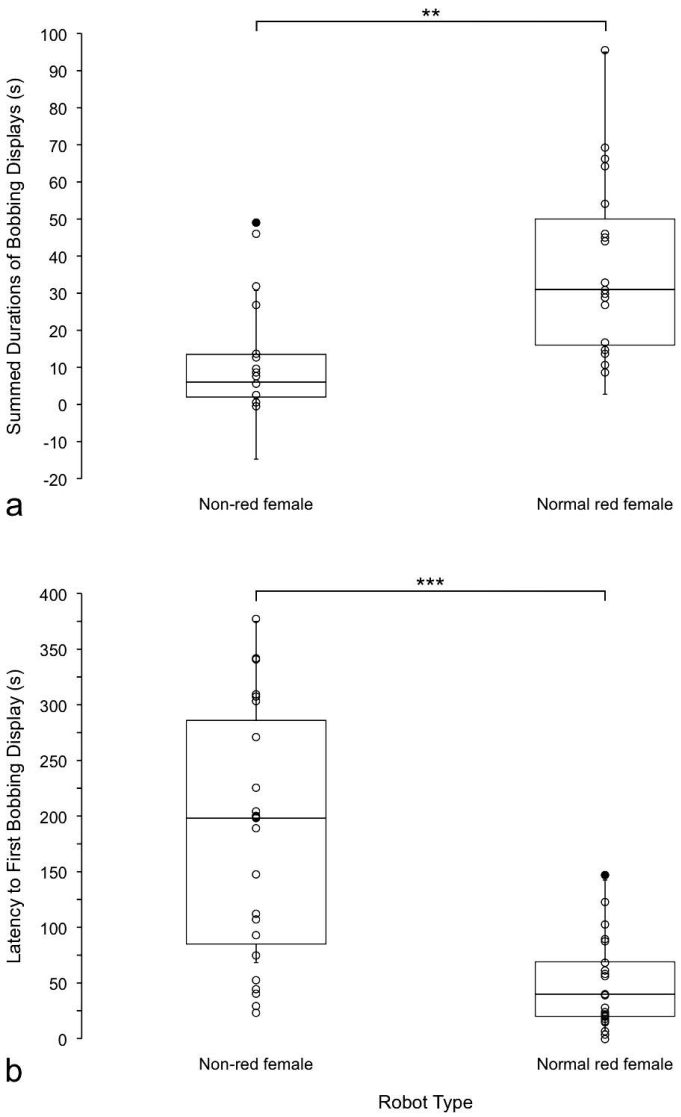

Fig. 3.—Summed durations of (a) bobbing displays and (b) latency to first bobbing display performed by adult *Microlophus bivittatus* (both sexes) during repeated measures trials in response to robots with and without normal breeding coloration. Each panel shows box plots overlain with complete distributions of raw (untransformed) data as open circles (each depicting a subject's response). In each box plot, the central 50% of the data distribution (i.e., the interquartile range; IQR) is contained within the box, with the horizontal line in each box representing the median. The end caps of the error bars (whiskers) are shown as the 1st quartile minus 1.5 IQR and the 3rd quartile plus 1.5 IQR. Filled circles indicate minimum and maximum outliers. ** = $P \leq 0.01$, *** = $P \leq 0.0001$.

Discussion

Although relatively uncommon in vertebrates, hormonally controlled sex-specific breeding coloration occurs in a diversity of lizard taxa (Cooper and Greenberg 1992; Olsson et al. 2013). In this study, we presented free-ranging male and female San Cristóbal Lava Lizards with robots intended

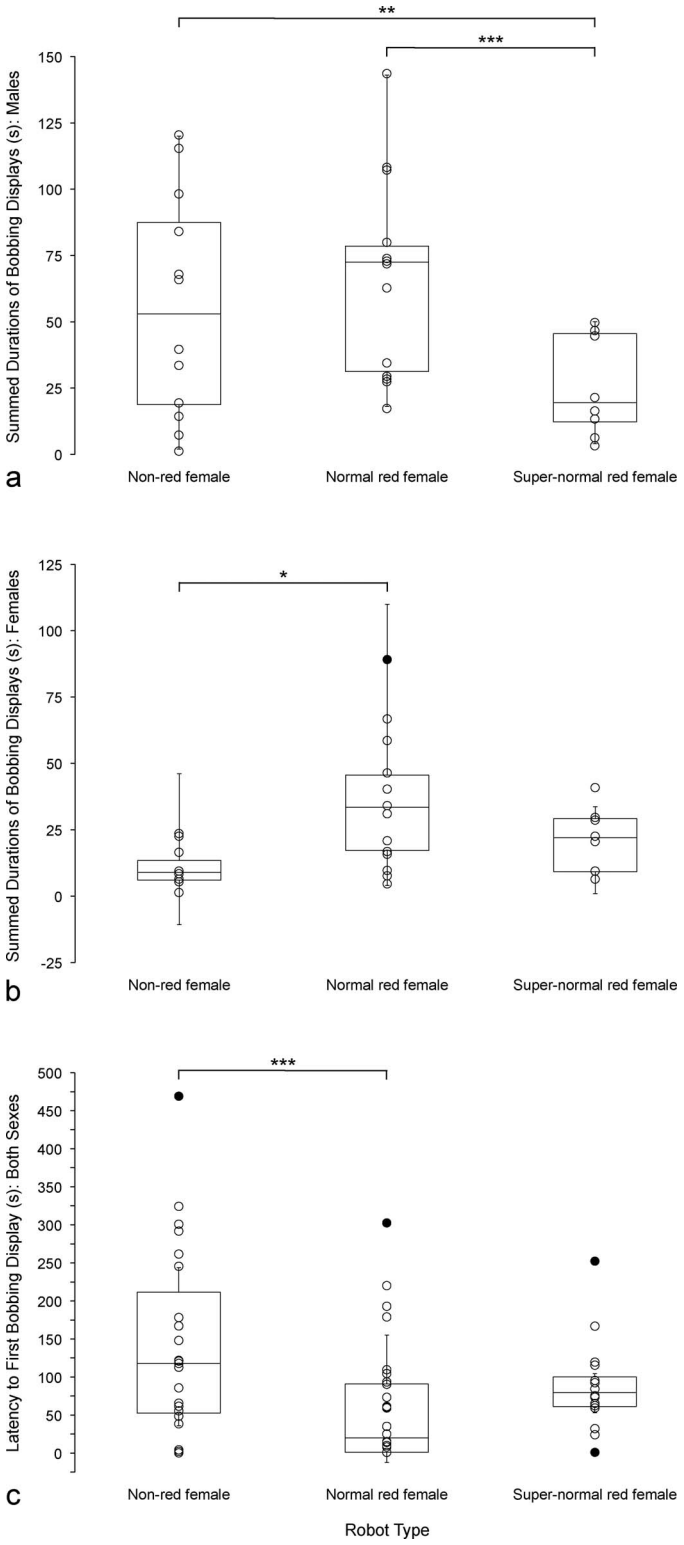

FIG. 4.—Summed durations of bobbing displays performed by male (a) and female (b) *Microlophus bivittatus* and latency to first bobbing display (c; both sexes), during single-stimulus trials in response to robots showing different amounts of breeding coloration. Parameters of box-whisker plots as described for Fig. 3. * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$.

to mimic conspecific females that (1) lacked nuptial coloration, (2) exhibited a normal configuration of nuptial coloration, or (3) expressed an extent of nuptial coloration beyond that occurring in our study species. In contrast to most prior experiments, we tested the effects of female nuptial color manipulation on display behavior in both sexes.

When presented with two stimuli in sequence, *M. bivittatus* subjects exhibited a clear pattern of responses: irrespective of sex or presentation order, subjects engaged in more display behavior and showed shorter display latencies to the side of a robot exhibiting normal female nuptial coloration than to the plain (non-red) side of the same robot. These findings are consistent with our predictions that a robot with conspecific female nuptial coloration would evoke more display, as well as shorter display latencies, than would the same robot lacking nuptial color. We interpret the subjects' stronger responses to the presence of female nuptial coloration as reflecting increased sexual attraction in males and increased aggression in females. These results are commensurate with conclusions from observational and experimental studies of female breeding coloration in other species (e.g., Hagar 2001; Chan et al. 2009; Stuart-Fox and Goode 2014). Whereas female nuptial coloration (early in its expression) stimulates male courtship by signaling female receptivity (Cooper and Greenberg 1992), its elicitation of aggression among breeding females might reflect competition for acquisition or defense of good oviposition sites (Yedlin and Ferguson 1973; Werner 1978).

In our second experiment, new subjects were each presented with one robot resembling a conspecific female whose nuptial coloration either was absent (non-red stimulus), typical (normal red stimulus), or exaggerated (super-normal red stimulus). Similar to subjects' responses in Experiment 1, both sexes displayed more frequently and exhibited shorter display latencies to the robot with normal female nuptial coloration than to the plain robot. However, the sexes differed in the proportion of display elicited by the super-normal red stimulus as compared to the other two forms of the stimulus.

Males performed the least amount of display to the super-normal red stimulus. This finding runs counter to our prediction and indicates that increased body coverage of female nuptial coloration diminished rather than heightened male motivation to display. One potential explanation for this outcome is that males correctly perceived the super-normal color pattern to be aberrant for a conspecific female. Alternatively, because fully expressed nuptial coloration in female lizards is positively correlated with increased female rejection display behavior (e.g., Carpenter and Ferguson 1977; Cooper and Greenberg 1992), male subjects might have viewed the probability of successful copulation with the super-normal stimulus to be particularly low. For females, the amount of display elicited by the super-normal red stimulus did not differ from that evoked by the non-red and normal red stimuli. This outcome likewise failed to support our prediction, as elaborating female nuptial coloration beyond the range of conspecific variation did not result in proportionally stronger responses.

Future Directions

In creating our super-normal stimulus for Experiment 2, we extended *M. bivittatus* female nuptial coloration from the

flanks and sides of the neck to encompass the entire head. Although this manipulation accomplished our goal of supernormality, it did not allow us to distinguish the effect of increasing nuptial coloration coverage area from that of a nonconspecific color pattern. These two factors could be disentangled in a future experiment by manipulating body location of nuptial coloration independently of coverage area. In addition, results from previous studies of lizards have shown that females with newly emerged nuptial coloration were less likely to exhibit courtship rejection than were females with fully expressed nuptial coloration; likewise, males have been shown to exhibit more-intense interest in females exhibiting early expression of nuptial coloration than in females with fully expressed nuptial coloration (e.g., Cooper and Greenberg 1992). Given that stimuli in the majority of prior experiments on lizard female nuptial coloration consisted of painted live conspecifics whose behavior could not be controlled, similar manipulations using robotic stimuli could provide further insights into how conspecifics perceive different stages of female nuptial color expression.

Acknowledgments.—We thank J. P. Muñoz, Administrador e Investigador, and S. Sotamba of the Galápagos Science Center (San Cristóbal). We are grateful to G. Quezada and M. Yepez of Galápagos National Park (San Cristóbal) for their support and permission to conduct research on Lava Lizards. DLC and JMM contributed equally to this study.

Supplemental Materials

Supplemental material associated with this article can be found online at https://doi.org/Herpetologica-D-16-00056.SF1; https://doi.org/Herpetologica-D-16-00056.SV1; https://doi.org/Herpetologica-D-16-00056.SV2; https://doi.org/Herpetologica-D-16-00056.SV3; https://doi.org/Herpetologica-D-16-00056.SV4; https://doi.org/Herpetologica-D-16-00056.SV5. Below is a list of captions for the supplementary videos:

Video S1.—A male *Microlophus bivittatus* subject exhibiting species-specific displays in response to displays by the normal red female robot.

Video S2.—A male *Microlophus bivittatus* subject approaching the super-normal red female robot while exhibiting two-bob displays and a species-specific display.

Video S3.—A female *Microlophus bivittatus* subject exhibiting species-specific displays in response to displays by the normal red female robot.

Video S4.—A female *Microlophus bivittatus* attacking and biting the normal red female robot, followed by two species-specific displays.

Video S5.—A male *Microlophus bivittatus* subject attempting to grasp the neck–head region of the non-red female robot, as occurs prior to normal copulation.

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Accepted on 30 April 2017
Associate Editor: Ryan Taylor