

Visual mate choice in poison frogs

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We investigated female mate choice on the basis of visual cues in two populations of *Dendrobates pumilio*, the strawberry poison frog, from the Bocas del Toro Archipelago in Panama, Central America. Mate choice experiments were carried out by presenting subject females of each of two morphs of this species (orange and green) from two different island populations (Nancy Key and Pope Island) with object frogs (one of each morph) under glass at one end of a terrarium. Recorded calls were played simultaneously from behind both object frogs. The experiments were carried out under two light regimes: (i) white light, and (ii) relatively monochromatic filtered blue light. Subject females from each population displayed a significant preference for their own morph under white light, but not under blue light. These results indicate that female *D. pumilio* use visual cues in mate choice, and suggest that colour may be the visual cue they use.

Keywords: poison frogs; Dendrobatidae; colour; visual; mate choice

1. INTRODUCTION

Anuran amphibians have played a central role in research on mating strategies and mate choice (Ryan 1994). Studies of mate choice in frogs have provided key empirical evidence for both sensory exploitation (Ryan & Rand 1990) and the handicap hypothesis (Welch *et al.* 1998). This research has all concerned mate choice for acoustical cues. It remains an open question whether visual cues, which play a large role in mate choice in fishes (e.g. Houde & Endler 1990), and birds (e.g. Hill 1990), are used for mate choice in any species of frog. While nocturnal species might not be expected to use such cues, many species of frogs (particularly in the tropics) are partially or completely diurnal (Duellmann & Trueb 1986). There has been very little research on the role of visual cues in the social behaviour of these frogs. Here we report evidence for mate choice on the basis of visual cues in a poison frog (*Dendrobates pumilio*, family Dendrobatidae) from the Bocas del Toro Archipelago in Panama, Central America.

Intraspecific differentiation in the poison frog *D. pumilio* of the Bocas del Toro Archipelago is one of the most dramatic cases of divergence among populations ever discovered (Daly & Myers 1967; Myers & Daly 1976, 1983). Different populations of this frog display completely different coloration and patterns from island to island and among isolated populations on the mainland, including red, orange, yellow, green, dark blue and black and white morphs, and various forms of spotting and speckling (Myers & Daly 1983). Myers & Daly (1976) argued that these different populations (with the exception of populations from east of the Valiente peninsula) are all members of a single species, on the basis of similarities in calling

characteristics. There is little variation among populations in note repetition rate, note duration, and area of emphasized frequency of the call among populations, and these call parameters vary more within populations than between them (Myers & Daly 1976). Genetic analysis of population differentiation suggests that these populations diverged very recently (Summers *et al.* 1997).

We investigated whether females of two different colour morphs from two islands in this archipelago, Pope Island (green morph; figures 1a and 2), and Nancy Key (orange morph; figures 1b and 3), prefer members of their own population in the context of mate choice, when only visual cues are available to differentiate between potential partners in courtship.

2. METHODS

Females were captured in the field, and kept in terraria at the Smithsonian Tropical Research Institute in Panama City. Females that were the subject of mate choice experiments (hereafter referred to as 'subject females') were placed in single 10 US gal (1 gal = 3.79 dm³) terraria and allowed to acclimatize to their environment for a week. On one end of each terrarium there were plants, hollow branches and pools, whereas the other end was empty. The bottom of each terrarium was covered with leaf litter. Subject females spent most of their time on the side of the terrarium with the plants and pools. Four such 'choice chamber' terraria were maintained simultaneously.

Mate choice experiments were carried out by placing one frog (hereafter referred to as 'object frogs') of each morph underneath an overturned clear glass (5 cm in diameter) at one end of the terrarium, approximately 20 cm apart (well within the visual range of these frogs). Experiments were not begun unless the subject female was on the opposite side of the terrarium from where the glasses were to be placed. We used standard, optically clear drinking glasses of the same size and shape,

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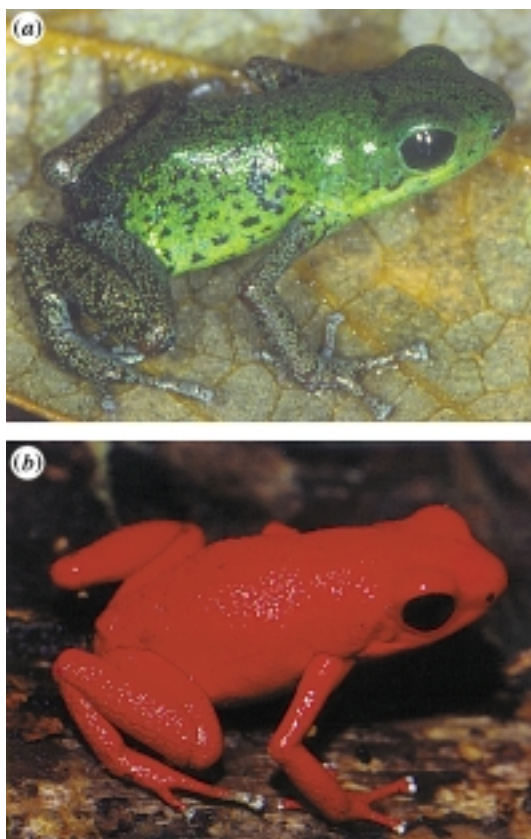


Figure 1. (a) Green morph of *Dendrobates pumilio* from Pope Island in the Bocas del Toro Archipelago, Panama. (b) Orange morph of *D. pumilio* from Nancy Key, Bocas del Toro, Panama.

which have no spectral absorption in the range covered by figures 2 and 3. The object frogs were matched for snout-vent length (within 1 mm).

Advertisement calls from different populations are very similar (Myers & Daly 1976), so we used calls from males of the orange morph for experiments with both the orange and green subject morph females. The same call was played at the same volume from each speaker during the experiments, so acoustic cues for mate choice were minimal or absent. During the trials, advertisement calls (Wells 1978) of the orange morph recorded in the field with a Marantz model PMD430 tape recorder (Marantz Europe B. V., Eindhoven, The Netherlands) at Nancy Key were played from speakers either 2 m behind and above the terraria (Nancy Key females) or inside the terraria behind the glasses containing the frogs (Pope Island females). For unknown reasons, the Pope Island (green) females were less responsive to calls played outside of the terraria than the Nancy Key (orange) females. This was not due to the call source (green versus orange morph calls), because calls recorded from green morph males on Pope Island were not more effective in eliciting a response from the green females. The sides for each morph were switched between consecutive trials, and the speakers were switched between trials for experiments with the green morph females (in which the speakers were in the terraria).

Subject female response to each object frog was measured as the amount of time the female spent orientated towards each one once she had approached within 15 cm of the frogs, and as the amount of time she spent in close proximity to each one, during a 1 h trial. To define close proximity, each overturned

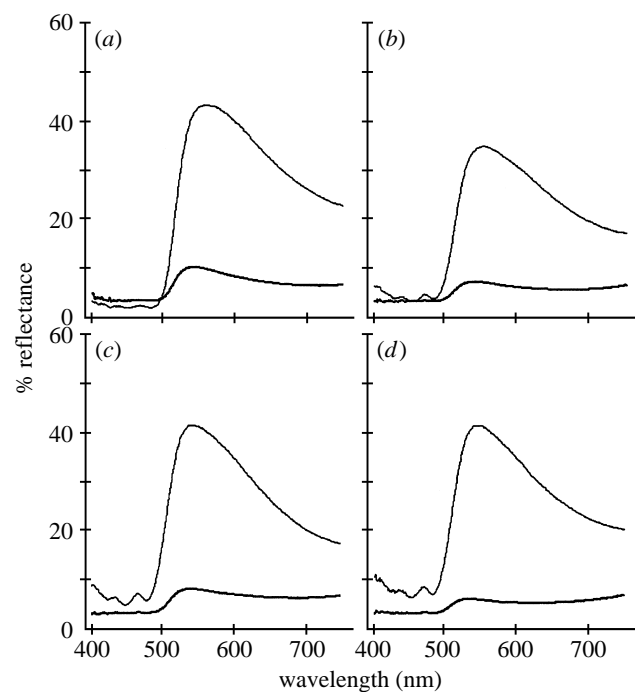


Figure 2. Average reflectance spectra from skin of four individuals of *Dendrobates pumilio* collected from Pope Island. Reflectance measurements were carried out in Dr Cronin's laboratory at the University of Maryland Marine Biotechnology Center in Baltimore, Maryland, using an Ocean Optics 2000 spectrometer (Ocean Optics, FL, USA). Each panel represents data from a single frog; the dark trace is from the dorsal surface (averaged from five or six separate measurements taken from the head and back regions), and the light trace is from the ventral surface (average of two or three scans from the belly region). All individuals are adult females.

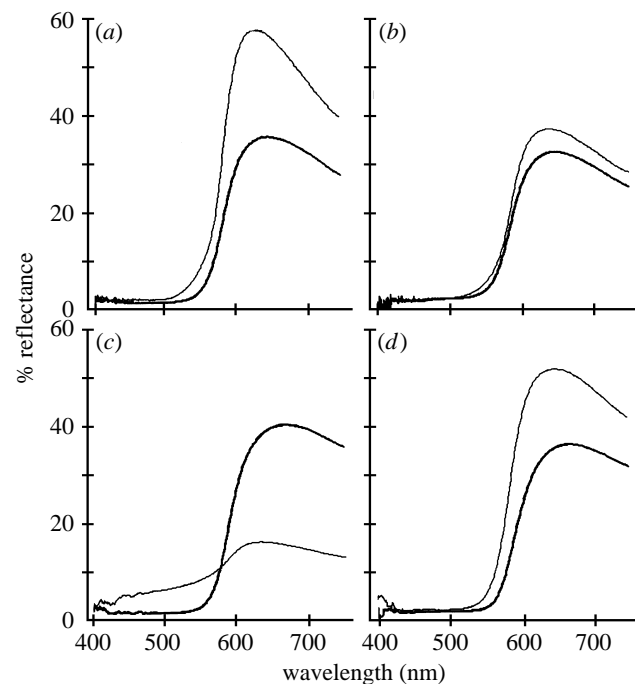


Figure 3. Similar to figure 2, except that data are from four individual *Dendrobates pumilio* from Nancy Key. All individuals are adult females except for (d) which was an adult male.

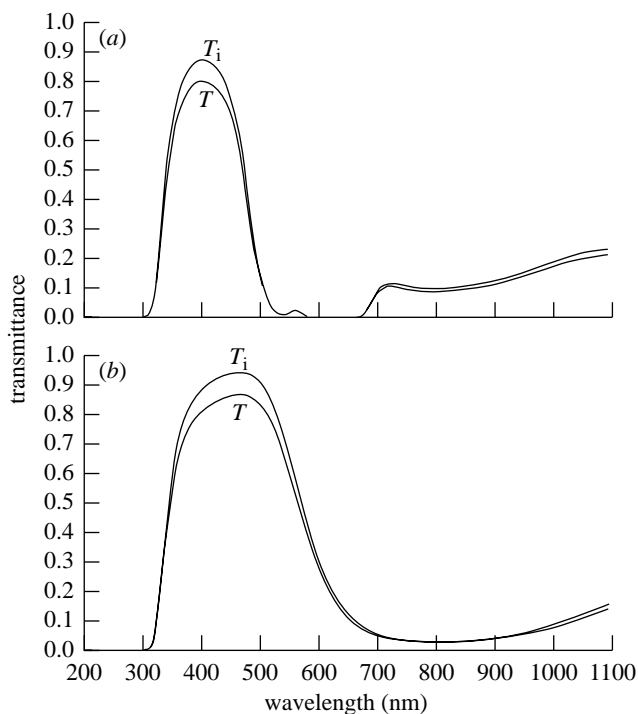


Figure 4. Spectral transmittance for (a) Schott BG 12 filter and (b) Schott BG 23 filter (BES Optics 1999). T = transmittance, T_i = internal transmittance.

glass was placed on an overturned Petri dish, and the subject female was considered to be in close proximity if she had at least two limbs on the Petri dish.

Two lighting regimes were employed in the experiments: white light and filtered blue light. Experiments under white light employed both 40 W overhead fluorescent lights and 25 W incandescent lights. Experiments to test for mate choice under conditions of reduced spectral information employed 60 W incandescent lights shone through two types of spectral filters (Schott filters from BES Optics, PA, USA): direct overhead lighting using a BG 12 blue-green filter, and side lighting using a BG 23 blue-green filter. These filters do not transmit wavelengths in the orange and red part of the visual spectrum. Spectral transmission for each filter is shown in figure 4. Unfortunately overall light intensity declined under the filtered light regime. However, the object frogs were clearly visible to human observers under the blue light, and most responding subject females tried to stroke the object frogs through the glass, indicating that they could perceive the object frogs under the blue light regime.

Female courtship behaviour in *D. pumilio* and closely related species is obvious and distinctive, and has been observed and described in a number of studies, both in the field and in captivity (e.g. Limerick 1980; Zimmermann & Zimmermann 1988; Zimmermann 1990; Summers 1992; Prohl 1995, 1997). Female courtship begins with approach and observation, after which the female will approach more closely and follow the male. Females will also touch the male during courtship, stroking and nudging him. Males call, move away, and eventually lead females to oviposition sites in the leaf litter. Females do not normally affiliate with other adults outside the context of courtship (this is not a social species), and so courtship behaviour provides an unambiguous assay of mate choice in this species.

Females were only recorded as responding during a trial if they orientated towards each of the object frogs during the

experiment. This ensured that they perceived each object morph, and hence were in fact making a choice. In most of the experiments, the female would try to make contact with one or both of the object frogs through the glass, attempting to stroke them in a manner similar to that seen previously during courtship in this species. In one case the subject female actually oviposited at the base of the overturned glass containing the object frog of her own morph, indicating that the female was in fact attempting to mate with that frog. Females were not observed to oviposit at any other time during their stay in the terraria, and eggs were not found in terrarium inspections.

In the first three trials (with orange morph females) male frogs were used as the object frogs in the choice experiments. Unfortunately, males would call when approached by the subject female, and their calls were clearly audible through the glass. Orange females displayed a preference for their own morph, but it was not clear that male calling behaviour was not influencing their choice. For this reason, subsequent experiments employed female object frogs, and the results reported here exclude the results using males. Object females under the glass did not reciprocate courtship by the subject female.

Some females were used as the object frogs in more than one experiment. However, within the experiments on each colour morph, each experiment for a particular subject employed a different pair of object frogs. Five green females and seven orange females were used as object frogs in the seven orange morph experiments, and six green females and seven orange females were used as object frogs in the eight green morph experiments carried out under white light. All blue light experiments were replicas of a white light experiment, except for the lighting regime.

Trials in which the female responded were scored for orientation and proximity (see above) by a researcher unfamiliar with these frogs, using a black and white video monitor so that differences in colour could not be perceived. The total amount of time spent orientated towards and in proximity to each frog was combined for analysis. Relative responsiveness was calculated as the response of the subject female to its own morph divided by the total time spent orientated towards or in the proximity of either frog.

3. RESULTS

Females responded to the object frogs in only 27 out of 144 experiments (19%). In most cases, the subject female did not react to the calls, and did not approach the object frogs. This is consistent with field observations of behaviour in this species. In the majority of interactions between males and females in the field in this and closely related species (e.g. Prohl 1995; Summers 1999), the female simply ignores the male and his calls. Females provide substantially more parental investment than males in this species; they produce nutrient-rich eggs and carry out an intensive regime of parental care (Weygoldt 1980; Brust 1993). Hence, female *D. pumilio* are relatively cautious and coy about mating. However, females that did respond typically took an active interest in both object frogs. In most trials the females moved back-and-forth between the two frogs, but spent more time courting one or the other object frog.

First, we compare the preference subject females displayed for object frogs from their own island under white light, relative to the preference they displayed

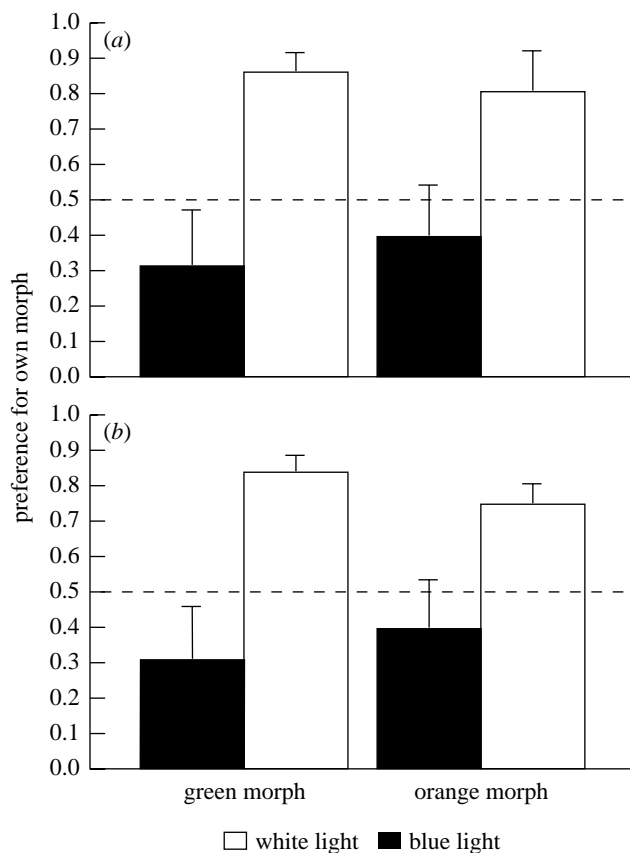


Figure 5. (a) Histograms showing the results of mate choice experiments for each colour morph for paired trials under white and blue light regimes. The dotted line represents no preference. (b) Histograms showing the results of all mate choice experiments (unpaired data) for each colour morph, including results from trials under white light which did not have a matching result under blue light. Bars indicate standard errors.

under blue light. Considering only those experiments where results were obtained for the same female under both white and blue light, subject females (both morphs combined) displayed a significant preference for frogs from their own island under white light (relative to the preference those same females displayed under blue light) in paired tests (paired t -test, $n=8$, d.f. = 7, $t=4.134$, $p=0.0044$). Green females from Pope Island displayed a significant preference for their own morph (figure 5a, paired t -test, $n=5$, d.f. = 4, $t=3.234$, $p=0.0319$). Orange females from Nancy Key did not display a significant preference for their own morph (figure 5a, paired t -test, $n=3$, d.f. = 2, $t=2.249$, $p=0.154$), although sample size was small and there was a trend in the predicted direction.

Considering data from all experiments, including experiments under white light with females that did not respond under blue light, a significant preference was displayed by females from both islands combined (unpaired t -test, $n=23$, d.f. = 21, $t=-5.050$, $p<0.0001$), and by females from Pope Island (figure 5b, unpaired t -test, $n=12$, d.f. = 11, $t=-3.944$, $p=0.0023$). A significant preference was also displayed by the females from Nancy Key (figure 5b, unpaired t -test, $n=11$, $t=2.987$, $p=0.0174$).

Subject females displayed a significant preference for their own morph under white light, relative to a baseline

of 50%, or no preference (one-sample t -test: for all subject females combined, $n=15$, d.f. = 14, $t=8.047$, $p<0.0001$; for Pope Island females (figure 5b), $n=8$, d.f. = 7, $t=6.965$, $p=0.0002$; for Nancy Key females (figure 5b), $n=7$, d.f. = 6, $t=4.630$, $p=0.0036$).

In contrast, all subject females combined displayed a significant preference for the opposite morph under blue light ($n=8$, d.f. = 7, $t=3.213$, $p=0.0148$). Separate analysis did not reveal a significant preference under blue light by females from Pope Island (figure 5b, $n=5$, d.f. = 4, $t=1.968$, $p=0.1204$), or from Nancy Key (figure 5b, $n=3$, d.f. = 2, $t=2.744$, $p=0.111$).

There was no significant tendency for females to prefer the frog placed on a particular side of the tank (unpaired t -test, $n=15$, d.f. = 13, $t=0.568$, $p=0.58$). For the females from Pope Island, there was no significant tendency to prefer the frog placed in front of a particular speaker in the tank (unpaired t -test, $n=8$, d.f. = 6, $t=-0.259$, $p=0.805$). Finally, there was no significant relationship between the size (snout-vent length) difference between the two object frogs, and the preference expressed by the subject female (linear regression: for Pope Island females, $n=5$, $r^2=0.064$, $t=0.451$, $F=0.203$, $p=0.6825$; for Nancy Key females, $n=7$, $r^2=0.182$, $t=1.529$, $F=2.338$, $p=0.187$).

4. DISCUSSION

These results demonstrate that females from each island prefer to court frogs from their own population, compared with members of another population. Under conditions in which the width of the spectrum of visible wavelengths available to the frogs is reduced, females no longer exhibit this preference. This demonstrates that female preference under white light is made on the basis of visual cues. The most obvious difference between these morphs is colour. Unfortunately light intensity declined with the use of the blue filters, and we were not able to avoid this disparity. Hence, it is possible that females use some other visual cue to recognize members of their own population, which they could not detect under the darker conditions of the blue light experiments. Nevertheless, our experiments suggest that differences in colour are the most likely visual cues used by these frogs.

There is behavioural evidence for colour vision in a variety of anurans, including *D. pumilio* (Hailman & Jaeger 1974). There is microspectrophotometric evidence that dendrobatid frogs have colour vision: preliminary research in J. C. Partridge's laboratory (personal communication) indicates that dendrobatids (including *D. pumilio*) have several different cone classes ranging in peak sensitivity from less than 450 nm to beyond 550 nm. Hence, it is likely that these frogs are quite capable of detecting the differences in colour between different morphs.

This is the first experimental demonstration that visual cues are important to frogs during courtship. Visual stimuli were a likely candidate for mate choice cues in this species because they are diurnal, and mating involves a long, complex courtship sequence in which the female appears to follow the male using both visual and acoustic cues (Zimmermann & Zimmermann 1988; Prohl 1995, 1997).

Mate choice on the basis of visual cues in general and colour in particular may be more common in anurans

than has previously been appreciated. In the poison frogs, long courtship is common, with the male and female moving together over the forest floor during the day. Females should have ample opportunity to assess male coloration in most species of poison frogs. Females in those species that have been studied frequently reject males after actively following them for some time (e.g. Limerick 1980; Summers 1992, 1999; Prohl 1995). It seems likely that coloration could play a role in the mate choice decisions of these females.

If female *D. pumilio* do in fact prefer to court members of their own population based on colour differences, then why should this be the case? There are two likely explanations: either divergence in colour drove divergence in female preference, or divergence in female preference drove divergence in colour. Divergence in coloration could drive female preference through a form of sensory bias: females may become more attuned to the colour of the males from their local population, and might evolve a preference for that colour because they are more easily able to detect it.

It is also possible that female choice may have driven colour divergence in these populations of *D. pumilio*, rather than the reverse (Summers *et al.* 1997). Sexual selection theory suggests that female preferences can drive rapid diversification among populations of species with female parental care, such as *D. pumilio* (West-Eberhard 1983). There is circumstantial evidence in favour of this hypothesis for diversification among the populations of *D. pumilio* in the Bocas del Toro populations (Summers *et al.* 1997).

We thank the scientific and administrative staff of the Smithsonian Tropical Research Institute for logistical support and advice. We thank Instituto Nacional de Recursos Naturales Renovables (INRENARE) of the Republic of Panama for research and collection permits. We thank A. S. Rand, S. McRae, H. Gray, E. Bermingham, H. Heying, M. J. Ryan, T. Halliday and two anonymous reviewers for comments, advice and discussion. The photograph of the green frog morph from Pope Island is the work of Marco Guerra.

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