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Analysis of Bobbing Displays in Four Species of Galápagos Lava Lizards Using Conventional and Novel Quantitative Methods

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Abstract: Some of the most striking behaviors of animals are displays used in courtship, territorial behavior, and in defense against predators. Among reptiles, lizards exhibit enormous diversity in the stereotyped motion patterns of their bobbing displays. Although the bobbing displays of numerous lizard species have been described to date, those of the Galápagos Lava Lizards (*Microlophus* spp., Tropiduridae) have received comparatively little attention. Here, we analyze bobbing displays from four of the nine lava lizard species: *Microlophus albemarlensis*, *M. bivittatus*, *M. grayii*, and *M. indefatigabilis*. Field-recorded displays first were standardized to a common amplitude scale, and attributes of display duration and head amplitude were measured. Next, a discrete Fourier transform (DFT) was computed for each display, and data were gathered on 13 frequency and amplitude variables. Then, we conducted a principal components analysis on the Fourier transform–based variables, and used discriminant function analyses (both standard and permuted) to test the strength of species specificity in display structure. Results showed that displays with simpler structure (*M. albemarlensis* and *M. grayii*) were more often correctly assigned to species than were displays with more complex structure (*M. bivittatus* and *M. indefatigabilis*). We offer predictions for further tests on the bobbing displays of additional species of Galápagos lava lizards, and suggest that DFT represents a promising tool for the analysis of animal motion displays.

Key words: Fourier transform; Motion signals; Signature display; Two-bob display; Visual communication

The diversity of animal signals in nature has fascinated biologists since Darwin (1871). Some of the most intriguing signals appear to have arisen via the ritualization of behaviors as displays to intra- and interspecific receivers (Bradbury and Vehrencamp 2011). Displays often are conspicuous events and occur in contexts such as courtship (Prum 1994; Frith and Beehler 1998; Clark et al. 2017), male–male competition (Clutton-Brock and Albon 1979; Andersson 1994; Berglund et al. 1996), and antipredator behavior (Hasson et al. 1989; Leal 1999; Cooper et al. 2004; Caro 2005; Gómez-Serrano and López-López 2017).

Many types of visual displays have been described for reptiles (e.g., Vliet 1989; Gillingham et al. 1995) and they are particularly diverse in lizards (Carpenter and Ferguson 1977). Among the Iguania (sensu Pyron et al. 2013), numerous species engage in displays where the head and forequarters, or all four limbs, are displaced vertically in a stereotyped sequence of movements termed headbobs or pushups. We refer collectively to these signals as bobbing displays. Such displays are thought to communicate information as diverse as species identity, territory ownership, physical condition, and reproductive state (Carpenter and Ferguson 1977; Jenssen 1977, 1978; Losos 2009). Published descriptions of lizard bobbing displays are abundant, so we do not attempt to summarize that literature here (e.g., Ord and Blumstein 2002; Macedonia and Clark 2003; Martins et al. 2004; Ord and Martins 2006). Despite the impact of Galápagos reptiles on Darwin's (1859) conception of evolution, lava lizards (*Microlophus* spp., Tropiduridae) remain a group whose bobbing displays have received relatively little attention (but see Carpenter 1966; Clark et al. 2015).

Lava lizards in the Galápagos Islands comprise nine allopatric species in two distinct clades arising from different mainland South American *Microlophus* ancestors (Kizirian et al. 2004; Benavides et al. 2009). The Western clade contains seven species, three of which are included in the present study (*M. albemarlensis*, *M. grayii*, and *M. indefatigabilis*). The smaller Eastern clade contains only two species, one of which is included in our study (*M. bivittatus*). Phylogenetic relationships among the lava lizards are well established and the sequences of island colonization have been reliably reconstructed (Kizirian et al. 2004; Benavidas et al. 2009). Results from such work allow us to view known differences in lava lizard bobbing displays from an evolutionary perspective (Carpenter 1966; Clark et al. 2015).

Carpenter (1966) showed that lava lizards exhibit considerable diversity in bobbing display structure (i.e., the vertical head motion depicted in a time vs. amplitude display action pattern [DAP] line graph). Yet the underlying causes of this variability remain unclear. Species recognition is a frequent explanation for display variation among sympatric congeners (Carpenter and Ferguson 1977; Jenssen 1977; Clark et al. 2015). Because lava lizards appear to have evolved in isolation and are allopatric in distribution (Carpenter 1966; Kizirian et al. 2004; Benavidas et al. 2009; but see Clark et al. 2015), species recognition cannot be a driving force behind interspecific bobbing display variation. Moreover, recent phylogenetic comparative analysis indicates that neither sexual selection nor island colonization order has had a detectable impact on lava lizard bobbing display diversity (Clark et al. 2015). Therefore, genetic drift is a likely driver of evolutionary change in lava

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FIG. 1.—Representative adult males of the four species of lava lizards (*Microlophus*) examined in this study. An example display action pattern (DAP) graph of each species' signature display is shown in the upper left corner of each photo.

lizards inhabiting the Galápagos Islands (Carpenter 1966; Jordan and Snell 2008).

Historically, bobbing displays have been quantified by measuring the durations of temporal units (Carpenter and Grubitz 1961). This method was initially established to document the four-legged pushup display of Ornate Tree Lizards (*Urosaurus ornatus*). As change in amplitude between flexed and extended leg positions was uniform throughout the display of this species, no substantial amplitude variation was present to document. In many lizard species, however, head/body amplitude varies throughout their bobbing displays. To address this issue, the original method of Carpenter and Grubitz (1961) has been extended to include measurements of head amplitude changes as well as (in some studies) temporal relationships among unit durations (Ord and Martins 2006; Clark et al. 2015; Vicente 2018). Here, we propose a new approach to bobbing display analysis in which displays are not partitioned into units, but instead are analyzed as entire waveforms using the Fourier transform. Whereas research on animal acoustic (Baeckens et al. 2019; Mcloughlin et al. 2019), electric (Crampton et al. 2008), and visual (color patterns: Stoddard and Osorio 2019) signals has relied on Fourier transformation to analyze these signals for many decades, our study is the first to utilize this method in the analysis of lizard bobbing displays.

In this study, we analyze bobbing display structure in four of the nine Galápagos lava lizard species using a suite of conventional and novel methods. First, we standardized the bobbing displays in our sample to a common amplitude scale of 0–1 (i.e., 0–100%). Next, we divided displays into temporal units from which unit durations and relative amplitudes of bobbing peaks were measured. Then, independent of unit-based calculations, we used an analytical approach to calculate Fourier transforms for each display to summarize display structure. Data from these variables were entered into a principal components analysis (PCA) to produce a smaller subset of normally distributed and uncorrelated variables. Finally, we used two forms of discriminant function analysis (standard and permuted DFA) to assess how well our derived variables assigned bobbing displays to the correct species. As Fourier transformation has not been used previously in the analysis of lizard bobbing displays, we made no a priori predictions regarding potential differences in DFA classification success among our four study species. Based on results of the work presented here, however, we make specific predictions about DFA classification success for

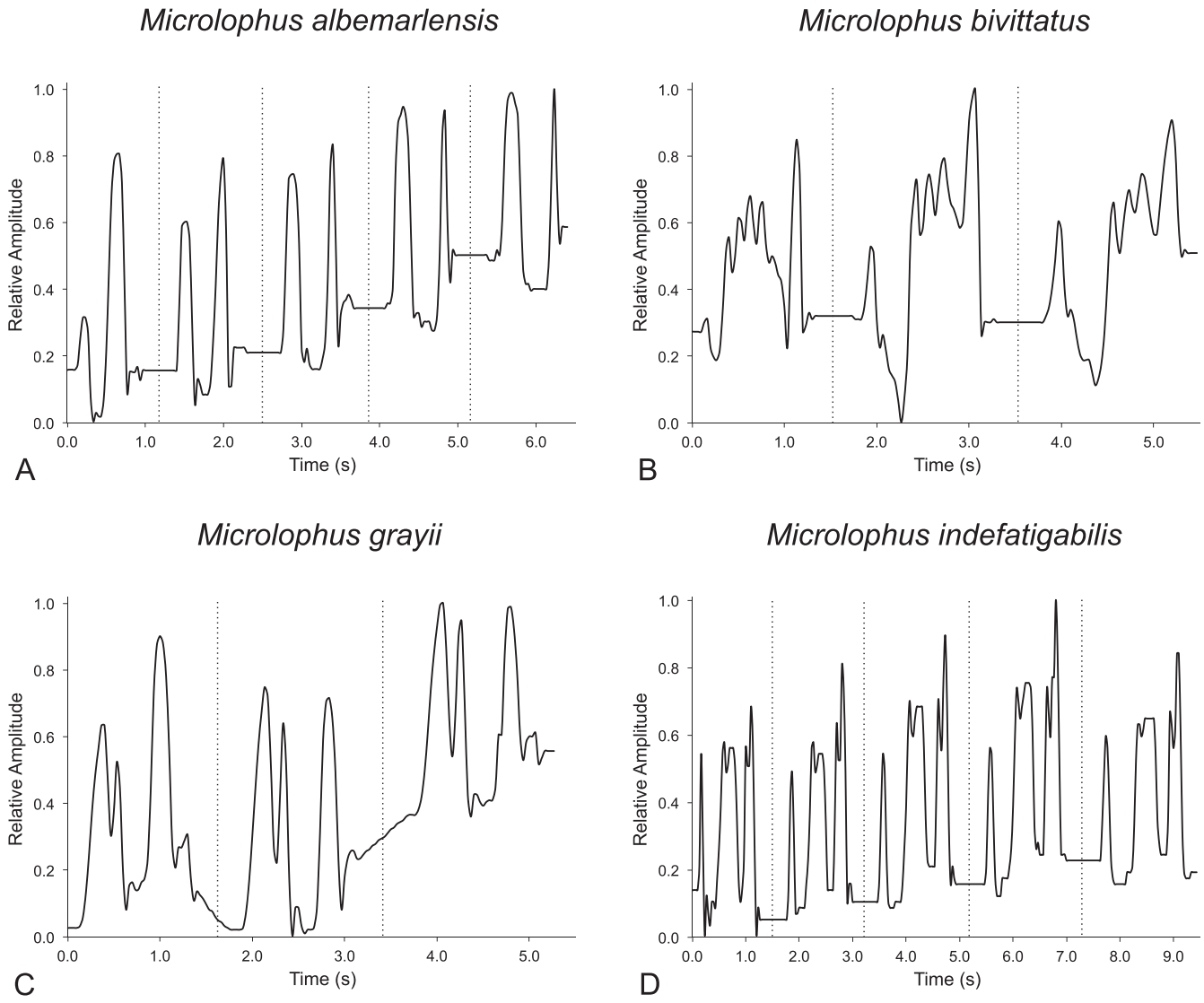

Fig. 2.—Structure of representative signature display volleys from each of four species of lava lizards: (A) *Microlophus albemarlensis*, (B) *M. bivittatus*, (C) *M. grayii*, (D) *M. indefatigabilis*. Vertical dotted lines separate sequential iterations of displays.

bobbing displays of Galápagos lava lizards that await further study.

Materials and Methods

Subjects and Study Areas

Microlophus albemarlensis (Fig. 1a) is endemic to the Galápagos islands of Isabella and Fernandina (Benavides et al. 2009). Bobbing displays of *M. albemarlensis* were video recorded in the vicinity of Muro de las Lagrimas on Isabella (Wall of Tears; 0°57′58.30″S, 91°00′45.95″W; in all cases, datum = WGS84). *Microlophus bivittatus* (Fig. 1b) is endemic to the island of San Cristóbal and, unlike our other study species, is a member of the Eastern clade of lava lizards. We recorded bobbing displays from *M. bivittatus* around Puerto Baquerizo Moreno (0°53′45.13″S, 89°36′24.70″W). *Microlophus grayii* (Fig. 1c) is endemic to the island of Floreana, and we recorded bobbing displays in the vicinity of Puerto Velasco Ibarra (1°16′27″S, 90°29′13″W). *Microlophus indefatigabilis* (Fig. 1d) is endemic to the islands of Santa Cruz and Santa Fe (Benavides et al. 2009). For this species, we recorded bobbing displays near the Charles Darwin Research Station on Santa Cruz (0°44′32″S, 90°18′13″W).

Display Data Collection and Visualization

We gathered display data from video recordings of experiments in which lizard robots were presented to subjects. Details of these experiments, including equipment used and protocols followed for commencing and terminating trials, can be found elsewhere (Macedonia et al. 2013, 2015; Clark et al. 2015, 2016, 2017, 2019). Subject signature displays (bobbing displays that exhibit taxon-specific structure and that occur in multiple contexts) were produced singly and in volleys (i.e., display series: Jenssen 1977). In this study, we consider displays performed within 3 s of each other to be part of the same volley (Fig. 2). Video clips of single displays and display volleys were recorded with identifying information, and were imported into Macintosh GraphClick (v3, Arizona Software, Switzerland) for mea-

surement. In GraphClick, vertical (y-axis) motion of the head was tracked frame-by-frame by placing the cursor over a subject's eye and clicking the computer mouse. The recorded y-axis coordinates then were exported to Microsoft Excel (v14.7, Microsoft Corporation, Redmond, WA, USA) for further processing (see below).

Because our subjects were free ranging, they could not be recorded at a standard focal distance. We therefore standardized (normalized) all bobbing displays to a common amplitude scale of 0 to 1 by first subtracting the smallest (y-axis coordinate) value from each value in a display, and then dividing each resulting value by the largest value in the display. The standardized values then were plotted as DAP graphs, which, depending on their structure, were partitioned into either three or five display units (Fig. 3). We assigned odd numbers (e.g., 1, 3, 5) to units in which the head was moving up and down in large amplitude sweeps, and even numbers to units in which the head either was motionless or contained one or two small bobs. We then measured display unit durations and peak amplitudes of large bobs in odd-numbered units. Display unit duration measurements had a time resolution of 0.033 s and peak amplitude measurements had a resolution of 0.01 (i.e., 1%). We chose not to measure amplitudes of small bobs, in light of their variable presence and our uncertainty that they might arise merely as dampened oscillations of large bobs.

For 12 adult males each of *M. albemarlensis*, *M. bivittatus*, and *M. indefatigabilis*, we chose 2 signature displays of sufficiently high video quality to conduct detailed analyses of display structure, resulting in a set of 24 displays per species. Because we were only able to obtain 1 signature display for 5 of our 12 adult male *M. grayii* subjects that possessed the most common time-amplitude morphology (Fig. 3c), 5 other males provided 3 displays each and 2 males provided 2 displays each, resulting in a total of 24 displays. Among our four study species only *M. bivittatus* regularly performed a second type of bobbing pattern—a brief two-bob display (Clark et al. 2017). In contrast to signature displays, two-bob displays never were produced in volleys and always were followed immediately by a change in body orientation or by locomotion (Fig. 4). We provide descriptive statistics for this display type from 12 *M. bivittatus* males (2 displays each).

Fourier Transformation of Bobbing Displays

The oscillatory nature of bobbing displays makes them qualitatively comparable to a series of trigonometric functions. We computed the discrete Fourier transform (DFT) for each display using the fast Fourier transform (FFT) algorithm in MATLAB (vR2016b). Fourier transformation partitioned the signal into a collection of sinusoidal waves of different frequencies and amplitudes. The sum of these sine functions recreates the original bobbing display. We plotted the DFT of each signal using the frequency domain, in which the horizontal axis represented the frequency and the vertical axis represented the amplitude.

The greatest amplitude in the DFT of a bobbing display tends to occur at a relatively low frequency. Higher frequencies generally correspond to lower amplitudes and represent noise in the signal. To reduce inclusion of this spurious noise, we chose an amplitude threshold, of 75% of the mean amplitude. Below this threshold amplitude was assumed to be negligible. We confirmed that this procedure had not removed important traits of bobbing displays by comparing the reverse Fourier transform of the simplified signal to the original signal.

TABLE 1.—Names and definitions of the 13 Fourier transform–based variables used to quantify signature displays in lava lizards (*Microlophus*). These variables comprise one primary measure (Variable 1) and three identical sets of secondary variables (Variables 2–5, 6–9, and 10–13) measured at three nonoverlapping amplitude ranges (0–5, 5–10, and 10–15 Hz, respectively).

Variable number	Variable	Description
1	Principal frequency	Frequency corresponding to peak amplitude
2	Peak frequency	Frequency corresponding to highest amplitude from 0 to 5 Hz
3	Partial sum	Partial sum of amplitudes from 0 to 5 Hz
4	Percentage of sum	Proportion of partial sum of amplitude to total sum amplitude from 0 to 5 Hz
5	Mean amplitude	Mean amplitude from 0 to 5 Hz
6–9	Peak frequency, partial sum, percentage of sum, mean amplitude 5–10 Hz	Same as Variables 2–5, but for 5–10 Hz
10–13	Peak frequency, partial sum, percentage of sum, mean amplitude 10–15 Hz	Same as Variables 2–5, but for 10–15 Hz

The overall feature anticipated to distinguish displays by species was the frequency corresponding to the maximal amplitude (i.e., the principal frequency), as it describes the most prominent trigonometric function composing the signal. To further characterize display transforms, we divided the frequency domain into low- (0–5 Hz), medium- (5–10 Hz), and high- (10–15 Hz) frequency regions. Within each of these three regions we extracted four variables: the peak frequency, the sum of region amplitudes, the proportion of this sum with respect to the total sum of amplitudes, and the region's mean amplitude (Table 1).

Comparative Analysis of Signature Display Structure

Exploratory analysis in SPSS (v21.0, IBM Inc., Armonk, NY, USA) revealed that most of our 13 Fourier transform–based variables (Table 1) were correlated, nonnormally distributed, or both. Therefore, we used principal components analysis (PCA with varimax rotation) to obtain a smaller number of uncorrelated, normally distributed variables. We saved the principal component (PC) scores and entered them into a canonical DFA to determine the accuracy with which the 96 signature displays could be correctly assigned to species. We also ran a cross-validated leave-one-out DFA, in which the display being classified was not used in generating the discriminant functions. We then used chi-squared goodness-of-fit tests in VassarStats (Lowry 2014) for each species to determine if the observed versus expected classification results differed significantly from chance.

To minimize the effects of autocorrelation that can result from the inclusion of multiple samples per subject in a DFA (Mundry and Sommer 2007), we conducted a permuted DFA (pDFA) in R (v3.3.2; R Core Team 2016) using a

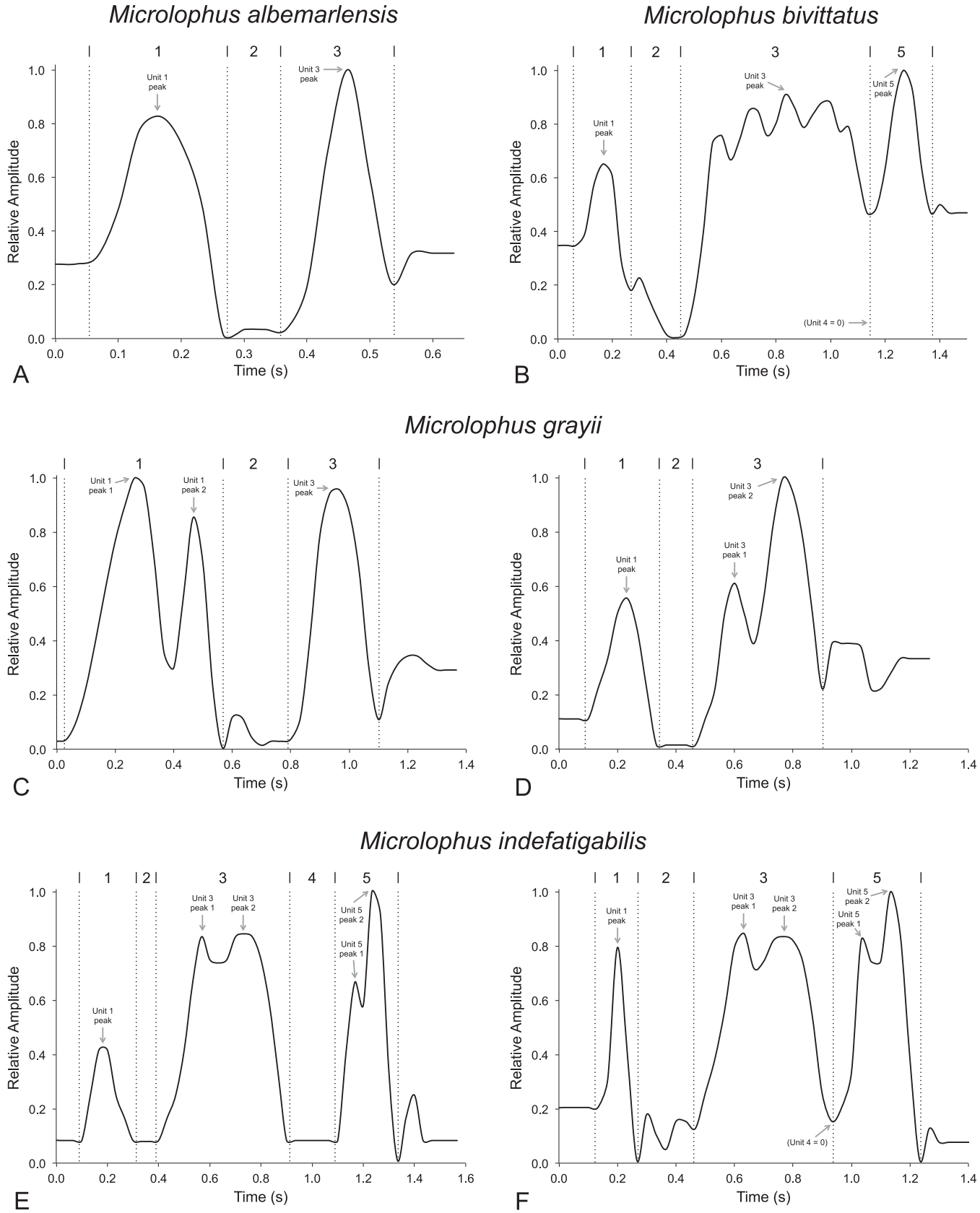

FIG. 3.—Representative signature displays from each of four species of lava lizards (*Microlophus*), with landmarks for measuring unit durations and peak amplitudes. Displays are divided into the numbered units shown at the top of each graph. Vertical dotted lines indicate the start and end of each display unit. Arrows (and accompanying labels) indicate bob peaks measured for amplitude. The simplest display was produced by *M. albemarlensis* (A), and consisted of two large, single-peaked bobs (Units 1 and 3). In *M. bivittatus* (B), displays consisted of a single bob (Unit 1) followed by a series of 3–6 rapid shallow bobs (Unit 3), and concluded with a large single bob (Unit 5). Displays of *M. grayii* (C) comprised a large double bob (Unit 1) followed by a large single bob (Unit 3). Occasionally this species (D) exhibited a large single bob (Unit 1) followed by a large double bob (Unit 3) or, more rarely, two or three large single bobs (not shown). Only the *M. grayii* typical display form shown in (C) was quantified. In *M. indefatigabilis* (E, F), displays included a large single bob (Unit 1) followed by two large double bobs (Units 3 and 5).

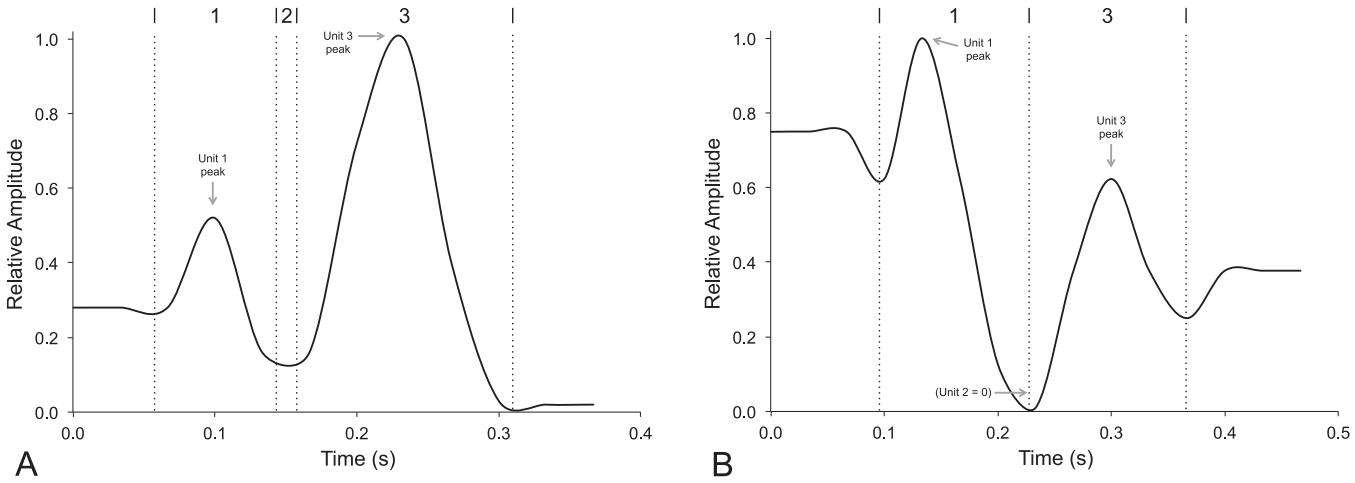

FIG. 4.—Two exemplars of the *Microlophus bivittatus* two-bob display (A, B), which is structurally and functionally distinct from this species' signature display (shown in Figs. 1–3).

function provided by Mundry (2015) that is based on lda in the R package MASS (Venables and Ripley 2002). In our pDFA, the control factor (subject) was nested within the test factor (species). We set the pDFA to select one display randomly from each subject (48 total displays) to create the discriminant functions, a selection process that was iterated 100 times and then averaged the subsequent classifications. To determine whether average classification success differed significantly from the random assignment of displays to species (i.e., the null hypothesis), the pDFA permuted the displays among species 1000 times. Like a standard DFA, pDFA generates results for a cross-validated (cross-classified) analysis; in our case, 48 displays were used to create the discriminant functions that classified the remaining 48 displays.

RESULTS

Descriptive statistics for landmark variables in our samples highlight species differences and similarities in bobbing displays (Tables 2–6). Because many of our display unit-based variables were nonnormally distributed, we report median, range, and coefficient of variation (CV) for unit durations and display peak amplitudes. The PCA conducted on our Fourier transform–based variables extracted five components with eigenvalues >1.0, which explained nearly 87% of the variation in display structure (Tables 7 and 8). Entering these five components into a DFA produced three discriminant functions, the first two of which explained nearly all (99.4%) of the variation (Table 9). Whereas *M. albemarlensis* and *M. grayii* exhibited virtually no overlap in discriminant function space, *M. bivittatus* and *M. indefatigabilis* were comingled and showed substantial overlap with *M. grayii* (Fig. 5). In this analysis, DF1 was the most important function in accounting for over 80% of the variance (Table 9). The most important principal components contributing to DF1 were PC2 and PC3 (Tables 7 and 8), where PC2 consisted primarily of MidPartSum and MidPercSum (combined variance ≈ 22%) and PC3 consisted largely of LowPeakFreq and PrincFreq (combined variance ≈19%).

The DFA correctly assigned displays to the species that produced them 81.3% of the time in the original classification, and 72.9% of the time in a cross-validated classification (Table 11). Chi-squared goodness-of-fit tests showed that observed classification success was significantly greater for each species than expected by chance (original DFA, chi-square $\geq$ 17.0, df = 3, $P \leq 0.007$; cross-validated DFA, chi-square $\geq$ 9.33, df = 3, $P \leq 0.025$). A greater number of correct assignments were made for the structurally simpler displays of *M. albemarlensis* and *M. grayii* than for the more complex displays of *M. bivittatus* and *M. indefatigabilis*. Although classification success for *M. albemarlensis* and *M. grayii* was identical in the original and cross-validated analyses, success decreased for *M. bivittatus* and *M. indefatigabilis* in the cross-validation analysis and most misclassifications were with each other (Table 11).

To compare the performance of pDFA with DFA, we initially ran the pDFA using the PC scores for all 96 cases (4 species × 12 subjects × 2 displays) to create the discriminant functions. Overall classification success was identical to that of our DFA at 81.3% (pDFA does not report classification success by group [i.e., species]). Finally, using the PC scores from one randomly chosen display of each subject (48 total cases), pDFA results revealed that displays were correctly assigned to species 80.0% of the time in the original classification (chance level = 44.0%; $P = 0.001$) and 72.5% of the time in the cross-classification (chance level = 35.1%; $P = 0.001$).

TABLE 2.—*Microlophus albermarlensis* signature display. Twelve subjects contributed two displays each. CV = coefficient of variation.

Display unit durations	Unit 1	Unit 2	Unit 3	Total
Median	0.233	0.184	0.200	0.633
Range	0.200–0.333	0.067–0.300	0.133–0.266	0.467–0.767
CV	13.170	40.848	17.265	16.909
Standardized peak amplitudes	Unit 1 peak		Unit 3 peak	
Median	1.000		0.952	
Range	0.772–1.000		0.533–1.000	
CV	7.615		15.937	

TABLE 3.—*Microlophus bivattatus* signature display. Twelve subjects contributed two displays each. CV = coefficient of variation.

Display unit durations	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Total
Median	0.167	0.134	0.633	0.000	0.200	1.167
Range	0.067–0.333	0.000–0.300	0.400–0.766	0.000–0.267	0.166–0.333	0.900–1.500
CV	35.277	72.176	16.390	161.246	19.422	10.837
Standardized peak amplitudes	Unit 1 peak		Unit 3 peak		Unit 5 peak	
Median	0.536		0.823		1.000	
Range	0.099–0.949		0.570–1.000		0.788–1.000	
CV	41.369		14.606		5.284	

Discussion

We analyzed bobbing displays from four species of Galápagos Lava Lizards using conventional and novel approaches. Although species specificity in signature display structure is readily visible (Figs. 2 and 3), our ability to demonstrate this specificity statistically was variable. Our finding that displays with comparatively simple structure exhibited the highest DFA classification success indicates that the Fourier transform–based analytical approach is less effective when bobbing displays exhibit complex morphology. This hypothesis could be tested in future research using displays of additional lava lizard species. For example, we can predict that the structurally simple signature display of *M. delanonis* will exhibit greater DFA classification success than will the more complex signature display of *M. habeli* (see Clark et al. 2015 for illustrations of these species' bobbing displays). In future studies, we intend to test the effect of additional and different Fourier transform–based variables on DFA classification success.

An alternative explanation for the finding that the displays of *M. albemarlensis* and *M. grayii* were more often correctly classified than were those of *M. bivittatus* and *M. indefatigabilis* is that the latter two species exhibited greater variability in the structure of their displays. Descriptive statistics showed that Unit 4 durations in the signature displays of *M. bivittatus* and *M. indefatigabilis* had high CVs compared to those of their other display units and to unit durations of the other two species. However, we do not know how much of the variability observed in Unit 4 durations was captured by our Fourier transform–based variables; nor do we know how much of that variation carried through to the PCA and, in turn, to the DFAs. Although PC2 and PC3 were weighted most heavily on the first discriminant function (>80% of the variation; Tables 9 and 10), the CVs for those components (see Table S1 in the Supplemental Materials available online) revealed no detectable pattern among the study species that would explain, for example, the comparatively poor classification of *M. indefatigabilis* displays. Discerning why DFA is more effective at classifying the displays of some species over others becomes increasingly difficult with each transformation away from the original variables.

TABLE 4.—*Microlophus grayii* signature display. Twelve subjects contributed 1–3 displays each. CV = coefficient of variation.

Display unit durations	Unit 1	Unit 2	Unit 3	Total
Median	0.500	0.233	0.300	1.067
Range	0.400–0.633	0.167–0.300	0.234–0.400	0.900–1.167
CV	12.127	13.908	14.461	7.207
Standardized peak amplitudes	Unit 1 peak 1	Unit 1 peak 2	Unit 3 peak	
Median	0.829	0.822	1.000	
Range	0.200–1.000	0.340–1.000	0.800–1.000	
CV	23.449	20.910	6.409	

Comparison of Fourier Transform–Based Analysis with Display Unit Analysis

Ideally, we would have been able to compare DFA classification success of the Fourier transform–based analysis with that of the unit-based analysis directly. We did not conduct a DFA of the display unit data, however, because we were unwilling to presume that display units were homologous among the four study species. To present one example, if one compares the signature display of *M. albemarlensis* (Fig. 3a) with that of *M. bivittatus* (Fig. 3b), it is uncertain whether *M. albemarlensis* Unit 3 is homologous with *M. bivittatus* Unit 3, or Unit 5, or either of these units. Nevertheless, we do not think that unit-based and Fourier transform–based approaches to bobbing display analysis are inherently incomparable. Comparisons among taxa should be possible in cases where it is reasonably clear that the units being compared are homologous. For example, minor structural differences exist between the signature displays of *M. indefatigabilis* on Santa Cruz and on Santa Fe, as well as between those of *M. jacobi* on Santiago and on Rabida (see Carpenter 1966 and Clark et al. 2015 for bobbing display illustrations). In such cases, we can be certain that we are comparing the same display units between taxa.

Evolution of Bobbing Displays in Galápagos Lava Lizards

On an evolutionary time scale, evidence exists that lizard bobbing display structure can change quickly (e.g., Martins et al. 2004). Therefore, the presence of shared derived traits in bobbing displays should likely be discernable in recently diverged taxa. Our study included two species (*M. bivittatus* and *M. albemarlensis*) that diverged from other *Microlophus* within the past million years (Benavides et al. 2009). First, *M. bivittatus* and *M. habeli* comprise the small Eastern clade of lava lizards. Both species' signature displays include a shallow series of three or more rapid bobs that is unique to this sister group (Carpenter 1966; Clark et al. 2015, 2016). Because *M. bivittatus* and *M. habeli* are estimated to have diverged only about 0.4 MYA (Benavides et al. 2009), the mutual possession of this trait indicates that it was present in their last shared common ancestor. Second, the two terminal taxa in the larger seven-species Western clade, *M. albemarlensis* and *M. pacificus*, are estimated to have diverged approximately 0.7 MYA (Benavides et al. 2009). The simple signature display of *M. albemarlensis* (Figs. 1–3) differs from *M. pacificus* only in that the latter species' display is prefixed with one or two small

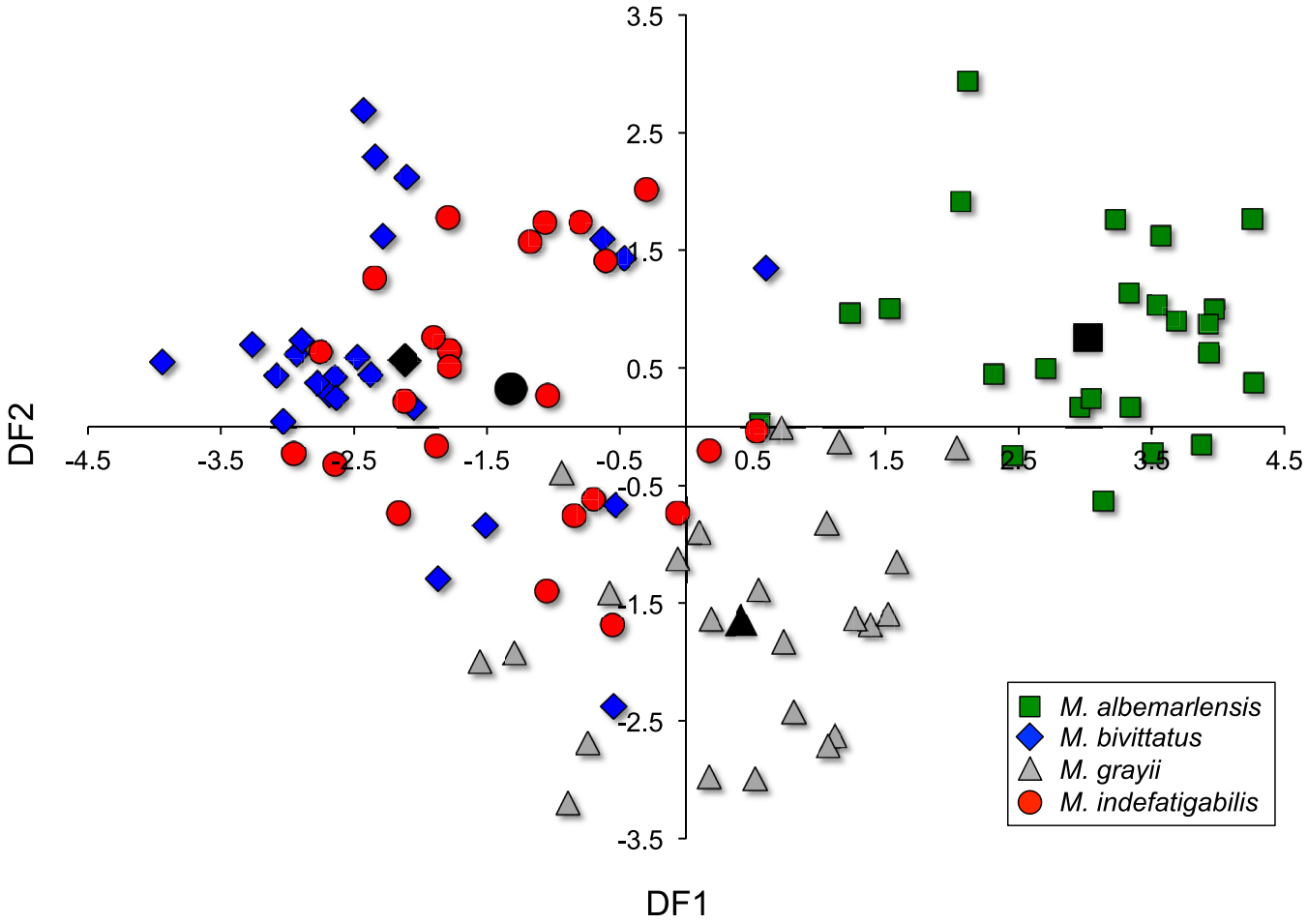

FIG. 5.—Scatterplot of scores from the first two of three functions in a discriminant function analysis (DFA) of PCA scores derived from 13 Fourier transform–based variables quantifying the study species' signature displays. DF1 explains over 80% of the variation in the distribution of scores, and DF2 explains nearly all the remaining variation. Although *M. albemarlensis* (squares) exhibits no overlap with *M. bivittatus* (diamonds), and minimal overlap with *M. grayii* (triangles) and *M. indefatigabilis* (circles), overlap exists between *M. bivittatus* and *M. indefatigabilis*. Distribution centroids for each species are shown as larger black symbols. A color version of this figure is available online.

TABLE 5.—*Microlophus indefatigabilis* signature display. Twelve subjects contributed two displays each. CV = coefficient of variation.

Display unit durations	Unit 1	Unit 2	Unit 3	Unit 4	Unit 5	Total
Median	0.200	0.217	0.467	0.050	0.333	1.300
Range	1.333–0.267	0.100–0.400	0.366–0.633	0.000–0.233	0.233–0.466	1.000–1.700
CV	20.683	36.674	15.909	107.106	17.183	14.501
Standardized peak amplitudes	Unit 1 peak	Unit 3 peak 1	Unit 3 peak 2		Unit 5 peak 1	Unit 5 peak 2
Median	0.559	0.781	0.769		0.769	1.000
Range	0.364–0.795	0.636–0.946	0.636–0.857		0.636–1.000	none
CV	18.887	10.059	9.438		11.181	0.000

TABLE 6.—*Microlophus bivittatus* two-bob display. Twelve subjects contributed two displays each. CV = coefficient of variation.

Display unit durations	Unit 1	Unit 2	Unit 3	Total
Median	0.133	0.000	0.167	0.333
Range	0.066–0.200	0.000–0.233	0.100–0.267	0.233–0.466
CV	24.896	175.488	30.436	18.253
Standardized peak amplitudes	Unit 1 peak		Unit 3 peak	
Median	0.698		1.000	
Range	0.520–1.000		0.623–1.000	
CV	21.700		8.793	

TABLE 7.—Eigenvalues, percent of variance, and cumulative percent of variance accounted for by the five principal components extracted from 13 Fourier transform–based variables representing display structure in lava lizards (*Microlophus*).

	Rotated sums of squared loadings		
Principal component	Eigenvalues	Percent of variance	Cumulative %
PC1	2.854	21.953	21.953
PC2	2.834	21.802	43.755
PC3	2.458	18.907	62.662
PC4	2.024	15.566	78.228
PC5	1.090	8.386	86.614

TABLE 8.—Relationships between the rotated principal components and the 13 Fourier transform–based variables. Variables with most heavily weighted factor loadings (i.e., those with an absolute value > 6) are shown in bold type. Abbreviations refer to variables defined in Table 1, as follows: Low = 0–5 Hz; Mid = 5–10 Hz; Hi = 10–15 Hz; PrincFreq = Principal frequency; PeakFreq = Peak frequency; PartSum = Partial sum; PercSum = Percentage of sum; MeanAmp = Mean amplitude. Example: HiPartSum = Partial sum of amplitudes from 10–15 Hz.

	Principal component				
Variable	1	2	3	4	5
HiPercSum	**0.953**	–0.057	0.071	–0.116	0.036
HiPartSum	**0.949**	–0.037	–0.072	0.030	–0.074
HiMeanAmp	**0.754**	0.235	0.434	–0.160	0.161
HiPeakFreq	**–0.647**	0.098	0.366	0.000	0.121
MidPartSum	–0.045	**0.945**	–0.006	0.096	–0.141
MidPercSum	–0.072	**0.933**	0.166	–0.098	0.018
MidMeanAmp	0.083	**0.655**	0.581	–0.244	0.177
LowPeakFreq	–0.038	–0.015	**0.890**	0.040	–0.131
PrincFreq	–0.056	0.312	**0.785**	–0.311	0.121
LowMeanAmp	–0.024	0.193	0.074	**0.912**	–0.028
LowPartSum	–0.10	–0.398	–0.455	**0.717**	–0.070
LowPercSum	–0.164	–0.526	–0.373	**0.679**	0.036
MidPeakFreq	–0.038	–0.061	–0.012	–0.030	**0.976**

TABLE 9.—Discriminant function analysis of the Fourier transform–based variables used to measure bobbing displays in lava lizards (*Microlophus*): Eigenvalues and percent of variance show the magnitude and proportion, respectively, of each function's discriminating ability.

Function	Eigenvalue	Percent of variance	Cumulative %
1	4.054	80.3	80.3
2	0.963	19.1	99.4
3	0.031	0.6	100.0

TABLE 10.—Discriminant function analysis of the Fourier transform–based variables used to measure bobbing displays in lava lizards (*Microlophus*): Standardized canonical discriminant function coefficients show the relationship (weighting) of each function to the input principal components (PC).

	Function		
	1	2	3
PC1	0.068	0.369	–0.038
PC2	1.218	0.237	–0.049
PC3	1.090	–0.405	–0.251
PC4	0.369	–0.658	0.749
PC5	0.365	0.825	0.422

bobs (Carpenter 1966; Clark et al. 2015, 2016). By comparison, lava lizard species that have been separated for longer time periods tend to exhibit displays with substantially different structure (see Clark et al. 2015).

Origin, Distribution and Function of Two-Bob Displays in Lava Lizards

One issue worth pursuing in future research is whether two-bob displays are performed by unstudied lava lizard species and, if so, whether those displays are performed in the same context (i.e., immediately prior to movement). Although species in the present study other than *M. bivittatus* occasionally performed a bobbing display prior to locomotion, such bobbing displays were invariably signature displays. In light of the sister-group relationship of *M. bivittatus* and *M. habeli*, and their relatively recent divergence (Kizirian et al. 2004; Benavides et al. 2009), we predict that the only other lava lizard species that performs two-bob displays should be *M. habeli*.

Curiously, the *M. bivittatus* two-bob display exhibits structural and/or contextual similarities to bobbing displays in some distantly related lizard taxa. For example, males in many lizard species approach females in courtship with repeated short bursts of movement where, just prior to movement, a shuddering display is performed. In *Anolis carolinensis*, this display consists of paired bobs (Orrell and Jenssen 2003) that superficially resemble the *M. bivittatus* two-bob display. Similarly, whereas shuddering displays in *Sceloporus graciosus* (Kelso and Martins 2008) and *S. undulatus* (Nava et al. 2012) have been reported to serve a male courtship function exclusively, Ruby (1977) found that both sexes of *S. jarrovi* produce this display in the nonbreeding season and that it signals an elevated probability of attack. Interestingly, shuddering displays in *S. undulatus hyacinthinus* were most frequently performed just prior to and during locomotion (Rothblum and Jenssen 1978). We speculate that the *M. bivittatus* two-bob display is an abbreviated shuddering display that has been liberated from the context of courtship and that now functions to signal impending movement.

We think it interesting that the two-bob display is so similar in structure to the *M. albemarlensis* signature display. Analyses of lava lizard phylogenetic relationships (Kizirian et al. 2004; Benavides et al. 2009; Pyron et al. 2013) suggest

TABLE 11.—Discriminant function analysis of the Fourier transform–based variables used to measure bobbing displays in lava lizards (*Microlophus*): Discriminant function classification results. Correct species assignments are in bold text.

Original analysis[a]	Predicted group membership				
Species	*M. albemarlensis*	*M. bivittatus*	*M. grayii*	*M. indefatigabilis*	Total
M. albemarlensis	**23 (95.8%)**	0 (0%)	1 (4.2%)	0 (0%)	24 (100%)
M. bivittatus	0 (0%)	**19 (79.2%)**	1 (4.2%)	4 (16.7%)	24 (100%)
M. grayii	1 (4.2%)	1 (4.2%)	**22 (91.7%)**	0 (0%)	24 (100%)
M. indefatigabilis	0 (0%)	5 (20.8%)	5 (20.8%)	**14 (58.3%)**	24 (100%)
Cross-validated[b]	Predicted group membership				
Species	*M. albemarlensis*	*M. bivittatus*	*M. grayii*	*M. indefatigabilis*	Total
M. albemarlensis	**23 (95.8%)**	0 (0%)	1 (4.2%)	0 (0%)	24 (100%)
M. bivittatus	0 (0%)	**15 (62.5%)**	1 (4.2%)	8 (33.3%)	24 (100%)
M. grayii	1 (4.2%)	1 (4.2%)	**22 (91.7%)**	0 (0%)	24 (100%)
M. indefatigabilis	0 (0%)	8 (33.3%)	6 (25%)	**10 (41.7%)**	24 (100%)

[a] In the original analysis 81.3% of cases were classified correctly to species.

[b] Of cross-validated cases, 72.9% were classified correctly to species.

that this resemblance in display form might be convergent, because *M. albemarlensis* and *M. bivittatus* are members of different clades. Yet, until bobbing displays of all Galápagos lava lizard species have been studied in detail, the possibility that two-bob displays are present elsewhere within the group cannot be excluded.

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Supplemental Material

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Literature Cited

Andersson, M. 1994. Sexual Selection. Princeton University Press, USA.

Baeckens, S., D. Llusia, R. García-Roa, and J. Martín. 2019. Lizard calls convey honest information on body size and bite performance: A role in predator deterrence? Behavioral Ecology and Sociobiology 73:87. DOI: https://doi.org/10.1007/s00265-019-2695-7

Benavides, E., R. Baum, H.M. Snell, H.L. Snell, and J.W. Sites, Jr. 2009. Island biogeography of the Galápagos lava lizards (Tropiduridae: *Microlophus*): Species diversity and colonization of the archipelago. Evolution 63:1606–1626.

Berglund, A., A. Bisazza, and A. Pilastro. 1996. Armaments and ornaments: An evolutionary explanation of traits of dual utility. Biological Journal of the Linnean Society 58:385–399.

Bradbury, J.W., and S.L. Vehrencamp. 2011. Principles of Animal Communication, 2nd ed. Sinauer Associates, Inc., USA.

Caro, T.M. 2005. Antipredator Defenses in Birds and Mammals. University of Chicago Press, USA.

Carpenter, C.C. 1966. Comparative behavior of the Galápagos lava lizards (*Tropidurus*). Pp. 269–273 in The Galápagos: Proceedings of the Galápagos International Scientific Project (R.I. Bowman, ed.). University of California Press, USA.

Carpenter, C.C., and G.W. Ferguson. 1977. Variation and evolution of stereotyped behavior in reptiles. Pp. 335–554 in Biology of the Reptilia, vol. 7: Ecology and Behaviour, A (C. Ganz and D.W. Tinkle, eds.). Academic Press, USA.

Carpenter, C.C., and G.G. Grubitz, III. 1961. Time-motion study of a lizard. Ecology 42:199–200.

Clark, D.L., J.M. Macedonia, J.W. Rowe, M.A. Stuart, D.J. Kemp, and T.J. Ord. 2015. Evolution of displays in Galápagos lava lizards: Comparative analyses of signalers and robot playbacks to receivers. Animal Behaviour 109:33–44.

Clark, D.L., J.M. Macedonia, J.C. Gillingham, J.W. Rowe, H.J. Kane, and C.A. Valle. 2016. Why does conspecific display recognition differ among species of Galápagos lava lizards? A test using lizard robots. Herpetologica 72:47–54.

Clark, D.L., J.M. Macedonia, J.W. Rowe, K. Kamp, and C.A. Valle. 2017. Responses of Galápagos lava lizards (*Microlophus bivittatus*) to manipulation of female nuptial coloration on lizard robots. Herpetologica 73:323–330.

Clark, D.L., J.M. Macedonia, J.W. Rowe, M.R. Austin, I.M. Centurione, and C.A. Valle. 2019. Galápagos lava lizards (*Microlophus bivittatus*) respond dynamically to displays from interactive conspecific robots. Behavioral Ecology and Sociobiology 73:136.

Clutton-Brock, T.H., and S.D. Albon. 1979. The roaring of red deer and the evolution of honest advertisement. Behaviour 69:145–170.

Cooper, W.E., Jr., V. Pérez-Melado, T.A. Baird, J.P. Caldwell and L.J. Vitt. 2004. Pursuit deterrent signaling by the Bonaire whiptail lizard *Cnemidophorus murinus*. Behaviour 141:297–311.

Crampton, W.G.R., J.K. Davis, N.R. Lovejoy, and M. Pensky. 2008. Multivariate classification of animal communication signals: A simulation-based comparison of alternative signal processing procedures using electric fishes. Journal of Physiology (Paris) 102:304–321.

Darwin, C. 1859. On the Origin of Species by Means of Natural Selection. Murray, UK.

Darwin, C. 1871. The Descent of Man and Selection in Relation to Sex. Murray, UK.

Frith, C.B., and B.M. Beehler. 1998. The Birds of Paradise. Oxford University Press, UK.

Gillingham, J.C., C. Carmichael, and T. Miller. 1995. Social behavior of the tuatara, *Sphenodon punctatus*. Herpetological Monographs 9:5–16.

Gómez-Serrano, M.A., and P. López-López. 2017. Deceiving predators: Linking distraction behavior with nest survival in a ground-nesting bird. Behavioral Ecology 28:260–269.

Hasson, O., R. Hibbard, and G. Ceballos. 1989. The pursuit deterrent function of tail-wagging in the zebra-tailed lizard (*Callisaurus draconoides*). Canadian Journal of Zoology 67:1203–1209.

Jenssen, T.A. 1977. Evolution of anoline lizard display behavior. American Zoologist 17:203–215.

Jenssen, T.A. 1978. Display diversity in anoline lizards and problems of interpretation. Pp. 268–285 in Behavior and Neurology of Lizards: An Interdisciplinary Conference (N. Greenberg and P.D. Maclean, eds.). National Institutes of Mental Health, USA.

Jordan, M.A., and H.L. Snell. 2008. Historical fragmentation of islands and genetic drift in populations of Galápagos lava lizards (*Microlophus albemarlensis* complex). Molecular Ecology 17:1224–1237.

Kelso, E.C., and E.P. Martins. 2008. Effects of two courtship display components on female reproductive behaviour and physiology in the sagebrush lizard. Animal Behaviour 75:639–646.

Kizirian, D., A. Trager, M.A. Donnelly, and J.W. Wright. 2004. Evolution of Galápagos lava lizards (Iguania: Tropiduridae: *Microlophus*). Molecular Phylogenetics and Evolution 32:761–769.

Leal, M. 1999. Honest signalling during prey–predator interactions in the lizard *Anolis cristatellus*. Animal Behaviour 58:521–526.

Losos, J.B. 2009. Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles. University of California Press, USA.

Lowry, R. 2014. VassarStats: Website for Statistical Computation. Available at http://www.vassarstats.net. Archived by WebCite at http://www.webcitation.org/6Rc2xYZ9h on 5 August 2014.

Macedonia, J.M., and D.L. Clark. 2003. Headbob display structure in the naturalized *Anolis* lizards of Bermuda: Sex, context, and population effects. Journal of Herpetology 37:266–276.

Macedonia, J.M., D.L. Clark, R.G. Riley, and D.J. Kemp. 2013. Species recognition of color and motion signals in *Anolis grahami*: Evidence from responses to lizard robots. Behavioral Ecology 24:846–852.

Macedonia, J.M., D.L. Clark, Z.N. Brown, S. Gensterblum, L. McNabb, A.B. Myrberg, B.D. Myrberg, M.F. Petroche, and A. Karson. 2015. Responses of *Anolis grahami* males to manipulations of species identity and components of displays in lizard robots. Herpetologica 71:110–116.

Martins, E.P., A. Labra, M. Halloy, and J.T. Thompson. 2004. Large-scale patterns of signal evolution: An interspecific study of *Liolaemus* lizard headbob displays. Animal Behaviour 68:453–463.

Mcloughlin, M.P., R. Stewart, and A.G. McElligott. 2019. Automated bioacoustics: Methods in ecology and conservation and their potential for animal welfare monitoring. Journal of the Royal Society Interface 16:20190225. DOI: https://doi.org/10.1098/rsif.2019.0225

Mundry, R. 2015. pDFA User's Manual (informal). Available from the author at roger_mundry@eva.mpg.de.

Mundry, R., and C. Sommer. 2007. Discriminant function analysis with nonindependent data: Consequences and an alternative. Animal Behaviour 74:965–976.

Nava, S.S., L. Moreno, and D. Wang. 2012. Receiver sex differences in visual response to dynamic motion signals in *Sceloporus* lizards. Behavioral Ecology and Sociobiology 66:1357–1362.

Ord, T.J., and D.T. Blumstein. 2002. Size constraints and the evolution of display complexity: Why do large lizards have simple displays? Biological Journal of the Linnean Society 76:145–161.

Ord, T.J., and E.P. Martins. 2006. Tracing the origins of signal diversity in anole lizards: Phylogenetic approaches to inferring the evolution of complex behaviour. Animal Behaviour 71:1411–1429.

Orrell, K.S., and T.A. Jenssen. 2003. Heterosexual signalling by the lizard *Anolis carolinensis*, with intersexual comparisons across contexts. Behaviour 140:603–634.

Prum, R.O. 1994. Phylogenetic analysis of the evolution of alternative social behavior in the manakins (Aves: Pipridae). Evolution 48:1657–1675.
Pyron, R.A., F.T. Burbrink, and J.J. Wiens. 2013. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. BMC Evolutionary Biology 13:1–53.
R Core Team. 2016. R: A Language and Environment for Statistical Computing, Version 3.3.2. Available at https://cran.r-project.org/. R Foundation for Statistical Computing, Austria.
Rothblum, L., and T.A. Jenssen. 1978. Display repertoire analysis of *Sceloporous undulatus hyacinthinus* (Sauria: Iguanidae) from southwestern Virginia. Animal Behaviour 26:130–137.
Ruby, D. 1977. The function of shudder displays in the lizard *Sceloporus jarrovi*. Copeia 1977:110–114.
Stoddard, M.C., and D. Osorio. Animal coloration patterns: Linking spatial vision to quantitative analysis. American Naturalist 193:164–186.
Venables, W.N., and B.D. Ripley. 2002. Modern Applied Statistics with S, 4th ed. Springer, USA.
Vicente, N.S. 2018. Headbob displays signal sex, social context and species identity in a *Liolaemus* lizard. Amphibia-Reptilia 39:203–218.
Vliet, K.A. 1989. Social displays of the American alligator (*Alligator mississippiensis*). American Zoologist 29:1019–1031.

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