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NO PREFERENCE FOR MORE COLORFUL OR SHOWIER MALES AMONG FEMALE BROWN ANOLES

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ABSTRACT

Female choice is an important sexually selective agent shaping ornament evolution. We investigated brown anole female preferences by quantifying female temporal associations with tanks containing size-matched males that differed in dewlap color and behavioral display intensity during two reproductive seasons. We found that female temporal association with tanks was not associated with male's dewlap color or display intensity in either summer. We also found no evidence that females associate with empty tanks, in either summer, as might be expected if females avoid harassment.

INTRODUCTION

'Pre-copulatory' female choice (coined by Anderson 1994 to distinguish from 'cryptic female choice' as described by Eberhard (1996) is an important selective mechanism that drives the evolution of sexual characters in a variety of animals (Andersson 1994). In pre-copulatory female choice, females use phenotypic trait variation among males to aid in mate choice decisions. This choice is apparent (and observable) because females spend time near males while they are making assessments of phenotypically varying males. Female choice has been shown to select for male size, territory size and quality, male reproductive behavior, tail length, call / song pitch, rate, duration and loudness, and ornament color in many animals (summarized by Andersson 1994).

Brown anoles (*Anolis sagrei*) are a Dactyloid lizard (*sensu* Nicholson et al. 2012) whose behavior exemplifies the polygynous territorial mating system. A dult males defend a space and/or access to several females (Tokarz 1998; Tokarz et. al. 2002) and mate with more than one female (Calsbeek and Manorcha 2006). Female home ranges often overlap one or more male's territories (Schoener and Schoener 1980; Tokarz 1998).

Anolis lizards are well known in the biological literature for their secondary sex characters, because adult males possess a colorful and extendable dewlap is displayed in intra- and inter-sexual contexts (Jenssen et al. 2000; Vanhooydonck et al. 2005; Jenssen 1970; Sigmund 1983). Furthermore, the ethological aspects of male displays are well understood (Jenssen 1977, McMann and Paterson 2003a, b; Paterson and McMann 2004). Head bob frequency, dewlap extension frequency and push-up frequency are important components of brown anole displays (Tokarz 1985; McMann and Paterson 2003a, b; Paterson and McMann 2004). Steffen and Guyer (2014) found that both dewlap color and display frequency (i.e. showiness) predicted contest success for territories as well as mates in brown anoles. These contests have yet to be fully understood, however, and because female home ranges overlap with several male's territories, speculation about the influence of female choice in Dactyloid evolution has dotted the literature (e.g., Sigmund 1983; Tokarz 1995, 1998).

Here, we perform behavioral observation trials during two consecutive reproductive seasons to investigate female temporal association with males who possess more colorful dewlaps or display more frequently. We predicted that if female choice was important to brown anole mating success, free-ranging females should move toward and spend more in front of a preferred male (i.e. a male that is more colorful or that displays more). We also considered the possibility that male dominance is intense and females avoid male's attentions to reduce harassment. We predicted that females would show temporal associations with empty tanks if male dominance was severe and females avoid harassment.

MATERIALS AND METHODS

Male and female brown anoles were collected May 6-10, 2011, and May 20-22nd, 2012 in Hillsborough County, FL, USA by Glades Herp employees, a reptile trade supplier. Lizards were shipped overnight to a lab at Penn State Behrend in Erie, PA., and cared for according to IACUC protocol (# 36766). Males and females were measured with a metal ruler to the nearest mm, and weighed to 0.001 grams using an electronic balance. Males larger than 39.0 mm and females larger than 34.0 mm were considered adults (see Licht, Gorman, 1970; Lee et al. 1989). Lizards were sprayed with water daily, fed crickets three times each week (3 per feeding) and meal worms ad libitum. All food items were dusted with Repta-vite vitamin powder (Zoo Med laboratories, San Luis Obispo, CA) before being offered to a lizard. Full spectrum fluorescent bulbs (Vitalite T8, 32 watt) were suspended 30.5 cm above each terrarium top. The laboratory was maintained at 32.2 °C, and relative humidity was maintained between 40-60%. No lizard was used in trials more than once.

We size-matched (nearest 0.5 mm) pairs of males and placed each individual male into two of 3 separate 37.9 liter (50.8 × 20.4 × 30.5 cm^3) terraria arranged next to a 208.2 L tank (91 x 45.72 x 45.72 cm^3) that contained an oviductal female (Figure 1). The outside walls of each tank were lined with green construction paper to facilitate visual detection of each male's red and yellow dewlap (Endler 1992). These individual green-walled tanks placed adjacent to each other prevented males from seeing each other and allowed us to study female mate choice without any chance of male-male competition affecting a female's preference. It also forces the focal female to seek out the male if she wishes to associate with him (Hill 2002; Burley et al 1982). All adult females were assumed to be reproductively active because females produce several single-egg clutches per month throughout the reproductive season (Andrews and Rand 1974; Lee et al. 1989). We measured the color of the dewlap in two distinct regions: the center (appears red to the unaided human eye) and the margin (appears yellow or white to the unaided human eye). Spectral measurements were taken with an Ocean Optics S2000 UV-visible spectrometer (OOIBase32 software) 1 day before initiation of the experiment (always starting at 10.00 h CST) on lizards that showed no signs of imminent shedding. All reflectance data were generated relative to a white reflectance standard and were taken in a dimly lit laboratory with no windows. We placed a small black rubber stopper on the tip of the reflectance probe, creating a 2-mm gap between the probe tip and the dewlap, ensuring a constant distance between probe and dewlap. To measure dewlaps with the spectrometer, we placed each lizard ventral side up on a flat black table and immobilized the animal with two pieces of athletic tape: one placed across its belly and the other across its mandible. The dewlap was maximally extended by grasping it with a small clamp and adjusting the height of the clamp via its attachment to a horizontal metal arm on a ring stand. We placed the spectrometer probe at a 90° angle, flush with the exposed skin of the dewlap. We measured spectral reflectance along the center and margin of the dewlap, taking six non-overlapping spectral measurements per dewlap region and averaging them for each lizard. Spectral measurements were gathered as percent reflectance at 1-nm wavelength increments from 300–700 nm (representing the lower range of photon absorption by UV-sensitive cones; Fleishman, Loew & Leal, 1993). Spectral measurements were smoothed using CLR, version 1.0 (Montgomerie copyright 2008). Each smoothed file was standardized (mean reflectance subtracted as described by Cuthill et al., 1999) and then reduced to the means of 20-nm bandwidths. Principal components analysis (PCA) was performed on these standardized spectral files and two PC's were found to be important in each dewlap regions spectral variation. The resulting PC coefficients were graphed against wavelength to describe spectral shape in the dewlap center and edge. Each spectral PC was interpreted using the following wavebands: short wavebands = 300-474 nm (which includes UV and blue spectra; medium wavebands = 475-599 nm (which includes green and yellow spectra); long wavebands = 600-700 nm (which includes orange and red spectra).

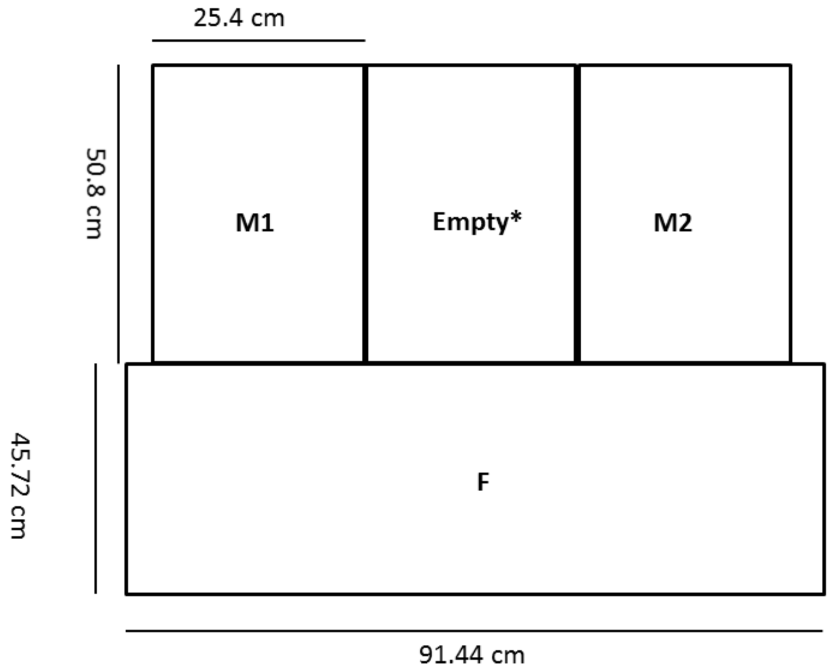

Figure 1. Female movement and overt female choice tank schematic diagram., viewed from above. M1 = tank containing male number 1 in size-matched dyad, M2 = tank containing male number 2 in size-matched dyad. Empty * = tank with no lizard present. The distribution of size-matched males in each tank was randomized. F = tank containing a female whose movement across the tank was video recorded and ethologically quantified and analyzed.

The lizards were video recorded (SONY Handycam DCRSX45), and observed at a later date. The frequency and duration of relevant male behaviors were quantified using an event-recorder program called Etholog (v. 2.2, 2006). Male behaviors such as dewlap extension frequency (# dewlap extensions per minute), head-bob frequency (# head-bobs per minute), and push-up frequency (# push-ups per minute) were quantified with Etholog.

Female preference was measured as female time associated with males of varying dewlap colors. We quantified the temporal association of females by summarizing the times spent in front of one of three 37.9 liter terraria (50.8 x 20.4 x 30.5 cm^3 (Figure 1). One of the 37.9 liter terraria was empty and its position was randomly assigned. The empty terraria served as a control tank to test if females preferred to spend a majority of time in front of a tank with no males, as a proxy for harassment avoidance. The other terraria contained males that differed in dewlap color and display frequency.

We used a k-ratios Chi-square test (Zar 1999) to determine if observed female tank associations (as measured via time spent in front of each tank) matched the tanks containing males who display the most or have the most colorful dewlap. We also used the k-ratios Chi-square test to determine if observed female tank associations matched empty tanks, to see if females avoided harassment.

RESULTS

Spectral analyses—In 2011 dewlaps, 97.18 % of the center's variation was reduced to 3 PC's (**Figure 2 a & b)**. PC1 represented low-to-medium wavelength reflectance (340-580 nm) relative to UV (300-340 nm) and long wavelength absorption (580-680 nm) and explained 74.83 % of the variation. PC2 represented medium-to long wavelength reflectance (520-700 nm) relative to short and medium wavelength absorbance (300-520 nm) and explained 15.91 % of the variation. PC3 represented short (300-420 nm) and long wavelength reflectance (600-680 nm) relative to medium-wavelength absorption (420-600 nm) and explained 6.44 % of the variation.

94.44% of the dewlap edge variation was reduced to two PC's. PC1 represented medium-to long wavelength reflectance (500-700 nm) relative to low-to-medium wavelength absorption (300-500 nm) and explained 89.38 % of the variation. PC 2 represented low-to-medium wavelength reflectance (300-540 nm) relative to medium-to-long wavelength absorption (540-700 nm) and explained 5.06 % of the variation.

In 2012 dewlaps, 92. 5% of the variation of the center was reduced to 2 PC's (**Figure 2 c&d)**. PC 1 represented low to medium wavelength reflectance (300-560 nm) relative to medium to long wavelength absorption (560-700 nm) and explained 80.02% of the variation. PC2 represented medium to long wavelength reflectance (~550-700 nm) relative to medium & low wavelength absorption (300 – 550 nm) and explained 12.35% of the variation of the dewlap spectra.

Spectral variation of the dewlap edge was reduced to two PC's that explained 93.43 % of the variation in 2012. PC1 of the dewlap center represented medium-to-long wavelength reflectance (500-700 nm) relative to medium to short wavelength absorbance (300-500 nm) and explained 87.33 % of the variation of the dewlap center. PC2 represented long wavelength absorbance (520-700 nm) relative to low to medium wavelength reflectance (300-520 nm) and explained 6.10 % of the spectral variation.

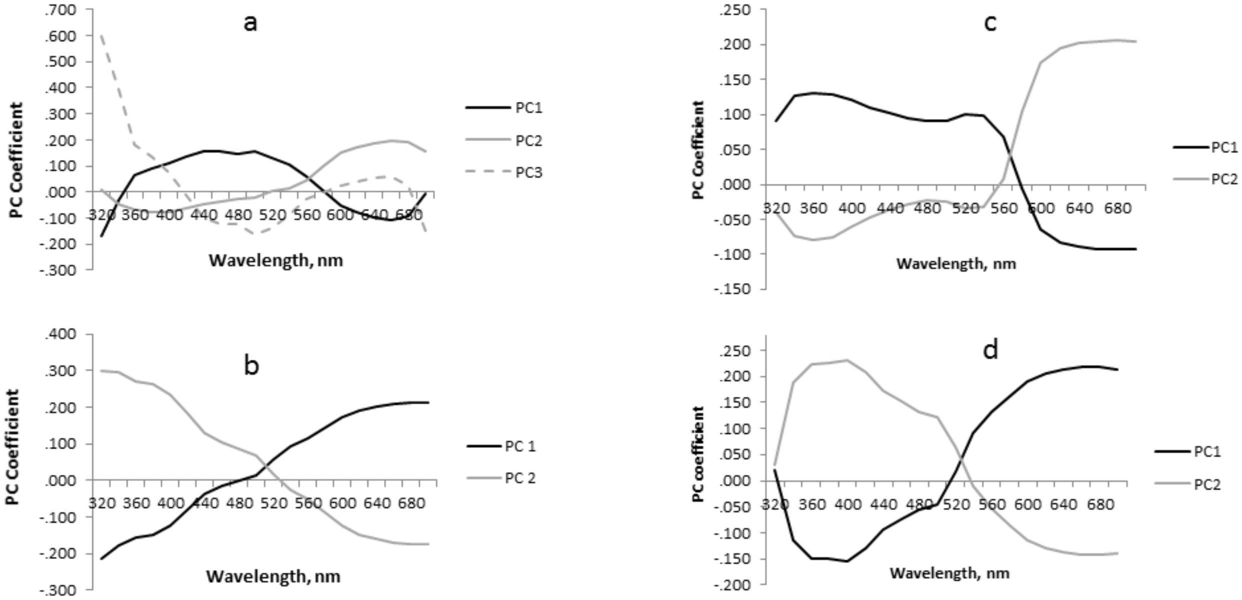

Figure 2a-d. Spectral variation of male brown anole dewlaps in 2011 (a = center, b = edge), and 2012 (c = center, d = edge) transformed and expressed as PC coefficients. 2011 dewlap center PC1 represented 74.83 % , center PC2 represented 15.91 %, and center PC3 represented 6.44 % of the spectral variation. Dewlap edge PC 1 represented 89.38 % of the spectral variation and edge PC2 represented 5.06 % of the spectral variation. 2012 dewlap center PC1 represented 80.02 % and center PC2 represented 12.35 % of the spectral variation. Dewlap edge PC 1 represented 87.33 % of the spectral variation and edge PC2 represented 6.10 % of the spectral variation.

Female temporal association with males—females showed variation in time spent in front of the right, center, and left tanks in each year, but that variation did not correlate with time spent in front of the male with the richer dewlap center (2011 PC 1 $X^2 = 0.553$, $P > 0.05$; PC 2 = 0.608, $P > 0.05$, PC 3 = 0.501; 2012 PC1 $X^2 = 0.569$, $P > 0.05$; PC 2 = 0.520, $P > 0.05$), or the dewlap edge (2011 PC 1 $X^2 = 0.569$, $P > 0.05$; PC 2 = 0.520, $P > 0.05$; 2012 PC1 $X^2 = 0.553$, $P > 0.05$; PC 2 = 0.608, $P > 0.05$) see Table 1.

The variation in time spent in in front of the right, center, and left tanks did not correlate with time spent in front of males that displayed more. (e.g. 2011 HB rate $X^2 = 0.498$, $P > 0.05$, 2012 HB rate $X^2 = 0.492$; 2011 DE rate $X^2 = 0.441$, $P > 0.05$ 2012 DE rate $X^2 = 0.469$, see Table 1) or tanks that contained no males (i.e. 2011 empty tank, $X^2 = 0.536$, $P > 0.05$; 2012 empty tank, $X^2 = 0.542$, $P > 0.05$) (Table 1).

Year	Trait	Trait	L	C	R	X^2	P
2011	Center spectra	Color PC 1	11(13)	7(9)	7(3)	0.530	> 0.05
		Color PC 2	11(13)	7(9)	7(3)	0.530	> 0.05
		Color PC3	10(13)	8(9)	7(3)	0.501	> 0.05
	Edge spectra	Color PC 1	15(13)	3(9)	7(3)	0.733	> 0.05
		Color PC 2	6(13)	8(9)	11(3)	0.536	> 0.05
	Behavior	Empty tank	6(13)	11(9)	8(3)	0.536	> 0.05
		HB rate (# / min)	17(7)	9(12)	6(8)	0.498	> 0.05
		DEW rate (# / min)	16(7)	9(12)	8(8)	0.441	> 0.05
2012	Center spectra	Color PC 1	7(15)	13(8)	7(4)	0.569	> 0.05
		Color PC 2	7(15)	10(8)	10(4)	0.520	> 0.05
	Edge spectra	Color PC 1	6(15)	12(8)	9(4)	0.553	> 0.05
		Color PC 2	6(15)	14(8)	7(4)	0.608	> 0.05
	Behavior	Empty tank	11(15)	6(8)	10(4)	0.542	> 0.05
		HB rate (# / min)	19(6)	7(12)	11(9)	0.492	> 0.05
		DEW rate (# / min)	9(6)	5(12)	4(9)	0.469	> 0.05

Table 1. Chi-square results demonstrating that female temporal associations with right, center, and left tanks containing size-matched males do not associate with tanks containing more colorful, showy Brown Anole males or tanks where no males are present (i.e. empty tanks). Numbers outside and inside parentheses = observed (expected). Numbers = numbers of trials. Edge = dewlap edge, Center = dewlap center, Empty tank = test for avoidance of males, Behavior, HB rate = tank containing male with greater head bob rate, number per minute; DEW rate = tank containing male with greater dewlap extension rate, number per minute.

DISCUSSION

Female brown anoles did not show preferences for more colorful males. Tanks that females spent the most time in front of did not correspond to tanks that contained the most colorful males. When females choose males, females should show longer durations of spatial association with males whom they prefer. For example, females affiliate with more colorful males in guppies, *Poecilia reticulate*, and this affiliation with a particular male is a reliable proxy of a male's ultimate reproductive success (Kodric-Brown 1993). In house finches females prefer the reddest males and they show this by spending the most time in front of the most colorful male's Plexigas compartment (Hill 1990).

Female brown anoles did not show preferences for males who displayed more. In contrast to our study, a recent field study of brown anoles showed that females associated with males who were more active, regardless of territory quality (Flanagan and Bevier 2014). However, in Flanagan and Bevier's study, males were not visually isolated from each other and females were not offered male-free space. Our analysis used glass tanks that had been visually modified to ensure that males were displaying to females, and not to nearby males. In addition, we offered females male-free space to investigate the possibility that male dominance is so intense that females avoid male harassment, and to test this possibility we always presented females with a randomly placed, male-free space. Female showed no temporal association with empty tanks in this study.

Very few lizards show female preference as the primary influence in male (or female) reproductive success. One notable exception to this infrequency is Broad-headed skinks (Cooper and Vitt 1988). In these lizards, females prefer larger males, and larger males defend mate access (Cooper and Vitt 1988). Lizards more commonly show male dominance. Male dominance has been found to be an important determinant of mate success in Wall lizards (Huyghe et al. 2005), Crotaphytine lizards (Husak et al. 2006), Phrynosomatids (Zucker 1994), Eublepharid geckos (Kratovich and Frynta 2002), and Agamid lizards (Whiting et al. 2006).

In at least two lizards (Iberian Rock Lizards, López et. al. 2002; Side-blotched lizards, Hews 1990), as well as several fish, frogs, and birds a combination of male dominance and female choice are determinants of reproductive success (summarized in Andersson 1994). Previous research on brown anoles had not eliminated this possibility because male brown anole lizards perform courtship displays in front of females, as well as rival males. Steffen and Guyer (2014) found that behavior and dewlap color were important predictors of copulation success (i.e. coitus) with females across years. The present findings, along with those of Steffen and Guyer (2014) suggest that pre-copulatory female mate choice is not a selective force in copulation and male reproductive success in brown anoles.

It remains possible that a female's choice becomes apparent over a longer time association scale, such that pre-copulatory preferences are not made until females have had repeated exposure with multiple males. It also remains possible that cryptic (post-copulatory) female choice may act as an intersexual selective mechanism that maintains armament / ornament size and color in brown anoles (as first suggested by Tokarz 1998; Tokarz et al. 2005). Female brown anoles can store sperm from up to 4 males (Calsbeek et al. 2007). Females show post-copulatory fertilization bias to determine hatchling sex and body size is used as a cue for sperm sex sorting (Calsbeek and Bonneaud 2008). Larger males are more dominant in social contests for territories (Tokarz 1985) but determining how sperm sex sorting might be related to social contests and sexual selection is an area for future research.

In summary, speculation about the influence of precopulatory female choice in Dactyloid ornament evolution has dotted the literature (e.g., Sigmund 1983; Tokarz 1995, 1998) because female home ranges often overlap one or more male's territories (Schoener and Schoener 1980; Tokarz 1998). Here we provide behavioral and observational evidence that females do not show temporal associations with males who are more colorful or who display more, as might be expected according to hypotheses about pre-copulatory female choice. We also show that females do not show associations with empty tanks, as might be expected if females avoid harassment due to intense male competition.

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