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Species Recognition in *Anolis grahami* (Sauria, Iguanidae): Evidence from Responses to Video Playbacks of Conspecific and Heterospecific Displays

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Abstract

The visual displays of male anoles (*Anolis* sp.) are usually viewed as characters that facilitate species recognition. This presumption stems largely from the extensive variation in the head bobbing movements and dewlap colouration in males of this genus. However, few experimental data exist that illuminate the importance of these visual signals for species recognition. We presented adult male *Anolis grahami* with colour-videotaped sequences of displays from conspecifics and from three heterospecific species of anoles. The congenerics (*A. carolinensis*, *A. conspersus*, and *A. sagrei*) were chosen on the basis of their phylogenetic and geographical relationships with *A. grahami*. Stimulus males were filmed at a constant distance and orientation from the video camera, and edited sequences of their displays were presented at a standard distance from subjects. Video playbacks allowed us to equalize the quantity of display performed by each stimulus species, and to eliminate stimulus/subject interactions as well as inter-trial variation in stimulus behaviour. Results revealed that the subjects responded more strongly to conspecific than to heterospecific displays on all behavioural variables examined, but there was no indication of discrimination among the displays of the three congeneric species.

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Introduction

Comprising over 250 species, lizards of the iguanid genus *Anolis* have undergone an extraordinary adaptive radiation throughout tropical and subtropical America, and the Caribbean Islands. One explanation for this diversity is that the head-bobbing and dewlap-pulsing displays of adult males serve as reproductive

isolating mechanisms by providing species-identification cues important for mate choice (RAND & WILLIAMS 1970; CREWS & WILLIAMS 1977; WILLIAMS & RAND 1977). The head-bobbing movements of male anoles are species-specific (JENSSEN 1977, 1978) and have been found to differ at both the subspecies and population level (JENSSEN 1981). Colour patterns found on the dewlap, an extendable throat fan, are similarly diverse (RAND & WILLIAMS 1970; WILLIAMS & RAND 1977; FLEISHMAN 1992; FLEISHMAN et al. 1993). Taken together, these visual displays of motion and colour function to deter rival male conspecifics and to attract female conspecifics (JENSSEN 1977).

For several reasons, discrimination of conspecifics from heterospecifics should be at a premium for many sympatric *Anolis* species. Given that interspecific resource competition is expected to be less intense than intraspecific competition (SCHOENER 1968), heterospecifics will rarely impose the same level of threat for resources as will conspecifics. Contests over resources take time and energy, and species should discriminate those taxa that represent a threat from those that do not. Although a simple recognition cue (e.g. body size) might suffice on the smaller 'two-species' Caribbean islands (GORMAN 1968; LAZELL 1972; LOSOS 1990, 1992), the larger islands contain sympatric assemblages of nearly a dozen *Anolis* species (WILLIAMS & RAND 1977; WILLIAMS 1983). Such high species diversity might require more elaborate species recognition cues, such as those provided by interspecific variation in head-bobbing pattern and dewlap colouration.

The ability to discriminate conspecifics from heterospecifics might also decrease predation risk by reducing the number of displays given to inappropriate audiences. Like the acoustic displays of male frogs that attract both mates and frog-eating bats (RYAN et al. 1981; TUTTLE & RYAN 1981; RYAN & RAND 1982), the conspicuous visual displays of male anoles may attract predators (FLEISHMAN 1985; MARLER & MOORE 1988; ENDLER 1992). Many *Anolis* species are preyed upon by kestrels (JOHNSTON 1975; CRUZ 1976), non-raptorial birds (e.g. cuckoos: CRUZ 1973, 1975), snakes (GRANT 1940; FLEISHMAN 1985, 1986, 1988a), and even by other anoles (RAND & ANDREWS 1975; STAMPS 1983; FITCH & HENDERSON 1987). Thus, for male anoles to reduce predation risk they should typically restrict their displays to conspecific audiences (but see ORTIZ & JENSSEN 1982).

In addition, infertile matings resulting from breeding with genetically-incompatible congenerics could compromise lifetime reproductive success, particularly for females. Cues to *Anolis* species identity, made available through male visual displays, could aid in female mate choice, thereby providing a driving force for the co-evolution of traits expressed by males and the preference exhibited for them by females.

To date, experimental tests of species recognition in anoles have relied on contest outcomes between displaying males. There are disadvantages to using live males as stimuli, however. For example, subjects and stimuli interact and influence each other's responses (McMANN 1993). Furthermore, live-stimulus males do not behave in precisely the same manner in different trials, even when facing the same opponent. This uncontrolled variation can be eliminated by creating a standard stimulus.

JENSSEN (1970) created 16-mm film loops of edited male *Anolis nebulosus* displays, and presented these stimuli to female conspecifics. He found that females preferred a filmed normal-male display to the same display run in reverse, and to a display with head bobs removed and dewlap pulses added. The behaviour of live males could not have been manipulated in such a manner, and the demonstration that *A. nebulosus* females were sensitive to fine details of intraspecific visual display was possible only through the use of a standard stimulus.

Although JENSSEN's (1970) experimental technique was innovative at the time, film is an inherently difficult medium for manipulating patterns of motion and colour. Perhaps for these reasons, filmed stimuli have not become widely adopted in studies of animal communication.

Fortunately, recent advances in video technology have provided a new avenue for studies of species recognition and other problems in visual perception (CLARK & UETZ 1990, 1992, 1993; EVANS & MARLER 1991, 1992; EVANS et al. 1993a, b; McQUOID & GALEF 1993; MACEDONIA et al. 1994).

In this paper, species recognition is assessed from the behavioural responses of male *Anolis grahami*, a Jamaican anole, to video playbacks of conspecific and heterospecific displays. Three congeneric species, *A. conspersus*, *A. sagrei*, and *A. carolinensis*, served as heterospecific stimuli to examine potential effects of phylogeny and familiarity on subjects' responses.

Methods

Subjects and Stimulus Males

Adult male *Anolis grahami grahami* (UNDERWOOD & WILLIAMS 1959) were collected between 15 and 18 Jan., 1993, in Discovery Bay, Jamaica. A total of 25 males served as experimental subjects. Video-recordings from two of these 25 males provided display sequences ('clips') that were used in creating the conspecific video stimuli. The *A. grahami* males at Discovery Bay are similar in colour to the Negril population described by JENSSEN (1981), in possessing a light to medium greenish body, light to dark vermiculations on the head and anterior trunk, and an orange dewlap with a yellow border. The proximal half of the tail is sky blue grading into magenta (cf. JENSSEN 1981), and the distal half is greenish brown. Mean (±SE) snout-vent length (SVL) for the 25 subjects was 63 ± 0.77 mm, and 67 and 68 mm, respectively, in the two males used for video stimuli.

A. conspersus is considered to be the closest living relative of *A. grahami* (GRANT 1940; WILLIAMS 1976; WILLIAMS & RAND 1977). These two species exhibit similar body sizes, shapes and colour patterns, but different tail and dewlap colours. *A. conspersus* males possess yellowish-green bodies with light to dark vermiculations on the entire trunk. Their tails are the same colour as their bodies and their dewlaps are deep blue. *A. conspersus* occurs only on Grand Cayman (GRANT 1940; WILLIAMS 1976), thus, this species is allopatric with *A. grahami*. Adult male *A. conspersus conspersus* were collected in George Town, Grand Cayman, between 8 and 13 Jan., 1993, and video-recordings of one of these males (SVL = 67 mm) provided the display clips for this species.

A. sagrei is less closely related to *A. grahami* than is *A. conspersus* (UNDERWOOD & WILLIAMS 1959), though recent phylogenetic analyses suggest a close affinity between *A. sagrei* and the Jamaican anoles (GUYER & SAVAGE 1992). *A. sagrei* differs from *A. grahami* both in size and colouration: *A. sagrei* is smaller, with a tan body and, in our population, a chocolate-brown dewlap. The two species are sympatric at Discovery Bay, and it is likely that our *A. grahami* subjects were familiar with *A. sagrei* and its displays. Two *A. sagrei* males (SVL's = 45 and 51 mm) provided the display clips for this species. Although these males were collected on North Bimini, they were similar in size and colouration to those in Discovery Bay.

A. carolinensis is neither closely related nor sympatric with *A. grahami*, and the two species differ

in body shape and colouration. *A. carolinensis* has a longer, sharper snout than *A. grahami*, and typically exhibits a uniformly yellowish-green body with a pink dewlap. Video-recordings of two males (both SVL's = 60 mm) provided the display clips for this species. One of the males (stimulus 1) was purchased from a commercial animal supplier (Charles Sullivan Co., Nashville, USA), and had a light yellowish-green body with a pink dewlap. The other male (stimulus 2) was collected from an introduced population in Hawaii, and had a medium yellowish-green body with a purple dewlap.

Display structure has previously been described for *A. grahami* (JENSSEN 1981), *A. sagrei* (SCOTT 1984), and *A. carolinensis* (DE COURCY & JENSSEN 1994), but has not been illustrated for *A. conspersus* prior to the present report. Generally speaking, the displays of *A. grahami* (Fig. 1) seemed most similar to those of *A. sagri* (Fig. 2): the head bobs of both species typically lasted about 4–5 s and temporal overlap between head bobbing and dewlap pulsing was minimal. Both *A. grahami* and *A. sagrei* frequently pulsed the dewlap between episodes of head bobbing. Although the displays of *A. carolinensis* (Fig. 3) are usually given in 'volleys' (i.e. series), while those of *A. grahami* are not, both species possess head-bobbing patterns with a '3-part construction'. In *A. grahami* (from Discovery Bay and Negril; JENSSEN 1981) this typically consists of: 1. A pair of introductory bobs followed by a brief pause; 2. Two bobs performed in the same cadence as the introductory bobs; and 3. A crescendo of 3 smaller bobs. This pattern is obscured for *A. carolinensis* by the time scale used to display the entire stimuli (Fig. 3), but can be seen in 'display action pattern' graphs published elsewhere for this species (DE COURCY & JENSSEN 1994). *A. conspersus* also tends to produce head bobs in volleys, but the display structure differs dramatically from the other species in that a single bob element of brief duration is repeated a variable number of times (Fig. 4). Colour illustrations of these *Anolis* species can be found in SCHWARTZ & HENDERSON (1985).

Based on the above information, we made several predictions about the responses of *A. grahami* subjects to the videotaped displays of their congeners. If *A. grahami* relies on general morphological attributes like body shape and body coloration to discriminate conspecifics from heterospecifics, then subjects should respond more strongly to *A. conspersus* than to *A. sagrei* or *A. carolinensis*. By contrast, if behavioural cues like head-bobbing patterns are heavily emphasized in species recognition, then *A. grahami* should respond more strongly to *A. sagrei* or *A. carolinensis* than to *A. conspersus*. Finally, if prior experience plays an important role in conspecific/heterospecific discrimination, then *A. grahami* should respond more strongly to *A. sagrei* than to *A. conspersus* or *A. carolinensis*. This latter prediction would not be expected to hold, however, if *A. grahami* did not generally respond to the smaller *A. sagrei* under natural conditions. Since all four species have very different dewlap colours, we did not make any predictions about subjects' responses with respect to heterospecific dewlap colour.

Housing

All lizards were maintained in standard 70-container racks designed for housing laboratory mice at the University of California, Davis (UCD). Each plastic container (28.2 × 17.4 × 12.4 cm) held one lizard and included a perch (1.25-cm diameter wooden dowel) and paper-towel flooring. The room was kept on a 14L:10D light cycle, 50–60 % relative humidity, and a temperature of 30–31 °. The lizards were sprayed daily with deionized water and were fed vitamin-dusted crickets and waxworms alternately every 3 d. Subjects were maintained in this environment for at least 60 d prior to experimental trials to bring them into breeding-season condition (LICHT 1967).

Stimulus Construction

Staged display contests between pairs of males were conducted several days (*A. grahami* and *A. conspersus*) or weeks (*A. carolinensis* and *A. sagrei*) after the arrival of the lizards at UCD. Males were video-recorded through the long axis of a standard 38-l glass aquarium using a Panasonic AG-450 colour video camera (740 lines) and Ampex S-VHS 120 videotape. The aquarium was illuminated with two 250-W photo bulbs and contained two perches (1.25-cm diameter wooden dowels) from which the males displayed at one another. All walls of the aquarium except for the front face were covered on their inside surfaces with neutral grey card to eliminate reflections. Focal distance was adjusted so that displaying lizards would appear life-size on the monitor used for playbacks. Only displays occurring on the more distant perch were filmed so that the stimulus male always appeared to be displaying toward the camera (and hence, toward future subjects). Stimulus clips were edited from
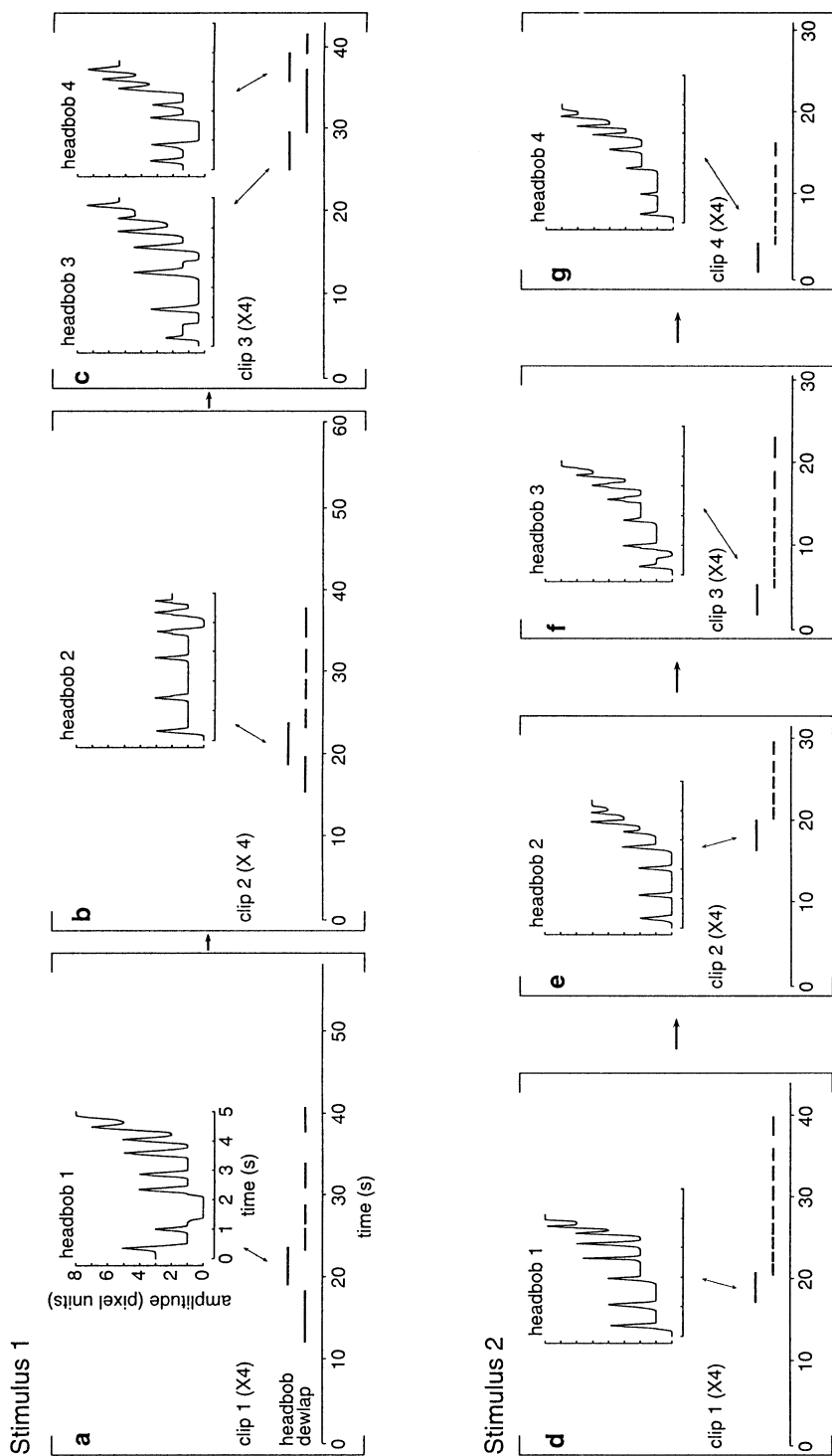
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Anolis grahami

Fig. 1: Flow diagram of components for *Anolis grahami* video-stimuli 1 and 2. Pixel units refer to y-axis changes in eye position of the lizard image during head bobs. Clip duration is indicated by the lower time scale in each clip illustrated. Horizontal dashes above the time scale indicate when the stimulus lizard was head bobbing and/or pulsing the dewlap. Dewlap retractions of less than one-half the dewlap diameter are not shown
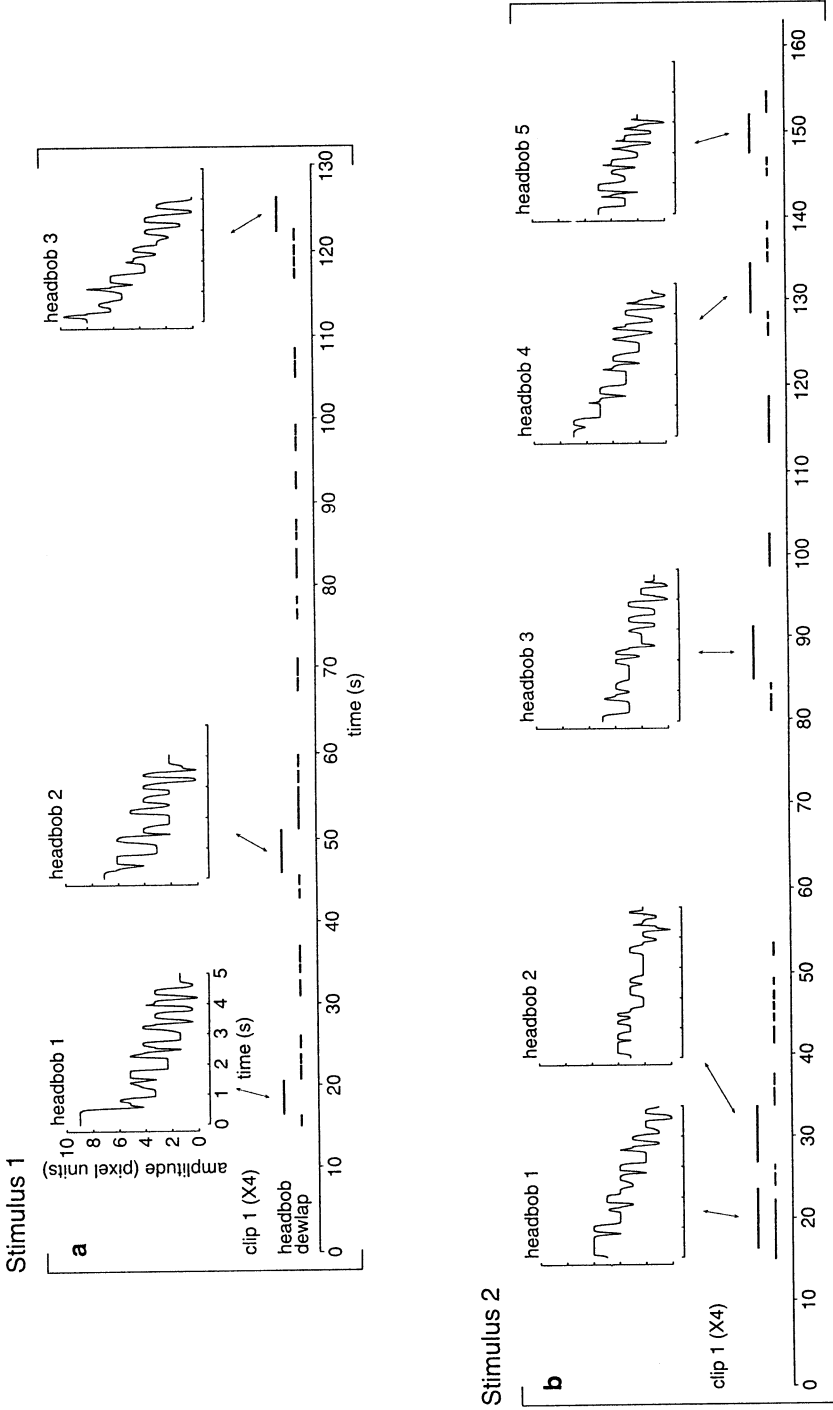
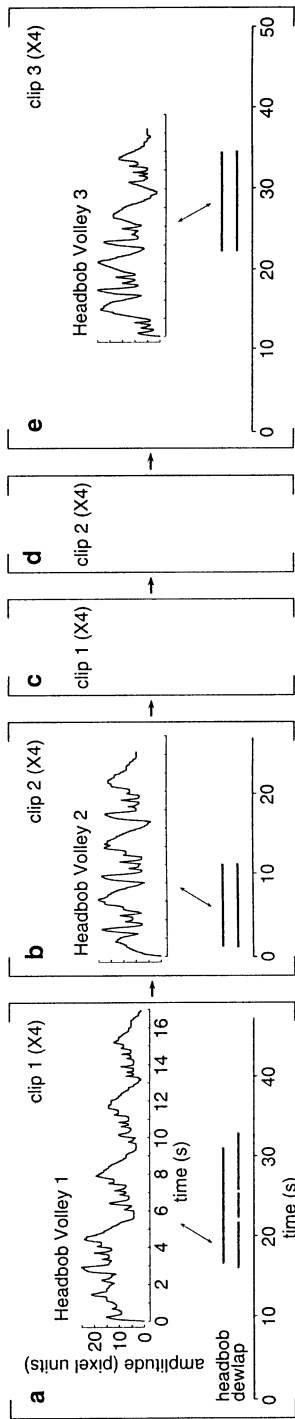*Fig. 2:* Flow diagram of components for *Anolis sagrei* video-stimuli 1 and 2. Legend as in Fig. 1
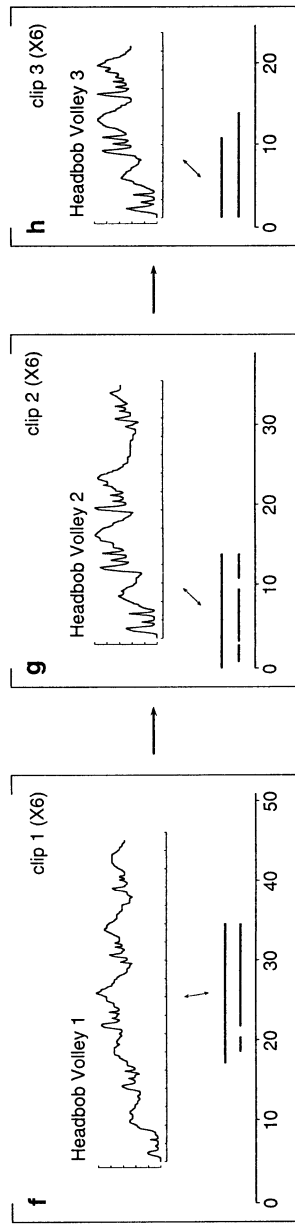
Fig. 3: Flow diagram of components for *Anolis carolinensis* video-stimuli 1 and 2. Clips c and d in Stimulus 1 are repeats of clips a and b, respectively. Legend as in Fig. 1
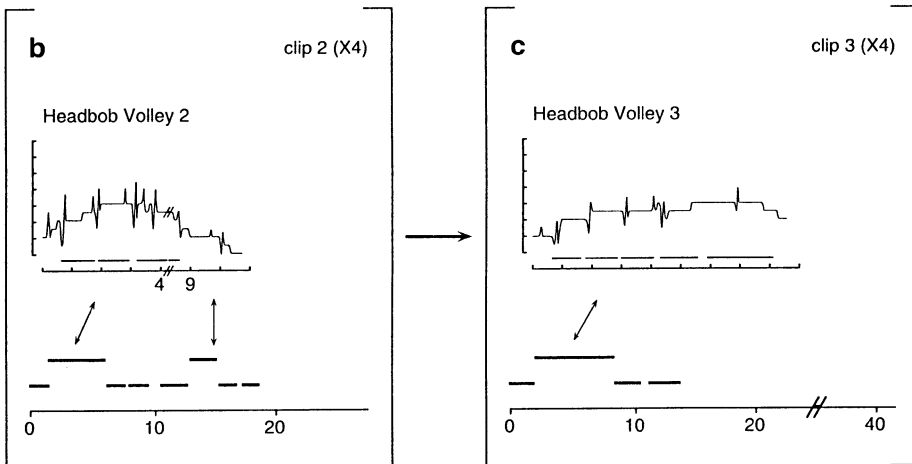
Anolis conspersus`

Stimulus 1

a clip 1 (X4)

amplitude (pixel units)

Headbob Volley 1

12
8
4
0

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
time (s)

headbob
dewlap

0   10   20   30   40   50   90
time (s)

b clip 2 (X4)

Headbob Volley 2

4 9

0   10   20

c clip 3 (X4)

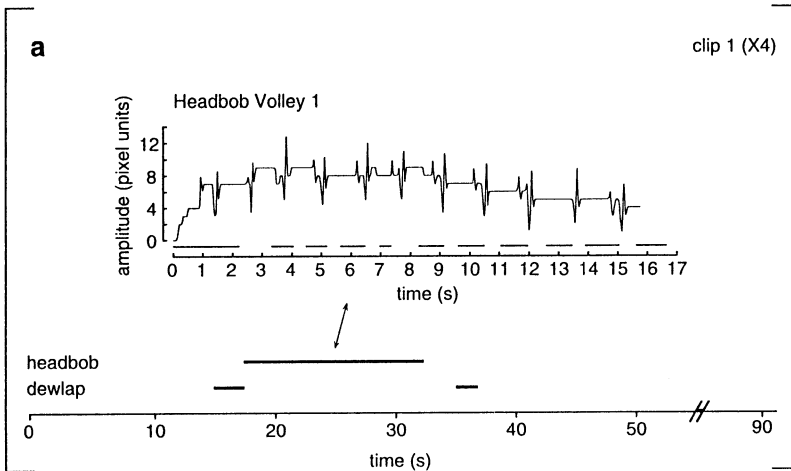Headbob Volley 3

0   10   20   40

Fig. 4: Flow diagram of components for *Anolis conspersus* video-stimulus 1. Due to the alternation of dewlap pulses and head bobs in this species, the locations of inter-bob dewlap pulses are shown in the upper part of the diagram for each clip. Clip duration axes are broken in a and c to reveal the details of the rapid head bobbing movements. In b, the broken x-axis indicates a natural pause of about 4 s in the display. During this pause the dewlap was held fully extended and the head remained motionless until bobbing was resumed. Legend as in Fig. 1

Table 1: Total running times and percentage of time spent displaying (head bobbing and dewlap pulsing) in video stimuli

Species and Stimulus#	Stimulus duration (s)	% display time/ stimulus duration	x̄ % display time/ stimulus duration
A. grahami #1	464.1	37	44
A. grahami #2	545.8	51	
A. sagrei #1	489.5	49	47
A. sagrei #2	643.3	44	
A. carolinensis #1	618.9	46	43
A. carolinensis #2	593.5	40	
A. conspersus #1	610.6	52	52

raw footage using Panasonic AG-7500 and AG-7510 recorder/player decks, and an AG-A750 edit controller. Although two stimuli, each of a different individual, were constructed for *A. grahami, A. sagrei,* and *A. carolinensis,* only one male *A. conspersus* displayed frequently enough to construct a stimulus comparable to those of the other species in terms of quality and amount of display.

The video stimuli were designed to match, as closely as possible, the durations of the head-bobbing/dewlap-pulsing displays across the stimulus species (Table 1) while maintaining species-specific attributes of these displays and a naturalistic appearance. Each stimulus consisted of 1–5 display 'clips' that were edited into a series, where each clip was repeated four times (six times in *A. carolinensis* stimulus #2) before the next clip in the series appeared (Figs. 1–4). We used a library of raw footage to produce seamless transitions from one display clip to the next. To maintain this naturalistic quality, the temporal locations of head bobbing and dewlap movements in each of the seven total video stimuli were allowed to vary. All stimuli began and ended with 15 s of the stimulus lizard resting calmly on the perch.

Although the video stimuli were matched for the duration of display as a proportion of stimulus running time (Table 1), the stimulus males varied in their use of several 'display modifiers' (*sensu* JENSSEN 1977, 1978). To document this variation, each clip of each stimulus was scored for sagittal compression of the body ('side flattening'), raised crest, and gorged throat. The potential effects of these modifiers on subjects' responses were examined statistically after the experiment was completed (see Results).

Computer-generated diagrams of stimulus lizard displays were created to illustrate species-specific patterns of head bobbing and dewlapping. A genlock ('SuperGen', Digital Creations), which is a device used to combine two image sources, was used to overlay a computer graphic (DeluxePaint IV, Electronic Arts) onto each stimulus image. A cursor was placed over the image of the lizard's eye (screen resolution = 320 × 200 lines) and vertical coordinates ('pixel units') were recorded for each video frame to transcribe the display patterns. These coordinate changes were compiled in a graph form (Figs. 1–4) using CricketGraph (Cricket Software).

Experimental Protocol

In groups of five, 25 *A. grahami* subjects were tested. Subjects were taken from their home cages and placed, one per aquarium, in the test chamber (see below). Pre-trial acclimation time was 46–50 h. On the first test day, subjects were presented with a stimulus from each of the four species in a unique random order. At the same time on the following day, subjects viewed each species' alternative stimulus in the reverse order. Subjects were tested in a 'round robin' fashion to maximize time between trials for each subject (approximately 45 min).

Playback Apparatus

Experimental trials were conducted inside a large (2.25 × 2.15 × 1.90 m) sound-attenuating chamber (Industrial Acoustics) lined with 10 cm thick Sonex echo-attenuating foam (Illbruck Inc.).

Table 2: Definitions of response variables scored

Behaviour	Definition
Dewlap pulse	One extension/retraction of the dewlap (via the hyoid and process retrobasilis (STAMPS & BARLOW 1973)
Head bobs	Stereotyped series of species-specific vertical head movements (Figs. 1–4)
Head tilt	Top of head is rotated toward shoulder nearest the stimulus, providing full view of skull breadth
Sagittal compression	Body is compressed sagittally, giving the impression of increased size when viewed laterally
Lateral presentation	Subject moves rapidly one or more body lengths, turns to present lateral view of sagittally compressed body, then (usually) tilts head toward stimulus
Gorged throat	Hyoid apparatus is lowered, increasing the dorso-ventral diameter of the throat and producing a sharp angular appearance where the skin is stretched taut against the distal end of the hyoid (STAMPS & BARLOW 1973)
Raised (nuchal) crest	Dorsal fold of muscular skin, running from the posterior $\frac{1}{3}$ of the skull to the interclavicle region, becomes erect over a period of several min
Lipsmack bouts	One-to-several consecutive lipsmacking movements
Yawn	Mouth opened to maximum width then closed

Two light fixtures, each containing two 122-cm full-spectrum fluorescent tubes (Vita-Lite, Duro-Test Corp.), were hung from the ceiling to provide illumination. These lights were on a timer that had the same 14L:10D light schedule as the room in which the lizards normally were housed. A heating/fan unit maintained air temperature at 30–31 ° in the test chamber. Humidity was not controlled, but the lizards were misted with water several times daily. Subjects were fed on the day prior to entering their test tanks.

Inside the test chamber, five 38-l aquaria were arranged in a 'fan' shape on a table. A perch (wooden dowel) was fixed to the side walls of each aquarium 10 cm above the aquarium floor and 7 cm from the back wall. The floor of each tank was covered with 150 grit sandpaper. Each aquarium had a screen top, and the side and back walls were covered with opaque card so that subjects could neither see their neighbours nor their own reflections. The front face of each tank was left uncovered during the acclimation period (see below), but was covered with an opaque card during pre-trial set-up on each test day.

The stimulus monitor (Sony BT-S901Y: 23 cm) was mounted on a tripod such that it could be panned from one subject to the next between trials. The monitor was encircled with branches and leaves to simulate a lizard displaying in a tree 1 m above the substrate. The stimulus lizards appeared at a height of 15 cm above the subjects' perches and at distances of 115–160 cm from subjects, depending on the subjects' locations in their tanks.

A colour video camera (Panasonic WV-CL320: 525 lines) fitted with a zoom lens (Canon model II) was mounted atop the stimulus monitor to record subjects' responses. A time code generator (Panasonic WJ-810) was coupled with the recording system to stamp a permanent running chronometer onto the test-session video tapes. During trials, stimuli and subjects were observed continuously outside the test chamber on video monitors (Panasonic CT-1383Y and CT-1331Y).

Data Analysis

Data on the subjects' responses (Tables 2, 3) were compiled by hand from frame-accurate scoring of the test-session videotapes. Durations of dewlap pulses and head bobs were measured and summed for each trial. Frequencies of head tilts, lateral presentations, gorged throats, lipsmacks, and yawns

Table 3: Responses of subjects measured/counted and analyses performed

Data type	Analysis type	Variables
1. Response latency	Latency to display in response to stimulus. If subject does not respond, latency = stimulus duration	Dewlap pulse, head bobs
2. Response duration	a. Σ duration of response to each stimulus species	Dewlap pulse, head bobs
	b. Same as 'a', but response durations represented as proportions of stimulus durations	Dewlap pulse
	c. Same as 'a', but only first 2.75 min of each stimulus were considered (see text)	Dewlap pulse
3. Response frequency	Frequency of response to a given stimulus species as a proportion of total response frequency	Dewlap pulse, head bobs, head tilt, lateral presentation, gorged throat, raised crest, lipsmacks, yawn

were counted and summed for each trial, and raised crest was scored at the end of each trial as being present or absent. Responses were counted as independent events if separated in time by more than 2 s (DE COURCY & JENSSEN 1994), which appeared to be a natural temporal division between bouts of dewlap pulsing (the most frequent response). Response latency was also measured for each trial. If a subject did not respond in a given trial with at least one of the behaviour patterns being scored, the response latency duration assigned to that subject for that trial was the duration of the stimulus shown in that trial.

Because the data on subjects responses were not normally distributed, non-parametric statistical analyses were conducted. $\bar{X} \pm SE$ were used only for descriptive purposes and for graphical depiction of the data. Friedman ANOVAs (two-tailed) were used to test responses of subjects across the four stimulus species. For significant ANOVAs ($p \leq 0.05$), Wilcoxon matched-pairs signed-ranks tests were subsequently used for pairwise comparisons. Given that, in most cases, all six possible pairwise comparisons were made for each variable, the level of significance was adjusted to 0.01 to protect against the increased probability of type I error (SOKAL & ROHLF 1981). In light of the *a priori* prediction that conspecific stimuli would elicit stronger responses and shorter response latencies than heterospecific stimuli, the pairwise conspecific/heterospecific comparisons were one-tailed, whereas the heterospecific/heterospecific comparisons were two-tailed.

Results

Of the 14 *A. grahami* subjects that responded to the video playbacks with head bobbing or dewlap pulsing (all subjects that responded with head bobs also dewlapped), the identity of the stimulus species strongly influenced whether or not subjects displayed (Cochran Q test: n = 14, df = 3, Q = 18.1, $p \leq 0.001$). Pairwise tests showed that more subjects responded to the conspecific stimuli than to any of the heterospecific stimuli (Fisher exact test: $p \leq 0.001$ in all three comparisons). To avoid redundancy, we will point out here that no pairwise

comparisons of responses to the three heterospecific stimuli were significant for this or any other test. Hence, all pairwise tests refer only to conspecific/heterospecific comparisons. Six subjects displayed to a single stimulus species only, and in every case the stimulus was conspecific (Cochran Q test: n = 6, df = 3, Q = 18.0, p ≤ 0.001).

The latency to the first head bob or dewlap pulse differed significantly among the stimulus species ($\overline{X} \pm$ SE: *A. grahami*, 254 ± 32 s; *A. carolinensis*, 496 ± 55 s; *A. conspersus*, 481 ± 51 s; *A. sagrei*, 470 ± 36 s; Friedman ANOVA: n = 14, $\chi^2 =$ 18.26, df = 3, p ≤ 0.0005). Subjects responded sooner to the conspecific stimuli than to any of the heterospecific stimuli (Wilcoxon test: *A. grahami* vs. *A. carolinensis*, T = 2.86, p ≤ 0.005; *A. grahami* vs. *A. conspersus*, T = 2.98, p ≤ 0.005; *A. grahami* vs. *A. sagrei*, T = 3.30, p ≤ 0.0005).

The amount of time subjects spent pulsing their dewlaps also differed significantly across the stimulus species (Friedman ANOVA: n = 14, $\chi^2 = 22.92$, df = 3, p ≤ 0.0001; Fig. 5 a), with subjects dewlapping for longer periods in response to conspecific than to heterospecific stimuli (Wilcoxon test: *A. grahami* vs. *A. carolinensis*, T = 3.17, p ≤ 0.001; *A. grahami* vs. *A. conspersus*, T = 3.05, p ≤ 0.001; *A. grahami* vs. *A. sagrei*, T = 3.23, p ≤ 0.001). Subjects likewise spent more time head bobbing in response to conspecific than to heterospecific stimuli, though this difference was not quite significant (Friedman ANOVA: n = 4, $\chi^2 =$ 7.45, df = 3, p ≤ 0.059; Fig. 5 b). The sample size for this comparison was, however, extremely small.

To test for possible effects of differences in stimulus running times on responses (see Methods, Table 1), the duration of dewlapping and head bobbing responses were converted to a proportion of the stimulus duration for each trial. These results were not qualitatively different from those above, in which subject display durations irrespective of differences in stimulus running times were considered (dewlapping: Friedman ANOVA: n = 14, $\chi^2 = 25.59$, df = 3, p ≤ 0.0001; Wilcoxon test: *A. grahami* vs. *A. carolinensis*, T = 3.17, p ≤ 0.001; *A. grahami* vs. *A. conspersus*, T = 3.30, p ≤ 0.0005; *A. grahami* vs. *A. sagrei*, T = 3.23, p ≤ 0.001; head bobbing: Friedman ANOVA: n = 4, $\chi^2 = 6.10$, df = 3, p ≤ 0.1).

We next examined whether the differential presence of several display modifiers (sagittal compression, raised crest, gorged throat: Table 2) affected the subjects' responses. The magnitude (or simply the presence) of these display modifiers was assessed for each clip of each stimulus (Table 4). Overall, conspecific stimuli contained more display modifiers in their clips than did the heterospecific stimuli. When considering only the first clips of the stimuli, however, some of the heterospecific stimuli exhibited more of the display modifiers than did the conspecific stimuli (Table 4).

To study the effects of display modifiers on subject responses, we focused on responses produced during the first clips of the video stimuli. Dewlap pulsing was chosen as the response variable for this analysis because it was the most frequent component of *A. grahami* display, and it was exhibited by all responding subjects. Dewlap pulsing was scored for the first 2.75 min of each trial, because
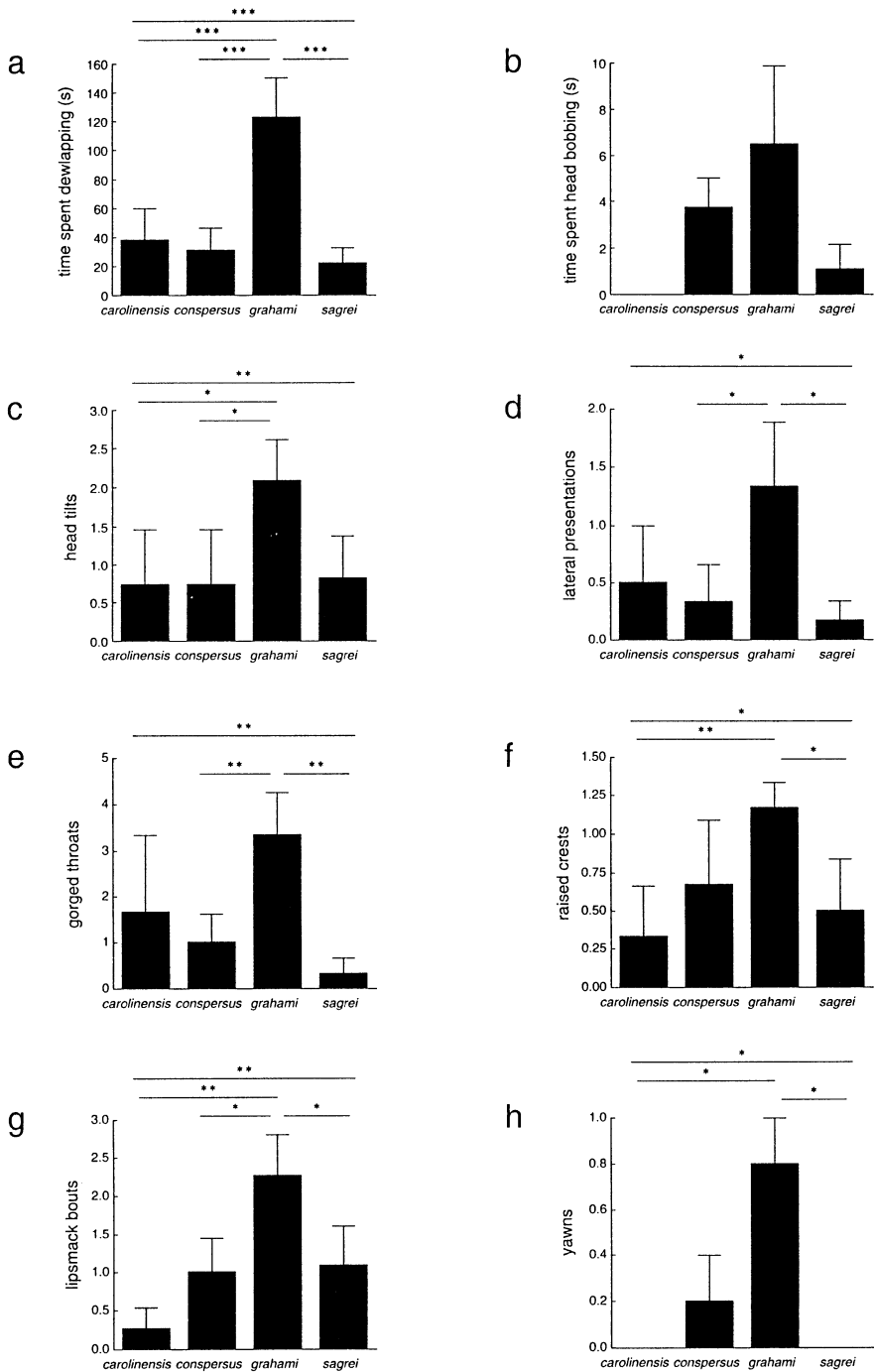
Fig. 5: Responses ($\overline{X}$ ± SE) of adult male *A. grahami* subjects to video playbacks of conspecific and heterospecific displays. *** p ≤ 0.001, ** p ≤ 0.01, * p ≤ 0.05. For overall comparisons (uppermost bar in each graph), alpha = 0.05; for pairwise comparisons, alpha = 0.01

Table 4: Assessment of display modifiers in video stimuli. Variable coding: sagittal compression of the body (1 = not compressed; 2 = slightly compressed, 3 = strongly compressed), raised crest (yes = nuchal crest raised to a height ≥ vertical diameter of the eye opening), gorged throat (yes = throat enlarged for at least 2 s by extension of the hyoid apparatus). Italics indicate additional statistical analysis for clip 1 of each stimulus (see text)

Stimulus	Clip	Body shape	Crest	Gorged throat
Anolis grahami #1	*1*	*3*	*no*	*no*
	2	3	yes	no
	3	3	yes	no
A. grahami #2	*1*	*3*	*no*	*yes*
	2	3	yes	yes
	3	3	yes	yes
	4	3	yes	yes
A. sagrei #1	*1*	*2*	*yes*	*no*
A. sagrei #2	*1*	*2*	*no*	*no*
A. carolinensis #1	*1*	*3*	*no*	*yes*
	2	3	no	no
	3	3	no	no
A. carolinensis #2	*1*	*3*	*no*	*yes*
	2	3	no	no
	3	3	no	no
A. conspersus #1	*1*	*3*	*no*	*no*
	2	2	no	no
	3	3	no	no

the durations of the first clips in the two *A. grahami* stimuli were 2.7 min and 2.8 min, respectively.

The results of this analysis were virtually identical to those presented above: subjects showed an overwhelming preference to pulse their dewlaps in response to conspecific stimuli (Friedman ANOVA: n = 13, χ^2 = 13.99, df = 3, p ≤ 0.005), and all conspecific/heterospecific pairwise comparisons were significant (Wilcoxon test: *A. grahami* vs. *A. carolinensis*, T = 2.67, p ≤ 0.005; *A. grahami* vs. *A. conspersus*, T = 2.31, p ≤ 0.01; *A. grahami* vs. *A. sagrei*, T = 2.58, p ≤ 0.005). Thus, the preference of subjects to respond to the conspecific images cannot be attributed to variation in display modifiers among the video stimuli.

Finally, subjects produced more display modifiers in response to conspecific than to heterospecific stimuli (see Fig. 5 c–h for pairwise comparisons): Friedman ANOVA, df = 3 in all cases; head tilts (n = 11, χ^2 = 10.83, p ≤ 0.01), lateral presentations (n = 6, χ^2 = 8.14, p ≤ 0.05), gorged throats (n = 6, χ^2 = 12.63, p ≤ 0.01), raised crests (n = 6, χ^2 = 9.55, p ≤ 0.05), lipsmack bouts (n = 11, χ^2 = 12.00, p ≤ 0.01), and yawns (n = 5, χ^2 = 8.60, p ≤ 0.05).

Discussion

The results of this study demonstrate that adult, male *Anolis grahami* subjects

responded sooner and more strongly to conspecific than to heterospecific video-taped displays on all behavioural variables examined. We interpret these results as indicating that subjects discriminated the video images of their own species from those of congeners. Although not all pairwise comparisons were statistically significant, in most cases this could be due to the small sample sizes (e.g. n = 4, 5, or 6) associated with certain responses (see above and Fig. 5).

Some of the variables scored, such as gorged throats, raised crests, and lateral presentations, are considered to be aggressive motor patterns in anoles (Jenssen 1977, 1978), and it may not seem too surprising that conspecific video stimuli were more likely than heterospecific stimuli to elicit these responses from subjects. However, conspecific stimuli also elicited more 'lipsmacks' and 'yawns' from subjects than did the heterospecific stimuli. These behaviour patterns do not appear aggressive, and although they could have communicative value, they may simply be involuntary autonomic consequences of sympathetic stimulation during threatening social situations. If so, responses like lipsmacks and yawns provide a type of behavioural assay that is fundamentally distinct from measurements of aggressive displays.

The most robust response of subjects to the video images was dewlap pulsing, a display that also does not appear to be particularly aggressive. For example, it was not uncommon for subjects to move to the back of their aquaria and pulse their dewlaps while 'looking over their shoulder' at the stimuli. We speculate that under natural conditions, dewlap pulsing may serve to acknowledge the presence of a displaying conspecific, rather than to signal willingness to engage in an aggressive interaction.

One possible reason for the relatively clear results obtained in this experiment is that the subjects were sufficiently distant from the stimuli. Under natural conditions, anoles often detect conspecifics at long distances; territorial male *Anolis aeneus* routinely display at neighbours or intruders 5–10 m away (J. A. Stamps, unpubl. data; Fleishman 1988b, 1992). After detecting one another, individuals then have the choice of whether or not to approach and engage in further social interactions.

It could be argued that the ability to distinguish conspecifics from heterospecifics is most important over relatively long distances, allowing individuals to avoid approaching a potential competitor or mate only to find, upon closer inspection, that it was a heterospecific. In contrast, when subjects are suddenly confronted at short distances by an aggressive-looking anole they may be inclined to respond defensively with less regard for species identity. For instance, when a conspecific intruder was presented at a short distance (40 cm) from *A. aeneus* subjects in a laboratory setting, subjects were most likely to respond with defensive (fan) or threat (gorged throat) displays, but when intruders were presented at 120–160 cm they were more likely to elicit a series of species-specific head-bobbing displays from subjects (Stamps & Barlow 1973). Thus, anoles may be more likely to exhibit species-specific display patterns in response to conspecifics located at relatively longer rather than shorter distances. Even though 160 cm is a small distance in relation to the territory diameters of many anoles, it is larger than the

40–50 cm that separate opponents in many captive studies of anoline aggressive behaviour (McMann 1993).

For example, prior to the present study, video techniques were used to examine species discrimination in two *Anolis* 'sibling species': *A. marcanoi* and *A. cybotes* (Macedonia et al. 1994). Results revealed a significant difference in the temporal patterning of subjects' responses to conspecific and heterospecific stimuli, but no differences were detected in variables such as response latency or amount of dewlap pulsing (variables shown in the present study being strong indicators of species discrimination). One possible explanation for the absence of more broadly based discrimination in the Macedonia et al. (1994) study is that the close phylogenetic affinity of the two stimulus species made the discrimination task more difficult. However, the video stimuli were presented at roughly 40 cm from subjects in that study, as compared to 115–160 cm in this study. This difference in the distances at which aggressively appearing video stimuli were encountered might have influenced the diversity of responses for which conspecific/heterospecific discrimination could be shown.

As video-playback experiments are still a novelty in the study of animal behaviour, a further caveat is in order. Subsequent to the work reported here, a second experiment was conducted with *A. grahami*. In this second series of trials, the 14 subjects who responded in the first experiment were shown the same video stimuli, but with the monitor colour turned off (i.e. in 'grey scale'). Intriguingly, no evidence of discrimination between conspecifics and heterospecifics was observed. It should be noted, however, that an important difference exists between the grey-scale image produced by a black-and-white video camera and monitor, and that produced by 'removing' the colour on a colour monitor. In a black-and-white system, the grey-scale image results from differences in brightness (luminance) levels associated with the colours of the actual image. However, a colour video monitor produces a grey-scale image by altering the colour amplitudes of the video pixels in a way that provides humans with the sensation of an achromatic (colourless) image. Consequently, the more a species' visual system differs from that of humans, the more unlikely this type of image will be perceived as grey scale. Colour vision in anoles is as yet poorly understood (Fleishman 1992), but differences from humans in the spectral responses of their retinal cones are evident (L. Fleishman & E. Loew unpubl. data). Thus, when using video playbacks to address questions about animal signals, the visual system of the species under study should, so far as is possible, be taken into account, in addition to video-equipment considerations.

Finally, it was interesting that *A. grahami* subjects gave no indication of discriminating among the three heterospecific *Anolis* stimuli. Given the predictions made at the outset, it would appear that gross attributes such as body shape, colouration, size, prior familiarity, or even general similarity in head-bobbing pattern, are not salient features in the discrimination process. It is possible, of course, that subjects did discriminate among the heterospecific stimulus species but responded to them as a single class of stimuli (i.e. non-conspecifics). Details of head-bobbing clearly differ among the stimulus species (Figs. 1–4), however,

and JENSSEN's (1970) film-loop-playback study demonstrated the sensitivity of anoles to manipulations of conspecific head-bobbing patterns. The four stimulus species in this study also differ dramatically in dewlap colour. Thus, our subjects may have been using details of head-bobbing patterns, dewlap colouration, or both in discriminating the videotaped displays of conspecifics from those of heterospecifics. The relative importance of these two traits for species recognition in *A. grahami* is currently being explored using digital-video techniques, which allow stimulus morphology and behaviour to be controlled and manipulated independently.

Acknowledgements

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