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Why Does Conspecific Display Recognition Differ Among Species of Galápagos Lava Lizards? A Test Using Lizard Robots

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Abstract: Discriminating between conspecific and heterospecific communication signals has important implications for evolution. The benefits of such discriminations are clear for sympatric congeners, but if conspecific display recognition (CDR) has a cost, it should be relaxed in species that have evolved in isolation. Of the nine lava lizard species (*Microlophus* spp.) endemic to the Galápagos Islands, all are thought to have evolved in allopatry and none overlap in geographic distribution. Although prior research failed to reveal CDR in male *M. grayii* on Floreana Island, male *M. indefatigabilis* on Santa Cruz Island showed a response bias toward conspecific displays under the same experimental conditions. In the present study we tested for CDR in two additional species: *M. albemarlensis* on Isabella Island and *M. bivittatus* on San Cristóbal Island. Subjects were presented with computer-controlled robots that either performed the conspecific display, a reversed-inverted conspecific display, or a display from an *Anolis* lizard species. Results revealed evidence of CDR in male *M. albemarlensis*, but no such evidence in male *M. bivittatus*. Interestingly, bathymetric data provide a potential explanation for CDR in *M. indefatigabilis* and *M. albemarlensis:* a land bridge between Santa Cruz and Isabella might have resulted in secondary contact and reinforcement in these two species during Pleistocene glacial maxima. Another possibility is that intraspecific selection for discrimination among males has caused heterospecific displays to be viewed as low-fidelity conspecific displays. Nevertheless, our findings are consistent with CDR being lost early in Galápagos *Microlophus* evolution, as predicted for allopatric speciation, and re-emerging later only where selection favored the discrimination of male displays.

Key words: Allopatric speciation; Communication; Head-bobs; *Microlophus*; Push-ups; Tropiduridae

A fascinating attribute of animal communication systems is the diversity of displays that have evolved to attract mates and intimidate rivals (Bradbury and Vehrencamp 2011). Stereotyped displays that facilitate discrimination of conspecifics from sympatric congeners are particularly widespread in vertebrates (Ryan and Rand 1993; Ptacek 2000; Ord and Stamps 2009), and a reduction in fitness resulting from interspecific mating might be a major driver of display differentiation (Brown and Wilson 1956; Hoskin et al. 2005). For example, reproductive character displacement in mate recognition signals has been documented in diverging populations where they have come into secondary contact (Lemmon 2009; Crampton et al. 2011; Ng and Glor 2011; Lambert et al. 2013). Recognition of species-specific display traits can serve as an effective prezygotic reproductive barrier and should be particularly important for females (Butlin 1987; Servedio 2004). Similarly, displays that facilitate competitor recognition can arise from interspecific competition, where agonistic character displacement of male phenotypic traits reduces fitness costs associated with inappropriate aggression toward heterospecifics (Grether et al. 2009; Okamoto and Grether 2013). In the absence of advantages associated with discrimination of conspecifics from other congeners, as can occur in allopatric speciation, relaxation of conspecific display recognition (CDR) is anticipated (Wellenreuther et al. 2009).

Males in many species of lizards regularly perform territorial head-bob or push-up displays (hereafter, displays) that usually consist of abrupt vertical motions of the head and torso (Carpenter and Ferguson 1977). These stereotyped displays exhibit species-specific, and sometimes population-specific, structure (Jenssen 1977; Martins and Lamont 1998; Martins et al. 1998, 2004; Ord et al. 2001, 2002; Ord and Martins 2006). Such visual displays might be important in male agonistic competition for discriminating conspecific competitors from sympatric congeners (Jenssen 1970; Carpenter and Ferguson 1977; Ord and Stamps 2009). As territorial iguanians, the Galápagos lava lizards (*Microlophus* spp.) consist of nine species that vary in body coloration and in the structure of their motion displays (Carpenter 1966; Stebbins et al. 1967). Galápagos lava lizards comprise eastern (two species) and western (seven species) radiations that have arisen from separate colonizations of the Galápagos from the South American mainland (Benavides et al. 2009). All Galápagos lava lizard species are presumed to have evolved in isolation from congeners, and no two species coexist on the same island (Kizirian et al. 2004; Benavides et al. 2009). Thus, the forces driving CDR in sympatric congeners are unlikely to explain display diversification in taxa that have evolved in allopatry (Clark et al. 2015). If CDR is costly, selection for it should have been relaxed in the evolution of the Galápagos lava lizards.

Computer-controlled robots have been employed to address questions about the evolution of behavior in vertebrates as diverse as fish (Faria et al. 2010), frogs (Narins et al. 2003, 2005; Taylor et al. 2008; Halfwerk et al. 2014a,b), birds (Patricelli et al. 2002, 2006; Goth and Evans 2004), and squirrels (Rundus et al. 2007; Partan et al. 2009, 2010). Robots likewise have been used in studies of stereotyped display behavior in lizards (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). For example, field presentations of robotic stimuli have demonstrated CDR in *Anolis grahami*

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(Macedonia et al. 2013, 2015)—a species that has evolved in sympatry with numerous congeners on Jamaica (Jackman et al. 2002). By comparison, the Galápagos lava lizards provide a unique opportunity to test for CDR in species that have evolved in isolation from congeners.

Previously, two species in the western radiation of Galápagos lava lizards—*M. grayii* on Floreana Island and *M. indefatigabilis* on Santa Cruz Island—were presented with lizard robots possessing a conspecific appearance and that performed either conspecific or congeneric displays (Clark et al. 2015). Whereas male *M. grayii* exhibited no evidence of discriminating conspecific displays from those of *M. indefatigabilis,* male *M. indefatigabilis* exhibited a strong bias in responding to conspecific displays. Presentations of robots with conspecific coloration to *M. occipitalis* on the coast of Ecuador also have provided equivalent evidence for CDR in this species (Clark et al. 2015). *M. occipitalis* is the sister species of the eastern radiation of the Galápagos *Microlophus* (Benavides et al. 2009); therefore, the presence of CDR in the outgroup (*M. occipitalis*) and in the ingroup (*M. indefatigabilis*) indicates that it could represent an ancestral character state.

At present, too few species of Galápagos *Microlophus* species have been examined to know whether CDR is rare or is common in these lizards. Given that selection for CDR should be relaxed in species that have evolved in isolation, we postulated previously that CDR in *M. indefatigabilis* could have resulted from secondary contact (Clark et al. 2015). Specifically, a land bridge between the islands of Santa Cruz and Isabella exposed during Pleistocene glacial maxima (Jordan and Snell 2008; Geist et al. 2014) could have facilitated such contact.

In this study, we extend our previous work by examining CDR in two additional species of Galápagos lava lizards: *M. albemarlensis*—a member of the western radiation; and *M. bivittatus*—a member of the eastern radiation. Each species was presented with lizard robots that exhibited conspecific body coloration and that performed one of three types of display: (1) conspecific display, (2) a computer-modified reversed-and-inverted (R-I) conspecific display, and, (3) display from a distantly related taxon (*Anolis*). We predicted previously that *M. albemarlensis* on Isabella Island should exhibit evidence of CDR (Clark et al. 2015), and by extension, *M. bivittatus* on San Cristóbal Island should fail to show evidence of CDR.

Materials and Methods

Subjects and Study Area

Microlophus albemarlensis is endemic to the Islands of Isabella and Fernandina in the Galápagos archipelago (Benavides et al. 2009). Males are patterned in shades of brown, black, white, and gray, sometimes with pinkish or copper overtones. In addition, two whitish lateral stripes that run the length of the torso add to an overall cryptic appearance (Fig. 1a). Like most lizards, *M. albemarlensis* exhibits male-biased sexual size dimorphism (SSD), measured as male snout–vent length (SVL) divided by female SVL (male SVL = 70.85 mm, n = 48; female SVL = 70.00 mm, n = 47; SSD = 1.12; data from Carpenter 1966: table 1). *Microlophus bivitattus* is endemic to the Island of San Cristóbal. Male *M. bivittatus* are quite similar in overall coloration to male *M. albemarlensis,* except that the *M. bivittatus* ventrum is yellowish and the brown tones of the body tend to be darker than those of *M. albemarlensis* (Fig. 1b). As with *M. albemarlensis*, male *M. bivittatus* (SVL = 69.60 ± 1.57 mm, n = 38) are larger than conspecific females (SVL = 60.00 ± 0.81 mm, n = 22; SSD = 1.16; DLC and JWR, personal observations).

Robot Construction and Display Programming

We briefly summarize robot construction and display programming methods here, because complete details are provided elsewhere (Clark et al. 2015; Macedonia et al. 2015). Adult male robot bodies (~75 mm SVL) were hand-carved from wood and secured by eyelets to a pushrod. A servomotor was connected to the pushrod to control the vertical display motions of the robot. Color-patterned coverings (skins) for the robot bodies were constructed from photos of live adult male lizards standing in profile. Using Adobe Photoshop®, a skin was sized to fit one side of a robot body and a mirror image was created to fit the other side of the body. The entire skin was inkjet-printed onto a photo-quality, stretchable, sticky-backed fabric, which was cut from the surrounding material and adhered to the wooden body. Latex hind limbs and a tail were produced with Plaster-of-Paris impression molds using a preserved *Microlophus* specimen. The latex limbs and tail were painted and then glued in place on the robot body (see images of subjects being tested with robots in the Supplemental Materials available online, Figs. S1 and S2.).

We modeled *M. albemarlensis* and *M. bivittatus* displays after Carpenter's (1966) representative Display Action Pattern (DAP) graphs (Carpenter and Grubitz 1961) for these species. The *Microlophus* displays were carefully replicated from the DAP graphs in the construction of a midi controller file (Logic Pro v9.1 for Macintosh OS). Constructed displays were checked for fidelity to Carpenter's published DAP graphs using the iPad IOS application Video Physics (Vernier Software and Technology, LLC). Two versions of each species' display were constructed: a typical version and a reversed-inverted (R-I) version. We created the R-I versions to present some subjects with a display that was identical in structure to the conspecific display, but whose performance by the robot was reversed in time, and whose motion pattern was inverted relative to the normal display (i.e., head raises were head drops and vice versa). The R-I stimulus offers control over display complexity when presenting subjects with nonconspecific displays, because displays from different species are unlikely to exhibit identical degrees of complexity. In addition, we programmed robots to perform a display from one of two heterospecific *Anolis* species that had been employed in a prior study (Macedonia et al. 2015). These heterospecific displays were used to test the perceptual boundaries of what subjects viewed as legitimate signals, because these stimuli bore no resemblance to conspecific display in structure. Given that *M. albemarlensis* exhibits one of the simplest displays among the Galápagos lava lizards (Fig. 2a), we used the simple sinusoidal display of *Anolis extremus* (Fig. 2e) as our heterospecific treatment for this species. The display of *M. bivittatus* is more complex by comparison (Fig. 2c), so for this species we used the display of *A. grahami* (Fig. 2f) as our heterospecific treatment. Although the *A. grahami*

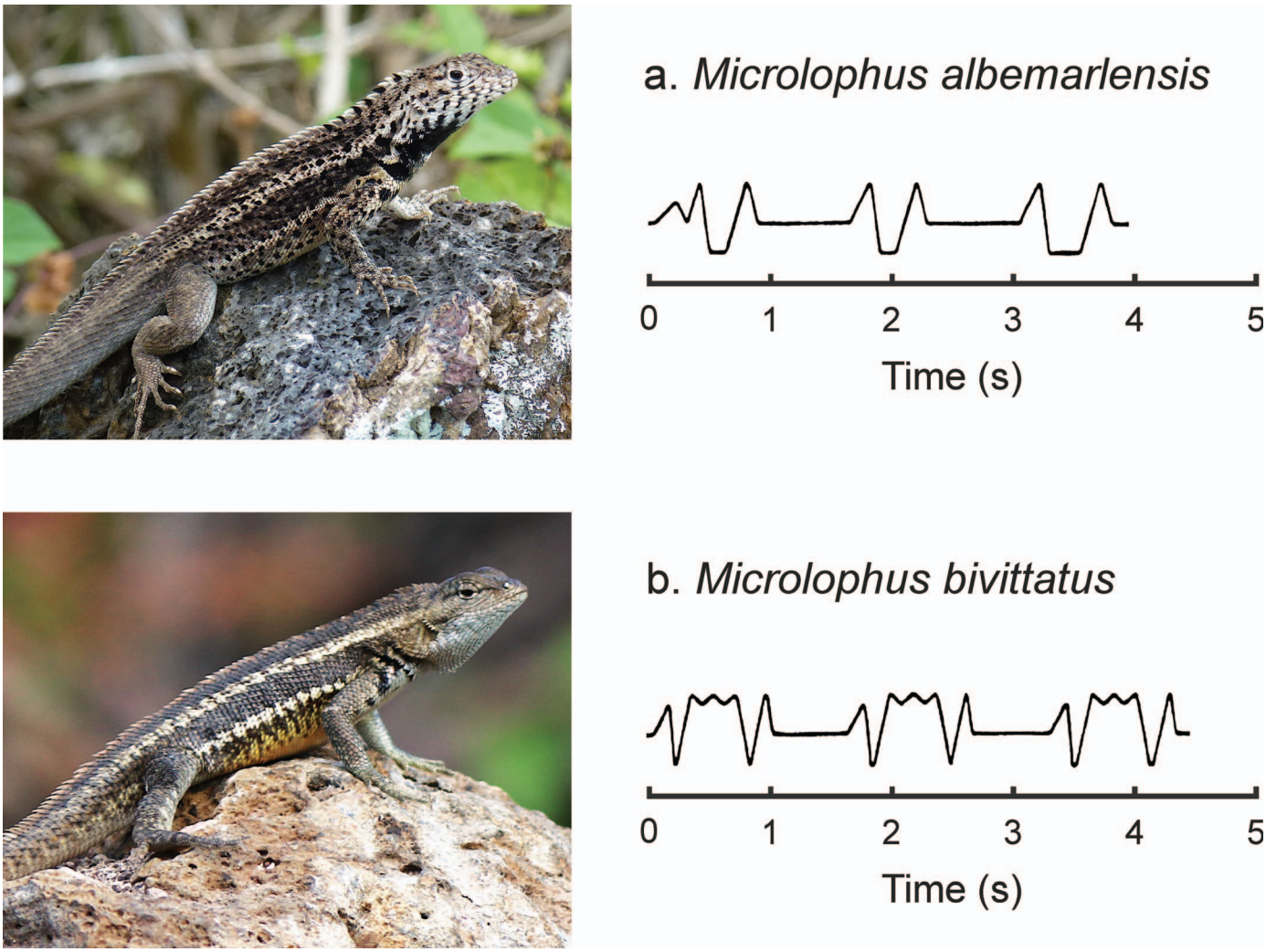

Fig. 1.—Representative adult male Galápagos lava lizards used as subjects in this study: (a) *Microlophus albemarlensis* and (b) *M. bivittatus*.

display is not as complex as that of *M. bivittatus,* the former species' square-wave display pattern bears no structural resemblance to the latter. Therefore, we suggest that the *A. grahami* display can be used to probe the limits of what *M. bivittatus* considers a display that necessitates a response.

To present a display sequence to subjects in field trials, the Logic Pro midi controller file was exported to an iPod touch using the Line 6 MIDI Mobilizer. A typical stimulus sequence consisted of three consecutive displays that together lasted 4 s followed by a 30-s pause. This cycle was iterated over a total trial period of 8.5 min. A single *A. grahami* display (Fig. 2f) lasted 4 s, so only one display was performed in each display–pause sequence. In addition, because our *M. bivittatus* display (Fig. 2c, d) was slightly greater in duration (4.5 s) than those of our other stimuli (4 s), interdisplay pauses were reduced by 0.5 s to match the combined display–pause sequence durations of all stimuli.

Robot Presentation Protocol

We presented robot stimuli to 50 adult male *M. albemarlensis* and to 60 adult male *M. bivittatus* in the vicinities of Muro de las lagrimas (Wall of Tears; 0°57′58.30″S, 91°00′45.95″W; datum = WGS84 for all coordinates) on Isabella, and Puerto Baquerizo Moreno (0°53′45.13″S, 89°36′24.70″W) on San Cristóbal, respectively. Robot presentations were conducted from 19 February to 7 March 2015 daily between 0900 and 1700 h in temperatures of 25–30°C under clear to partly cloudy skies. We searched for adult male lizards (visual estimate of ≥65-mm SVL) by walking along dirt roads and paths while visually scanning lava rock walls and rock outcrops for potential subjects. When a suitable male was located, we positioned a robot parallel to the subject at a distance of ~1–3 m. To record responses, a digital video camera on a tripod was set ~2 m behind the stimulus robot and in line with the subject. Our robot-camera configuration allowed us to capture the image of the subject and robot in the same field-of-view. If a subject did not flee or display during the setup period (~2–3 min), the robot display sequence was triggered (from an iPod touch), thus initiating the trial. Each subject received only a single trial. Treatments were chosen in a constrained random fashion, where each subject witnessed only one display type and all three display types were presented to different lizards in sequence before a given display type was repeated. To avoid testing subjects more than once, we did not search for lizards in locations where we already had conducted trials.

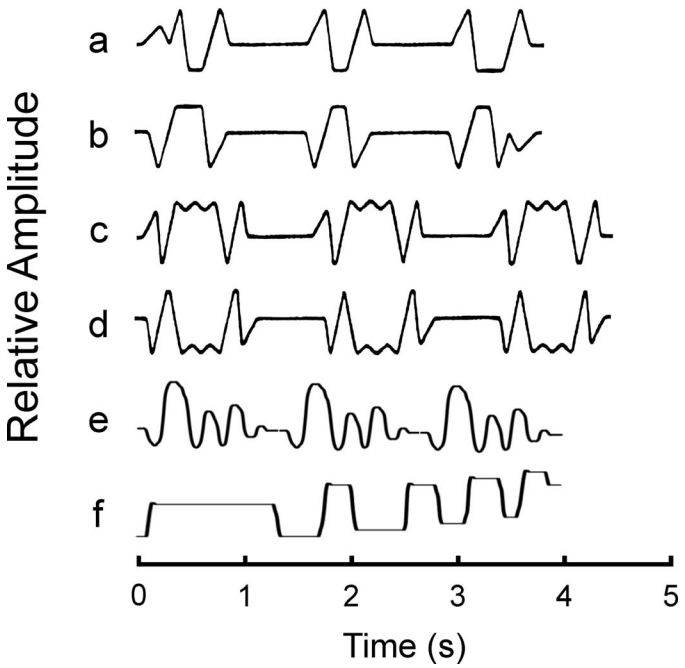

FIG. 2.—Display action pattern stimuli used in robot presentation trials: (a) series of three *Microlophus albemarlensis* displays; (b) same series as in (a) but reversed in time and inverted, such that downward head motions are upward motions and vice-versa; (c) series of three *M. bivittatus* displays; (d) same series as in (c) but reversed in time and inverted; (e) series of three *Anolis extremus* displays; (f) single *A. grahami* display.

Statistical Analysis

We used two measures to quantify responses of adult male subjects to robot presentations: (1) latency to the subject's first display in a trial, and, (2) the summed duration of all displays performed by the subject in a trial. Nonresponding subjects were excluded from analyses. Trial recordings were scored by an observer who was naïve to the experimental treatments. Not all subjects that displayed during a trial continued to do so through the end of the trial, and some subjects moved out of view of the video camera for brief periods. To be included in the analyses, subjects were required to be visible for a minimum of 80% of the trial (>90% of subjects were visible for ≥95% of their respective trial durations). To normalize trial durations across subjects, time spent displaying in response to the robot was converted to the proportion of the trial for which the subject remained attentive and was observable in the trial video (Clark et al. 2015). For each species, we used one-way analyses of variance (ANOVA) to test the null hypothesis that latency to display and total display duration did not differ among the three experimental treatments. The distributions of the two response variables met the assumptions of normality for both species following log-transformations (confirmed with Shapiro–Wilk tests). For significant ANOVAs, post hoc pairwise comparisons were made among the three treatments using the Fisher Least Significant Difference test. Simulations have shown that this test produces acceptable Type I error rates in comparisons that involve four or fewer treatments (Keselman et al. 1979). Statistical tests were conducted in SPSS (v21.0, IBM Inc., Armonk, NY).

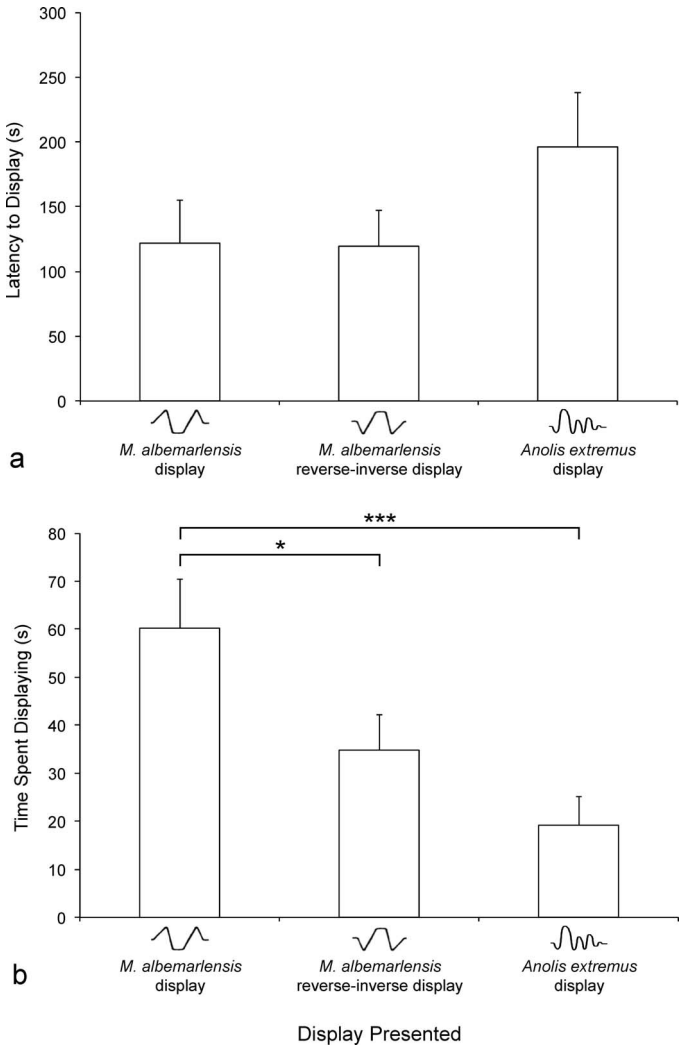

FIG. 3.—(a) Latency to first display and (b) total duration of displays by *Microlophus albemarlensis* adult male subjects in response to robots with conspecific coloration performing particular displays. Bars represent means + 1 SE; * $P \leq 0.05$, *** $P \leq 0.001$.

RESULTS

Experiment 1: Robot Presentations to *Microlophus albemarlensis*

Of the 50 *M. albemarlensis* adult male subjects that received robot presentations, 46 responded with one or more displays. An ANOVA did not detect any significant differences in response latency among the three experimental treatments ($F_{2,43} = 1.19$, $P = 0.32$; Fig. 3a). In contrast, display duration differed among treatments ($F_{2,43} = 6.31$, $P < 0.01$), with the conspecific stimulus ($n = 17$) eliciting more total time performing displays than did either the conspecific R-I stimulus ($n = 16$, $P < 0.05$) or the heterospecific stimulus ($n = 13$, $P = 0.001$; Fig. 3b).

Experiment 2: Robot Presentations to *Microlophus bivittatus*

Of the 60 *M. bivittatus* adult male subjects that received robot presentations, 49 responded with one or more displays. As in *M. albemarlensis*, response latency was similar among treatments in *M. bivittatus* ($F_{2,46} = 1.21$, $P = 0.31$; Fig. 4a). Moreover, whereas display duration differed among treatments ($F_{2,43} = 4.05$, $P = 0.02$), subjects spent less time

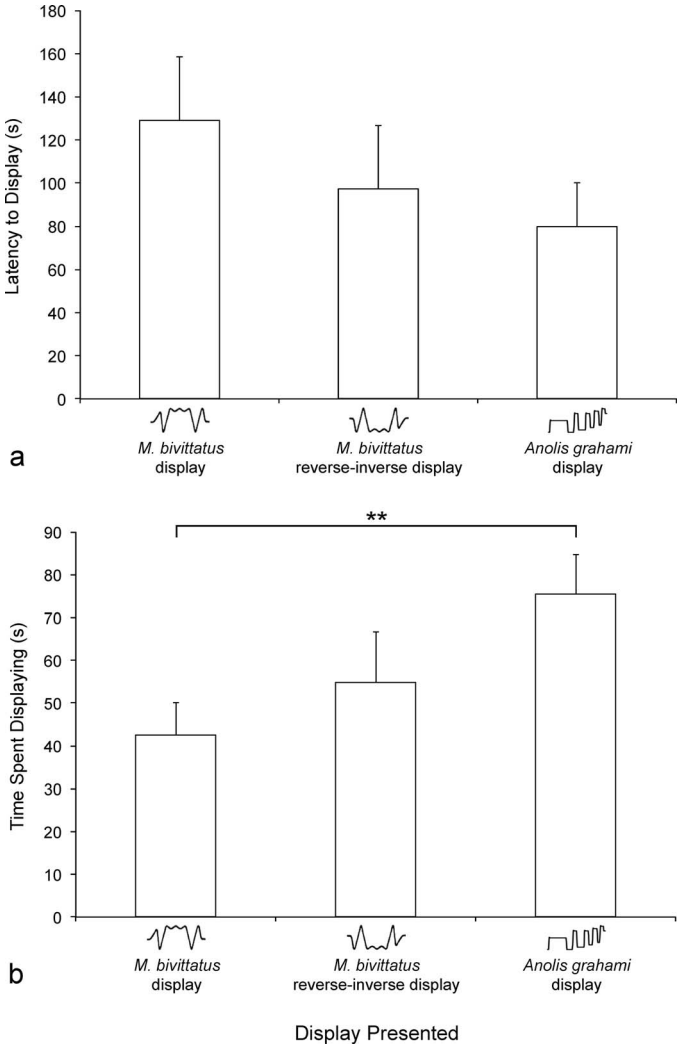

FIG. 4.—(a) Latency to first display and (b) total duration of displays by *Microlophus bivittatus* adult male subjects in response to conspecific robots performing particular displays. Bars represent means + 1 SE; ** $P \leq 0.01$.

displaying in response to the conspecific stimulus ($n = 19$) than to the conspecific R-I stimulus ($n = 13$). Subjects also responded less to the conspecific stimulus than to the heterospecific stimulus ($n = 18$, $P = 0.01$; Fig. 4b).

DISCUSSION

As shown in prior experiments with *M. indefatigabilis* on Santa Cruz Island and *M. occipitalis* in coastal Ecuador (Clark et al. 2015), adult male *M. albemarlensis* in the present study exhibited a response bias in favor of robots that performed conspecific display (compared with presentations of an unfamiliar display). In addition, by presenting two display types that differed in degree of similarity to the conspecific display, we have shown that *M. albemarlensis* males are sensitive to the details of display structure. Unlike the heterospecific (*A. extremus*) display, which consisted of smooth sinusoidal headbobs, the conspecific R-I stimulus was identical in structure to the normal display but with its execution reversed and vertical bobbing motions inverted. The intermediate level of response to the conspecific R-I treatment indicates that *M. albemarlensis* subjects considered this display to be more like the conspecific display than they did the heterospecific display.

In contrast to *M. albemarlensis*, adult male *M. bivittatus* on San Cristóbal Island did not exhibit a response bias for conspecific display, providing no evidence for CDR. To date, only one other *Microlophus* species tested—*M. grayii* on Floreana Island—has failed to show a bias for conspecific display over heterospecific display. Unlike *M. grayii* subjects, however, whose total display durations were nearly identical in response to conspecific and congeneric (*M. indefatigabilis*) display (Clark et al. 2015), male *M. bivittatus* responded most strongly to a display type that had nothing in common structurally with conspecific display (Figs. 2f, 4b). At present, we can offer no compelling explanation for the fact that *M. bivittatus* subjects responded most strongly to the *A. grahami* display. We speculate, however, that the conspicuous square-wave structure of this display might have been perceived as a type of supernormal stimulus (Tinbergen and Perdeck 1951). This possibility could be tested in future research by presenting *M. bivittatus* subjects with a series of square-wave and sinusoidal display stimuli that varied in vertical-motion amplitude, velocity, and acceleration (Fleishman 1986).

Interpreting the Presence or Absence of CDR in Galápagos Lava Lizards

Benavides et al. (2009) presented a colonization sequence for the Galápagos lava lizards based on the combination of DNA sequence data and geological dates for the origin of each island in the volcanic archipelago. Molecular data have established that the Galápagos lava lizards comprise two radiations that arose from different South American *Microlophus* ancestors. The eastern radiation contains only two species: *M. bivittatus* (San Cristóbal Island) and *M. habeli* (Marchena Island). San Cristóbal Island was colonized directly by lizards arriving from South America and is approximately two million years older than Marchena Island, which was colonized from San Cristóbal Island (Benavides et al. 2009). Importantly, molecular data indicate that the mainland species, *M. occipitalis*, is the sister taxon to the eastern Galápagos radiation (Benavides et al. 2009; Fig. 5). Having demonstrated a bias for conspecific display in *M. occipitalis* (Clark et al. 2015), we interpret the absence of CDR in *M. bivittatus* as the loss of this recognition. Given that *M. bivittatus* and *M. habeli* diverged ~400 kya (Benavides et al. 2009; Fig. 5) we predict that, similar to *M. bivittatus*, *M. habeli* will fail to exhibit CDR.

In contrast to the eastern radiation of Galápagos lava lizards, the common *Microlophus* ancestor of the western radiation is unclear (Benavides et al. 2009). Genetic data indicate that Española Island (*M. delanonis*) was the first island in the western radiation to be colonized, and that lizards on this island served as the stock for independent colonization of Santa Cruz Island (*M. indefatigabilis*) and Floreana Island (*M. grayii*; Benavides et al. 2009). Previously, we showed that *M. indefatigabilis* on Santa Cruz exhibited strong evidence of CDR, whereas *M. grayii* on Floreana showed no evidence of discriminating between conspecific and congeneric displays (Clark et al. 2015). Note that the apparent absence of CDR in some species does not necessarily indicate an inability to discriminate conspecific from nonconspecific displays. Rather, display differences

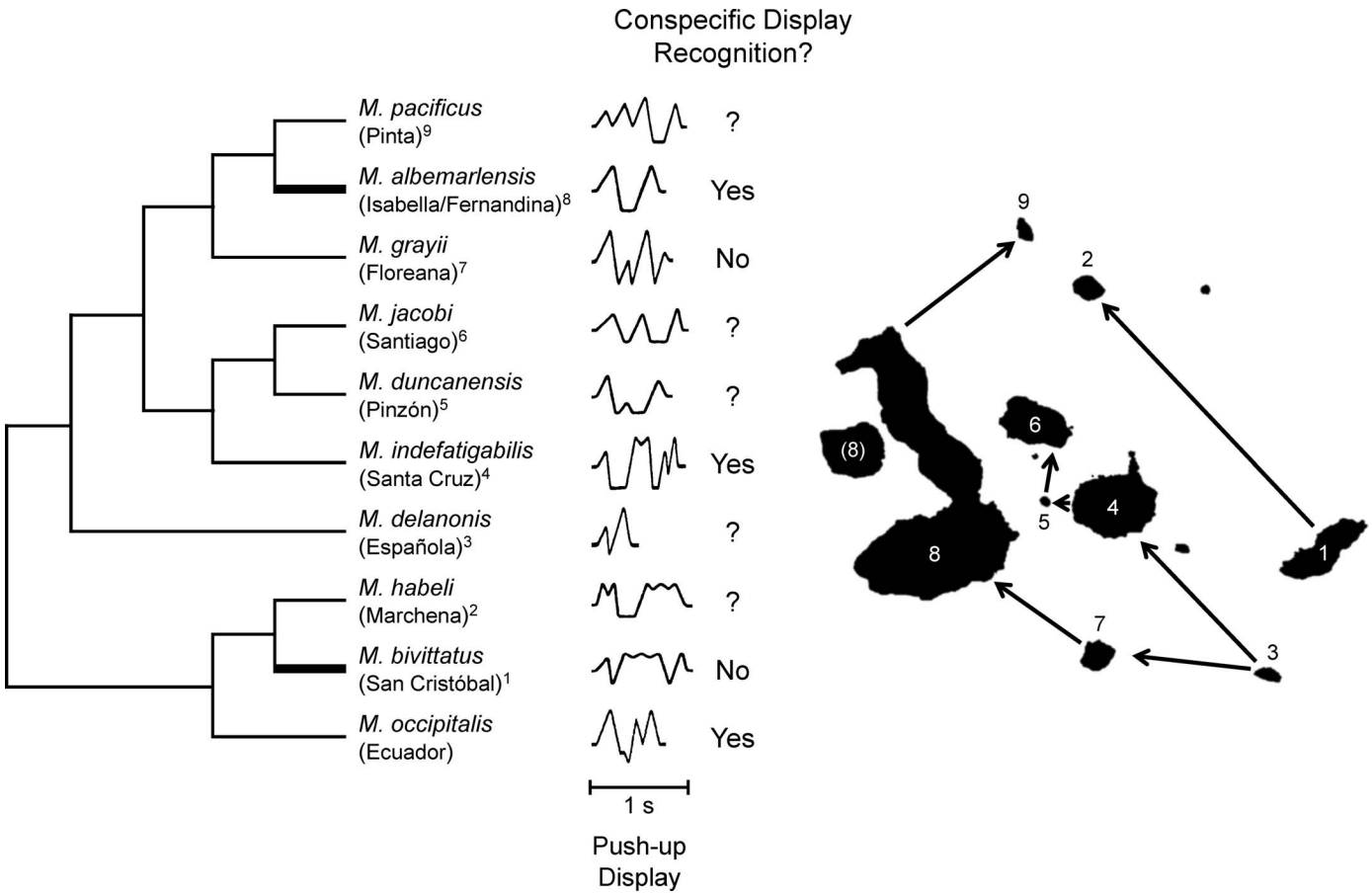

FIG. 5.—Phylogeny of the Galápagos lava lizards and the putative ancestor of the eastern radiation, *Microlophus occipitalis,* from Ecuador (based on Benavides et al. 2009). Thick branches indicate species tested in the present study. Display action patterns are adapted from Carpenter (1966). Conspecific-display recognition summarizes conclusions from the present study and Clark et al. (2015). Numbers on map of the Galápagos Islands correspond to superscripts that follow island names in the phylogeny diagram. *Microlophus albemarlensis* occurs on two large islands—Isabella and Fernandina—denoted on map as "8" and "(8)," respectively. Arrows on map depict colonization sequences (following Benavides et al. 2009).

simply might lack functional significance for these species, which is an anticipated outcome of evolution in isolation from congeners.

Given that Isabella Island (*M. albemarlensis*) was colonized directly from Floreana Island ~700 kya (Benavides et al. 2009), an absence of CDR in *M. grayii* on Floreana Island might have implications for its presence in *M. albemarlensis*. To date, the Galápagos *Microlophus* species that have been shown to exhibit CDR occur on only two islands for which a land bridge could have been exposed during Pleistocene glacial maxima: Santa Cruz Island and Isabella Island (Jordan and Snell 2008; Geist et al. 2014). During the past ~615 ky, sea levels have been sufficiently low (−120 to −130 m compared with present levels; Bintanja et al. 2005) to facilitate exposure of land bridge at least four times between these two islands. As a potential explanation for the presence of CDR in *M. indefatigabilis,* we suggested previously that a land bridge between Santa Cruz Island and Isabella Island could have led to secondary contact between *M. indefatigabilis* and *M. albemarlensis* (Clark et al. 2015). Our demonstration of CDR in *M. albemarlensis* in the present study is consistent with this hypothesis, as is the apparent absence of CDR *M. bivittatus*. Lack of historical gene flow between *M. indefatigabilis* and *M. albemarlensis* (Benavides et al. 2009) does not necessarily argue against secondary contact, because postspeciation mating between these two species could fail to produce viable offspring that would leave traces of introgression. Too few species of Galápagos lava lizards have been tested for occurrence of CDR for our results to constitute strong evidence for secondary contact between *M. indefatigabilis* and *M. albemarlensis*. Nevertheless, this hypothesis makes the explicit prediction that future experiments will fail to reveal CDR in the five species remaining to be tested (Fig. 5).

An alternative hypothesis is that CDR is an extension of intraspecific discrimination of male displays, in which heterospecific displays are perceived as low-fidelity conspecific displays, and discrimination of display quality is crucial for reproductive success. In this scenario, enhanced discrimination of variable male quality could favor fine-level evaluations of a sexually selected trait (e.g., motion display performance or ornamental coloration). Given that female mate choice has rarely been shown in iguanians, such a phenomenon might be anticipated to emerge in the context of male–male competition (see Clark et al. 2015). Our robot presentation experiments cannot, however, address an intraspecific display discrimination hypothesis. Additional explanations also might be possible, despite the fact that phylogenetic distribution of traits such as display complexity (Fig. 5), ornamental color expression, and sexual size

dimorphism have revealed no discernable pattern (Clark et al. 2015) or relationship to CDR. Therefore, future investigations should seek not only to investigate CDR in as-yet untested *Microlophus* species, but also should focus on intraspecific variation in traits that have arisen through sexual selection.

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Supplemental Material

The following photographs are associated with the online version of this article:

Fig. S1.—Adult male *Microlophus bivittatus* observing a *M. bivittatus* robot on San Cristóbal Island. Figure can be found at http://dx.doi.org/10.1655/Herpetologica-D-15-00040.S1.

Fig. S2.—Adult male *Microlophus albemarlensis* observing a *M. albemarlensis* robot on Isabella Island. Figure can be found at http://dx.doi.org/10.1655/Herpetologica-D-15-00040.S2.

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