ORIGINAL PAPER

Looking on the bright side: females prefer coloration indicative of male size and condition in the sexually dichromatic spadefoot toad, *Scaphiopus couchii*

Tatiana Vásquez · Karin S. Pfennig

Abstract Females across many taxa commonly use multiple or complex traits to choose mates. However, the functional significance of multiple or complex signals remains controversial and largely unknown. Different elements of multiple or complex signals may convey independent pieces of information about different aspects of a prospective mate (the "multiple messages" hypothesis). Alternatively, multiple or complex signals could provide redundant information about the same aspect of a prospective mate (the "redundant" or "back-up" signal hypothesis). We investigated these alternatives using spadefoot toads, *Scaphiopus couchii*. Spadefoot toads primarily use calls to attract their mates, but males also exhibit sexually dimorphic coloration. We investigated whether male coloration is indicative of male size, condition, or infection status by a socially transmitted monogenean flatworm. We found that male coloration and dorsal patterning predicts male size and condition but not infection status. Moreover, when we presented females with a choice between a bright male model and a dark male model, we found that females preferred the bright model. Because aspects of males' calls are also associated with male size and condition, we conclude that coloration is a potentially redundant indicator of male phenotype. We suggest that coloration could enhance mate choice in conjunction with male calling

T. Vásquez · K. S. Pfennig (✉)
Department of Biology, University of North Carolina,
Chapel Hill, NC 27599, USA
e-mail: kpfennig@email.unc.edu

Present address:
T. Vásquez
Biology Department, Chaffey Community College,
Rancho Cucamonga, CA 91730, USA

behavior by providing females with a long distance cue that could enable them to identify prospective mates in a noisy chorus environment where the discrimination of individual calls is often difficult. Generally, such redundant signals may facilitate mate choice by enhancing the quality and accuracy of information females receive regarding prospective mates.

Introduction

When courting females, males often use multiple complex traits (reviewed in Candolin 2003; Hebets and Papaj 2005). In their displays to females, males may combine visual cues with acoustic, chemical, or tactile cues (Andersson 1994; Candolin 2003; Hebets and Papaj 2005). However, the functional significance of multiple complex traits remains unclear. Two primary hypotheses have been proposed to explain the use of multiple traits in mate choice: the "redundant" (or "back-up") signal hypothesis and the "multiple messages" hypothesis (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005).

The redundant signal hypothesis argues that multiple traits provide females with the same type of information about a single aspect of the male's suitability as a mate. For example, multiple aspects of a signal may reveal species identity (Kihslinger and Klimley 2002; Hankison and Morris 2003), or multiple aspects of a male display may indicate that a male is in good condition (Birkhead et al. 1998; Grether et al. 1999; see also discussion by Candolin 2003; Jawor and Breitwisch 2004). Such redundant

information may provide females with enhanced accuracy about mate quality: The use of back-up signals may enhance detection of desirable mates, reduce assessment errors, or better enable females to discriminate against cheater males (Møller and Pomiankowski 1993; Johnstone 1996; Rowe 1999; Candolin 2003; Partan and Marler 2005). The redundant signal hypothesis therefore predicts that females will prefer male traits that are indicative of the same aspect of male quality.

The multiple messages hypothesis, by contrast, posits that multiple cues provide females with discrete information about different qualities of a potential mate. For example, one cue may provide information about the male's species identity, whereas a second trait may indicate whether the male is in good condition (Crawford et al. 1997; Gerhardt and Huber 2002; Hankison and Morris 2002). By using multicomponent signals in this way, females can assess males on the basis of several properties, all of which may be critical to a female's mate choice decision. Alternatively, individual females may attend to a particular cue depending on their own circumstances. Regardless, the multiple messages hypothesis predicts that females will prefer male traits that are associated with different aspects of the male phenotype.

In anurans, the most prominent feature of male courtship is usually male calling behavior (Gerhardt and Huber 2002). These signals are generally thought to be the key means by which males attract females or compete with other males (Gerhardt and Huber 2002). Although acoustic signals are themselves potentially complex traits with different aspects of a male's call indicating male size, condition, or species identity (Gerhardt 1982, 1991; Gerhardt and Huber 2002), anurans may also glean information using visual cues (Buchanan 1994; Summers et al. 1999; Burrowes 2000; Sheldon et al. 2003; Abrunhosa and Wogel 2004; Rosenthal et al. 2004; Hartmann et al. 2005; Hirschmann and Hödl 2006; Krishna and Krishna 2006; Narins et al. 2005). In particular, in some anuran systems, male coloration may play a key role in mate choice (e.g., Summers et al. 1999; Burrowes 2000; Sheldon et al. 2003). Indeed, 32 anuran species are sexually dimorphic in coloration (reviewed in Hoffman and Blouin 2000), a pattern that may reflect sexual selection on male coloration. However, relatively little work has been conducted to understand the maintenance of color and pattern diversity in anurans (Hoffman and Blouin 2000).

We sought to assess the potential role of color in mate choice using the spadefoot toad, *Scaphiopus couchii*, which is sexually dichromatic with males often possessing brighter coloration than females (Stebbins 2003). *S. couchii* occurs throughout the southwestern USA and northern Mexico. Breeding by *S. couchii* takes place primarily on a single night after a rainstorm (Bragg 1965), although it can occur during the day (K. Pfennig, personal observation). Breeding aggregations form in temporary rain-filled pools where males call to attract females.

S. couchii adults are host to an endoparasitic monogenean flatworm, *Pseudodiplorchis americanus* (Tinsley and Earle 1983; Tinsley and Jackson 1986; Tinsley 1989, 1990). *P. americanus* initially infects the lungs, potentially causing long-lasting damage (Tinsley et al. 2002) before moving to the host bladder, where it resides and feeds on host blood (Tocque 1993; Tocque and Tinsley 1994). Parasite infection reduces hematocrit levels and fat reserves important for survival during the toad's aestivation period (Tocque 1993; Tocque and Tinsley 1994). Parasite larvae are released from infected hosts at breeding aggregations. Consequently, parasite infection is transmitted socially among adults at the breeding aggregations (Tinsley 1989, 1990).

Female *S. couchii* can potentially use aspects of the males' calls to assess prospective mates. Male call duration is positively correlated with male condition, and dominant frequency is inversely correlated with male size (Pfennig and Tinsley 2002). Because males that are heavier for a given body size also have larger testes for a given size, such males may better fertilize a female's clutch (Pfennig and Tinsley 2002). Moreover, larger males sire offspring with enhanced survival (Woodward 1987). Thus, females are expected to prefer males that are larger or in better condition. Parasitized males are in better condition, and they possess longer calls than unparasitized males (Pfennig and Tinsley 2002), however. Consequently, females that prefer good-condition males may enhance the likelihood that they will mate with a parasitized male. If females are more likely to acquire infection from their mates rather than from other infected individuals in the breeding aggregation, females might trade-off the benefits of selecting good-condition or larger mates with the risk of parasite infection.

When females are given a choice of male calls that are long in duration (indicative of good-condition males that are more likely parasitized) vs male calls that are short in duration (indicative of poor-condition males that are least likely parasitized), female preference depends on the females' own infection status (Pfennig and Tinsley 2002). In particular, parasitized females, as a group, are random in their preferences for long vs short calls, whereas unparasitized females prefer long calls (Pfennig and Tinsley 2002). Thus, based strictly on preferences of call duration, whether females increase their chances of mating with parasitized males appears to depend on their existing infection.

If, however, females used additional signals in mate choice, females might be able to identify males that are both better-condition mates and free of infection. One such additional signal may be coloration. Male *S. couchii* are highly variable in dorsal coloration and pattern. Males can

range from light yellow-green with some spotting but very light (or no) patterning, to yellow-green with a heavy dark green mottled pattern (Fig. 1). This latter phenotype is very similar to that of females, which are typically yellow-green with a dark green mottled pattern.

For this study, we sought to determine whether male color is indicative of male condition, size, or infection status. Moreover, we evaluated whether male color was correlated with call duration, a character females may use in mate choice (Pfennig and Tinsley 2002). We then determined if females potentially assess coloration during mate choice.

Materials and methods

Male size, condition, infection status, and call duration

In 1999 and 2000, 68 males (20 in 1999 and 48 in 2000) were collected from breeding aggregations in populations near Portal, AZ, and their color analyzed (details of color analysis are below). Each male's snout–vent length (SVL) and mass were measured within 24 h of capture. In 1999, the captured males were dissected, and their infection status by *P. americanus* was determined (detailed methods are

Fig. 1 *Scaphiopus couchii* pairs at a natural breeding aggregation. Note coloration of males relative to females and differences between males in coloration. Male at *left* is an extremely bright male. Male at *right* is intermediate in coloration. Males can also closely resemble females in coloration and pattern. Photo by David Pfennig. (To see this image in color, go to Behav Ecol Sociobiol online)

provided in Pfennig and Tinsley 2002). To determine infection status by *P. americanus* in 2000, each toad was illuminated against a fiber optic light source to look for adult parasites in the toad's bladder. This method is reliable to determine parasite infection because the skin of *S. couchii* is transparent under light and parasites are clearly visible in the toad's bladder (Pfennig and Tinsley 2002).

To obtain a standardized measure of mass, we saved the residuals of a cubic regression of mass on SVL. We refer to these residuals as "condition" hereafter; males that are heavier for a given body size will have higher values of this condition index than males that are lighter for a given body size. Males that are in better condition may have larger testes and longer calls but may be more often parasitized than males in poorer condition (Pfennig and Tinsley 2002).

We used correlation analysis to relate these measures of male size and condition to male color and pattern (see below). In 1999, mating calls of 19 of the above 20 males were also recorded before their collection, and their calls were analyzed for a previous study (Pfennig and Tinsley 2002). Because we also wished to evaluate whether male call duration (length of a single call in seconds) was associated with male coloration, we related male color and pattern to their call duration as described below. Details of the methods for call analysis are provided in Pfennig and Tinsley 2002.

Male coloration and pattern analysis

We used two approaches to analyze male coloration. First, we analyzed scanned photos of each male using Adobe Photoshop CS v. 8 (Adobe Systems, San Jose, CA) to measure variation in color intensity among the males with respect to three primary colors: red, green, and blue. Second, we assessed variation in pattern intensity among the males using two assays. We used ImageJ software v. 1.37g (US National Institutes of Health) to measure variation in the distribution of gray intensity for the males. We also presented photos to naïve human observers who rated each male's dissimilarity from the females' phenotype of a dark mottled pattern on the dorsum. The details of each analysis are provided below.

To obtain images of the males collected above, we photographed the males within 24 h of capture. Each male was placed on a table approximately 27 cm below the camera. Photographs were taken using a Canon EOS Elan camera equipped with a Canon 35–105 mm (1:4.5–5.6) zoom lens and a ring flash set at 1/60 s, F 4.0 shooting Kodachrome 64-color transparency film. Because of slight differences in lighting, however, exposure levels of the males differed between years. The resulting negatives were then scanned with an EPSON 2480 Photo scanner (with resolution set at 2,391×2,382 dpi) to produce digital images.
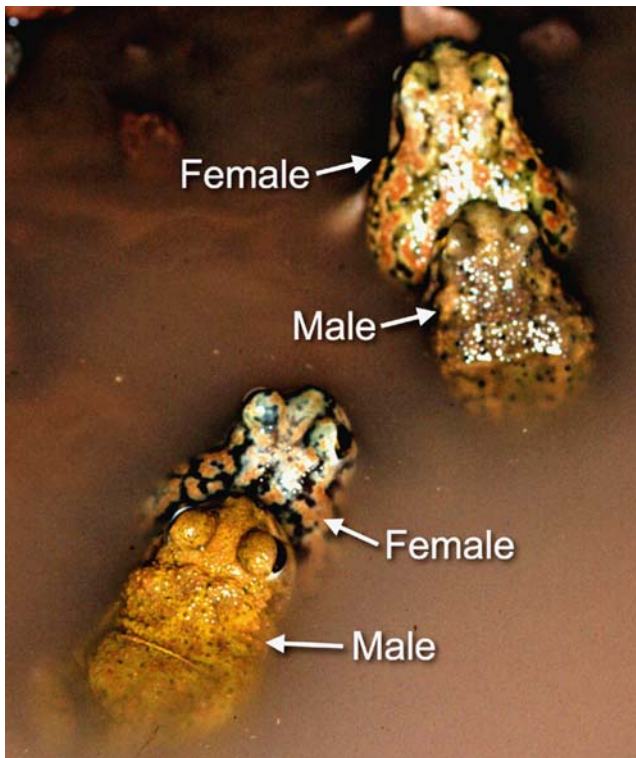
We measured red–green–blue (RGB) color parameters for all males using Adobe Photoshop. To do so, each photograph was overlaid with a grid within Photoshop that was divided into 1-cm squares. Using the marquee selection tool and the histogram option for each of 12 randomly chosen squares on a specimen's dorsum, Photoshop generated a mean intensity of luminosity, red, green, and blue for each square. The 12 values of each color parameter were then averaged to obtain mean values of red, green, and blue for each male. Because of differences in light level between years and slight variation among photos in illumination and reflection from the specimens, we standardized each image by dividing each mean color parameter by the image's mean luminosity (Villafuerte and Negro 1998). Doing so controlled for differences in exposure and allowed us to assess the relative intensity of red, green, and blue among the males.

These intensity measures of red, green, and blue are not strictly independent, however. We therefore used a principal components analysis to reduce these variables to two principal components (referred to as PC1 and PC2, hereafter) that were independent multivariate measures of RGB color variation in the males. These principal components were those that had eigenvalues greater than 1, and together, they described more than 85% of the variation in the data.

To assess the toads' dorsal patterning, we performed two analyses. First, we used ImageJ v. 1.37g to measure variation in gray intensity. Males with mottling similar to a female pattern would be expected to have higher variation in gray intensity than males with little or no mottled pattern. We converted all of the photos from RGB color to 8-bit grayscale using ImageJ. We then generated three vertical transects approximately 300 pixels in length laid at random locations along the side of the toad. We ascertained the mean and standard deviation of the distribution of gray pixels along each transect. We then calculated the mean coefficient of variation of gray pixels for the three transects for each male (Zar 1984). We use this measure as one assay of variation in dorsal pattern.

In a second approach to describing male patterning, we obtained ratings of male pattern from four human observers who were naïve to the study objectives to characterize each male's dissimilarity from the females' typical phenotype. Observers viewed printed images of each male and independently rated each male on a scale of 0 to 2. A score of 0 indicated that a male exhibited a strongly mottled pattern resembling that possessed by females. A score of 2 indicated the absence of mottling on the male and therefore a high dissimilarity to a female's patterning. A score of 1 indicated that the male was intermediate in mottling intensity. The scores of each of the four observers were averaged for each male, and this mean provided us with our

measure of pattern intensity that was an index of dissimilarity from the typical female pattern.

Because our data did not meet parametric assumptions, we used nonparametric Spearman correlation analysis in all tests of association (Zar 1984). We used Wilcoxon normal approximation tests (Zar 1984) for all comparisons of means between parasitized and unparasitized males.

Female preference tests for male color

Females were collected near Portal, AZ, and then transported to the University of North Carolina, where they were housed according to methods in Pfennig 2000. We tested a total of 26 females. All were in reproductive condition (eggs were visible underneath the females' skin).

To assess female mate preferences for male coloration, we presented females with two similarly sized clay models that differed in coloration. One mimicked an extreme "bright" male with no dorsal mottling and the other mimicked an extreme "dull" male with dark dorsal mottling (Fig. 2a). Testing of females was conducted in a darkened room that was sufficiently illuminated by dim white light to observe the females' behaviors.

Each female was placed on a platform in the center of a wading pool filled approximately half-full with water. The central platform on which the female was placed was equidistant between two platforms set 180° apart at the edges of the pool. We placed a speaker on each of these two platforms, so that the speakers were 180° and 1.4 m apart. Two additional platforms were set at 90° from the speakers so that they were directly in front of and behind the central platform on which the female was initially placed (i.e., there were a total of five platforms in the arena). These served as neutral areas for the female where she could leave the water if desired without approaching the speakers.

A clay model was placed directly in front of a speaker so that the dorsum of the model faced outward away from the speaker. From the two speakers, we broadcast 31 s of identical S. couchii calls at identical rates antiphonally, so that the only difference between the sides was the male model. The call stimuli were synthesized using Audacity (an open source software project coordinated by Source-Forge.net) to generate calls consisting of average call properties for the populations from which the females had been collected (see Pfennig 2000 for call distributions). The models were switched after each female was tested to control for side biases. Each female was used only once in the experiment.

At the start of each trial, a female was placed in an opaque container for an acclimation period of 10 min. We began playing the call stimuli at the start of this period. At the end of the acclimation interval, the female was released and allowed to move freely around the arena while the
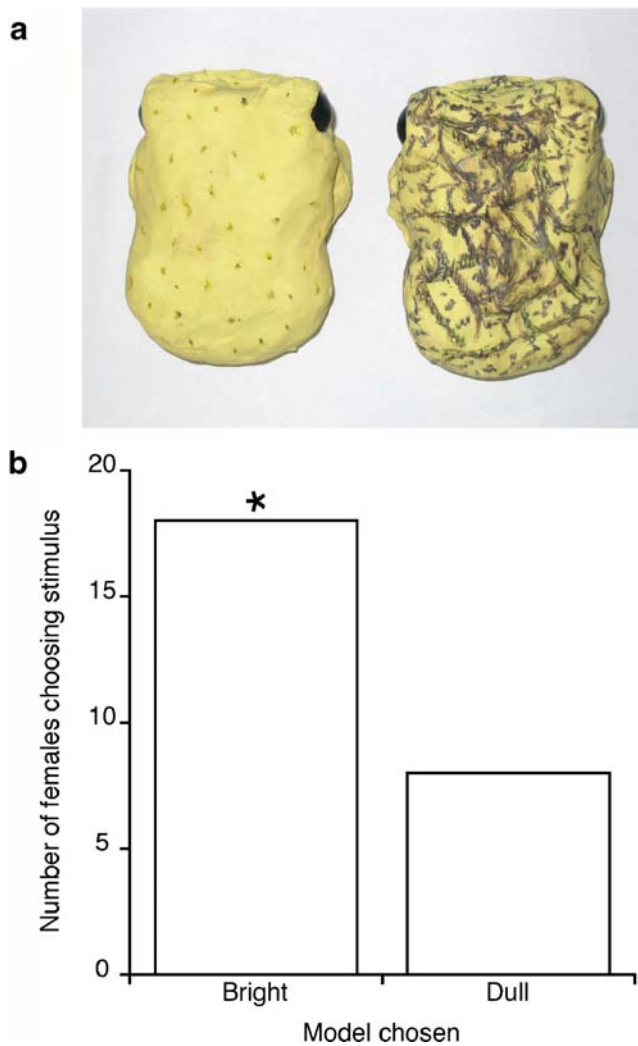
Fig. 2 **a** Alternative male models presented to females. **b** Number of females choosing the "bright" model (on the *left* in **a**) vs the "dull" model (on the *right* in **a**). The *asterisk* indicates that females significantly preferred the bright model. (To see this image in color, go to Behav Ecol Sociobiol online)

stimuli continued to play. When a female came within one body length of or touched a clay model, she was scored as preferring that stimulus. Scoring preference in this way is a reliable bioassay of female preference because female spadefoot toads initiate pair formation when they closely approach a male (Pfennig 2000; Pfennig and Tinsley 2002). We used a binomial test to determine if females significantly preferred the bright model more often than expected by a random 1:1 expectation.

Results

We found that aspects of both male coloration and pattern were associated with both male condition and size. In terms of coloration, both SVL and condition were negatively correlated with PC1 (SVL: $r_s=-0.31$, $N=68$, $P=0.009$;

condition: $r_s=-0.29$, $N=68$, $P=0.018$; Fig. 3). This principal component described decreasing red intensity and increasing green and blue intensity. By contrast, SVL and condition showed no strong association with PC2 (SVL: $r_s=-0.21$, $N=68$, $P=0.092$; condition: $r_s=-0.18$, $N=68$, $P=0.13$). This principal component was strongly associated with decreasing green intensity and increasing blue intensity.

Moreover, we found that larger, better-condition males were most dissimilar in dorsal pattern from females. Indeed, when we assessed whether SVL and condition were as-
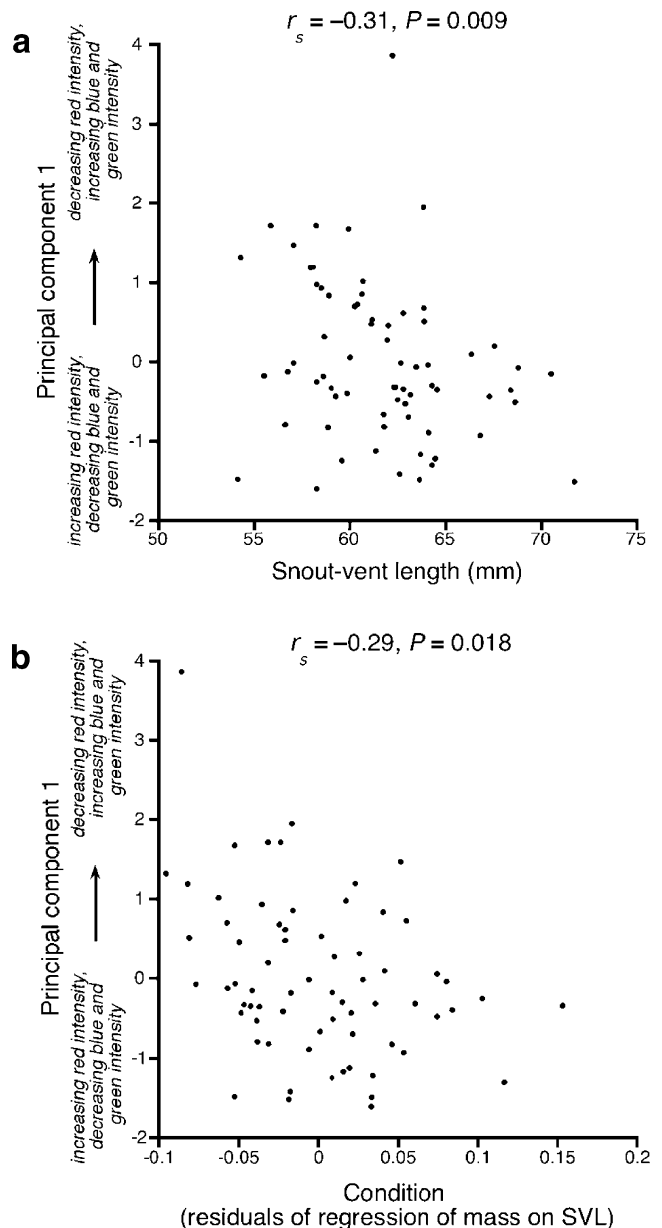

Fig. 3 The association between **a** male snout–vent length and **b** male condition with principal component 1 (*PC1*), a combined measure of red, green, and blue color intensity. In both analyses, a nonparametric Spearman correlation coefficient is reported that is not sensitive to outliers in the data

sociated with variation in dorsal patterning (as measured by the coefficient of variation in gray scale intensity), we found that larger and better-conditioned males showed less variation (i.e., they were less mottled in pattern; SVL: $r_s=-0.36$, $N=68$, $P=0.002$; condition: $r_s=-0.37$, $N=68$, $P=0.002$). Our measures using human observers corroborated these results: Both SVL and condition were significantly, positively correlated with our index of dissimilarity to female pattern (SVL: $r_s=0.31$, $N=68$, $P=0.01$; condition: $r_s=0.38$, $N=68$, $P=0.002$).

Not surprisingly, male coloration and male patterning seem to be associated: Variation in dorsal patterning was positively correlated with PC1 ($r_s=0.48$, $N=68$, $P<0.0001$) but not PC2 ($r_s=-0.002$, $N=68$, $P=0.98$). Likewise, males rated as least dissimilar from females by the human observers had higher values of PC1 (there was no relationship with PC2; PC1: $r_s=-0.43$, $N=68$, $P=0.0002$; PC2: $r_s=0.05$, $N=68$, $P=0.67$).

When we compared coloration and patterning between parasitized ($N=10$) and unparasitized ($N=58$) males, we found no clear evidence of differences between these different groups. Parasitized and unparasitized males did not differ in either PC1 or PC2 (Wilcoxon normal approximation; PC1: $Z=-0.93$, $P=0.35$; PC2: $Z=-0.23$, $P=0.82$). Although unparasitized males exhibited greater dorsal variability than parasitized males (mean coefficient of variation [±SE] for unparasitized males: 22.1% [0.01]; parasitized males: 17.6% [0.02]), the difference between the groups was not significant (although marginally so: Wilcoxon normal approximation $Z=-1.81$, $P=0.070$). Moreover, we found that parasitized and unparasitized males did not differ in their index of pattern dissimilarity to the female phenotype (Wilcoxon normal approximation $Z=0.39$, $P=0.76$). Thus, color and patterning does not appear to provide females with clear information regarding male infection status.

Finally, when we investigated whether male color or pattern were correlated with male call duration, we found no associations. Male call duration was not significantly associated with our measures of color (PC1: $r_s=-0.24$, $N=19$, $P=0.32$; PC2: $r_s=-0.33$, $N=19$, $P=0.17$). Similarly, call duration was not associated with variation in male dorsal pattern ($r_s=-0.37$, $N=19$, $P=0.11$) or our index of pattern dissimilarity from the female phenotype ($r_s=0.05$, $N=19$, $P=0.86$).

When we presented females with the "bright" male model that was dissimilar from females in patterning vs the "dull" male model that possessed patterning resembling that of a female (Fig. 2a), we found that females preferred the bright model. Of 26 females tested, 18 (69%) preferred the bright model, and eight (31%) preferred the dull model (Fig. 2b), a pattern that differed significantly from random 1:1 expectation (binomial test: $P=0.01$).

Discussion

We found that male dorsal coloration (including both color intensity and patterning) potentially indicates both male size and condition in *S. couchii*. Based on the associations between male size and condition with PC1, the coloration of larger and better-condition males included higher intensities of red while tending to include less blue and green. Moreover, larger and better-condition males were less variable in dorsal pattern and were rated as most dissimilar from females. Although coloration predicts male condition and size, however, it apparently does not serve as an indicator of infection status. For their part, females preferred the male model that possessed no mottled pattern (Fig. 2), indicating that females may use coloration in their mate choice decisions to identify larger, better-condition mates.

Although the focus of mate choice studies in anurans is often on acoustic cues, some anurans appear to use coloration in mate choice even in species that breed at night (see "Introduction"). Indeed, color discrimination in amphibians is well established (Fite 1976). Anurans possess trichromatic vision, which enables them to discriminate among blue, green, and red (Gordon and Hood 1976; Aho et al. 1993), and experiments in dark and light environments indicate that amphibians' visual performance is similar in both illumination conditions (Aho et al. 1993; Kicliter and Goytia 1995). Moreover, recent work by Roth and Kelber 2004 with nocturnal helmet geckos (*Tarentola chazaliae*) demonstrates that nocturnal vertebrates potentially have color vision even under dim moonlight intensities that would render humans color blind. They speculate that frogs and toads might be similarly capable of color vision at night (Roth and Kelber 2004), which underscores the need for additional work on visual signaling in nocturnal anurans.

A major drawback of our study, however, is that we currently know nothing about the use of coloration in *S. couchii* under natural illumination conditions. The populations of *S. couchii* from which we sampled typically breed at night, but they have also been observed to breed during the day (K. Pfennig, personal observation). The natural conditions under which visual signals may be used by *S. couchii* is therefore potentially widely variable, and further work is needed to ascertain the degree to which these signals are detectable and utilized by females under natural conditions. Moreover, the current analysis is somewhat crude in that we lacked quantitative measures of color (e.g., via spectroradiometry) and standards for correcting the measures of colors across the different photos (e.g., see Villafuerte and Negro 1998), which would have enabled us to more accurately assay male coloration. However, our methods were effective for describing the *relative* variation

in coloring among the sampled males and thereby indicated how color is associated with other aspects of male phenotype (i.e., SVL and condition). Our findings therefore provide a springboard for a more detailed analysis quantifying male coloration, the mechanisms underlying variation in male color, and its evolutionary significance.

Another factor not considered in this study is the role of male coloration in male–male competition. Male *S. couchii* exhibit satellite behavior, and males that resemble females may experience less aggression from other males. Consequently, they may have enhanced success as satellites. Additional studies are required to discover how male coloration mediates male–male competition in this system.

Despite these drawbacks, our study has shown that *S. couchii* females can potentially discriminate between males on the basis of coloration. As we discuss below, our results suggest that color and patterning may be important but redundant signals used by *S. couchii* females during mate choice.

In *S. couchii*, it is unclear how females may benefit in their preference of brighter males. Because such a preference would increase their chances of mating with larger or better-condition mates, females that prefer bright males may obtain enhanced fertilization success or enhanced growth of their offspring. Indeed, in *S. couchii*, males that are heavier for a given body size also have larger testes for a given size, suggesting that better-condition males may better fertilize a female's clutch (Pfennig and Tinsley 2002). Moreover, larger males appear to sire offspring with enhanced survival (Woodward 1987).

In contrast to the findings for male size and condition, we found no clear evidence that coloration is indicative of infection status. Parasitized and unparasitized males did not differ in either PC1 or PC2, which were our measures of combined red, green, and blue intensity among the males. Moreover, although unparasitized males were less variable in dorsal pattern than parasitized males, the difference was not statistically significant. This result could be attributed to lack of statistical power, but our findings that parasitized and unparasitized males also did not differ in their scores of dissimilarity to females suggests that the lack of difference between the groups is genuine. Thus, our results suggest that females likely cannot use color as a reliable indicator of male infection status.

The finding that male coloration was associated with male size and condition but not infection status suggests that coloration is associated with male traits that are also associated with aspects of male calls. Recall that male condition is also positively correlated with call duration and male size is inversely correlated with call dominant frequency (Pfennig and Tinsley 2002). Based on these results, *S. couchii* females do not appear to use color to gain additional information about a prospective mate's quality

over what is available in male calls. The results are contrary to the prediction of the multiple messages hypothesis, which posits that females use multiple male courtship signals to obtain different types of information about a prospective mate (Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003; Hebets and Papaj 2005). Instead, our findings suggest that coloration is redundant with aspects of male calls as an indicator of male size and condition. Such redundancy in male courtship signals may be common (e.g., Birkhead et al. 1998; reviewed in Candolin 2003; Hebets and Papaj 2005; Loyau et al. 2005), but the functional significance of using multiple or complex redundant signals in mate choice remains largely unexplored (Candolin 2003; Hebets and Papaj 2005).

One explanation for females' use of redundant signals in mate choice is that doing so may increase a female's ability to accurately identify a high-quality mate. In particular, when females face difficulty assessing male signals in one signaling context (e.g., because of noise or interference), redundant signals may provide females with an alternative source of information (i.e., redundant signals serve as "back-up" signals; Møller and Pomiankowski 1993; Johnstone 1996; Candolin 2003; Hebets and Papaj 2005; Partan and Marler 2005). In species that attend breeding aggregations (as is the case with many anurans), for example, females may face especially difficult circumstances for discriminating among males using acoustic cues because of the interference that results from many conspecifics (and often heterospecifics as well) signaling simultaneously (Doolan 1981; Schwartz 1987; Arak et al. 1990; Farris et al. 1997; Cooley and Marshall 2001; Gerhardt and Huber 2002; Hettyey and Pearman 2003). Indeed, female ability to accurately discriminate or detect males is often compromised as signal to noise ratios decrease, and in some circumstances, females may only be able to detect the males nearest to them when relying on acoustic signals (Schwartz and Gerhardt 1989; Wollerman 1999; see also discussion and review in Rowe 1999; Gerhardt and Huber 2002; Wollerman and Wiley 2002a, b; Partan and Marler 2005).

As a consequence of relying on a signal that is displayed in a noisy context, females may more often make mistakes in mate choice or, alternatively, spend more time sampling males (Wollerman and Wiley 2002a, b; Candolin 2003). Either alternative will increase the costs of choice and may select for the use of alternative signals that are less susceptible to environmental interference (Rowe 1999; Partan and Marler 2005). Because *S. couchii* breeds in dense choruses that often consist of several species, male coloration may provide *S. couchii* females with an informative cue in a less noisy communication channel that allows for detection of individual males over a longer distance than acoustic signals. Coloration may thereby aid females in the detection and localization of prospective

mates. Consequently, female preferences for bright males may enable females to move more efficiently through breeding choruses or spend less time assessing males. By doing so, they may avoid male harassment and possible forced copulation. Moreover, by assessing males from a distance and minimizing the time spent moving among males that are possibly infected by *P. americanus* at the breeding aggregation, females might thereby reduce their exposure to infective *P. americanus* larvae swimming in the water column of the breeding aggregation (Tinsley 1989, 1990).

Although coloration may be redundant with call duration in that both indicate male condition, we found that the two traits were not correlated. Such a finding may result from lack of statistical power. However, the finding may also suggest that multiple indicator traits could provide females with independent routes for assessing condition. Indeed, the two traits may be regulated by different mechanisms, both of which are associated with condition. For instance, call duration may reveal current male energy reserves, whereas male color may change with age (and males in better condition may live longer) or reflect increased testosterone (Richards 1982; Hayes and Menendez 1999; better-condition males have larger testes, Pfennig and Tinsley 2002). Regardless, the lack of association of these two traits suggests that they can evolve independently of each other. Consequently, population variation in both the relative expression of these two traits and female strength of preference for each trait may arise depending on the relative efficacy with which females can use each trait to identify fitness-enhancing mates.

In sum, our findings suggest that male coloration may play an important role in mate choice in *S. couchii* in particular and in sexually dichromatic anurans more generally. This study underscores the need for additional investigations of how animals, such as anurans, use complex signals in multiple sensory modalities during mate choice. Perhaps most critically, this work suggests that multiple signals may provide redundant information about a prospective mate. Further studies are needed, however, to determine how and why females use multiple signals to select mates. Such studies are crucial for understanding why complex courtship behaviors evolve.

References

Abrunhosa PA, Wogel H (2004) Breeding behavior of the leaf-frog *Phyllomedusa burmeisteri* (Anura: Hylidae). Amphib Reptil 25:125–135
Aho AC, Donner K, Helenius S, Larsen LO, Reuter T (1993) Visual performance of the toad (*Bufo bufo*) at low-light levels—retinal ganglion-cell responses and prey-catching accuracy. J Comp Physiol A Sens Neural Behav Physiol 172:671–682
Andersson M (1994) Sexual selection. Princeton Univ. Press, Princeton
Arak A, Eiriksson T, Radesater T (1990) The adaptive significance of acoustic spacing in male bush crickets *Tettigonia viridissima*—a perturbation experiment. Behav Ecol Sociobiol 26:1–7
Birkhead TR, Fletcher F, Pellatt EJ (1998) Sexual selection in the zebra finch *Taeniopygia guttata*: condition, sex traits and immune capacity. Behav Ecol Sociobiol 44:179–191
Bragg AN (1965) Gnomes of the night: the spadefoot toads. University of Pennsylvania Press, Philadelphia, PA
Buchanan BW (1994) Sexual dimorphism in *Hyla squirella*—chromatic and pattern variation between the sexes. Copeia 1994:797–802
Burrowes PA (2000) Parental care and sexual selection in the Puerto Rican cave-dwelling frog, *Eleutherodactylus cooki*. Herpetologica 56:375–386
Candolin U (2003) The use of multiple cues in mate choice. Biol Rev 78:575–595
Cooley JR, Marshall DC (2001) Sexual signaling in periodical cicadas, *Magicicada* spp. (Hemiptera: Cicadidae). Behaviour 138:827–855
Crawford JD, Cook AP, Heberlein AS (1997) Bioacoustic behavior of African fishes (Mormyridae): potential cues for species and individual recognition in *Pollimyrus*. J Acoust Soc Am 102:1200–1212
Doolan JM (1981) Male spacing and the influence of female courtship behavior in the bladder cicada, *Cystosoma saundersii* Westwood. Behav Ecol Sociobiol 9:269–276
Farris HE, Forrest TG, Hoy RR (1997) The effects of calling song spacing and intensity on the attraction of flying crickets (Orthoptera: Goryllidae: Nemobiinae). J Insect Behav 10:639–653
Fite KV (1976) The amphibian visual system, a multidisciplinary approach. Academic, London
Gerhardt HC (1982) Sound pattern-recognition in some North American treefrogs (Anura, Hylidae)—implications for mate choice. Am Zool 22:581–595
Gerhardt HC (1991) Female mate choice in treefrogs—static and dynamic acoustic criteria. Anim Behav 42:615–635
Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans: common problems and diverse solutions. University of Chicago Press, Chicago
Gordon J, Hood DC (1976) Anatomy and physiology of the frog retina. In: Fite KV (ed) The amphibian visual system, a multidisciplinary approach. Academic, London, pp 29–86
Grether GF, Hudon J, Millie DF (1999) Carotenoid limitation of sexual coloration along an environmental gradient in guppies. Proc R Soc Lond Ser B Biol Sci 266:1317–1322
Hankison SJ, Morris MR (2002) Sexual selection and species recognition in the pygmy swordtail, *Xiphophorus pygmaeus*: conflicting preferences. Behav Ecol Sociobiol 51:140–145

Hankison SJ, Morris MR (2003) Avoiding a compromise between sexual selection and species recognition: female swordtail fish assess multiple species-specific cues. Behav Ecol 14:282–287

Hartmann MT, Giasson LOM, Hartmann PA, Haddad CFB (2005) Visual communication in Brazilian species of anurans from the Atlantic forest. J Nat Hist 39:1675–1685

Hayes TB, Menendez KP (1999) The effect of sex steroids on primary and secondary sex differentiation in the sexually dichromatic reedfrog (*Hyperolius argus*: Hyperolidae) from the Arabuko Sokoke forest of Kenya. Gen Comp Endocrinol 115:188–199

Hebets EA, Papaj DR (2005) Complex signal function: developing a framework of testable hypotheses. Behav Ecol Sociobiol 57:197–214

Hettyey A, Pearman PB (2003) Social environment and reproductive interference affect reproductive success in the frog *Rana latastei*. Behav Ecol 14:294–300

Hirschmann W, Hödl W (2006) Visual signaling in *Phrynobatrachus krefftii boulenger*, 1909 (Anura: Ranidae). Herpetologica 62:18–27

Hoffman EA, Blouin MS (2000) A review of colour and pattern polymorphisms in anurans. Biol J Linn Soc 70:633–665

Jawor JM, Breitwisch R (2004) Multiple ornaments in male northern cardinals, *Cardinalis cardinalis*, as indicators of condition. Ethology 110:113–126

Johnstone RA (1996) Multiple displays in animal communication: 'backup signals' and 'multiple messages'. Phil Trans R Soc Lond Ser B Biol Sci 351:329–338

Kicliter E, Goytia EJ (1995) A comparison of spectral response functions of positive and negative phototaxis in 2 anuran amphibians, *Rana pipiens* and *Leptodactylus pentadactylus*. Neurosci Lett 185:144–146

Kihslinger RL, Klimley AP (2002) Species identity and the temporal characteristics of fish acoustic signals. J Comp Psychol 116:210–214

Krishna SN, Krishna SB (2006) Visual and acoustic communication in an endemic stream frog, *Micrixalus saxicolus* in the Western Ghats, India. Amphib–Reptil 27:143–147

Loyau A, Saint Jalme M, Cagniant C, Sorci G (2005) Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). Behav Ecol Sociobiol 58:552–557

Møller AP, Pomiankowski A (1993) Why have birds got multiple sexual ornaments. Behav Ecol Sociobiol 32:167–176

Narins PM, Grabul DS, Soma KK, Gaucher P, Hödl W (2005) Cross-modal integration in a dart-poison frog. Proc Natl Acad Sci USA 102:2425–2429

Partan SR, Marler P (2005) Issues in the classification of multimodal communication signals. Am Nat 166:231–245

Pfennig KS (2000) Female spadefoot toads compromise on mate quality to ensure conspecific matings. Behav Ecol 11:220–227

Pfennig KS, Tinsley RC (2002) Different mate preferences by parasitized and unparasitized females potentially reduces sexual selection. J Evol Biol 15:399–406

Richards CM (1982) The alteration of chromatophore expression by sex hormones in the Kenyan reed frog, *Hyperolius viridiflavus*. Gen Comp Endocrinol 46:59–67

Rosenthal GG, Rand AS, Ryan MJ (2004) The vocal sac as a visual cue in anuran communication: an experimental analysis using video playback. Anim Behav 68:55–58

Roth LSV, Kelber A (2004) Nocturnal colour vision in geckos. Proc R Soc Lond B Biol Sci 271:S485–S487

Rowe C (1999) Receiver psychology and the evolution of multicomponent signals. Anim Behav 58:921–931

Schwartz JJ (1987) The function of call alternation in anuran amphibians—a test of 3 hypotheses. Evolution 41:461–471

Schwartz JJ, Gerhardt HC (1989) Spatially mediated release from auditory masking in an anuran amphibian. J Comp Physiol A Sens Neural Behav Physiol 166:37–41

Sheldon BC, Arponen H, Laurila A, Crochet PA, Merila J (2003) Sire coloration influences offspring survival under predation risk in the moorfrog. J Evol Biol 16:1288–1295

Stebbins RC (2003) A field guide to western reptiles and amphibians, 3rd edn. Houghton Mifflin, New York

Summers K, Symula R, Clough M, Cronin T (1999) Visual mate choice in poison frogs. Proc R Soc Lond Ser B Biol Sci 266:2141–2145

Tinsley RC (1989) The effects of host sex on transmission success. Parasitol Today 5:190–195

Tinsley RC (1990) The influence of parasite infection on mating success in spadefoot toads, *Scaphiopus couchii*. Am Zool 30:313–324

Tinsley RC, Earle CM (1983) Invasion of vertebrate lungs by the polystomatid monogeneans *Pseudodiplorchis americanus* and *Neodiplorchis scaphiopodis*. Parasitology 86:501–517

Tinsley RC, Jackson HC (1986) Intestinal migration in the life cycle of *Pseudodiplorchis americanus* (Monogenea). Parasitology 93:451–469

Tinsley RC, Cable J, Porter R (2002) Pathological effects of *Pseudodiplorchis americanus* (Monogenea: Polystomatidae) on the lung epithelium of its host, *Scaphiopus couchii*. Parasitology 125:143–153

Tocque K (1993) The relationship between parasite burden and host resources in the desert toad (*Scaphiopus couchii*), under natural environmental conditions. J Anim Ecol 62:683–693

Tocque K, Tinsley RC (1994) The relationship between *Pseudodiplorchis americanus* (Monogenea) density and host resources under controlled environmental conditions. Parasitology 108:175–183

Villafuerte R, Negro JJ (1998) Digital imaging for colour measurement in ecological research. Ecol Lett 1:151–154

Wollerman L (1999) Acoustic interference limits call detection in a Neotropical frog *Hyla ebraccata*. Anim Behav 57:529–536

Wollerman L, Wiley RH (2002a) Background noise from a natural chorus alters female discrimination of male calls in a Neotropical frog. Anim Behav 63:15–22

Wollerman L, Wiley RH (2002b) Possibilities for error during communication by neotropical frogs in a complex acoustic environment. Behav Ecol Sociobiol 52:465–473

Woodward BD (1987) Paternal effects on offspring traits in *Scaphiopus couchi* (Anura: Pelobatidae). Oecologia 73:626–629

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall, Englewood Cliffs