

Did sexually dimorphic dorsal coloration evolve by a pre-existing bias in males in the lizard *Sceloporus minor*?

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Abstract Theory and empirical evidence indicate that male secondary sex traits can evolve by co-option of pre-existing biases in females. However, relatively few studies have explored whether male pre-existing biases could drive the evolution of traits important in male contests. Male spiny lizards (*Sceloporus*) are characterized by the expression of sexually dimorphic blue throat and abdominal patches. These features are revealed to conspecifics during social interactions, and variation in ventral color can predict the outcome of male contests in some species of spiny lizards. In *Sceloporus minor*, males in some populations also express bright blue color on the dorsal surfaces. Given the significance of blue color in intrasexual signaling in other species of *Sceloporus*, blue dorsal color may have evolved in *S. minor* by co-option of a male sensory bias for the color blue. We tested this hypothesis in a population that exhibits an ancestral phenotype for male dorsal color (brown/orange), and lacks males with bright blue dorsal coloration. Resident territorial males were presented with one of three types of intruder males manipulated in dorsal color by painting. Orange males mimicked the ancestral dorsal phenotype found at the study site; blue males resembled those from a population with the derived (blue) form of this trait; and green males represented a novel stimulus control. If blue dorsal color evolved in *S. minor* in part due to co-option of a male sensory bias, we predicted that resident males would exhibit either increased or decreased levels of aggression to blue intruders relative to controls. We found no difference in resident aggressive behavior across all treatments, thus failing to support the predictions of a pre-existing bias. We discuss these findings in the context of social behavior in *Sceloporus*, and propose directions for further study in this species.

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Introduction

Male secondary sex traits evolve by two major mechanisms: the expression of female mating preferences (mate choice) and outcomes from contests between males (contest competition; Andersson 1994). In male contest competition, males engage in contests for access to a limited resource (e.g., food or mates) and assess the quality of opponents by evaluating traits that predict opponent resource holding potential (RHP; Andersson 1994). Typically, males with more elaborate traits defeat their less showy rivals, even in the absence of direct physical contact (Andersson 1994). In female choice, females exhibit a preference for certain males as a function of variation in male traits; typically, males with more elaborate traits are preferred as mates.

A pre-existing sensory bias is a bias in the sensory system that generates a preference for certain stimuli over others (Endler and Basolo 1998). The evolution of male traits in response to pre-existing female sensory biases is termed sensory exploitation (Ryan and Rand 1993) or sensory traps (Christy 1995), depending on whether the bias was adaptive in a different context prior to the evolution of the male trait (Endler and Basolo 1998). Pre-existing bias models (including sensory exploitation and sensory traps) are distinct from other major classes of models for female choice in that female preferences evolve prior to the evolution of preferred male traits (Ryan and Rand 1993). As a consequence, the pre-existing bias hypothesis can only be tested within the context of a phylogeny (Endler and Basolo 1998). Basolo (1990, 1995a) identified several conditions that should be met to conclude that a pre-existing bias is present in a lineage, and thus potentially available for signalers to exploit in the context of mate acquisition. First, within a clade of closely related taxa, males of one species or population should express a secondary sex trait that females use in mate choice. Second, males in a closely related species should lack this trait entirely, or exhibit only a reduced form. Third, females from populations where males express the ancestral form of the trait should mate preferentially with males with the derived form of the trait (or a synthetic analog). The extent to which the first condition is requisite has been disputed, however; if preferences confer high costs to females, such preferences may be only weakly expressed in extant derived taxa ('chase-away' hypothesis; Holland and Rice 1998).

Empirical studies have found evidence of pre-existing biases in a wide range of taxa, including arthropods (Proctor 1991, 1992; Sakaluk 2000; Córdoba-Aguilar 2002; Stålhandske 2002), fish (Basolo 1990, 1995a, b, 2002; Rodd et al. 2002; Macias Garcia and Ramirez 2005; Tobler 2006), amphibians (Ryan et al. 1990; Ryan and Rand 1993; Karuzas et al. 2004), birds (Madden and Tanner 2003), and primates (Miller et al. 2004). Other studies have found no support for pre-existing biases (Olsson 2001; Borgia and Keagy 2006), or failed to confirm existing support following revision to the background phylogenetic hypothesis (Ron 2008). While there is debate about the strength of existing evidence for sensory biases and their role in sexual selection (e.g., Fuller et al. 2005), the view that pre-existing biases represent a potentially important mechanism for the evolution of male secondary sex traits under female choice is widely supported (e.g., Endler and Basolo 1998; Arnqvist 2006; Rodríguez 2009). Similar models have been applied to other signaling contexts, including aposematism (Smith 1977), predator/prey strategies (Fleishman 1992; Nelson et al. 2010), and floral pollination syndromes (Schaefer and Ruxton 2009), suggesting that much of signal diversity may be explained by receiver biases.

Pre-existing biases of male contest traits

A relatively unexplored question is whether signals that are important in male–male contest competition could also arise via pre-existing biases (i.e., male biases of male contest traits). Endler (1992) noted that there is no obvious restriction to this possibility, since the traits used in signals to rivals should be subject to the same design constraints as those used in mate attraction. Nevertheless, relatively few studies have directly addressed this issue (Ryan and Rand 1998; Quinn and Hews 2000). One interesting aspect of the hypothesis of pre-existing biases of male contest traits is that multiple predictions might be consistent with this hypothesis. For example, if displays of the novel signal reduce aggression in opponents lacking the signal, both high- and low-quality males (i.e., cheaters) could achieve high fitness. Trait reliability could be established if a correlation between RHP and signal quality then evolved rapidly (Maynard Smith and Harper 2003). Alternatively, males that produce the signal might generate increased aggression from rivals. If only males with high RHP produce the signal, and these males win contests against aggressive but poor-quality fighters, then the signal could be maintained as a reliable indicator of male quality. In sum, multiple outcomes could be consistent with male pre-existing biases of male traits; however, the key prediction is that opponents with the derived form of the trait would elicit different levels of aggression relative to controls.

Since many male traits can serve to both attract females and deter rivals, evidence of pre-existing biases of male contest traits may be more readily detected in taxa where male contest competition is especially intense and where female choice of traits is relatively weak. Members of the genus *Sceloporus* (Squamata: Phrynosomatidae) would seem to fit these criteria. The genus *Sceloporus* is a diverse assemblage of diurnal New World lizards (Wiens and Reeder 1997) characterized by the use of bright blue ventral patches in stereotyped social displays (Carpenter 1978; Wiens 1999). Adult males defend territories that typically contain multiple females (Abell 1999; Haanel et al. 2003a) and attempt to exclude rivals from access to females (Ruby 1978). In most species only males possess colorful abdominal and throat patches (Wiens 1999), features revealed to conspecifics during courtship and aggressive interactions (Cooper and Burns 1987). There is relatively little evidence of overt female mate choice in spiny lizards (e.g., Quinn 2001) but good evidence that the outcome of male–male competition predicts mating success (e.g., Haanel et al. 2003b).

Given that blue color badges were probably present in the common ancestor of *Sceloporus* (Wiens 1999), and appear to be relevant to the social behavior of at least some extant species (Cooper and Burns 1987), blue color may have played an important and possibly fundamental role in the social evolution of spiny lizards in general (Wiens et al. 1999). In this context, a study by Quinn and Hews (2000) with the striped plateau lizard *S. virgatus* is particularly intriguing. In *S. virgatus*, males lack blue abdominal patches that phylogenetic reconstructions indicate were present in a recent common ancestor (Wiens 1999). Quinn and Hews (2000) paired male *S. virgatus* with opponents whose abdominal patches were manipulated for color and pattern by painting. They found that males paired with opponents painted blue on the abdomen (but not other colors) were significantly more likely to retreat following presentation of these patches by an opponent (Quinn and Hews 2000). The response by males of *S. virgatus* to males with the replaced blue signal may be analogous to the ‘residual preference’ pre-existing bias model of female choice (Basolo 1995a). In this model, trait and preference co-occur at some point in time, but the trait is subsequently lost. Females retain the preference for the lost trait, and this bias is available for males to co-opt in the future (Basolo 1995a). In *S. virgatus*, males may have retained a response to blue as an aggressive signal, following a secondary loss of the signal.

The results of Quinn and Hews (2000) do not demonstrate evolution as a function of a sensory bias, but do suggest that males of *S. virgatus* exhibit a sensory bias that could be exploited by males in the future. By extension, it is therefore conceivable that other species of *Sceloporus* could retain evidence of the historical evolution of blue badges as a function of male sensory biases.

Study species

The minor lizard *Sceloporus minor* Cope is a common and conspicuous inhabitant of boulders and rock outcroppings throughout the southern Chihuahuan Desert of central México (Chrapliwy 1964). Like most spiny lizards, adult males exhibit bright blue ventral and throat coloration (Smith 1936). However, the species is distinguished by exceptional variation in sexually dimorphic dorsal color. Males in populations in Hidalgo, Querétaro, and Tamaulipas exhibit a bright blue head, legs and tail; some males express a uniform cobalt blue dorsum whereas other males express shoulder patches that range from light yellow to dark red (Martin 1958; Chrapliwy 1964; Stephenson 2010). In the northern and western parts of its range (e.g., states of San Luis Potosí and Zacatecas), male dorsal color may be gray, yellow, brown, or orange (e.g., Webb and Axtell 1994; Wiens et al. 1999). Phylogenetic analyses of *S. minor* indicate that populations with bright, cobalt blue males are derived from populations that lack this phenotype; moreover, blue dorsal color evolved at last twice from a brown/orange ancestral condition (Wiens et al. 1999; Wiens and Penkrot 2002; Fig. 1). If male dorsal coloration is important in male contest competition in *S. minor*, bright blue dorsal color could conceivably have evolved via a pre-existing sensory bias for this trait in males (Wiens et al. 1999). One consideration is that dorsal color in *S. minor* is not necessarily a monomorphic trait; as Wiens et al. (1999) noted, not all males in at least some populations these authors scored as blue actually express a uniform blue dorsum (see also Stephenson 2010). Nevertheless, the expression of bright, cobalt blue dorsal color in males—on the back (i.e., the dorsum, sensu Stephenson 2010), head, legs, and tail—is clearly present in only a subset of populations in this species.

We tested whether resident adult males from a population in southwestern San Luis Potosí that express the hypothesized ancestral condition of dorsal color (brown/orange: Wiens et al. 1999) respond differently to intruder males painted to mimic the derived bright

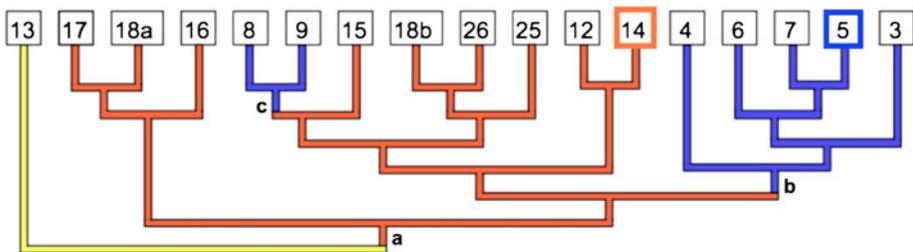


Fig. 1 Hypothesized evolutionary relationships of populations of *Sceloporus minor* showing major dorsal color phenotypes sensu Wiens et al. (1999). Orange (clade a, with the exception of clades b and c) corresponds to the ‘brown’ character state of Wiens et al. (1999), and indicates populations that express a phenotype varying from brown to orange. Blue (clades b, c) indicates populations where males express bright blue dorsal color on some or all dorsal surfaces. Yellow (outgroup) represents hypothesized ancestral dorsal color character state for *S. minor*. The study site of Escalerillas corresponds to Population 14, and the source population for the referential derived blue dorsal color phenotype (La Manzana) corresponds to Population 5. Phylogeny modified from Wiens et al. (1999) (Color figure online)

blue phenotype characteristic of populations in northwest Hidalgo state relative to control groups. We predicted that if a male pre-existing bias explains the existence of bright blue dorsal color in *S. minor*, blue-painted males would receive either increased or decreased aggression relative to both orange males (to mimic the putative ancestral phenotype) and green males (a novel stimulus control). The addition of the novel stimulus control group is important in this design, as a difference in aggression between blue and orange males would not by itself be good evidence of a sensory bias. Instead, if there was no difference in aggressive behavior by residents toward blue males as opposed to green males, this would suggest that any observed difference between blue and orange males is more likely due to resident males responding to an unfamiliar stimulus, rather than by sensory stimulation to the color blue per se.

Materials and methods

Research was conducted near the community of Escalerillas, San Luis Potosí, México (22°5′N, 101°4′W) from 11 October to 23 November 2007. This period coincides with the expected peak of male breeding behavior in this population (Ramírez-Bautista et al. 2008). Escalerillas is located about 3 km from the community of Colonia Insurgentes, the location of Population 14 in Wiens et al. (1999). The town of La Manzana, Hidalgo (20°52′N, 99°13′W), which served as a reference population for derived male color, is located about 2 km from Population 5 in Wiens et al. (1999).

Experimental treatments

Resident males ($N = 27$) and intruder males ($N = 29$) were collected at separate sites in the Escalerillas area, since previous interactions between lizards may predict the outcome of future contests (Forster et al. 2005). In all cases, resident and intruder pairs were found at locations separated by at least 500 m, a dispersal distance almost twice as great as that observed for any individual in another population (La Manzana) of *S. minor* (B. Stephenson, unpublished data). Lizards were captured by noose on their territories, and brought to a nearby residence that served as a field station within a few hours. Both resident and intruder males were measured for color in a darkroom using a portable spectrometer (see “Appendix 1” for additional details). After collection of spectral data to measure natural dorsal color, males were administered a small dose (0.07–0.11 cc) of 2% lidocaine by subcutaneous injection. Following onset of anesthesia, lizards were measured for mass and snout-vent length (SVL) following the trait definitions and measurement protocols of Stephenson (2010). An index of body condition was calculated as $[\text{mass}^{1/3}/\text{SVL}]$ (Olsson 1994). Resident males were then marked by applying a unique sequence of colored beads to the base of the tail (Fisher and Muth 1989). Following recovery from anesthesia, marked residents were released back onto their territories. Residents were always released the day after capture, thus helping minimize the potential for territory takeover by neighboring males (Patterson 2002), and possible associated confounding effects on resident behavior. Intruder males were not marked with beads, but instead were assigned to one of three paint treatment groups (Fig. 2a–c). Intruders painted blue on the dorsum (Fig. 2a) were designed to mimic the derived phenotype present in some males (blue morphs: Stephenson 2010) from La Manzana (Fig. 2d). Orange intruder males (Fig. 2c) were painted to resemble the most common dorsal phenotype found in males at Escalerillas (Fig. 2e). A third class of intruder was painted green (Fig. 2b), a phenotype

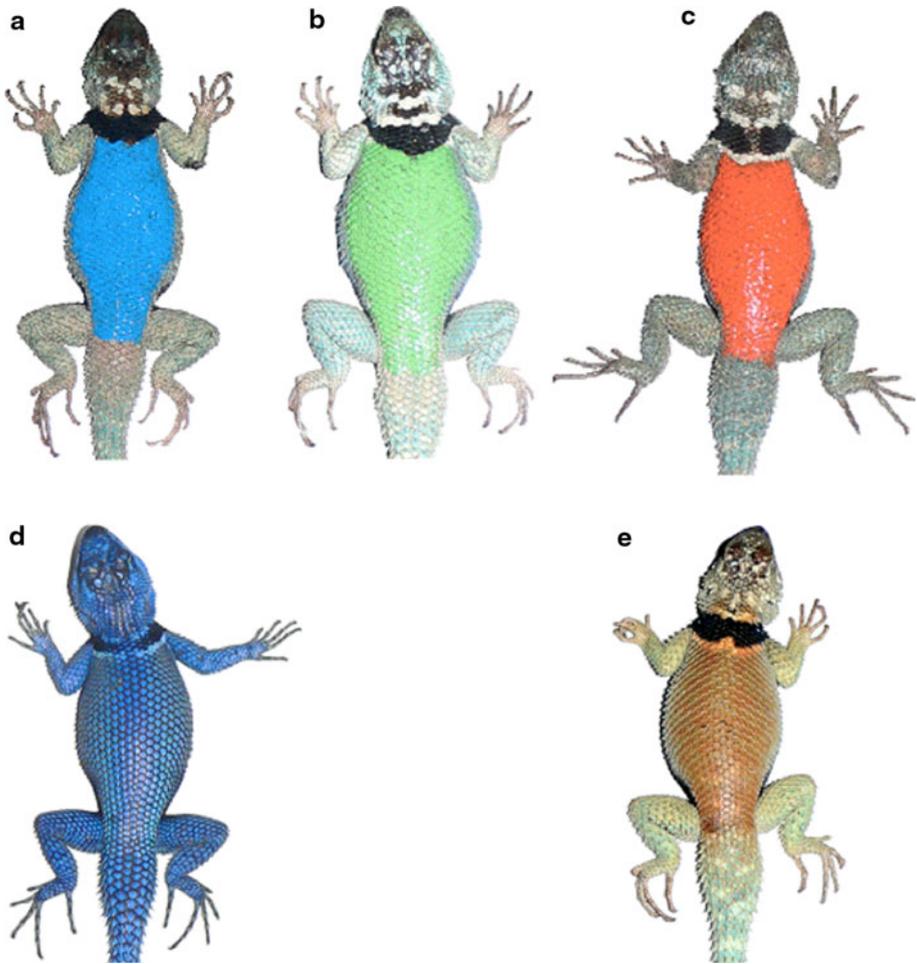


Fig. 2 a–c Examples of painted male treatments used in staged introductions. **a** Blue, **b** green, **c** orange. **d** Unpainted blue morph male from La Manzana, Hidalgo. **e** Unpainted orange male from Escalerillas, San Luis Potosí (Color figure online)

that does not occur in *S. minor* (Wiens et al. 1999), its sister taxon *S. serrifer* (Martínez-Méndez and Méndez-de la Cruz 2007; Wiens et al. 2010) or in other squamate reptiles found at the study site (Grant and Smith 1959; B. Stephenson, unpublished data). Change in color of the dorsum following application of paint was confirmed by spectroradiometry after drying (Fig. 3). All paints used were of a brand certified non-toxic to humans (Chromacryl; Stuart-Fox and Johnston 2005).

Experimental trials

Trials were conducted 1–3 days following capture of intruders. Intruder males were introduced onto the territory of residents with the aid of a telescoping fishing pole and a 1-m long tether (Cooper and Burns 1987). The fishing pole attained a length of approximately 5 m when fully extended, and so the minimum distance by researchers to each

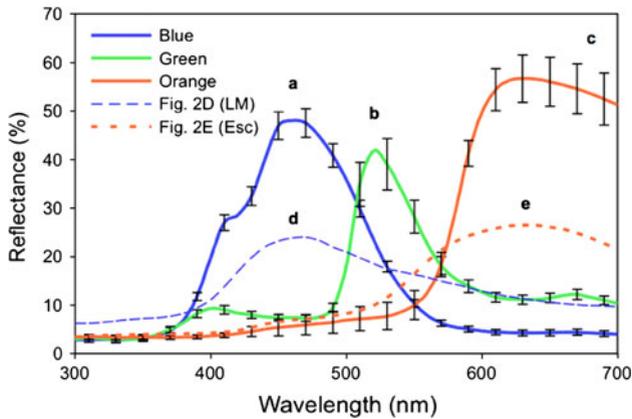


Fig. 3 Mean reflectance spectrum for each of three paints used in intruder experiments: *blue* (a), *green* (b), and *orange* (c). Reflectance measured following application of paint to dorsum of intruder males, and expressed relative to a 99% white standard. Spectra plotted as average of medians of 10 nm bins; *standard error bars* plotted at 20 nm increments for clarity. $N = 9$ males/paint treatment. Spectrum from representative *blue* male from La Manzana (Fig. 2d) and *orange* male from Escalerillas (Fig. 2e) provided for comparison (d, e) (Color figure online)

resident male at the start of each trial was 3–5 m. After the targeted resident male was sighted, the selected intruder lizard was placed onto the same boulder (or boulder complex) at a spot 1–3 m away from the resident male. A trial started once the intruder male was judged to be clearly within the line of sight of the resident male. If the resident was determined to have not seen the intruder after 20 min following placement of the intruder male on the boulder, the trial was postponed for 24 h or canceled entirely. Most resident males retreated to crevice refuges following initial approach by researchers, but usually reappeared where first seen within 10 min. Due to time constraints, marked males were rejected for use as residents if they failed to re-emerge from crevices within the line of sight of observers within approximately 1 h on at least two separate occasions. Trials lasted up to 20 min, but were preemptively stopped if sustained physical contact was maintained for more than 60 s, or if the risk of injury to either lizard was considered excessive (e.g., sustained biting, retreating to a boulder crevice with tether). Experiments were recorded using a video camera (Panasonic PV-GS300) positioned 3–5 m from the resident male at the start of the trial. Responses of residents to intruders were scored by viewing videotapes of each trial. At the conclusion of each trial, intruder lizards were cleaned of stimulus paint, marked with a small white paint dot at the base of the tail to indicate previous use, and released at the site of capture no more than 3 days after collection. Each resident male and intruder male was used in only one trial. Resident males were not recaptured following trials, and so retained their beads after the conclusion of experiments. However, observations from a marked population of *S. minor* in Hidalgo indicate that most lizards naturally shed their beads over the course of a year, with no obvious effect on behavior within or across years (B. Stephenson, unpublished data).

Statistics

Rates of all display variables were adjusted in proportion to the length of the trial and the proportion of time the resident was visible to the camera during the trial. Principal

Table 1 Ethogram of behavior patterns scored from videotaped trials of *Sceloporus minor* at Escalerillas

| Behavior | Description |
|------------|--|
| Headbob | One or two rapid and continuous up-and-down vertical movements of the head followed by pause of 0.5 s or more. May be performed while walking or standing still. A headbob sequence is a continuous series of headbobs with a pause of <3 s between consecutive headbobs. |
| Pushup | Slow, exaggerated vertical movement of the head, usually accompanied by an extension of the forelimbs that results in an elevation of the anterior half of the torso. Cannot be performed while walking. A pushup sequence is a continuous series of pushups with a pause of <3 s between consecutive pushups. |
| Throat Fan | Extension of the throat in the vertical plane exposing the blue throat fan (dewlap). |
| Fullshow | Lateral compression that exposes the blue abdominal patches accompanied by an extension of the throat fan. |
| Hop | Short hop, often in a lateral direction, that results in at least two but usually all four limbs lifted into air. |
| Chase | A. Sprinting towards an opponent that results in displacement of the opponent, or B. Sprinting towards opponent <2 s after opponent runs in opposite direction. |
| Escape | Sprinting away from opponent. |
| Straddle | Placement of body across midsection of opponent, typically in a longitudinal orientation. |
| Headbutt | Push of opponent lizard with head (often around midsection) without biting. |
| Bite | Opponent lizard is seized with jaws. |

components analysis (PCA) was used to transform 16 behavioral display variables to a smaller subset of orthogonal variables. The broken-stick criterion advocated by Jackson (1993) was used to determine the number of PCs to retain for analysis. Because data failed to meet conditions for parametric tests, we used the non-parametric Kruskal–Wallis and Mann–Whitney tests in hypothesis testing. Statistics were performed using SYSTAT 12 (SYSTAT Software, Inc.) and JMP 8 (SAS Institute, Inc.). For all tests, alpha was set to 0.05 with Bonferroni adjustment for multiple tests (Rice 1989).

Results

Following release, 26/27 (96%) marked resident males were resighted at least once, and all exhibited behavior consistent with normal territorial activity. Of those located, 23/26 (88%) were found at or near their capture site within 72 h of release. Two of the remaining three males were subsequently located but failed to meet behavioral criteria necessary to carry out a trial (i.e., failing to reappear within 1 h of researcher approach on two separate occasions) and so were not used. Overall, 24 successful trials were performed with eight trials per treatment group.

Resident males and intruder males did not differ in body size (SVL: Mann–Whitney $U = 340$, $P = 0.28$) or condition (Mann–Whitney $U = 348$, $P = 0.22$). No difference was found between the three resident male groups in body size (SVL: Kruskal–Wallis $H = 5.08$, $P = 0.08$) or condition (Kruskal–Wallis $H = 2.13$, $P = 0.34$). Similarly, there was no relationship between paint treatment and intruder male body size (Kruskal–Wallis $H = 2.31$, $P = 0.31$) or condition (Kruskal–Wallis $H = 0.45$, $P = 0.80$).

We first tested for differences in resident male aggressive behavior using a subset of behavioral variables (Tables 1, 2) hypothesized a priori to be particularly informative with

respect to differences in male agonistic behavior. Of six response variables examined, there was a trend towards a difference in *Latency to first fullshow*; painted blue intruder males tended to receive a resident male's first full show more quickly following detection of the intruder by the resident than orange or green intruder males. However, this difference was not significant following Bonferroni adjustment.

We then extended the analysis to include 16 display variables (Tables 1, 3), and used PCA to generate a smaller number of orthogonal variables for use in additional statistical tests. The first principal component (PC1) of the behavioral response scores was found to explain 37.9% of the variation among behavioral variables. All display variables except *Escapes/min*, *Latency to first approach*, *Latency to first display*, and *Latency to first fullshow*

Table 2 Summary data for tests of differences in resident male responses to intruders using a preselected subset of untransformed behavioral variables

| Variable | <i>H</i> | <i>P</i> |
|---------------------------|----------|--------------|
| Headbobs/min | 0.24 | 0.88 |
| Pushups/min | 1.36 | 0.50 |
| Fullshows/min | 4.78 | 0.09 |
| Latency to first approach | 4.14 | 0.13 |
| Latency to first display | 1.14 | 0.56 |
| Latency to first fullshow | 7.08 | <i>0.029</i> |

Kruskal–Wallis *H* tests used for all comparisons. *N* = 8 for each treatment. All *P* values are unadjusted and shown in italics when *P* < 0.05

Table 3 Factor loadings for PC1 and descriptive statistics for all 16 scored response variables of resident males to intruders in staged introduction trials

| Variable | PC1 loadings | Intruder male color | | |
|---------------------------|--------------|---------------------|-----------|------------|
| | | Blue | Green | Orange |
| Headbobs/min | 0.960* | 2.3 ± 0.6 | 1.8 ± 0.5 | 1.9 ± 0.7 |
| Headbob sequences/min | 0.878* | 0.7 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.2 |
| Headbobs/sequence | 0.656* | 2.8 ± 0.5 | 2.8 ± 0.4 | 2.2 ± 0.2 |
| Pushups/min | 0.834* | 1.5 ± 0.3 | 1.1 ± 0.2 | 1.6 ± 0.3 |
| Pushup sequences/min | 0.832* | 0.6 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 |
| Pushups/sequence | 0.199 | 2.0 ± 0.5 | 2.9 ± 0.3 | 2.4 ± 0.2 |
| Throat fans/min | 0.433 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.0 ± 0.0 |
| Fullshows/min | 0.805* | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.3 |
| Chases/min | 0.419 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.0 |
| Bites + Headbutts/min | 0.663* | 0.5 ± 0.3 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| Straddles/min | 0.684* | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.1 |
| Hops/min | 0.402 | 0.7 ± 0.4 | 0.3 ± 0.2 | 0.0 ± 0.0 |
| Escapes/min | −0.345 | 0.1 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Latency to first approach | −0.224 | 0.0 ± 0.0 | 2.0 ± 0.7 | 2.3 ± 1.4 |
| Latency to first display | −0.151 | 0.4 ± 0.2 | 0.8 ± 0.5 | 1.5 ± 0.6 |
| Latency to first fullshow | −0.486 | 2.8 ± 2.5 | 6.9 ± 2.9 | 10.8 ± 3.2 |

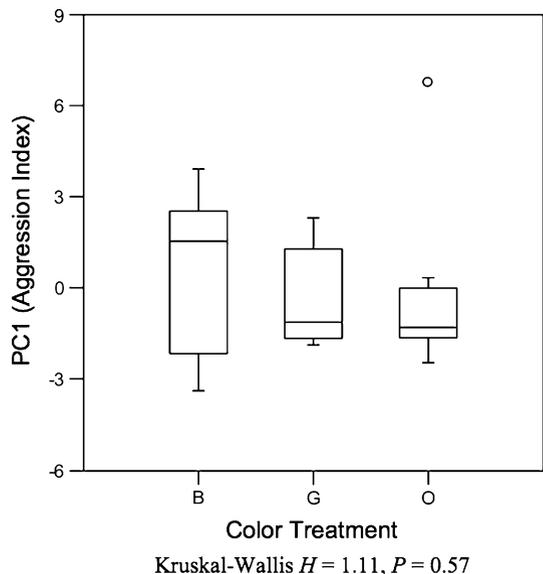
Means reported ± SE. *N* = 8 for each treatment. Variables significantly correlated with PC1 following sequential Bonferroni adjustment indicated by (*)

(Tables 1, 3) loaded positively onto PC1, indicating that PC1 describes variation in resident male aggressive behavior (Table 3). In addition, there was a significant positive correlation of PC1 with eight display variables: *Headbobs/min*, *Headbob sequences/min*, *Headbobs/sequence*, *Pushups/min*, *Pushup sequences/min*, *Fullshows/min*, *Bites and Headbutts/min*, and *Straddles/min* (Tables 1, 3). There was also a trend towards a positive correlation of PC1 with *Chases/min* and *Throat fans/min*, and a trend towards a negative correlation of PC1 with *Latency to first fullshow* (Table 3), but these relationships were non-significant following sequential Bonferroni adjustment. PC2 and PC3 explained 14.3% and 11.6% of the remaining variation in behavioral variables; however, only PC1 was retained for analysis, since PC2 explained less variation than that required (14.8%) under the broken-stick model (Shaw 2003). A test for differences in PC1 score by intruder color was non-significant (Kruskal–Wallis $H = 1.11$, $P = 0.57$; Fig. 4), indicating that resident males did not differ in the intensity of aggressive behavior directed towards intruder males of any color type. Removal of a single extreme outlier (Fig. 4) from the Orange treatment group did not change the outcome (Kruskal–Wallis $H = 1.79$, $P = 0.41$). Finally, we also tested whether resident males differed in aggressive behavior towards blue and orange males only, which could provide justification for repeating the entire experiment using a larger sample size. However, no significant differences in resident male behavior towards these two groups alone were found (see “Appendix 2”).

Discussion

Endler (1992) proposed that animal signals evolve in predictable directions due to bio-physical constraints on signal design, changes in the physical features of the environment, or the sensory properties of receivers, a phenomenon he termed sensory drive. One component of sensory drive is sensory bias (Endler and Basolo 1998), which posits that

Fig. 4 The effect of male intruder color on resident aggression in male *S. minor* at Escalerillas. $N = 8$ for all treatment groups



secondary sex traits can evolve solely in response to preceding changes in the sensory properties of receivers, rather than via any intrinsic correlation between the expressed trait and receiver benefits (Ryan and Rand 1993). This idea was developed partly to account for the observation that females can exhibit preferences for traits that do not occur within their population or species (Burley and Symanski 1998) but which appear in closely related taxa (Basolo 1995a). Although mate choice in males has received less attention than female choice, there is evidence for male pre-existing biases of female secondary sex traits (Basolo and Delaney 2001; Basolo 2002).

An intriguing question is whether traits important in male contest competition could evolve the same way. Males of many lizards make use of conspicuous visual traits in social interactions, and variation in these display traits has been shown to predict contest outcome (Olsson 1994; Stapley and Whiting 2006) and reproductive success (Anderholm et al. 2003). We tested the hypothesis of male pre-existing biases of male contest traits in the lizard *Sceloporus minor*. Males in at least two populations have independently evolved bright blue dorsal coloration, whereas males with the ancestral form of this trait lack bright blue color (Wiens et al. 1999). Blue color is relevant to the sexual signaling of other species of *Sceloporus* (Cooper and Burns 1987; Quinn and Hews 2000) and its expression on the abdomen and throats of males probably represents the ancestral character state for the genus (Wiens 1999). Thus, males that express bright blue color on the dorsum might exploit a pre-existing sensory bias in males (Wiens et al. 1999). Nevertheless, we found no effect of intruder color on aggressive behavior of males in a population with the ancestral phenotype (Fig. 4; Tables 2, 3). As sample size was small ($N = 8$ for all treatments), we subsequently tested the response of resident males to only blue and orange males. A significant difference in aggressive response between these two groups only would not by itself indicate a sensory bias, but might provide a rationale for repeating this experiment in Escalerillas (with appropriate controls) using a larger sample size. However, there was no significant difference in aggressive behavior directed towards males of these two groups alone (“Appendix 2”).

Could meaningful differences in resident male behavior be obscured following transformation of the raw variables by PCA? In a preliminary analysis, we tested for differences across intruder treatments in a subset of untransformed variables we suspected might be especially relevant with respect to the hypothesis: *Headbobs/min*, *Pushups/min*, *Fullshows/min*, *Latency to first approach*, *Latency to first display*, and *Latency to first fullshow* (Table 2). However, Kruskal–Wallis tests of each of these six variables revealed no significant differences following correction for multiple tests (Table 2). This finding is consistent with analysis of PC1 (Fig. 4), derived from all 16 of the display variables measured in this study.

Finally, although males with a blue dorsum sensu Wiens et al. (1999) were not seen at Colonia Insurgentes (Wiens et al. 1999) or during an initial visit to Escalerillas in 2006 by one of us (BPS), one pale blue male was seen during this experiment and served as a resident in one trial. Subsequent color analysis of this individual revealed similarities in several spectral attributes to those of blue males from La Manzana (Stephenson 2010), despite a dramatically different appearance to human observers. In addition, at least one other report has documented pale blue *S. minor* elsewhere in the region (Grant and Smith 1959). These observations are not incongruent with the hypothesized pattern of dorsal color evolution in this species (Wiens et al. 1999), but do suggest that color variation among males at Escalerillas is more complex than previously understood (see Stephenson 2010 for detailed analysis of color variation at this site).

Future directions

In this study, we selected two populations sampled by Wiens et al. (1999) characterized by the expression of brown/orange dorsal color as the ancestral character state, and differing in dorsal color expression. Specifically, we asked whether males from Population 14 (i.e., Escalerillas), which exhibit the ancestral dorsal color state, exhibit a response to cobalt blue dorsal color, a derived phenotype present in males from Population 5 (i.e., La Manzana; Fig. 1). This test assumes that any pre-existing bias present in the most recent common ancestor of these two populations would be retained in males from Escalerillas. Although we found no support for this hypothesis, it is conceivable that a sensory bias present in the common ancestor of Population 5 and 14 was lost in the subsequent evolutionary history of the subclade containing Population 14 (Fig. 1). Evidence of within-species variation in retention of sensory biases is not without precedent in the literature; for example, sailfin mollies exhibit geographic variation in the retention of sensory biases for male swords (Witte and Klink 2005). In this regard, it is notable that blue dorsal color evolved a second time in the common ancestor of Populations 8 and 9. This trait is absent from males in Population 15, the sister taxon to the clade containing Populations 8 and 9 (Wiens et al. 1999; Fig. 1). Thus, a repeat of this experiment using males from Population 15 would be informative with respect to conclusions about the importance (or lack thereof) of male pre-existing biases in the evolution of male dorsal coloration in *S. minor*.

Alternatively, any responses by male *S. minor* to blue dorsal color in conspecific males may have evolved simultaneously with or subsequently to its expression in the two clades where this phenotype presently occurs (Fig. 1; see also Ryan and Rand 1993), rather than preceded it (i.e., sensory bias). If blue dorsal color in males evolved via male contest competition for reasons other than sensory bias, we might expect that resident males from these populations (e.g., La Manzana) respond differently to intruder males from the same population manipulated for dorsal color. Such an approach could be addressed using a design similar to that described in this report. Future experimental and observational studies of *S. minor* should shed light on the extent to which this unusual trait evolved by sexual selection, natural selection, or a combination of these two forces.

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Appendix 1

Spectroradiometry

Males were measured for color using an Ocean Optics USB4000 spectrophotometer and pulsed xenon light source (PX-2) connected to a bifurcated radiance probe. Readings were recorded in terms of percent reflectance in ~ 0.2 nm steps, and expressed relative to a 99%

white standard. For both painted and unpainted males, readings were collected from five separate locations on both the left and right sides of the dorsum, for a total of 10 readings/male. Each reading represented an average of 100 scans at a given point, collected over an integration time of 16 ms. Output was analyzed with Spectrasuite software (Ocean Optics: Dunedin, FL). Painted intruder males were measured for dorsal color a second time after applied paint was allowed to thoroughly dry (typically at least 1 h following application). Following Stephenson (2010), a single spectrum (of ten collected/male) associated with the longest (i.e., reddest) wavelength of peak reflectance (hue: λ_{Rmax}) was selected for use in subsequent spectral analyses. Thus, means for blue, green, and orange males in Fig. 3 were derived from averaging a single spectrum per subject. Spectra from sample males from Escalerillas and La Manzana also represent the single spectrum with the longest hue (Fig. 3). Additional details on protocols for spectroradiometry and rationale for spectral analyses are described in Stephenson (2010).

Appendix 2

Comparisons of blue and orange males only

Restricting our analyses to blue and orange groups only revealed that resident males and intruder males did not differ in body size (SVL: Mann–Whitney $U = 154$, $P = 0.33$) or condition (Mann–Whitney $U = 144$, $P = 0.55$). Blue and orange resident male groups did not differ in body size (SVL: Mann–Whitney $U = 14$, $P = 0.06$) or condition (Mann–Whitney $U = 18$, $P = 0.14$), and there was no difference among treatment groups in intruder male body size (Mann–Whitney $U = 45$, $P = 0.17$) or condition (Mann–Whitney $U = 31$, $P = 0.92$). We conducted a separate PCA using the 16 display variables from blue and orange males only. The PC1 of the behavioral response scores explained 41.2% of the variation among behavioral variables, and was positively associated with all display variables except *Escapes/min*, *Latency to first approach*, *Latency to first display*, and *Latency to first fullshow* indicating that PC1 was correlated with aggressive behavior. Again, only PC1 was retained for analysis, since PC2 explained less variation (14.3%) than that required (14.8%) under the broken-stick model (Shaw 2003). A test for differences in PC1 score by intruder color was non-significant (Mann–Whitney $U = 40$, $P = 0.40$), indicating that resident males did not differ in the intensity of aggressive behavior directed towards blue or orange males alone. We reran this analysis (including performing a separate PCA) after removing a single extreme outlier from the Orange treatment group; however, this did not change the outcome (Mann–Whitney $U = 39$, $P = 0.20$).

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