Evolution of displays in Galápagos lava lizards: comparative analyses of signallers and robot playbacks to receivers

David L. Clark [a, *], Joseph M. Macedonia [b], John W. Rowe [a], Mark A. Stuart [a], Darrell J. Kemp [c], Terry J. Ord [d, e]

[a] Department of Biology, Alma College, Alma, MI, U.S.A.
[b] Department of Biology, Florida Southern College, Lakeland, FL, U.S.A.
[c] Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia
[d] Evolution and Ecology Research Centre, University of New South Wales, Kensington, NSW, Australia
[e] School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, NSW, Australia

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Differentiation in the structure of animal signals and displays among closely related species has intrigued biologists for centuries. Such divergence is often attributed to behavioural premating isolation, where sexual selection has favoured species recognition in environments containing sympatric congeners. However, in some cases closely related taxa have undergone speciation and display differentiation in isolation, in the apparent absence of benefits afforded by species recognition. Such is the case for the lava lizards (*Microlophus* spp.) endemic to the Galápagos Islands, where no two species overlap in distribution and all are thought to have evolved in allopatry from congeners. To test alternative evolutionary models, we used several phylogenetic comparative methods to assess how *Microlophus* displays have evolved. Results showed some potential for the influence of genetic drift, but little evidence of sexual selection (via male—male competition) or colonization history on the way displays have diverged among taxa. We then used lizard robots to test whether two representative Galápagos lava lizard species, *Microlophus grayii* and *Microlophus indefatigabilis*, would respond preferentially to a robot performing conspecific displays over those of the congener. We predicted that, in the absence of reinforcing selection, neither species would show a preference for conspecific displays. Results were mixed: whereas *M. grayii* showed no discrimination of conspecific over heterospecific displays, male *M. indefatigabilis* showed significantly stronger responses to their own displays than to those of the heterospecific. We then conducted the same experiment with a mainland congener, *Microlophus occipitalis*, to provide a broader view of potential responses across the group. Results revealed significant discrimination against heterospecific displays. We discuss our findings in light of hypotheses of signal differentiation, and suggest alternative interpretations for recognition of conspecific displays in species that are thought to have evolved in isolation.

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Animal signals and displays exhibit extensive variation in structure, modality and function (Bradbury & Vehrencamp, 2011). Particularly striking is the diversity in signal form that can exist among closely related taxa despite little difference in signal function. For example, signals produced by males to deter rivals or court females almost invariably differ in some aspect of their design, both at the level of species (e.g. Doucet, Mennill, & Hill, 2007; Marchetti, 1993; Podos, 2001; Seddon, 2005) and populations (e.g. Campbell et al., 2010; Irwin, Bensch, & Price, 2001; Potvin & Clegg, 2015). The most common explanations for the origins of such signal variation among taxa are differences in the selection pressures acting on signals, such as the intensity of sexual selection (Chen, Stuart-Fox, Hugall, & Symonds, 2013; Hunt, Snook, Mitchell, Crudgington, & Moore, 2012; Perez I de Lanuza, Font, & Monterde, 2013), properties of the environment that select for certain signal characteristics over others to improve signal transmission range (Derryberry, 2009; Kirschel et al., 2011; Ord, Stamps, & Losos, 2010; Tobias et al., 2010), or the presence of unintended receivers that select against conspicuous signalling (e.g. parasites: Bernal, Rand, &

* Correspondence: D. L. Clark, Alma College, Department of Biology, 614 W. Superior Street, Alma, MI 48801, U.S.A.
E-mail address: clarkd@alma.edu (D. L. Clark).

Ryan, 2006; Robert, Amoroso, & Hoy, 1992; predators: Endler, 1982; Morgans, Cooke, & Ord, 2014; Stuart-Fox & Ord, 2004). Although signal variation among closely related taxa is often interpreted to be adaptive, signal form can change over time for nonadaptive reasons as well, such as through genetic or cultural drift (Campbell et al., 2010; Luther & Baptista, 2009; Potvin & Clegg, 2015) and other stochastic factors that affect the trajectory of phenotypic evolution (Ord, 2012; Ord, Charles, & Hoffer, 2011; Wischmann, Floreano, & Keller, 2012).

Identifying the causes of signal differentiation is important not only for our understanding of signal evolution and adaptation, but also because social signals can be instrumental in instigating and maintaining reproductive isolation among populations: a vital ingredient for speciation (Coyne & Orr, 2004). Whether animal signals act as isolating barriers might depend on the process promoting signal differentiation among taxa, with adaptive divergence being potentially more likely to result in reproductive isolation (or more rapid isolation) than nonadaptive divergence (Coyne & Orr, 2004; Price, 2008; Sobel, Chen, Watt, & Schemske, 2010). Nevertheless, any change in signal form that affects attributes used in species recognition should be important in segregating taxa.

At the most basic level, receivers should discriminate conspecific from nonconspecific signals. However, meta-analyses of experimental studies of species recognition in a variety of species have revealed that discrimination is hard to predict (Ord, King, & Young, 2011). The common conception is that sympatric taxa should rarely respond to heterospecific mating or territorial signals, but in many taxa both sexes are found to respond as strongly to a heterospecific signal as one produced by members of their own species (reviewed in: Ord, King, et al., 2011; Ord & Stamps, 2009; Peiman & Robinson, 2010). Conversely, species that never interact with congeners (e.g. sole representatives of genera on islands) are assumed not to distinguish between conspecific and nonconspecific signals because there is no selection pressure for species recognition. Yet, a range of allopatric species have been found to discriminate against unfamiliar, heterospecific signals (Ord, King, et al., 2011).

The lesson from past empirical studies of species recognition is that the utility of social signals for reproductive isolation is dependent on a range of factors specific to the taxa in question (Ord, King, et al., 2011; Ord & Stamps, 2009). It also follows that discrimination against unfamiliar signals may vary widely among closely related taxa, depending on their present ecology and events in recent history (e.g. historic interactions with sympatric species). Classically, behaviour was considered to be central to the establishment and maintenance of reproductive isolation among populations (e.g. Mayr, 1963), but its primacy has come under scrutiny in recent years, with some depicting its role as secondary to ecological factors (e.g. Nosil, 2012; Streelman & Danley, 2003). Elucidating the role of social communication in segregating individuals into different reproductive populations is complicated. It requires assaying potential species identity cues in signals, identifying the factors that drive divergence in those attributes among closely related taxa (populations and species), and testing the response of receivers to signals that differ in those cues under appropriate ecological conditions (Mendelson & Shaw, 2012).

Islands provide geographically isolated populations that can be used to document processes of phenotypic differentiation, including social communication (e.g. Baker, Baker, & Tilghman, 2006; Potvin & Clegg, 2015; Uy, Moyle, & Filardi, 2009; Vanhooydonck, Herrel, Meyers, & Irschick, 2009). In rare cases, inter-island colonization (or the colonization of mainland environments) can also allow the direct study of secondary contact among previously isolated species (e.g. Grant & Grant, 2009, 2010). However, it often is difficult to attribute whether discrimination has occurred in these situations because of an a priori ability of individuals to recognize and subsequently discriminate against nonconspecific signals, or because postcontact selection has occurred through reinforcement (Coyne & Orr, 2004). In the context of animal communication, secondary contact can be simulated through the presentation to local allopatric populations of social signals used by congeners on a nearby island or on the mainland (Uy et al., 2009). This technique offers a powerful test of the importance of signal differentiation in reproductive isolation, because it simulates critical first contact among congeners, a factor typically overlooked in the study of natural secondary contact events (see Grant & Grant, 2009, for a rare exception), and, in addition, circumvents the potential confound of postcontact selection on discrimination abilities in receivers (Matute, 2015).

Lava lizards (genus *Microlophus*) are distributed throughout the Galápagos archipelago and comprise nine endemic species (Benavides, Baum, Snell, Snell, & Sites, 2009). Adult males on each island can vary in body coloration and the structure of a 'push-up' or 'headbob' display used in advertising territory ownership to rival males and conspecific females (Carpenter, 1966; Stebbins, Lowenstein, & Cohen, 1967; Fig. 1). Congeners do not coexist on any island, and populations on each island are assumed to have always been allopatric (e.g. Benavides et al., 2009; Kizirian, Trager, Donnelly, & Wright, 2004). This allopatric origin of Galápagos *Microlophus* species led Carpenter (1966) to suggest that interspecific variation in territorial displays probably arose by genetic drift. However, there are a number of other reasons why *Microlophus* territorial displays might differ among the islands, including all of the typical selection pressures commonly identified for animal communication more generally (e.g. differences in sexual selection). The *Microlophus* radiation in the Galápagos originated from two independent colonization events from mainland South America (Benavides et al., 2009). This fact adds another potential source of variation to signal design, given that the trajectory of signal evolution can depend on the peculiarities of the evolutionary ancestor from which taxa have originated (Ord, 2012; Ord, Charles, et al., 2011; Wischmann et al., 2012).

We used the Galápagos lava lizards as a model system to explore the potential causes of signal differentiation among taxa originating from the same and different evolutionary ancestors, and to determine the consequence of this signal variation for species recognition should species meet via secondary contact. To this end, our study has two parts. First, we used published 'display action pattern' (DAP) graphs that detail the vertical body movements present in territorial displays of adult males from 15 *Microlophus* taxa (Carpenter, 1966, 1977; Fig. 1). These displays included (1) all nine currently recognized Galápagos *Microlophus* species, (2) paired island populations for three of these species that vary in their period of isolation (0.07, 0.11 and 0.36 million years; Benavides et al., 2009) and (3) three *Microlophus* species from mainland Ecuador (Benavides et al., 2009; Kizirian et al., 2004; Fig. 1). From these graphs, we measured a range of display characteristics that have been shown to vary among closely related species of other iguanid lizards (Martins, 1993; Martins, Bissell, & Morgan, 1998; Martins, Labra, Halloy, & Thompson, 2004; Martins & Lamont, 1998; Ord & Martins, 2006; Ossip-Klein, Fuentes, Hews, & Martins, 2013). We used several complementary phylogenetic comparative methods to assess the potential mode of *Microlophus* display evolution and whether different attributes of displays have evolved independently or synchronously. We then tested several of the most obvious reasons why displays should differ among taxa, including differences in the strength of sexual selection (the likely intensity of competition for territories among males within taxa), evolution from different evolutionary ancestors (colonization history) and the potential impact of an ecological release following the colonization of the Galápagos islands (e.g.
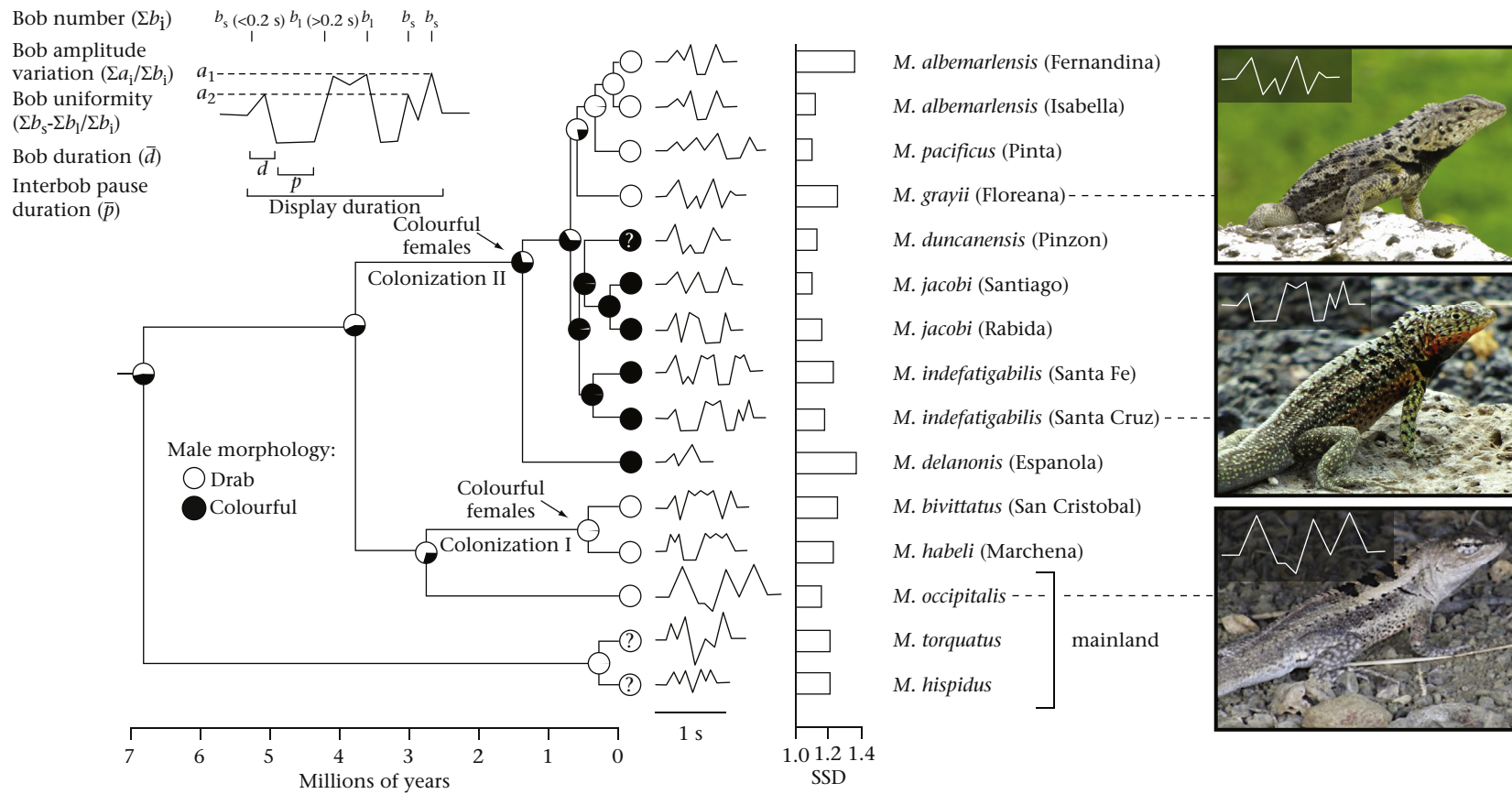
Bob number (Σb_i)

b_s (<0.2 s) b_l (>0.2 s) b_l b_s b_s

Bob amplitude variation ($\Sigma a_i / \Sigma b_i$) a_1

Bob uniformity ($\Sigma b_s - \Sigma b_l / \Sigma b_i$) a_2

Bob duration ($\bar{d}$) d p

Interbob pause duration ($\bar{p}$)

Display duration

Colourful females

Colonization II

Male morphology:
○ Drab
● Colourful

Colourful females

Colonization I

1 s

7   6   5   4   3   2   1   0
Millions of years

1.0 1.2 1.4
SSD

M. albemarlensis (Fernandina)
M. albemarlensis (Isabella)
M. pacificus (Pinta)
M. grayii (Floreana)
M. duncanensis (Pinzon)
M. jacobi (Santiago)
M. jacobi (Rabida)
M. indefatigabilis (Santa Fe)
M. indefatigabilis (Santa Cruz)
M. delanonis (Espanola)
M. bivittatus (San Cristobal)
M. habeli (Marchena)
M. occipitalis
M. torquatus   mainland
M. hispidus

Figure 1. The phylogeny of *Microlophus* lizards based on Benavides et al. (2009) and Pyron and Burbrink (2014). The line diagrams at the tips of the phylogeny depict the structure of adult male territorial displays as the vertical movements of the body over time (Carpenter, 1966, 1977). From these diagrams we scored six display characteristics (inset). The likelihood of evolutionary gains in conspicuous body colour are shown by the proportion of black in pie charts (males) or by arrows at associated nodes (females). The colours of male *M. duncanensis, M. torquatus* and *M. hispidus* were uncertain and were given tentative assignments based on the information available (see text for details). The Galapagos have been colonized by *Microlophus* lizards twice from mainland South America (Benavides et al., 2009; Kizirian et al., 2004) and the lineages originating from these separate colonization events are highlighted. The degree of sexual size dimorphism (SSD) exhibited within taxa is shown by the histogram. Photographs depict the three species tested in robot playback experiments.

escape from mainland predation: Blumstein & Daniel, 2005; release from stabilizing selection for species identity cues in allopatry: Amezquita, Flechas, Lima, Gasser, & Hödl, 2011). Note that although habitat types vary among Galápagos Islands, the general ecology of *Microlophus* is one of rock-dwelling lizards defending territories in open environments. This implies that differences in signalling environments have a low probability of being a major factor in display differentiation.

Second, we conducted robot playback experiments to two island species originating from the same colonization event (and subsequently controlling for gross differences in their evolutionary history; *Microlophus grayii* and *Microlophus indefatigabilis*) and a third species from mainland Ecuador (*Microlophus occipitalis*). All three species tested were allopatric from other *Microlophus*. For the Galápagos species, there has almost certainly never been a history of interactions with congeners (Benavides et al., 2009; Kizirian et al., 2004), whereas this was less likely to be the case for the mainland species, given the distribution of other *Microlophus* in Ecuador and neighbouring Peru (www.reptile-database.org). The use of robotic stimuli allowed the sophisticated manipulation of the behaviour and morphology of the displaying lizard, and also provided a means of presenting stimuli to free-living lizards in the same environment and ecological context where they normally communicate with one another. These robot playbacks were conducted in three experiments. All three species witnessed an adult male lizard robot that exhibited their own species' body colour pattern, but that performed a conspecific or heterospecific push-up display (experiment 1). In the case of one of the island species (*M. indefatigabilis*), playbacks also included the presentation of a robot that performed bouts of conspecific and heterospecific displays in consecutive order during the same playback presentation (experiment 2). In the last experiment, we presented the mainland species (*M. occipitalis*) with a robot male that performed a conspecific or heterospecific push-up display (as in experiment 1) and which exhibited the body colour pattern of a conspecific or a heterospecific (i.e. four experimental treatments).

Taken together, these experiments were designed to assay the responses of territorial males to the motion and morphological signals of conspecifics or closely related congeners, with the underlying assumption that males expressing an unusual territorial display or colour pattern will have difficulty in establishing a territory and subsequently acquiring matings with local females. This focus on male receivers rather than females was appropriate because, although there are some notable exceptions (e.g. Bleay & Sinervo, 2007; Hamilton & Sullivan, 2005; Wikelski, Carbone, Bednekoff, Choudhury, & Tebbich, 2001), evidence for direct female male choice to date has been scarce in iguanid lizards (Jenssen, Lovern, & Congdon, 2001; LeBas, 2001; Olsson & Madsen, 1995; Tokarz, 1995). Instead, reproduction is typically centred on males establishing exclusive territories that monopolize access to resident females (Jenssen et al., 2001). Females distribute themselves according to resource availability (Hews, 1990; Stamps, 1983) and have not been observed to move their territories if resident males are replaced by new males (e.g. Hews, 1993; LeBas, 2001). For these reasons, if female choice does occur in lava lizards, it is most likely secondary to male–male contests over territory. The role of social communication in reproductive isolation should therefore predominantly fall to the discrimination of territorial displays by male rivals (e.g. see also Uy et al., 2009). To place the responses of male lizards into the broader context of past experimental studies of species recognition, we converted responses to our robots into an effect size measure of the level of discrimination shown by each species, and compared these effects with the results of other studies published over the last 30 years (data compiled by: Ord, Charles, et al., 2011; Ord, King, et al., 2011; Ord & Stamps, 2009).

METHODS

Phylogenetic Comparative Analyses

Data

All data and sources used in comparative analyses are provided in Supplementary Table S1. Six display characteristics were measured from the DAP graphs for each of the 15 taxa reported in Carpenter (1966, 1977). These characteristics were the same as those previously used in other comparative analyses of lizard territorial displays (e.g. Martins, 1993; Ord & Martins, 2006) and are expected to encompass most of the variability in display form among species (see Ord & Martins, 2006). These display characteristics were as follows: the total duration of the territorial display (s); the mean duration of all individual bobs making up the display (s); the mean duration of the pause between each bob (s); the total number of bobs in the display; a measure of bob uniformity (i.e. the extent to which bob durations differed from one another); and a measure of the variability in the amplitude of each bob included in the display. Specific descriptions of how each of these characteristics were formally defined and measured are provided in Fig. 1.

We also categorized whether males and females of each taxon exhibited noticeable sexual (orange and red) coloration, given that morphological cues such as colour should provide additional species identity cues to augment those present in the territorial display (colour was coded as '0' = absent or weak, '1' = moderate to strong). These categorizations were based on direct observation of taxa in the field (*Microlophus albemarlensis*, *Microlophus bivattatus, M. grayii, M. indefatigabilis* and *M. occipitalis*) or from photographs sourced online that labelled the species and sex of the individual depicted (e.g. www.reptile-database.org). Male coloration in *Microlophus duncanensis*, *Microlophus torquatus* and *Microlophus hispidus* could not be reliably determined. We therefore tentatively assigned *M. duncanensis* as colourful and *M. torquatus* and *M. hispidus* as cryptic using text descriptions available online. As these assignments were difficult to verify for sex and accuracy of species identification, our analyses were conducted with alternative codings for these species as well. Although orange/red coloration is distributed widely in the Iguania (Cooper & Greenberg, 1992), we assumed for our analysis that occurrences within the genus *Microlophus* are not independently derived. We did not include black chin patches, shoulder stripes or dorsal crest bars under sexual coloration, as these markings also are consistent with a disruptive (crypsis) function (Stone, Snell, & Snell, 2003).

To index the likely strength of sexual selection operating in each taxon, we computed the sexual size dimorphism (SSD: male size/ female size) using snout–vent length (SVL) data from Carpenter (1966, 1977), combined with our own data for *M. grayii*, *M. indefatigabilis*, *Microlophus bivittatus* and *M. occipitalis* (range, median sample size: males = 21–56, 28; females = 7–31, 22; Rowe & Clark, n.d.). Male-biased sexual size dimorphism has been shown in a range of taxonomic groups to reflect the intensity of male–male competition (Andersson, 1994; Fairbairn, Blanckenhorn, & Székely, 2007), including lizards (Cox, Skelly, & John-Alder, 2003; Kratochvil & Frynta, 2002). SSD therefore has been used in a number of comparative studies on lizards as an estimate of the strength of sexual selection operating within taxa (Chen et al., 2013; Ord, Blumstein, & Evans, 2001; Ord & Garcia-Porta, 2012; Ord & Martins, 2006; Stuart-Fox & Ord, 2004).

The phylogeny we used was based primarily on the time-calibrated tree developed by Benavides et al. (2009) that included all of the species and populations of Galápagos lava lizards, as well as several mainland species (e.g. *M. occipitalis*). Benavides et al. (2009) also reconstructed the colonization history of the lava lizard radiation in the Galápagos and traced its origins to two separate

events from the mainland (Fig. 1). However, the phylogeny did not include the mainland species *M. torquatus* and *M. hispidus* that were part of our study. These species were positioned based on the time-calibrated tree of Pyron and Burbrink (2014). The species coverage of the Benavides et al. (2009) and Pyron and Burbrink (2014) trees overlapped, which allowed the placement of these two species with information on divergence times being retained.

Analyses

All comparative analyses were conducted using R v.3.1.1 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) unless stated otherwise. First, we applied several methods to estimate the mode of display evolution. At a general level, these methods measured the extent to which the evolution of each display characteristic has been dependent on phylogenetic history. We computed Blomberg's K (Blomberg, Garland, & Ives, 2003) and Pagel's λ statistics (Pagel, 1999) using the R package 'phytools' v.0.3-93 (Revell, 2012). In both cases, a value of 0 infers that a phenotypic trait has been potentially free to evolve independently of phylogeny. Conversely, values approaching 1 may reflect traits that have evolved closely with the phylogeny, such that variation among present-day species has accumulated through incremental changes consistent with the process of Brownian motion. The probability of these values being statistically distinguishable from zero was determined by randomization tests based on 10 000 simulations for K and a likelihood ratio test for λ. We also computed the phylogenetic half-life ($t_{1/2}$) and level of stochasticity that has occurred during signal differentiation (v_y) using the package SLOUCH v.1.2 (Hansen, Pienaar, & Orzack, 2008). Both values range from 0 to infinity (typically estimation stops at 1000). In the case of $t_{1/2}$, values close to zero are similar to K and λ in the respect that the evolution of the display characteristic has not been affected by phylogeny and bursts of change have potentially occurred over short periods of evolutionary time. Conversely, large values of $t_{1/2}$ can reflect strong phylogenetic inertia and incremental change over long periods of evolutionary time. Values of v_y that approach infinity imply that stochastic processes have been heavily influential in evolution such that the value of a display characteristic has changed widely and randomly over evolutionary time. In contrast, values that approach zero suggest that the display characteristic has either closely tracked phylogeny (when accompanied by high values of $t_{1/2}$) or closely tracked a moving adaptive optimum (when accompanied by low values of $t_{1/2}$). A confidence range for both $t_{1/2}$ and v_y was obtained by determining the range of parameter estimates within two likelihood units of the best estimate.

Second, we applied the phylogenetic principal components analysis in 'phytools', based on a composite estimate of λ (Revell, 2012). This analysis was used to evaluate which display characteristics might have evolved in concert or largely independently of one another. Display characteristics might have evolved together because they are targeted by the same selection pressure, are genetically or developmentally correlated in some way, or are viewed by receivers as essentially the same display attribute. With this in mind, we used the principal components to highlight representative display characteristics that are likely to summarize separate (evolutionary orthogonal) aspects of display variation among taxa, which then were used to test the fit of several evolutionary predictor models.

Third, we developed four evolutionary models that might account for the display variation evident within our study group of organisms: (1) an 'island effect' model that assumed display evolution has proceeded differently in taxa in the Galápagos compared to those on the mainland (e.g. resulting from ecological release following island colonization); (2) a 'colonization history' model

that assumed display evolution has been contingent on the particular evolutionary ancestor that initially colonized the Galápagos; (3) a 'male–male competition' model that assumed display variation reflects variation in the intensity of male–male competition among taxa, as measured by sexual size dimorphism (SSD often is positively correlated with increasing display complexity in lizards, presumably because of the need for improved opponent assessment; Ord et al., 2001; Ord & Garcia-Porta, 2012; Ord & Martins, 2006; Stuart-Fox & Ord, 2004); and (4) a 'null' model in which display variation among taxa was assumed to reflect Brownian motion and stochastic processes (drift) or some predictor variable not considered in the other models applied. These models were fitted to the data using SLOUCH, and the level of support for each model was evaluated using the second-order Akaike's Information Criterion (AIC$_c$). The model with the lowest AIC$_c$ value is the model that best fits the data (ΔAIC$_c = 0$), although any other model within at least two units of this model were considered to be equally compelling (ΔAIC$_c \leq 2.0$).

Finally, we used the program Mesquite v.3.01 (Maddison & Maddison, 2010) to reconstruct the evolutionary history of colour evolution in males and females using maximum likelihood and the Mk1 model (Lewis, 2001), which assumes gains and losses are equally probable. For reconstructions of male coloration, we conducted two analyses: one (the most plausible scenario) in which males of *M. duncanensis* were conspicuously coloured, while those of *M. torquatus* and *M. hispidus* were not, and another in which these colour codes were reversed.

Robot Playback Experiments

Study species

We selected target species for robot playbacks based on several criteria. First, we selected species that differed noticeably in the structure of their territorial displays (Fig. 1), particularly in bob uniformity and bob amplitude (i.e. variation that our comparative analyses inferred were traits that have been largely free to vary among taxa independently of phylogeny: see Results). Second, in an effort to control for broad differences in evolutionary history that might result from originating from different ancestors (e.g. Ord, 2012), the two island species were selected from a radiation arising from the same colonization event. Finally, we selected species that differed in male body coloration and degree of sexual size dimorphism, which are phenotypic characteristics found to vary among lizard taxa with intensity of sexual selection (see above).

Microlophus grayii is endemic to the island of Floreana, where males are cryptically patterned grey, white, brown and black (Fig. 1) and are substantially larger (SVL: mean $\pm$ SE $= 89.43 \pm 1.98$ mm; $N = 21$) than females (SVL: 70.82 ± 1.63 mm; $N = 17$; SSD $= 1.26$). *Microlophus indefatigabilis* is endemic to the island of Santa Cruz and several nearby islets, where adult males are striking in their ventral and lateral orange, yellow and black advertisement coloration (Fig. 1). In this species males are moderately larger (SVL: 70.00 ± 1.18 mm; $N = 56$) than females (SVL: 59.23 ± 1.27 mm; $N = 31$; SSD $= 1.18$). *Microlophus occipitalis* is endemic to coastal Ecuador, where males are cryptically coloured tan, brown and white, and exhibit a disruptive patterning of dark chevrons that run along their dorsal crest. Like *M. indefatigabilis*, male *M. occipitalis* are somewhat larger (SVL: 63.84 ± 1.49 mm; $N = 32$) than females (SVL: 54.88 ± 0.74 mm; $N = 26$; SSD $= 1.16$).

For *M. grayii*, we carried out robot presentations to 40 adult males from 25 February to 28 February 2012 on Floreana Island. Trials were conducted in the vicinity of Puerto Velasco Ibarra (1°16′27″S, 90°29′13″W) along rock walls and roads bordering the village. For *M. indefatigabilis*, we presented robots to 54 adult males

from 29 February to 7 March 2012 on Santa Cruz Island, in the vicinity of the Charles Darwin Research Station (0°44′32″S, 90°18′13″W). Lizard subjects were located on natural lava rock formations and on human-made structures such as lava rock walls and piles.

We conducted robot presentations to both species of Galápagos lava lizards daily between 0900 and 1700 hours in warm and relatively constant temperatures (25–30 °C) under clear to partly cloudy skies. As ours is not an ecological study, we cannot address whether factors such as territory quality, population density, food availability or other variables differed between Santa Cruz and Floreana in some manner relevant to our study. We can state at least that although earlier work (Stone et al., 2003) revealed greater parasite diversity on Santa Cruz than on Floreana, little to no difference was found between the two islands in the presence/absence of types of predators.

For *M. occipitalis*, we presented robots to 87 adult males from 3 May to 14 May 2014 in a dry forest–dune beach habitat along the western coast of mainland Ecuador near the fishing village of Puerto Lopez (1°32′45″S, 80°48′38″W). Lizards were found by walking trails while searching the vegetation and rocky outcrops for subjects.

Robot construction and programming

To control robot push-up displays, we secured a Futaba S9001 servomotor (F.I.C. American Corp., Carol Stream, IL, U.S.A.) inside plastic housing (25 × 15 × 10 cm high) that was painted a dark grey colour similar to that of local lava rocks. The servomotor housing also contained a Yost Engineering ServoCenter Midi v.1.2 (Portsmouth, OH, U.S.A.) control board that communicated midi controller messages to the servomotor. A Li-ion battery (Powerizer[®] model no. H4HCT18650902, BatterySpace.com/AA Portable Power Corp., Richmond, CA, U.S.A.) was used to power the robot and control system. In the field, midi controller messages were sent to the input of the ServoCenter from an iPod touch (model no. A1213, 1 Infinite Loop, Cupertino, CA, U.S.A.) using a Line 6 MIDI Mobilizer (Line 6, Inc., Calabasas, CA, U.S.A.).

Robot bodies were constructed from large wooden dowels that were carved to approximate an average-sized adult male lava lizard (~75 mm SVL, Rowe & Clark, n.d.). The body was secured posteriorly via a Micro E/Z hinge (model no. DUB937, Du-bro Products, Inc., Wauconda, IL, U.S.A.) and anteriorly by a small metal eyelet. We attached the eyelet to a pushrod that was connected to the servomotor. When activated, the servomotor controlled the up-and-down push-up display of the robot.

To produce life-like lizard models, 'skins' for the bodies of our study species were constructed using high-resolution photographs of live adult male lizards standing in profile. We removed backgrounds from the digital images using Adobe Photoshop[®] and sized the skins to fit the lizard models. Once a fit for one side of the body was obtained, we created a mirror image to fit the other side. The entire 'skin' was then printed onto a photo-quality, stretchable, sticky-back fabric (Dritz Printed Treasures, Prym Consumer USA Inc., Spartanburg, SC, U.S.A.) using an inkjet HP Deskjet 460 printer. We cut out the lizard skin image and adhered it to the model. Using a preserved *M. occipitalis* specimen, latex hindlimbs and a tail were produced with plaster-of-Paris impression moulds. The latex limbs and tail were painted and then glued in place at the posterior portion of the model (see Supplementary Material Figs S1–S3).

We note that we made no attempt to adjust the coloration of the 'skins' to our subjects' (likely) tetrachromatic visual system. Spectral sensitivity is unknown for *Microlophus*, and the uncertain phylogenetic relationship of the Tropiduridae to other lizard families (Townsend et al., 2011) ruled out a reasonable proxy for which spectral sensitivity is known (e.g. Macedonia, Clark, Riley, & Kemp,

2013). In addition, body coloration in male *M. grayii* and *M. occipitalis* is largely greyscale and cryptic in appearance, which should reduce the importance of any differences in appearance between human and *Microlophus* colour perception. Last, ultraviolet (UV) reflectance is low in males of our study species (generally less than 5%: Clark & Macedonia, n.d.), which largely avoids the difficulty of reproducing colours that include UV wavelengths. For these reasons we chose to avoid manipulating the colours in our photographs for output to the printer.

To program the robot, we obtained video recordings of territorial displays performed by five adult males of all three of our study species collected during previous work in 2010 and 2011. These videos were transcribed and used to generate a mean DAP graph for each species, which was then used to develop a midi controller file using Logic Pro (v.9.1 for Macintosh OS) software. For each study species, a display sequence consisted of three consecutive territorial displays followed by a 30 s pause, which was iterated for a total trial period of 8 min. To present a display sequence to subjects in field trials, we exported the Logic Pro midi controller file directly to an iPod touch using the Line 6 MIDI Mobilizer.

Experimental protocol

We searched for adult male lizards (≥65 mm SVL) by walking along paths and dirt roads, and by surveying rocky outcrops. When a subject was located, the robot was moved to a distance ~1–3 m perpendicular to the lizard's side-on view, so that the robot push-up displays were readily visible to the male. To record the behaviour of the test subject, we secured a digital video camera to a tripod and positioned it ~2 m behind the stimulus robot and in line with the subject such that we could capture the image of the robot and the subject in a single field of view. If the subject did not flee or display throughout the set-up period (ca. 2 min), we triggered the robot's display sequence from an iPod touch, thus initiating the 8 min trial (see Fig. S1 showing field set-up). We avoided repetitively searching areas for lizards in order to avoid testing the same subject more than once.

We used a single-presentation technique for all 40 *M. grayii* trials and for the first 24 of 54 *M. indefatigabilis* trials in which a subject responded to the stimulus robot. Test subjects were presented with a robot having a conspecific body colour pattern and performing either conspecific or heterospecific push-up displays. To control for order effects, we alternated which species' push-up display was shown to each new subject as we encountered them.

During the experimental trials with *M. indefatigabilis* it became apparent that subjects were attending preferentially to the conspecific stimulus, a response that we had not observed in trials with *M. grayii* males. The greater attention and more emphatic response of *M. indefatigabilis* subjects for the conspecific display led us to implement a matched-pairs ('stimulus switch') design for 30 male subjects. For stimulus switch playbacks, each subject was presented with a conspecific sequence and a heterospecific sequence of displays. We alternated which display type (conspecific or heterospecific) a given subject witnessed first in the sequence of trials. Thus, 15 subjects were presented with 8 min of conspecific display followed by 8 min of heterospecific display, and 15 subjects observed 8 min of heterospecific display followed by 8 min of conspecific display.

We followed the single-presentation protocol for all 87 trials in which an *M. occipitalis* subject responded to the stimulus. However, we expanded our initial experimental design to include four treatments, where each subject saw only one stimulus type: a robot having a conspecific (*M. occipitalis*) or heterospecific (*M. indefatigabilis*) appearance, and that performed either conspecific or heterospecific push-up displays. No animals were harmed

or handled during our study and all animal behaviour welfare guidelines were followed.

Subject responses

Responses to the robot stimuli by our subjects were quantified directly from video recordings. In each robot presentation trial we quantified two measures: (1) latency to the initiation of the first territorial display and (2) the summed duration of all territorial displays exhibited. Subjects that did not respond during a trial with at least one territorial display were excluded from analysis.

In addition to our two response measures, we created a ranked composite score for subjects' responses. This score was based on aggressive behaviours, originally described by Carpenter (1966), that can be expressed together or independently as follows: crest up (1 point): the nuchal and dorsal crests are raised exposing brightly coloured scales; gular inflation (1 point): the neck region becomes inflated and distended; lateral compression (1 point): the subject turns laterally (i.e. perpendicular) towards the stimulus, draws in its sides and stands tall, in an apparent attempt to appear larger (see Supplementary Videos S1, S3); and challenge display (4 points): this display includes crest up, gular inflation and lateral compression, plus the addition of an arched-back push-up display (see Supplementary Video S1). A score of 4 was the maximum permitted for any subject. For example, if a subject escalated during a trial to the challenge display, only this behaviour was considered in computing its total score. Composite scores were arcsine transformed prior to analysis. (Our robot presentation set-up is illustrated in Supplementary Figs S1–S3 and examples of male responses are shown in Videos S1–S4).

Statistical analysis

Trial durations varied among individuals, as some subjects moved out of view or fled before the scheduled end of a trial. We therefore converted the amount of time spent performing territorial displays during a trial to a proportion of the total stimulus presentation (playback) period. In experiments 1 and 2, nonnormal distributions of subject response latencies and durations failed to normalize with transformation. Thus, in experiments 1 and 2 we used the Wilcoxon two-sample test and the Wilcoxon matched-pairs signed-ranks test, respectively, to test for differences in subject responses between treatments. In experiment 2, responses were tested first for effects of stimulus order regardless of stimulus identity, and then tested for effects of stimulus type regardless of stimulus order. As the same data set was used in both tests, we adjusted our alpha level for significance from $\alpha = 0.05$ to $\alpha = 0.025$. We did not test latency to the first push-up in experiment 2, as subjects were not naïve when the second push-up display type was presented during trials. In experiment 3, subject response data again were non-normally distributed. However, a log transform normalized the proportion of time in trials that subjects spent performing push-up displays. We therefore used a one-way

ANOVA to compare subject responses among the four experimental treatments, and the Tukey HSD test for post hoc pairwise comparisons. As transforms failed to normalize push-up display latencies, we used a Kruskal–Wallis ANOVA to compare these responses among treatments. Statistical tests were conducted using JMP v.9.0 (SAS Institute Inc., Cary, NC, U.S.A.) and SPSS v.21.0 (IBM, Armonk, NY, U.S.A.).

To benchmark lizard responses relative to past studies of species recognition, for treatments in which a conspecific body was used in playbacks, we computed Cohen's *d* statistic for (1) latency to display and (2) the proportion of the presentation period a male spent displaying to the conspecific display relative to the nonconspecific display. This effect size was then combined for both display measures and converted into an *r* value for comparison with the data used in the meta-analyses of Ord and Stamps (2009) and Ord, Charles, et al. (2011) and Ord, King, et al. (2011). The data set covered 92 studies and 111 species (including mammals, birds, reptiles, amphibians, insects and noninsect invertebrates) in which the response of subjects had been tested to a conspecific and nonconspecific mating or territorial signal (acoustic, visual, olfactory or multimodal). The methods we used to convert lizard responses into a combined effect size were identical to those used by Ord and Stamps (2009) and Ord, Charles, et al. (2011) and Ord, King, et al. (2011).

RESULTS

Mode of Display Evolution

There was broad agreement among the three methods applied: the evolution of *Microlophus* territorial displays could be categorized as consisting of those characteristics that have been moderately affected by phylogenetic inertia (bob duration and interbob pause duration) and those that have largely been free to vary independently of phylogeny (display duration, bob number, bob uniformity and bob amplitude variation). Whether values were statistically distinguishable from 0 varied by method, but the evolution of bob duration was found consistently to have been significantly affected by phylogenetic history in randomization tests of *K* and likelihood ratio tests of λ, and had a support region for $t_{1/2}$ that overlapped moderate to high values of phylogenetic inertia (Table 1). In contrast, the evolution of bob uniformity and bob amplitude variation appeared to have been least affected by phylogeny: randomization tests of *K* and likelihood ratio tests of λ failed to distinguish estimates from zero, while the support region for $t_{1/2}$ (although wide) encompassed low values of phylogenetic inertia. Stochasticity during display differentiation seems to have been low for all characteristics, but especially so for bob duration and interbob pause duration, in which support regions were small and overlapped zero (Table 1).

Table 1
Alternative estimates of the mode of evolutionary differentiation in *Microlophus* territorial displays

Display characteristic	Blomberg's K		Pagel's λ		SLOUCH			
	K	P^1	λ	P^2	$t_{1/2}$	Support region	v_y	Support region
Display duration	0.21	0.031	0.00	1.000	70	10–∞	30	0–970
Bob duration	0.52	0.008	1.01	0.000	520	230–∞	10	0–50
Interbob pause duration	0.39	0.003	0.46	0.308	660	290–∞	10	0–40
Bob number	0.23	0.025	0.00	1.000	10	10–300	60	30–∞
Bob uniformity	0.15	0.138	0.00	1.000	10	0–870	20	10–∞
Bob amplitude variation	0.09	0.525	0.00	1.000	10	10–900	20	10–∞

$t_{1/2}$: phylogenetic half-life; v_y: level of stochasticity that has occurred during signal differentiation.
[1] Based on 100 000 simulations.
[2] Likelihood ratio test.

Axes of Display Evolution

Phylogenetic principal components highlighted three primary components that collectively summarized the vast majority of display variation among taxa (98%; Table 2). In general, bob number and display duration loaded positively on the first principal component, bob uniformity loaded almost exclusively on the second principal component, whereas display duration, bob duration and bob amplitude variation all loaded positively on the third component (Table 2). Interbob pause duration was not prominent on any of the three main component axes.

The first three components also were clearly dominated by three display characteristics: bob number, bob uniformity and bob amplitude variation, respectively. These three characteristics were subsequently selected for evolutionary model tests, justified (further) by the fact that these characteristics were those that appeared to have been free to evolve independently of phylogeny (see previous section and Table 1).

Predictors of Display Evolution

The evolutionary null model was found to be the best supported model for bob number, bob uniformity and bob amplitude variation. Support for the alternative models of island effect, colonization history and male–male competition were not compelling ($\Delta AIC_c > 2.0$; Table 3).

Reconstructions of Conspicuous Colour Evolution

Irrespective of whether *M. duncanensis* males are colourful or not, conspicuous male coloration most likely evolved once, and specifically in the radiation of Galápagos *Microlophus* that originated from the archipelago's second colonization event (Fig. 1). Such coloration appears to have been retained to various degrees in taxa that have diversified throughout the western islands of the archipelago, but probably lost in males of taxa that radiated through the eastern islands where present-day males are cryptically coloured. Alternatively, conspicuous male coloration was ancestral and subsequently lost in those lineages lacking conspicuous coloration today. However, the evidence for this alternative scenario was weak: reconstructions at nodes prior to colonization were either equivocal (root) or skewed to an absence of conspicuous coloration (nodes prior to colonization I and II).

Interpretation of female colour evolution is dependent on whether *M. torquatus* and *M. hispidus* females are confirmed to possess or lack sexual coloration. Assuming its absence, conspicuous coloration in females has evolved twice and in tight association with both colonization events of the Galápagos (Fig. 1). Alternatively, if sexual coloration is present in *M. torquatus* and *M. hispidus*

females, then conspicuous coloration is probably ancestral for all *Microlophus* and has been subsequently reduced in *M. occipitalis*.

Experiment 1: Conspecific versus Heterospecific Display, Single Stimulus Presentations

Microlophus grayii

Male *M. grayii* showed statistically indistinguishable responses to conspecific and heterospecific display stimuli. We were unable to detect a difference in latency to the first territorial display by *M. grayii* subjects in response to conspecific and heterospecific displays (Wilcoxon two-sample test: $\chi^2_1 = 0.005$, $P > 0.94$; Fig. 2). Likewise, we found no statistically distinguishable difference in the proportion of time subjects spent performing territorial displays in response to conspecific and heterospecific displays ($\chi^2_1 = 0.338$, $P > 0.56$; Fig. 2). Last, we failed to find a difference in our composite response score for subjects' responses to conspecific and heterospecific displays (mean score $\pm$ SE: to *M. grayii* display $= 1.95 \pm 0.35$, $N = 20$; to *M. indefatigabilis* display $= 1.90 \pm 0.42$, $N = 20$; $\chi^2_1 = 0.058$, $P > 0.80$).

Microlophus indefatigabilis

In striking contrast to *M. grayii* males, *M. indefatigabilis* males showed significantly stronger responses to conspecific than to heterospecific territorial displays. First, latency to the initial display was significantly shorter in response to conspecific than to heterospecific displays (Wilcoxon two-sample test: $\chi^2_1 = 3.97$, $N = 12$, $P < 0.04$; Fig. 2). Similarly, the proportion of time spent in display during a trial was significantly greater in response to conspecific than to heterospecific displays ($\chi^2_1 = 5.07$, $N = 12$, $P < 0.02$; Fig. 2). Finally, the composite response score of lizards was significantly greater for males presented with the conspecific display compared to males presented with the heterospecific display (mean score $\pm$ SE: to *M. indefatigabilis* display $= 3.08 \pm 0.39$, $N = 12$; to *M. grayii* display $= 1.75 \pm 0.50$, $N = 12$; $\chi^2_1 = 3.99$, $N = 12$, $P < 0.046$).

Table 3
Computed ΔAIC_c values from SLOUCH analyses for adaptive and nonadaptive models of evolutionary differentiation of *Microlophus* territorial displays

Evolutionary model applied	Bob number	Bob uniformity	Bob amplitude variation
Null	0.0	0.0	0.0
Island effect	3.7	3.5	3.8
Colonization history	7.8	8.2	8.3
Male–Male competition (SSD)	3.8	2.2	3.2

AIC_c: Akaike's Information Criterion; SSD: sexual size dimorphism (male:female snout–vent length).

Table 2
Phylogenetic principal components analysis of *Microlophus* display characteristics

Display characteristic	pPC1	pPC2	pPC3	pPC4	pPC5	pPC6
Display duration	**0.51**	−0.44	**0.51**	0.53	−0.04	0.01
Bob duration	−0.40	−0.30	**0.53**	0.60	0.31	−0.11
Interbob pause duration	−0.37	−0.42	−0.26	−0.13	−0.77	−0.12
Bob number[1]	**1.00**	−0.01	0.01	−0.02	0.00	0.00
Bob uniformity[1]	−0.16	**−0.97**	0.12	−0.10	0.01	0.00
Bob amplitude variation[1]	−0.22	0.38	**0.89**	−0.14	0.00	0.00
Standard deviation	0.46	0.22	0.16	0.07	0.02	0.01
Proportion of variance	0.72	0.17	0.09	0.02	0.00	0.00
Cumulative proportion of variance	0.72	0.89	0.98	1.00	1.00	1.00

$\lambda = 0.00$, $N_{taxa} = 15$. Prominent loadings (>0.5) are highlighted in bold.
[1] Display characteristics selected for hypothesis testing.
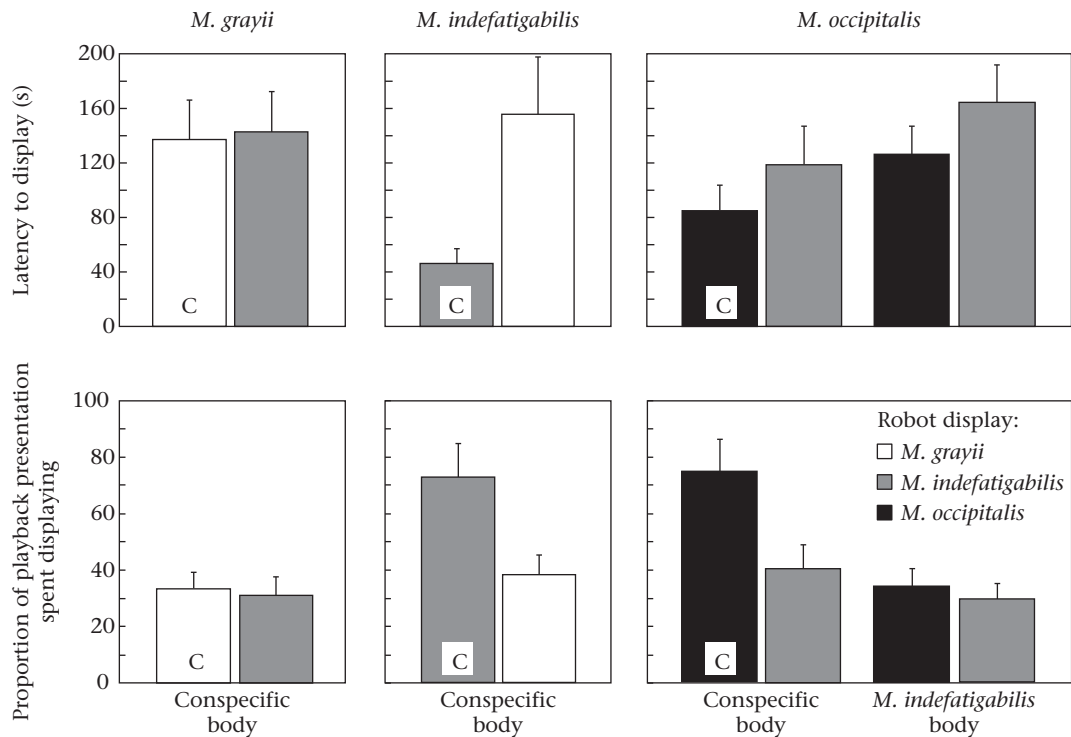
Figure 2. Mean ± SE latency to respond and amount of display elicited by a *Microlophus* robot from adult male lizards of *M. grayii* (Floreana Island), *M. indefatigabilis* (Santa Cruz Island) and *M. occipitalis* (mainland). Robot playbacks in which a conspecific body and conspecific display were presented are highlighted by 'C'.

Experiment 2: Conspecific versus Heterospecific Display, Stimulus-Switch Presentations

Microlophus indefatigabilis

We were unable to detect an effect of stimulus order on the proportion of each trial devoted to displays (Wilcoxon signed-ranks test: $S = 24.50$, $N = 30$, $P > 0.62$; Fig. S3). However, subjects spent significantly greater proportions of time performing displays in response to conspecific than to heterospecific displays ($S = 191.50$, $N = 30$, $P < 0.0001$; Fig. S4).

Experiment 3: Conspecific versus Heterospecific Display and Morphology, Single Stimulus Presentations

Microlophus occipitalis

Latency to the first display was shortest in response to the robot with a conspecific body and conspecific display, and differences in latency among treatments approached significance (Kruskal–Wallis test: $H_{83} = 7.3$, $N = 87$, $P = 0.063$; Fig. 2). Display duration proportion differed significantly among treatments (one-way ANOVA: $F_{3,83} = 7.18$, $N = 87$, $P < 0.001$; Fig. 2). Pairwise comparisons revealed that display duration proportion in the treatment in which robots had a conspecific morphology and performed a conspecific display differed from all other treatments (P value range in Tukey HSD tests: $P = 0.041$ to $P < 0.001$). No pairwise comparisons among the other three treatments were significant.

Lava Lizard Discrimination Compared to Past Studies of Species Recognition

The variation in the nature of responses to conspecific and nonconspecific signals in other taxonomic groups is extensive, but the heterospecific discrimination we documented for island *M. indefatigabilis* and mainland *M. occipitalis* were broadly similar to the bulk of findings from past studies: an effect quartile range of 0.05–0.40 for allopatric species (Fig. 3a) and 0.16–0.55 for sympatric species (Fig. 3b); 95% confidence range for *M. indefatigabilis* was 0.11–0.57 and *M. occipitalis* was 0.03–0.42 (see top panel in Fig. 3). Nevertheless, the lack of discrimination in island *M. grayii* was not unusual either, with the response of this species (−0.02) coinciding with a large number of other studies of allopatric taxa that also showed similar responses to both conspecific and nonconspecific stimuli (e.g. mode effect size = 0.05; Fig. 3a). There have been too few studies of species recognition in allopatric territorial species, which is the most relevant context for Galápagos *Microlophus*, to make a worthwhile direct comparison ($N = 4$ studies).

DISCUSSION

All lava lizards use push-up/headbob displays to advertise territorial ownership and resolve territorial disputes, but the types of movements that make up these displays varies widely among species, and in some cases among populations of the same species (e.g. *Microlophus jacobi* and *M. indefatigabilis*; Fig. 1). In particular, our analyses have shown that the number, duration and amplitude of bobs included in displays have evolved independently of each other and independently of phylogeny. Furthermore, the apparent lack of phylogenetic inertia in these display traits does not appear to reflect stochasticity in the evolutionary process. These characteristics therefore should be ideal candidates for cues on species identity because they vary among species and have evolved largely independently of each other, which should in turn allow greater complexity to be encoded in the overall display.

Just why this territorial display varies among Galápagos *Microlophus* species, however, remains unclear. Contingent effects from different colonization events have had little impact on the evolutionary trajectory of display form (Table 3). Neither has there been a detectable impact of ecological release, which is anticipated
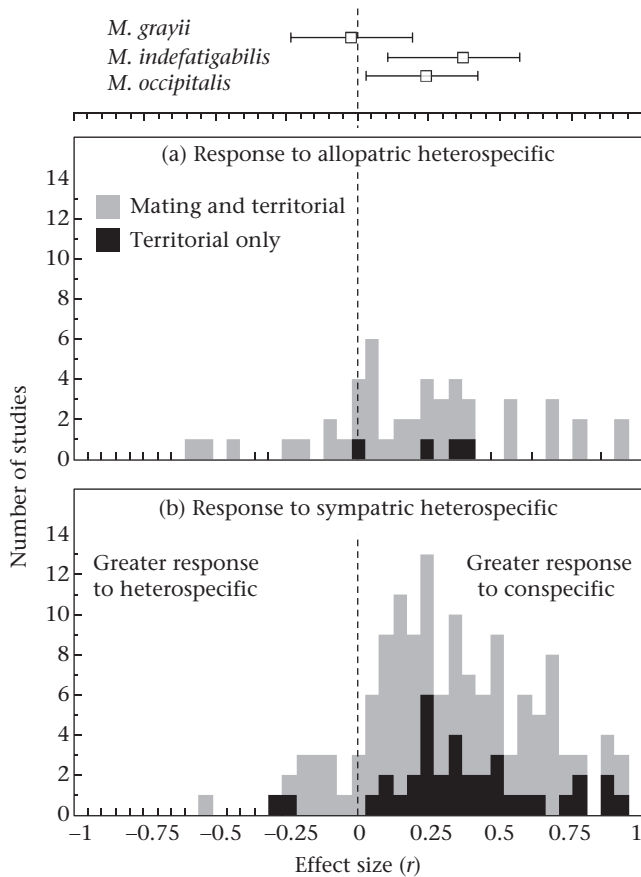
Figure 3. Discrimination of conspecific versus heterospecific territorial displays by adult male *Microlophus* lizards in robot playback experiments (uppermost panel) compared to effect sizes of other studies of species recognition that tested novel (a) or familiar (b) heterospecific signals. Positive values reflect a greater response to conspecific signals; negative values reflect a greater response to heterospecific signals; values of zero reflect equal or nondiscriminatory responses to conspecific and heterospecific signals. Data for *Microlophus* are estimated *r* values and associated 95% confidence intervals.

following dispersal from a mainland environment to an island (Losos & Ricklefs, 2009): predation on lizards is generally expected to be higher on the mainland than on islands (e.g. Andrews, 1979), and species are more likely to encounter sympatric congeners that can limit the opportunity of signal variation arising (because of stabilizing selection for species recognition; Amezquita et al., 2011). *Microlophus* lizards do differ widely in SSD, which implies the intensity of competition for territories and females also varies among taxa as well (Cox et al., 2003), but this feature had little predictive power for explaining display differentiation among taxa (Table 3).

It is possible that display diversification has arisen simply through genetic (or cultural) drift, as hypothesized by Carpenter (1966). However, this interpretation should be made cautiously because bob number, uniformity and amplitude are characteristics that appear to have experienced rapid bouts of evolutionary change in their history and low stochastic differentiation in the evolutionary process. This pattern is different to what would be generally expected for phenotypic differentiation via drift, which would tend to occur through the gradual accumulation of phenotypic change over long periods of evolutionary time. Instead, the pattern exhibited by bob number, uniformity and amplitude might be consistent with adaptive evolution (Butler & King, 2004; Hansen, 1997), but the interpretation of statistical parameters such as K and λ as representing explicit evolutionary phenomena must be

made carefully (e.g. see Revell, Harmon, & Collar, 2008). A literal interpretation of adaptive evolution from the value of these parameter estimates would, however, infer that the relative support for the null evolutionary model in our analyses reflects the poor fit of the other models considered, rather than display evolution via nonadaptive processes such as drift. In other words, display divergence among the taxa in our study might be due largely to some other variable not included in our analyses (e.g. differences in predation pressure among islands).

Irrespective of the underlying cause of *Microlophus* display diversification, robot playbacks confirmed that at least two of our study species (*M. indefatigabilis* and *Microlophus occipitalis*) responded preferentially to conspecific over nonconspecific display structure when the stimulus robot possessed conspecific coloration. As for our other study species, *M. grayii*, the level of responses to conspecific and nonconspecific displays was uniformly low. Lack of discrimination in past studies has sometimes resulted from recognition errors by receivers (i.e. mistaking a heterospecific signal for one produced by a conspecific; Ord, King, et al., 2011), but this does not seem to be the case here. The latency to respond and total amount of aggressive display in *M. grayii* elicited by a conspecific robot display were comparable to the slower response and shorter time spent displaying by the other two species to heterospecific stimuli. It is currently unclear whether the lack of a preferential response to conspecific display is related to the overall lower responsiveness of *M. grayii* males, or whether socioecological differences exist between *M. grayii* and the other species that may explain our results. This issue awaits further study.

The strong discrimination against unfamiliar territorial displays by the two other species tested, *M. indefatigabilis* (Santa Cruz Island) and *M. occipitalis* (mainland), was unanticipated, as both species are isolated from congeners and at least *M. indefatigabilis* is thought to have evolved in allopatry. There would appear to be little present-day adaptive benefit for these lizards to discriminate against unfamiliar territorial displays. Past studies of species recognition have shown that the behaviour of receivers to unfamiliar social signals are highly variable among species and are dependent on a combination of factors (Ord, King, et al., 2011). Indeed, the relative responsiveness to conspecific versus heterospecific displays in our study species was well within the range documented in similar studies published over the past 30 years on a diversity of taxonomic groups (Fig. 3). In the specific cases of *M. indefatigabilis* and *M. occipitalis*, we cannot rule out that discrimination against an unfamiliar territorial display does not reflect past selection on receivers that resulted in reinforcement on secondary contact. A history of interactions with congeners is possible for the mainland species given the presence of several other *Microlophus* in northwestern South America, but it is less clear how this might have occurred for *M. indefatigabilis* on Santa Cruz. One possibility is that a land bridge between Santa Cruz and Isabella might have been exposed during Pleistocene glacial maxima (for bathymetric maps, see Geist, Snell, Snell, Goddard, & Kurz, 2013; Jordan & Snell, 2008), which could have led to secondary contact between *M. indefatigabilis* and the resident species on Isabella, *M. albemarlensis*. Evidence that male *M. albemarlensis* respond preferentially to conspecific over nonconspecific displays in robot presentations would be consistent with this secondary contact hypothesis. More generally, playback experiments with other Galápagos *Microlophus* should provide better resolution of the origin and loss of conspecific display recognition.

The collective results of our comparative and playback studies show that species identity cues can evolve in animal signals and that they can elicit differential responses in receivers in the absence of contemporary selection pressure for species recognition. Whereas the specific mechanism promoting display differentiation

among lava lizards remains unknown, display evolution has occurred among populations isolated on different islands (and potentially through adaptation). The resulting differences in display pattern should function to promote reproductive isolation among taxa if secondary contact were to occur. This possibility would be especially interesting if a preference for the population-specific display pattern could be demonstrated between different island populations of the same species (e.g. *M. jacobi* and *M. indefatigabilis*; Fig. 1). In general, our results align with the classical notion that behavioural isolation among divergent or diverging taxa is of primary importance in reproductively segregating populations in the early stages of the speciation process (Mayr, 1963). Nevertheless, our data also show that closely related taxa can still differ in the probable functionality of their social signals for reproductive isolation.

Taken together, our findings support the conclusion, drawn by meta-analyses of species recognition in disparate taxa, that animals often show responses to conspecific and nonconspecific signals that do not conform to simplistic expectations based on sympatry and allopatry (Ord, King, et al., 2011; Ord & Stamps, 2009). Our study extends this general conclusion by highlighting that large differences in receiver responses to congeneric signals also can exist among species that share much of their general ecology, behaviour and evolutionary history. The Galápagos radiation of lava lizards promises to continue to provide a rich canvas for studying signal evolution among isolated, closely related taxa, as well as affording potential insights into the role of behavioural isolation in the process of animal speciation. In particular, the role of females in reinforcing reproductive barriers in a social system heavily centred around male territorial display would be an interesting avenue for future research.

Acknowledgments

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2015.08.002.

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