Comparison of Headbob Displays in Gray-Dewlapped and Red-Dewlapped Populations of Green Anoles (*Anolis carolinensis*)

Joseph M. Macedonia[1,2], David L. Clark[3], Lauren E. Cherry[1], Natasha E. Mohamed[1], and Bradley W. Bartel[1]

[1] Department of Biology, Florida Southern College, Lakeland, FL 33801, USA
[3] Department of Biology, Alma College, Alma, MI 48801, USA

ABSTRACT: Green Anoles (*Anolis carolinensis*) are comprised of red-dewlapped (RD) forms that are found throughout the southeastern USA and a gray-dewlapped (GD) form that is restricted to southwest Florida. Prior research has shown that RD *A. carolinensis* produce headbob displays of three distinct types that differ primarily in their temporal patterns. Based on known morphological, physiological, and genetic differences between GD and RD populations, we hypothesized that these populations also would differ in headbob display structure. To test this hypothesis we quantified 440 displays from 39 males (24 GD and 15 RD) and assigned displays to type using numerical decision criteria. Although comparison of the same display types between GD and RD males revealed differences in the durations of several homologous display units (i.e., bobs or interbob pauses), only one unit differed following statistical correction for multiple comparisons. By taking into account all display variation in both populations simultaneously, however, discriminant function analysis correctly assigned display units with high accuracy to population and display type. Nevertheless, differences in unit durations often were greater between two RD populations occurring within Florida than they were between our GD and RD study populations. Thus, despite our demonstration of differences in the display temporal structure between GD and RD forms of *A. carolinensis*, these differences appear to be no greater in magnitude than those observed between RD populations.

Key words: Contest behavior; Dewlap color; Population divergence

ANIMAL signals and displays evolve through the ritualization of behavior patterns where many striking examples involve the visual or acoustic sensory modalities (e.g., Bradbury and Vehrencamp 2011). A common type of visual signal in lizards is the push-up or headbob display, which comprises a series of stereotyped up–down movements used to attract mates and intimidate same-sexed rivals (e.g., Carpenter and Ferguson 1977; Jenssen 1977). These displays have been shown to exhibit substantial interspecific variation in iguanian genera such as *Microlophus* [= *Tropidurus*] (Carpenter 1966, 1977), *Sceloporus* (Carpenter 1978; Martins 1993), *Cyclura* (Martins and Lamont 1998), *Liolaemus* (Martins et al. 2004), and *Anolis* (Jenssen 1977, 1978). Whereas these displays typically exhibit species-specific form, distinctive structural and temporal variation have also been documented at the population level (Jenssen 1971, 1981; Martins et al. 1998; Lovern et al. 1999). In the genus *Anolis*, a second type of display involves the extension and retraction of the dewlap. Although anole dewlaps exhibit enormous species-specific variation in coloration (e.g., Nicholson et al. 2007), they sometimes also exhibit population-specific patterns (Ng and Glor 2011; Stapley et al. 2011; Glor and Laport 2012; Alfonso et al. 2013; Bienentreu et al. 2013).

Green Anoles (*Anolis carolinensis*) have become model organisms in studies of genomics (Alföldi et al. 2011), population genetics (Campbell-Staton et al. 2012; Tollis et al. 2012; Tollis and Boissinot 2014), neurobiology, endocrinology, and behavior (see Lovern et al. 2004 for a review). In addition, a number of studies have examined headbob behavior in this species, particularly those focused on display structure and usage (DeCourcy and Jenssen 1994; Jenssen et al. 2000, 2012; Orrell and Jenssen 2003) and display development (Lovern and Jenssen 2003). Variation occurs in dewlap coloration throughout the *A. carolinensis* distribution, but most populations exhibit dewlaps that are some shade of red (including pink and purplish-red or violet-red), which

we refer to collectively as red-dewlapped (RD). In contrast, members of a population restricted to southwest Florida exhibit greenish-gray dewlaps, which we refer to as gray-dewlapped (GD; Christman 1980; Vance 1991; Macedonia et al. 2003). The GD population differs from other *A. carolinensis* populations in several ways, including morphology and life history (Michaud and Echternacht 1995), physiology (Wade et al. 1983; Wilson and Echternacht 1987, 1990), and DNA sequences (Campbell-Staton et al. 2012; Tollis et al. 2012; Tollis and Boissinot 2014).

In a prior comparative study of headbob behavior, differences in male *A. carolinensis* display temporal structure were evaluated for three populations (Florida, Georgia, and Hawaii; Lovern et al. 1999). Although that study did not include subjects from the southwest Florida GD population, the authors remarked on the uniqueness of this population by asking "what might be the display structure and use by a small population of *A. carolinensis* in Florida whose communication signal includes a gray rather than pink dewlap [and] what is unique about the selective milieu of the gray-throated population, when all other populations across the southeastern United States are consistently pink-throated?" (Lovern et al. 1999:232). In the present study, we addressed this question by recording and analyzing headbob display structure in male GD *A. carolinensis* from southwest Florida. We compare our findings with RD males from central Florida, whose displays we also recorded and analyzed, as well as with data from a previously studied RD population in northeastern Florida (i.e., Lovern et al. 1999). In light of the documented distinctiveness of the GD form, we hypothesized that our GD study population would differ from RD populations of *A. carolinensis* in headbob display structure as well.

Materials and Methods

Study Subjects

We captured 40 GD adult male *A. carolinensis* from Punta Gorda, Florida and 24 RD adult males from Lakeland,

[2] Correspondence: e-mail, jmacedonia@flsouthern.edu

Florida. We initially sought to capture RD males as near to Punta Gorda as possible for comparing displays from the two dewlap color forms. In our search for RD males beyond the northern edge of the GD distribution in southwest Florida, however, we found only nonnative Brown Anoles (*Anolis sagrei*).

Housing and Video Recording

Following capture, each subject was housed individually in a 37.9-L glass terrarium (tank) for 5–7 d acclimation prior to video recording of staged male contests. Subjects were kept on a 14L:10D light cycle with a 100-W light bulb inside an aluminum reflector that was placed on the screened top of each tank as a light and heat source. The tanks housing lizards were misted thoroughly with water daily and subjects were fed calcium-dusted commercial crickets (*Acheta domestica*) every other day until satiated. Each tank also contained a perch made of roughened PVC pipe that ran the length of the tank and that was elevated approximately equidistant between the tank floor and screen top. Pairs of tanks were oriented with their long sides (and perches) parallel to one another. Cardboard was placed between tanks to prevent subjects from seeing each other prior to trials. A trial was initiated by removing the cardboard divider between adjacent tanks, thereby allowing subjects to visually communicate through the glass walls of their respective tanks. Interactions were video-recorded at ~2 m distance from subject tanks using two tripod-mounted digital camcorders (Sony Handycam DCR-SX63). Each camera was dedicated to a single tank and oriented at a right angle to that tank's long wall. Recordings were made at 30 frames/s (time resolution = 0.033 s) through the long sides of the tanks, which usually allowed both contestants to be seen in the trial footage. To maintain a minimum of disturbance to subjects, the room was darkened during trials except for the reflector bulbs that illuminated the interiors of the test subjects' tanks.

Data Acquisition and Measurements

Video recordings of trials were imported into Macintosh GraphClick (v.3, Arizona Software, Switzerland), where vertical (Y-axis) head motion was tracked frame-by-frame (vertical head motion comprises virtually all of the motion in *Anolis* headbob displays; Jenssen 1977). The Y-axis co-ordinate values were obtained by placing a cursor over the displaying subject's eye and clicking the mouse, which recorded the coordinate for that frame. This process was repeated for every frame in a display. The coordinate values for each display were exported to Microsoft Excel (v.14.4, Microsoft Corporation, Redmond, Washington) and plotted as a Display Action Pattern (DAP) graph, in which head elevation is represented on the Y-axis and elapsed time on the X-axis (Carpenter and Grubitz 1961). A screen capture was then made of each DAP graph and imported into Microsoft PowerPoint (v.14.4, Microsoft), where each display was positioned on its own page (slide). Vertical lines were overlaid onto each DAP image to delimit the beginning and end of each naturally occurring sequential unit (i.e., a headbob or an interbob pause). Following DeCourcy and Jenssen (1994), headbob units were assigned odd numbers and interbob pause units were assigned even numbers. Screen captures of these unit-demarcated DAP images were
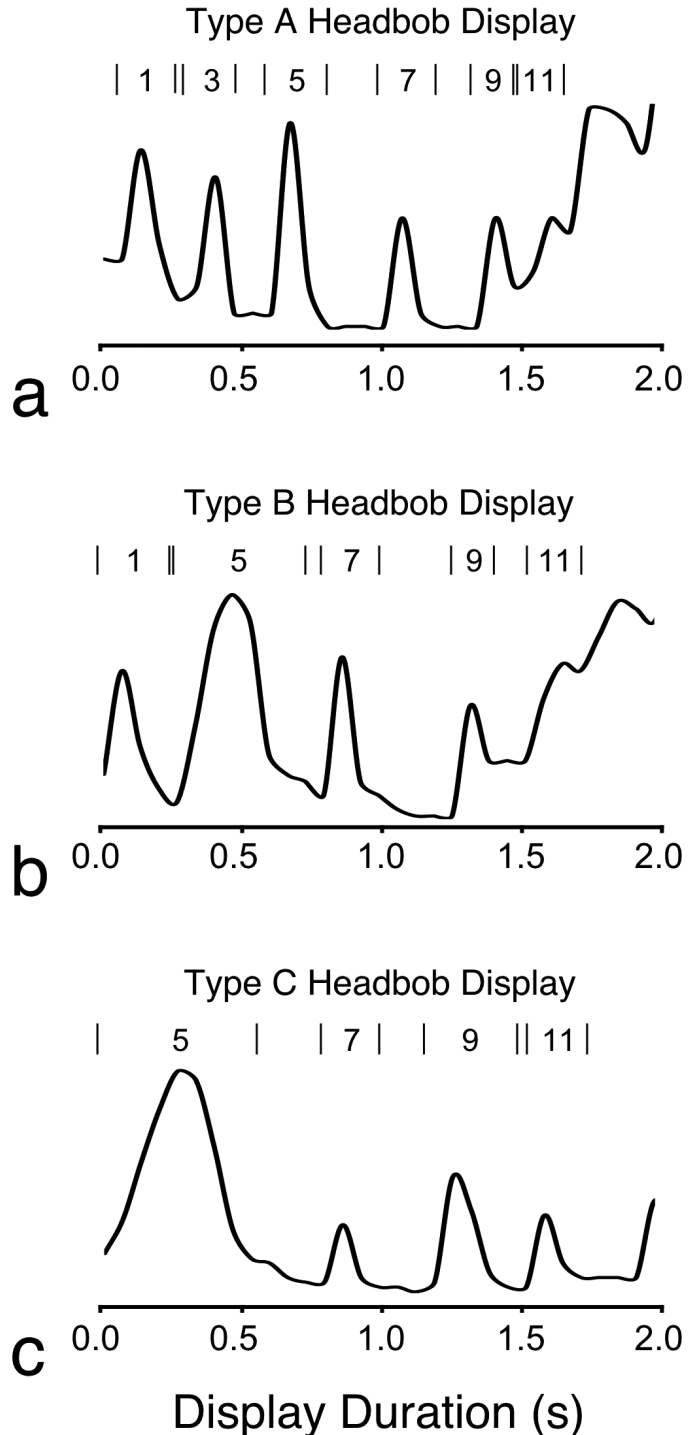

FIG. 1.—Examples of Type A, B, and C headbob displays from the gray-dewlapped population of *Anolis carolinensis*. The displays illustrated are from the same adult male. For clarity, only odd units (i.e., bobs) are numbered. See text for details.

taken and imported into ImageJ v1.43u (Rasband 2010). Here, the duration of each display unit was measured by first drawing a line between 0 s and 0.1 s on the X-axis of the DAP graph, then recording the distance in pixels covered by the line, and finally by opening the "set scale" window to enter the pixel distance, the known distance (0.1), and the unit of length (seconds). This series of actions calibrated the scale for measuring the duration of each display unit.

TABLE 1.—Descriptive statistics and results of Mann–Whitney *U*-tests on mean unit durations for Type A, B, and C displays from gray-dewlapped and red-dewlapped populations of *Anolis carolinensis* from Punta Gorda and Lakeland, Florida, respectively. The *P* values shown are not corrected for multiple tests (see text). We follow DeCourcy and Jenssen (1994) in assignment of display unit numbers. Intra-individual unit means are reported. Sample sizes (*n*) are numbers of subjects (GD,RD) performing at least one display of a given display type.

Display (*n*)	Unit	Gray-dewlapped $\bar{X} \pm$ SE (s)	Red-dewlapped $\bar{X} \pm$ SE (s)	*U*	*P*
Type A (24,15)	U1	0.27 ± 0.01	0.23 ± 0.02	114.0	0.06
	U2	0.14 ± 0.02	0.18 ± 0.03	221.5	0.24
	U3	0.20 ± 0.01	0.16 ± 0.01	84.5	0.01
	U4	0.16 ± 0.01	0.11 ± 0.01	153.0	0.85
	U5	0.19 ± 0.01	0.18 ± 0.01	90.5	0.06
	U6	0.13 ± 0.01	0.08 ± 0.01	91.0	0.01
	U7	0.16 ± 0.01	0.15 ± 0.01	158.0	0.54
	U8	0.10 ± 0.01	0.15 ± 0.02	252.0	0.04
	U9	0.14 ± 0.01	0.14 ± 0.01	190.0	0.79
	U10	0.05 ± 0.01	0.11 ± 0.01	318.0	0.001
	U11	0.17 ± 0.01	0.16 ± 0.01	117.0	0.14
	$\sum$U1–11	1.71 ± 0.03	1.70 ± 0.06	137.0	0.40
Type B (20,4)*	U1	0.19 ± 0.01	0.11 ± 0.01		
	U2	0.14 ± 0.01	0.20 ± 0.04		
	U5	0.31 ± 0.01	0.20 ± 0.03		
	U6	0.09 ± 0.01	0.07 ± 0.03		
	U7	0.17 ± 0.01	0.17 ± 0.02		
	U8	0.22 ± 0.01	0.12 ± 0.05		
	U9	0.15 ± 0.01	0.15 ± 0.02		
	U10	0.08 ± 0.01	0.16 ± 0.02		
	U11	0.17 ± 0.01	0.14 ± 0.03		
	$\sum$U1–11	1.52 ± 0.00	1.32 ± 0.00		
Type C (21,14)	U5	0.40 ± 0.01	0.37 ± 0.01	114.0	0.28
	U6	0.20 ± 0.01	0.23 ± 0.02	190.0	0.15
	U7	0.15 ± 0.01	0.14 ± 0.01	121.0	0.39
	U8	0.16 ± 0.01	0.17 ± 0.02	166.5	0.52
	U9	0.20 ± 0.01	0.17 ± 0.01	90.5	0.06
	U10	0.09 ± 0.01	0.10 ± 0.02	157.5	0.73
	U11	0.15 ± 0.01	0.13 ± 0.01	79.5	0.02
	$\sum$U5–11	1.35 ± 0.00	1.31 ± 0.00	129.5	0.57

* Sample sizes of Type B unit means for RD males were too small for statistical comparisons.

Following DeCourcy and Jenssen (1994), displays from our subjects were categorized as Type A, B, or C. The most-conspicuous difference among display types lies in the relative duration of headbobs at the beginning of the displays, where Type A displays contain three bobs of comparatively short duration, Type B displays contain one bob of short duration followed by one bob of long duration, and Type C displays contain a single bob of very-long duration (Fig. 1; DeCourcy and Jenssen 1994). Variation in the typical number of headbobs performed in each display type indicates a fusion (or splitting) of display units in the initial portions of the displays, which is reflected in the display unit-numbering scheme for this species (Fig. 1; see DeCourcy and Jenssen 1994; Lovern et al. 1999).

To classify displays, we created a decision criterion derived from previously published descriptive statistics (DeCourcy and Jenssen 1994; Lovern et al. 1999; Jenssen et al. 2000; Orrell and Jenssen 2003). We based our criterion on the quotient of the duration of the first bob in the display divided by the duration of the second bob. This heuristic method provided a simple and objective classification scheme that was consistent with visual sorting of displays, where a quotient of 0.8–2.0 = Type A display, a quotient <0.8 = Type B display, and a quotient >2.0 = Type C display. Although many displays in our sample could be readily classified to type by visual comparison of the first two bobs' durations, our quantitative criterion was useful in categorizing displays whose quotients fell close to the boundaries separating Type A from Type B displays and Type A from Type C displays.

Display Analyses

Following Orrell and Jenssen (2003), we calculated durations of the first 11 units in our subjects' Type A headbob displays, the first nine units in Type B displays, and the first seven units in Type C displays. From the 40 GD males and 24 RD males who experienced experimental contests, 24 GD and 15 RD males provided at least one display for analysis, for a total of 440 displays scored (displays per subject: $\bar{X} \pm$ SE = 4.5 ± 0.3). To avoid biasing our analyses in favor of individuals who performed more displays than did other individuals, we used unit means for each subject in our analyses. We analyzed headbob displays for stereotypy using descriptive statistics, including the coefficient of variation (CV; SD/$\bar{X} \times$ 100). We followed the convention that display units with a CV < 35% can be considered highly stereotyped (Barlow 1977). Headbob unit durations were compared between homologous units of the same display types for GD and RD populations using Mann–Whitney *U*-tests in VassarStats (Lowry 2014). As multiple display units were compared from the same displays, we applied sequential Bonferroni corrections to *P* values, thereby reducing the probability of Type 1 error (Gaetano 2013). Proportions of variance attributable to population, among subjects within population, and within subjects were calculated using nested analyses of variance

TABLE 2.—Mean display unit coefficients of variation (CVs) and overall mean CVs for Type A, B, and C displays from gray-dewlapped and red-dewlapped populations of *Anolis carolinensis* in Florida. We follow the convention of DeCourcy and Jenssen (1994) in assignment of display unit numbers. Unit CVs are grand means calculated from intra-individual means. Sample sizes (*n*) are numbers of subjects (GD,RD) performing at least one display of a given display type.

Display (*n*)	Unit	Gray-dewlapped CV (%)	Red-dewlapped CV (%)	*U*	*P*
Type A (24,15)	U1	24.9	32.1		
	U2	56.3	59.1		
	U3	25.4	22.5		
	U4	41.5	34.2		
	U5	15.9	20.2		
	U6	47.0	39.5		
	U7	19.9	15.0		
	U8	50.3	38.5		
	U9	18.7	15.6		
	U10	52.6	47.0		
	U11	21.1	32.2		
	$\sum$ U1–U11	34.0	32.4	128	0.35
Type B (20,4)*	U1	19.2	21.2		
	U2	44.4	37.5		
	U5	16.1	32.6		
	U6	58.2	82.3		
	U7	16.8	17.5		
	U8	27.0	79.4		
	U9	19.6	20.7		
	U10	48.6	19.3		
	U11	19.4	42.0		
	$\sum$ U1–U11	29.9	39.2		
Type C (21,14)	U5	16.0	12.9		
	U6	30.5	29.1		
	U7	20.2	17.7		
	U8	23.9	35.6		
	U9	22.5	10.7		
	U10	57.5	67.7		
	U11	22.7	16.8		
	$\sum$ U5–U11	27.6	27.2	121	0.52

* Sample sizes for Type B displays of RD males were too small to allow statistical comparison.

(ANOVA; McDonald 2009). Following earlier studies (Lovern et al. 1999; Macedonia and Clark 2003; Orrell and Jenssen 2003), we do not report *F*-test statistics and *P* values for the nested ANOVAS on account of the unbalanced nature of our data.

We used discriminant function analysis (DFA) to differentiate among display types from our study populations (Jenssen et al. 2000; Macedonia and Clark 2001). The DFA created linearly weighted combinations of our original continuous variables (i.e., means of subjects' display unit durations) which predicted membership to one of our categorical variables (i.e., population and display type). As DFA can be affected by multicollinearity and nonnormal variable distributions, we ran a second DFA using factors from a principal components analysis (PCA) as input variables to our DFA. The PCA generated linearly weighted combinations of our original variables such that each component was uncorrelated with any other component. Unlike DFA, PCA does not take into account group membership in creating functions (i.e., components were constructed without regard for population or display type). We rotated the components (varimax) from the initial solution to maximize their interpretability with respect to our original variables (display unit duration means), and components that exceeded Jolliffe's criterion (i.e.,
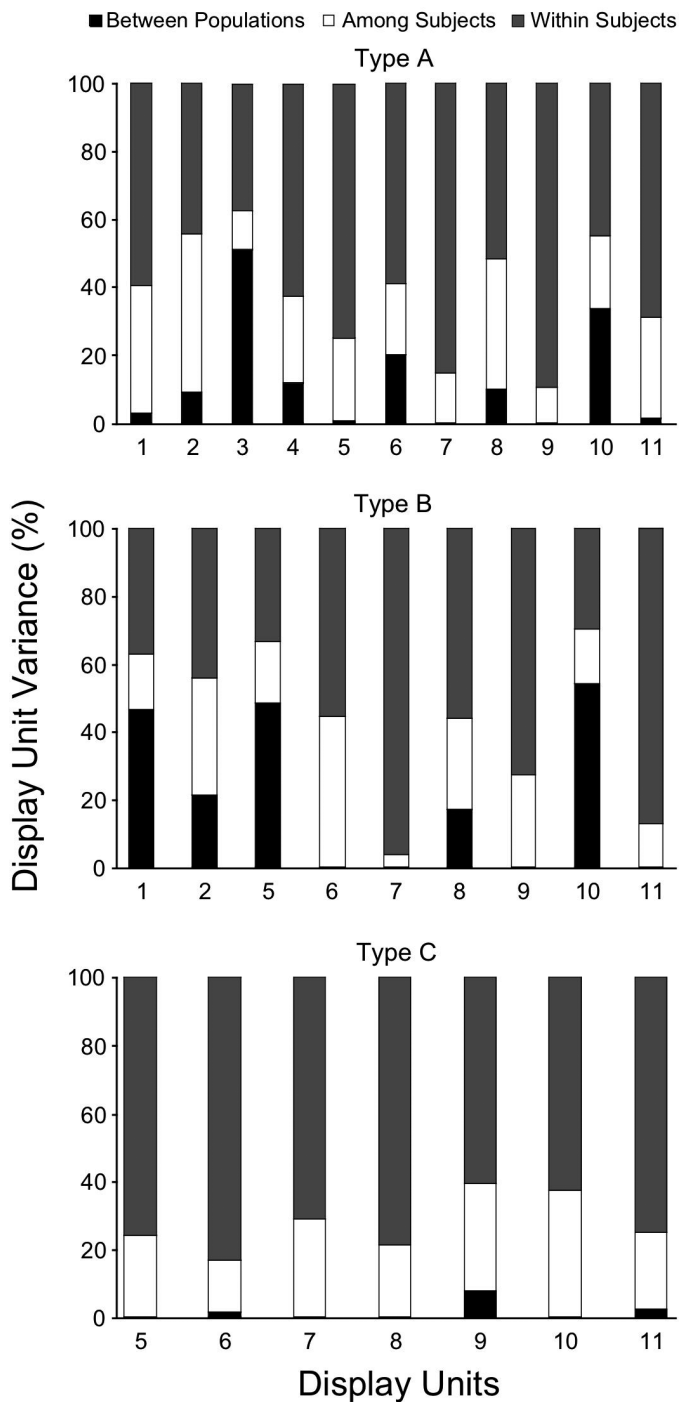

FIG. 2.—Display unit variance as determined by nested analyses of variance on Type A, B, and C displays. Bar charts show the hierarchical distribution of variation in unit durations attributable to population (gray-dewlapped and red-dewlapped), among subjects, and within subjects.

eigenvalues >0.7; Jolliffe 1986) were entered into a DFA. We conducted DFA and PCA in SPSS (v21.0, IBM Inc., Armonk, NY), and binomial probabilities of correct assignments to population and display type in the DFAs were computed in VassarStats (Lowry 2014).

We compared our data for headbob display unit durations with those previously published for an RD population in Palatka, Florida (Lovern et al. 1999). Although we could not test for specific differences between the two populations, as

TABLE 3.—Group membership classification assignments of five discriminant functions derived (A) directly from subject display unit means and (B) from six principal components generated from subject display unit means. GD = gray-dewlapped subjects, RD = red-dewlapped subjects; see text for descriptions of display types.

Display	GD Type A	GD Type B	GD Type C	RD Type A	RD Type B	RD Type C
A.						
Subjects (n)	24	20	21	15	4	14
No. correct	21	19	16	12	0	8
% correct	87.5%	95.0%	76.2%	80.0%	0.0%	57.1%
Exact P	1.3×10^{-10}	1.0×10^{-12}	1.2×10^{-7}	4.6×10^{-8}	0.85	2.0×10^{-4}
B.						
Subjects (n)	24	20	21	15	4	14
No. correct	18	19	16	10	0	8
% correct	75.0%	95.0%	76.2%	66.7%	0.0%	57.1%
Exact P	2.5×10^{-7}	1.0×10^{-12}	1.2×10^{-7}	9.0×10^{-6}	0.85	2.0×10^{-4}

only descriptive statistics were available from the literature, a visual comparison allowed us to assess whether the temporal display structure of our local RD population of *A. carolinensis* males was unusual in any way that might skew the comparison to our GD population. All response variables are reported as $\bar{X} \pm 1$ SE.

RESULTS

Display Temporal Structure and Stereotypy

Mann–Whitney *U*-tests of headbob display unit durations revealed several differences between our GD and RD populations (Table 1). Following sequential Bonferroni correction for *P* values, however, only one display unit differed between the two populations (Type A display, Unit 10, adjusted $P = 0.012$). Regarding stereotypy, the temporal structure of headbobs (odd units), but not interbob pauses (even units), was highly stereotyped for both populations (Table 2). Whereas odd unit CVs were <35% in every case (GD males, 19.89 ± 0.78; RD males, 21.98 ± 2.28), those of even units were much more labile (GD males, 44.82 ± 3.43; RD males, 47.43 ± 5.82).

Sources of Display Unit Variance

A nested ANOVA conducted on each of the three display types revealed the relative contributions in display structure of variation between populations, among subjects within populations, and within subjects (Fig. 2). In Type A displays, within-subject variance explained most of the variation in display structure ($61.7 \pm 4.9\%$) and was followed by among-subject variation within populations ($25.5 \pm 3.5\%$). Variance attributable to population differences in Type A display unit durations contributed relatively little overall ($12.8 \pm 4.9\%$), except for Units 3 and 10, where it explained a large amount of variation (51.0% and 33.5%, respectively). Similarly, within-subject variance in unit durations accounted for most of the variation in Type B displays ($50.9 \pm 8.0\%$), except for Units 1, 5, and 10, where population differences accounted for the majority of the variance (46.5–54.0%). In Type C displays, within-subject variation again accounted for the majority of variance in unit durations ($74.4 \pm 3.1\%$). Variance attributable to population differences in Type C display unit durations was low ($1.7 \pm 1.1\%$), and among-subject variation explained much of the variance not accounted for by within-subject variation ($25.9 \pm 2.8\%$).

Differentiation of Display Types from GD and RD Populations

A DFA returned five discriminant functions, where the first function accounted for 87.7% of total model variance and exhibited large function coefficients only for durations of Units 1 and 3. Cross-validation classification successfully predicted group membership (i.e., whether a display unit mean came from a GD or RD male Type A, B, and C display) in 76 of the 98 cases (77.6%), which is far greater than random chance (binomial test of overall classification success: exact $P = 1.0 \times 10^{-12}$). Correct group assignment was high for all cases except for RD Type B displays, where only four subjects provided display unit means and none of those four cases was correctly classified (Table 3A). In our second analysis, PCA returned six rotated components with eigenvalues greater than 0.7, where the first component (20.0% of the variance) was most heavily weighted (i.e., loadings >0.5 or <−0.5) on display Units 3, 6, 8, and 11, and the second component (18.1% of the variance) was weighted most heavily on display Units 1, 6, and 10. The six rotated components were entered into a DFA that produced five discriminant functions in which the first function accounted for 82.5% of total model variance. Cross-validation classification successfully predicted group membership in 71 of the 98 cases (72.4%), which is greater than chance alone (binomial test of overall classification success: exact $P = 1.0 \times 10^{-12}$). Correct group assignment was again high and was similar to the DFA conducted on the original variables (Table 3B).

Comparison of Two RD Populations

A visual examination across display types and populations indicated that, in most of the 24 display units compared, all three populations were similar in having unit durations well within 0.1 s of each other (Fig. 3). On the other hand, the northeastern RD population exhibited a number of substantially different unit durations from our Lakeland RD population, where unit values did not overlap. In many cases, unit durations were more similar between our GD and RD study populations than between the two RD populations (Fig. 3).

DISCUSSION

In this study, we have addressed the issue of display structure posed by Lovern et al. (1999), although a demon-
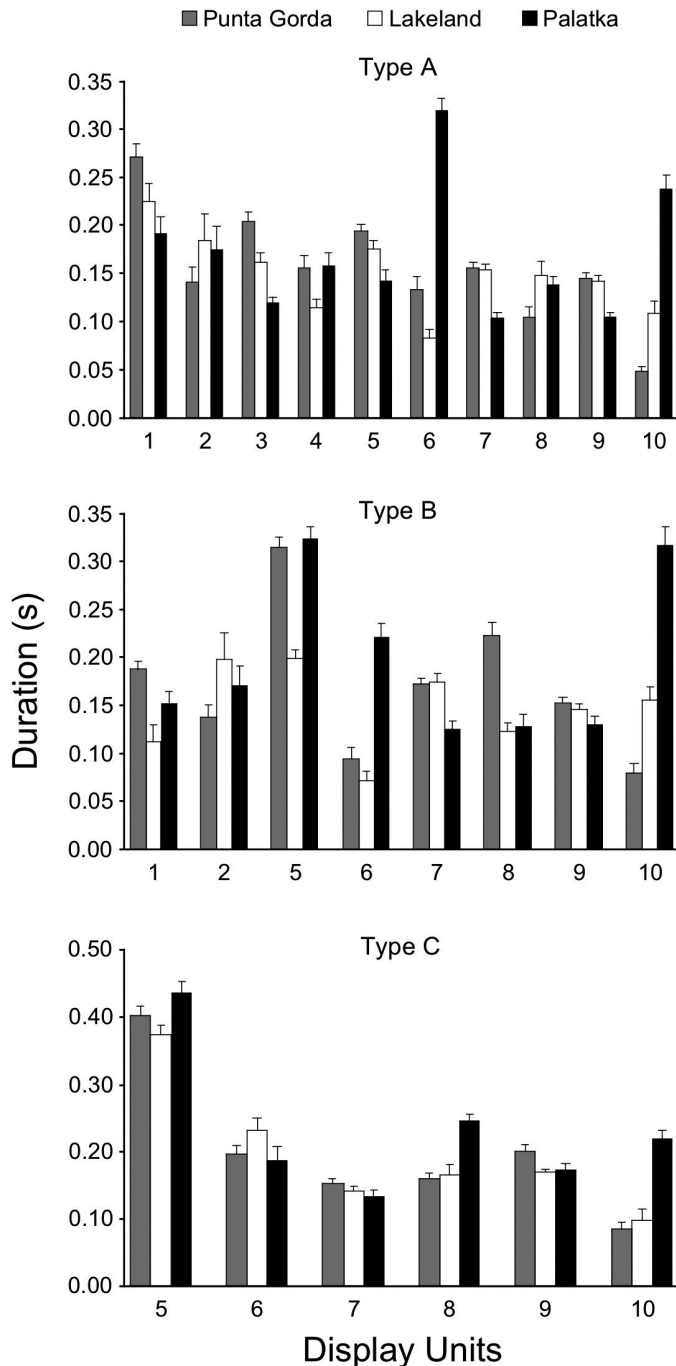
Fig. 3.—Comparison of unit durations in Type A, B, and C display types from our gray-dewlapped (GD; Punta Gorda, Florida) and red-dewlapped (RD; Lakeland, Florida) study populations with an RD population in Palatka, Florida. Data for the Palatka population are adapted from Lovern et al. (1999). Bars depict means + 1 SE.

stration of a unique selective milieu remains elusive. For example, previous work on dewlap coloration and habitat light from populations of *A. carolinensis* throughout Florida failed to uncover evidence that a gray dewlap would have a visibility advantage over a red dewlap (Macedonia et al. 2003). Nevertheless, based on unique genetic, physiological, and morphological variations, we hypothesized that headbob display temporal structure would be distinctive in the GD population. Test results for durations of individual units

revealed, however, that the duration of only a single headbob unit from one display type differed between our GD and RD populations. On its own, this result indicates that, despite the fact that the GD *A. carolinensis* population exhibits a number of unique attributes, headbob display structure is not one of them. When considering the mean durations of subject display units from all display types and both populations within the same analysis, however, most subjects' displays could be accurately assigned to the correct population and display type (Table 3). The only exception was RD Type B displays, which fared poorly in the cross-validation analyses, likely on account of the small sample size ($n = 4$). Therefore, our findings do indicate that, on the whole, GD headbob displays can be distinguished from RD headbob displays.

To ensure that the RD population we used for comparison was not unusual in headbob unit durations, we compared our results for this population (Lakeland, Florida) to those of another Florida RD population (Palatka, Florida; Lovern et al. 1999). Counter to expectation, our GD population and both RD populations exhibited similar durations for most—though not all—headbob display units across display types. When exceptions were observed, the RD population in northeast Florida, not our GD population, was most different. This distinctiveness was particularly evident in the long durations of certain interbob pauses (Units 6 and 10 of Type A and Type B displays; Fig. 3), a feature also noted by Lovern et al. (1999).

Although GD male *A. carolinensis* appear to have retained a headbob display structure that is seen in other populations, other characteristics of this population have led some researchers to suggest that it may warrant subspecies recognition (Christman 1980; Vance 1991). For example, an investigation of *A. carolinensis* allozyme diversity found that the GD population in Naples, Florida, possessed three unique alleles at eight polymorphic loci, making it the most distinctive among seven populations sampled between southern Florida and central Texas (Wade et al. 1983). These authors speculated that the GD population may have diverged from other populations when isolated during Pleistocene glacial minima or that the GD population may have arisen from an independent colonization event from Cuba (discussed in Macedonia et al. 2003).

In several recent phylogeographic studies of *A. carolinensis* in Florida, a southern Florida–Everglades clade was recovered that contains GD as well as RD populations (Campbell-Staton et al. 2012). In fact, Tollis and Boissinot (2014) showed that, whereas mtDNA sequences nest the GD population within a more-inclusive Everglades clade that also contains RD populations, nuclear DNA fails to resolve one southern Florida population from another. Molecular clock estimates indicate that inundation of peninsular Florida during peaks of Pleistocene sea-level rise ($\approx$2.15 million years ago) isolated the Everglades clade from other clades (Tollis and Boissinot 2014). Given that the GD population is part of a clade that contains RD populations, and that the clade exhibits multiple mtDNA lineages, it seems reasonable that population bottlenecks, caused by repeated fragmentation of peninsular Florida into islands, might have been relatively common and that the GD population originated in this insular environment.

In sum, despite the conserved nature of headbob display structure in *A. carolinensis*, the morphological, physiological,

and life history traits that are unique to the GD population will continue to invite further investigation. Among the unresolved questions is: How is the integrity of the GD population maintained? Even though a broad hybrid zone with RD populations occurs along much of the GD distribution, most males within the GD distribution exhibited gray dewlaps (fig. 1 in Christman 1980).

Might assortative mating through female mate choice play a role in maintaining the GD color morph? This question has been addressed in other *Anolis* species. For example, Jenssen (1996) tested the possibility of assortative mating between two parapatric sibling species in Haiti, *Anolis websteri* and *Anolis caudalis*, which exhibit distinguishable headbob displays (Jenssen and Gladson 1984). Despite dewlap color patterns that differ most profoundly in areas of secondary contact (i.e., reproductive character displacement of dewlap coloration; e.g., Lambert et al. 2013), *A. websteri* females failed to discriminate conspecific males from *A. caudalis* males (Jenssen 1996). Furthermore, the dichotomous choice experiment of MacDonald and Echternacht (1991) indicated that females from GD and RD populations did not preferentially associate with their respective males. Thus, if reproductive reinforcement is responsible for maintaining the integrity of the GD distribution, the factors that guide female mate choice remain unclear and await further investigation.

LITERATURE CITED

Alföldi, J., F. Di Palma, M. Grabherr, C. Williams, L. Kong, E. Mauceli, P. Russell, C.B. Lowe, R.E. Glor, and J.D. Jaffe, et al. 2011. The genome of the green anole lizard and a comparative analysis with birds and mammals. Nature 477:587–591.

Alfonso, Y.U., H.J. Morris, A. Gutiérrez, L. Rodríguez-Schettino, D. Denis, and J.E. Steffen. 2013. Dewlap color variation based on pterin and carotenoid pigments in three subspecies of *Anolis jubar* of the Cuban southern coast. Copeia 2013:201–205.

Barlow, G.W. 1977. Modal action patterns. Pp. 98–134 in How Animals Communicate (T.A. Sebeok, ed.). Indiana University Press, USA.

Bienentreu, J.F., A. Hertz, G. Kohler, and S. Lotzkat. 2013. Distribution extension for *Anolis salvini* Boulenger, 1885 (Reptilia: Squamata: Dactyloida) in western Panama. Check List 9:169–174.

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

Campbell-Staton, S.C., R.M. Goodman, N. Backström, S.V. Edwards, J.B. Losos, and J.J. Kolbe. 2012. Out of Florida: mtDNA reveals patterns of migration and Pleistocene range expansion of the Green Anole lizard (*Anolis carolinensis*). Ecology and Evolution 2:2274–2284.

Carpenter, C.C. 1966. Comparative behavior of the Galápagos lava lizards (*Tropidurus*). Pp. 269–273 (Paper 35) in The Galápagos: Proceedings of the Galápagos International Scientific Project (R.I. Bowman, ed.). University of California Press, USA.

Carpenter, C.C. 1977. The aggressive displays of three species of South American iguanid lizards of the genus *Tropidurus*. Herpetologica 33:285–289.

Carpenter, C.C. 1978. Comparative display behavior in the genus *Sceloporus* (Iguanidae). Contributions in Biology and Geology to the Milwaukee Public Museum 18:1–71.

Carpenter, C.C., and G.W. Ferguson. 1977. Variation and evolution of stereotyped behavior in reptiles. Pp. 298–422 in Biology of the Reptilia, vol. 18, Physiology E (C. Gans and D. Crews, eds.). University of Chicago Press, USA.

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

Christman, S.P. 1980. Preliminary observations on the gray-throated form of *Anolis carolinensis* (Reptilia: Iguanidae). Florida Field Naturalist 8:11–16.

DeCourcy, K.R., and T.A. Jenssen. 1994. Structure and use of male territorial headbob signals by the lizard *Anolis carolinensis*. Animal Behaviour 47:251–262.

Gaetano, J. 2013. Holm-Bonferroni sequential correction: An Excel calculator, Version 1.2. Available at http://www.researchgate.net/242331583_Holm-Bonferroni_Sequential_Correction_An_EXCEL_Calculator. Archived by WebCite at http://www.webcitation.org/6RgJhQr3J on 8 August 2014.

Glor, R.E., and R. Laport. 2012. Are subspecies of *Anolis* lizards that differ in dewlap color and pattern also genetically distinct? A mitochondrial analysis. Molecular Phylogenetics and Evolution 64:255–260.

Jenssen, T.A. 1971. Display analysis of *Anolis nebulosus* (Sauria, Iguanidae). Copeia 1971:197–209.

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. 269–285 in Behavior and Neurology of Lizards: An Interdisciplinary Colloquium (N. Greenberg and P.D. MacLean, Eds.). National Institutes of Mental Health, USA.

Jenssen, T.A. 1981. Unusual display behavior by *Anolis grahami* from western Jamaica. Copeia 1981:728–733.

Jenssen, T.A. 1996. A test of assortative mating between sibling lizard species, *Anolis websteri* and *A. caudalis*, in Haiti. Pp. 303–315 in Herpetology of the West Indies: A Symposium in Honor of Albert Schwartz (R. Powell and R.W. Henderson, Eds.). SSAR Contributions to Herpetology Series, vol. 12. Society for the Study of Amphibians and Reptiles, USA.

Jenssen, T.A., and N.L. Gladson. 1984. A comparative display analysis of the *Anolis brevirostris* complex in Haiti. Journal of Herpetology 18:217–230.

Jenssen, T.A., K.S. Orrell, and M.B. Lovern. 2000. Sexual dimorphisms in aggressive signal structure and use by a polygynous lizard, *Anolis carolinensis*. Copeia 2000:140–149.

Jenssen, T.A., S. Garrett, and W.J. Sydor. 2012. Complex signal usage by advertising male green anoles (*Anolis carolinensis*): A test of assumptions. Herpetologica 68:345–357.

Jolliffe, I. 1986. Principle Components Analysis. Springer-Verlag, USA.

Lambert, S.M., A.J. Geneva, D.L. Mahler, and R.E. Glor. 2013. Using genomic data to revisit an early example of reproductive character displacement in Haitian *Anolis* lizards. Molecular Ecology 22:3981–3995.

Lovern, M.B., and T.A. Jenssen. 2003. Form emergence and fixation of head bobbing displays in the green anole lizard (*Anolis carolinensis*): A reptilian model of signal ontogeny. Journal of Comparative Psychology 117:133–141.

Lovern, M.B., T.A. Jenssen, K.S. Orrell, and T. Tuchak. 1999. Comparisons of temporal display structure across contexts and populations in male *Anolis carolinensis*: Signal stability or lability? Herpetologica 55:222–234.

Lovern, M.B., M.M. Holmes, and J. Wade. 2004. The green anole (*Anolis carolinensis*): A reptilian model for laboratory studies of reproductive morphology and behavior. ILAR Journal 1:54–64.

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

MacDonald, D.M., and A.C. Echternacht. 1991. Red-throated and gray-throated *Anolis carolinensis*: Do females know the difference? *Anolis* Newsletter IV, Pp. 92–103. Archived by WebCite at http://www.webcitation.org/6S3KH5qAf on 23 August 2014.

Macedonia, J.M., and D.L. Clark. 2001. Headbob display analysis of the Grand Cayman anole, *Anolis conspersus*. Journal of Herpetology 35:300–310.

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., A.C. Echternacht, and J.W. Walguarnery. 2003. Color variation, habitat light, and background contrast in *Anolis carolinensis* along a geographical transect in Florida. Journal of Herpetology 37:467–478.

Martins, E.P. 1993. A comparative study of the evolution of *Sceloporus* push-up displays. American Naturalist 142:994–1018.

Martins, E.P., and J. Lamont. 1998. Evolution of communication and social behaviour: A comparative study of *Cyclura* rock iguanas. Animal Behaviour 55:1685–1706.

Martins, E.P., A.N. Bissell, and K.K. Morgan. 1998. Population differences in a lizard communicative display: Evidence for rapid change in structure and function. Animal Behaviour 56:1113–1119.

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.

McDonald, J.H. 2009. Handbook of Biological Statistics. Available at http://udel.edu/~mcdonald/statintro.html. Archived by WebCite at http://www.webcitation.org/6RgCVpl4x on 8 August 2014.

Michaud, E.J., and A.C. Echternacht. 1995. Geographic variation in the life history of the lizard *Anolis carolinensis* and support for the pelvic constraint model. Journal of Herpetology 29:86–97.

Ng, J., and R.E. Glor. 2011. Genetic differentiation among populations of a Hispaniolan trunk anole that exhibit geographical variation in dewlap colour. Molecular Ecology 20:4302–4317.

Nicholson, K.E., L.J. Harmon, and J.B. Losos. 2007. Evolution of *Anolis* lizard dewlap diversity. PLOS ONE 2:e274.

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

Rasband, W.S. 2010. ImageJ, v1.43u. U.S. National Institutes of Health, USA. Available at http://imagej.nih.gov/ij/.

Stapley, J., C. Wordley, and J. Slate. 2011. No evidence of genetic differentiation between anoles with different dewlap color patterns. Journal of Heredity 102:118–124.

Tollis, M., and S. Boissinot. 2014. Genetic variation in the green anole lizard (*Anolis carolinensis*) reveals island refugia and a fragmented Florida during the quaternary. Genetica 142:59–72.

Tollis, M., G. Ausubel, D. Ghimire, and S. Boissinot. 2012. Multi-locus phylogeographic and population genetic analysis of *Anolis carolinensis*: Historical demography of a genomic model species. PLOS ONE 7:e38474.

Vance, T. 1991. Morphological variation and systematics of the green anole, *Anolis carolinensis* (Reptilia: Iguanidae). Bulletin of the Maryland Herpetological Society 27:43–89.

Wade, J.K., A.C. Echternacht, and G.F. McCracken. 1983. Genetic variation and similarity in *Anolis carolinensis* (Sauria: Iguanidae). Copeia 1983:523–529.

Wilson, M.A., and A.C. Echternacht. 1987. Geographic variation in the critical thermal minimum of the green anole, *Anolis carolinensis* (Sauria, Iguanidae), along a latitudinal gradient. Comparative Biochemistry and Physiology Part A: Physiology 87:757–760.

Wilson, M.A., and A.C. Echternacht. 1990. A comparison of heat and cold tolerance of two morphs of *Anolis carolinensis* (Iguanidae) from southern Florida. Journal of Herpetology 24:330–333.