

# Geographic Variation in Male Sexual Signals in Strawberry Poison Frogs (*Dendrobates pumilio*)

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## Abstract

In this paper, we compare the advertisement calls of 207 neotropical strawberry poison frogs (*Dendrobates pumilio*) collected in 21 localities along a transect from northern Costa Rica to western Panama. Populations varied most in call duration and call rate, while pulse rate and duty cycle were less variable. Multivariate analyses showed that call variation followed a cline with higher call rates, shorter calls, lower duty cycles and higher pulse rates in the southeast. Body size decreased towards the southeast and explained most variation in dominant frequency, as well as some residual variation in call rate. We conclude that a combination of geography and morphology is largely responsible for call variation within this species. Two inferred bio-acoustic groups were roughly in accordance with two genetic groups, geographically separated in central Costa Rica. However, genetic distances among populations did not co-vary with call dissimilarity after correction for geographic distances. Thus, differences in calls between genetic groups are probably mainly a result of clinal variation. These findings agree with the general observation that bio-acoustic variation is often not (highly) associated with genetic divergence. Moreover, colour polymorphism observed among Panamanian populations was not reflected in a higher variability in call parameters relative to the monomorphic Costa Rican populations.

## Introduction

The sexual communication system of animals is important for successful reproduction at three levels: sexual signals (e.g. mating calls and colour patterns) facilitate mate detection; they serve as indicators for species identity; and they can indicate mate quality which might vary among conspecifics. Several studies have shown that among-population differences in sexual signals are common, and that they influence mate recognition (Claridge & Morgan 1993; Endler & Houde 1995; Pröhl et al. 2006). Various hypotheses have been proposed to explain this geographic variation in sexual signals (reviewed in Gerhardt & Huber 2002). Firstly, differences in habitat acoustics may lead to differences in signals. Several

studies have tested this hypothesis in anurans and found no correlation between call structure and habitat type (Zimmerman 1983; Penna & Solis 1998), but in the frog *Acris crepitans*, calls differed in structure between grassland and forest habitats (Ryan et al. 1990). Secondly, call variation may arise from variation in the presence of acoustically orienting predators and parasites. In katydids, it has been shown that individuals in populations with high predation or parasite incidences call less often, use simpler calls or use vibratory instead of acoustic signals than in populations with lower predation pressure (Belwood & Morris 1987). A third hypothesis is related to arbitrary differences in local preferences for certain mate traits, which has been documented in several taxa (Endler & Houde 1995; Griffith et al.

1999). In túngara frogs, most females prefer local calls over non-local calls, even when the non-local calls were from genetically similar populations, suggesting that arbitrary sexual selection explains variation in calls and preferences (Pröhl et al. 2006). Another hypothesis is that call differences arise from acoustic interaction with closely related species. Support for this hypothesis arises from documentation of reproductive character displacement, i.e. the pattern of geographic variation in which differences between the signals or preferences of two closely related species are greater in sympatry than in allopatry (Marquez & Bosch 1997; Höbel & Gerhardt 2003). Finally, call variation may also arise from genetic drift.

Anurans have long served as a focal study group for research on sexual selection and communication (Gerhardt & Huber 2002). Mate choice in this group is facilitated mainly by acoustic advertisement signals (Gerhardt & Huber 2002), although visual displays or colour pattern may also play a role (Hödl & Amézquita 2001; Summers et al. 1999). In this study, we examine patterns of variation in sexual signals in the neotropical strawberry poison frog (*Dendrobates pumilio*).

The study subject is a small, terrestrial and diurnal frog that inhabits tropical wet forests or forest-similar habitats in the eastern lowlands of Nicaragua, Costa Rica and Panama. Throughout, most of their range, the frogs are morphologically similar (red body with red, blue or black limbs), but they show amazing colour and pattern polymorphism in and around the Bocas del Toro Archipelago in Panama (e.g. blue, green, red, orange, with or without spots; Daly & Myers 1967; Summers et al. 2004). Conspicuous body colours have been interpreted as aposematism, as all populations studied possess toxic alkaloids (Myers & Daly 1983; Daly et al. 1987; Saporito et al. 2004). Additionally, frogs from Bocas del Toro have been shown to prefer their own colour morph in visual choice experiments, suggesting that colour may be important in mate choice (Summers et al. 1999). In this study, it was assumed that acoustic signals are an important component of sexual communication in *D. pumilio*.

Considerable variation in call parameters among individual males has been found within two populations of *D. pumilio* in Costa Rica (Pröhl 2003). Geographical call variation was not encountered in an earlier study, but this result was based on a smaller data set (Myers & Daly 1976). In contrast, geographic variation in calls and morphology was reported in the case of two other *Dendrobates* species or

species complexes which belong to the same species group. Although the study lacks a powerful statistical analysis, Meyer (1996) detected differences in advertisement call structure among populations in *Dendrobates granuliferus* from south to central Pacific Costa Rica. Lötters et al. (1999) reported local differences in advertisement calls in a complex of probably closely related *Dendrobates* species from Colombia and Ecuador.

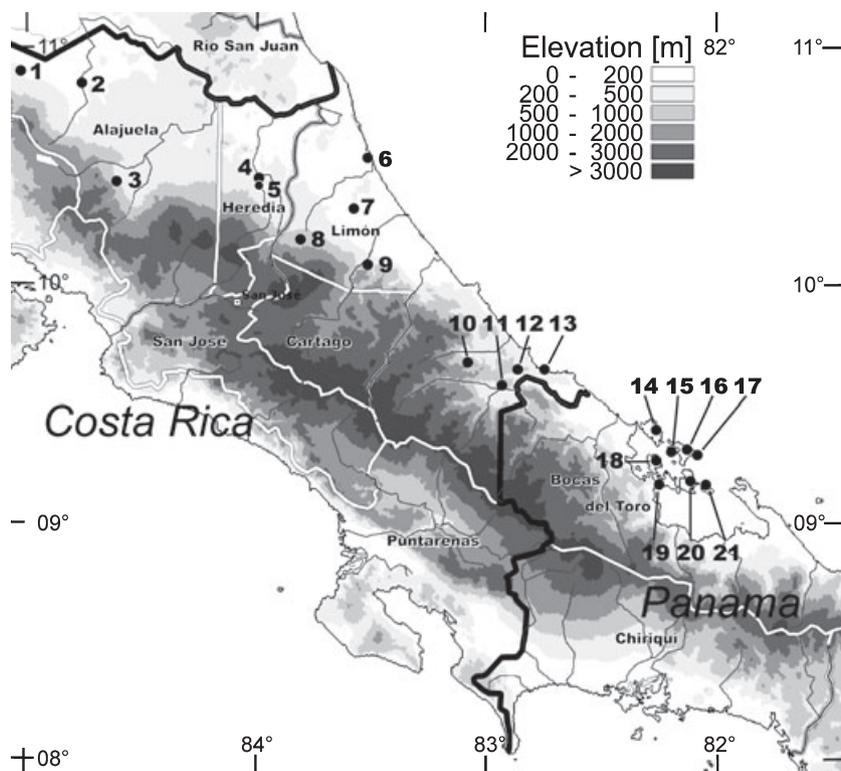
A recent phylogenetic study divided *D. pumilio* into two genetic groups: a north-western (NW) group consisting of populations from northern Costa Rica, and a south-eastern (SE) group comprising populations from Central and South Costa Rica as well as western Panama (Hagemann & Pröhl 2007). This study examines advertisement calls from 21 populations along a transect throughout most of the distribution range *D. pumilio*. We document geographic variation in call traits and evaluate which call parameters vary most within and among populations. Furthermore, we explore if variation in calls is correlated to genetic differentiation and geography. Additionally, we ask whether call and colour differences show similar patterns of geographic variation.

## Methods

### Study Area and Call Recording

Advertisement calls of *D. pumilio* were recorded in 21 populations (Fig. 1; 13 populations in Costa Rica and eight populations in Panama) during the years 1995 (populations 3, 10, 11 and 12), 1998 (populations 14, 15, 16, 18 and 20) and 2004 (populations 1, 2, 4, 5, 6, 7, 8, 9, 13, 17, 19 and 21). Each population was visited only once. An earlier study (Pröhl 2003) showed that calls do not differ significantly between years within the same populations. We therefore think that the observed call differences are not due to yearly effects, but represent examples of geographic variation. In general, there was a distance of at least 10 km between populations. Only La Selva and Puerto Viejo S. (2 km) and three Panamanian populations (6–8 km) were closer to each other. Except for Cauchero, all Panamanian populations were located on the Islands of the Bocas del Toro Archipelago. We sampled one population on each of the islands of the archipelago that we visited, except for Bastimentos island, where we recorded frogs from two populations (Pueblo Bastimentos and Punta Vieja).

The entire transect was approx. 380-km long and covered most of the species range, except populations in southern Nicaragua which were difficult to visit



**Fig. 1:** Distribution of sample localities in Costa Rica and Panama. Sample locations are arranged from the northwest to the south-east. 1 = Upala, 2 = Caño Negro, 3 = Fortuna, 4 = Puerto Viejo S., 5 = La Selva, 6 = Tortuguero, 7 = Pueblo Nuevo, 8 = Guápiles, 9 = Siquirres, 10 = Hitoy Cerere, 11 = Suretka, 12 = Bribri, 13 = Puerto Viejo T., 14 = Isla Colón, 15 = Isla Solarte, 16 = Pueblo Bastimentos, 17 = Punta Vieja, 18 = San Christóbal, 19 = Cauchero, 20 = Isla Popa, 21 = Isla Cayo de Agua. Populations 1–8 belong to the NW genetic group, populations 9–21 belong to the SE genetic group as found in Hagemann & Pröhl (2007).

because of a lack of infrastructure. In total, we recorded advertisement calls of 207 males, with a mean of 10 males per population ( $SD = 2.5$ ). We used a Sony professional walkman (WM-D6C; Sony Corporation, Tokyo, Japan) and a Sennheiser microphone (MHK 416 P48; Sennheiser, Wedemark-Wennebostel, Germany), placed approx. 1 m away from the males, to record the calls. We recorded frogs during the morning hours (6:00 AM to 12:00 PM) when they are most active. After each recording, we measured air temperature, and captured the frog to measure body size (SVL to the nearest 0.5 mm with a calliper) and body weight (to the nearest 0.05 g with a pesola spring scale). Because we did not capture frogs in Suretka and Fortuna, and because some frogs in other populations escaped, we have weight and size data for 167 of the 207 frogs we recorded. We spent 2–5 d in each population, and documented the longitude and latitude of all sample sites with a Garmin GPS 12. In most populations ( $n = 15$ ), we also collected toe clips from the recorded frogs and stored them in 96% alcohol for later genetic analysis.

### Sound Analyses

We analysed one advertisement call sequence for each male. We used the digital sound analysing

system Batsound Pro vs. 3.31 (Pettersson Elektronik, Uppsala, Sweden) to digitise calls at a sampling rate of 22 050 Hz. Call parameters are defined and illustrated in Pröhl (2003). Ten calls of each sequence were analysed by quantifying the following call properties: call rate (calls/s), call duration (ms) and pulse rate (pulses/s) using oscillograms. Dominant frequency was measured by using spectrograms (FFT length 256, Hanning Window). The duty cycle was calculated as call rate multiplied by average call duration (Taigen & Wells 1985; Gerhardt 1991). This indicates the percentage of time an acoustic signal was produced within the call sequence.

### Genetic Distances

After DNA extraction, three fragments of mitochondrial gene (Cytochrome *b*, Cytochrome Oxidase I and 16S rRNA) were amplified via polymerase chain reaction (PCR) and sequenced on the MegaBACE 1000 automated DNA Sequencer (Amersham Biosciences, Piscataway, New York, USA) of one frog per population. Consensus sequences were used to calculate K2P distances (Kimura 1980) with MEGA, version 3.0 (Kumar et al. 2004). For more details about DNA extraction, PCR and sequencing protocol see Hagemann & Pröhl (2007).

**Data Analysis**

Preliminary t-tests were used to establish whether the calls from two locations on the island Bastimentos (locations Pueblo Bastimentos and Punta Vieja, 7.3 km apart) and the calls from La Selva and Puerto Viejo S. (2 km apart) were significantly different, to decide whether these should be considered as one or two populations. In both cases, several call parameters differed between locations and consequently we treated them as different populations.

Some call parameters of *D. pumilio* seem to be temperature-dependent (Pröhl 2003). At the beginning of the statistical analysis, we calculated Pearson's product moment correlation coefficients to assess how call parameters change in relation to temperature. Four call parameters (call rate, call duration, pulse rate and dominance frequency) were temperature-dependent (Table 1). As ambient temperature varied greatly during the day and between locations [range<sub>overall</sub>: 22.7–31.8°C], overall regression coefficients (b) were calculated to adjust these call parameters to the mean temperature of 26.9°C (SD = 2.12) by using the equation  $Y_{adj} = y - (b \cdot t_{measured}) + (b \cdot t_{mean})$  with  $Y_{adj}$  = adjusted value of call parameter,  $y$  = original value of call parameter,  $b$  = regression coefficient and  $t$  = temperature. All following analyses were performed with temperature adjusted data.

**Table 1:** Pearson's product-moment correlation coefficients (r) between call properties and ambient temperature, and regression coefficient (b) of calls on temperature. n = 207 calls across the geographic distribution of the species in Costa Rica and Panama. P-values significant after Bonferroni adjustment are  $p < \alpha = 0.05/5 = 0.01$

	r	p	b	p
Call rate (1/s)	0.67	<0.001	0.41	<0.001
Call duration (ms)	-0.47	<0.001	-3.24	<0.001
Duty cycle (ms/s)	0.028	0.69	0.001	0.51
Pulse rate (1/ms)	0.32	<0.001	0.008	<0.001
Dom. Frequency (kHz)	0.55	<0.001	0.12	<0.001

We used one-way ANOVAS to test for differences between populations in every call parameter. We calculated post hoc Tukey's tests for unequal sample sizes to ascertain which population pairs differed in which call parameters. Coefficients of variation (CV = SD\*100/mean) were calculated for every call parameter within and among populations to find out which call parameters are most distinctive. Within-populations CVs are based on the mean and SD over all recorded males in every population. The mean within-population CV (CV<sub>w</sub> mean, given in Table 2) is calculated by averaging all within-population CVs. Among-population CVs (CV<sub>a</sub>) are based on the grand mean and SD over all populations. Among-population CVs were initially calculated for all populations combined.

To compare the call diversity in monomorphic and polymorphic populations of *D. pumilio*, we calculated among population CVs for: (1) monomorphic NW Costa Rican populations with red dorsum and blue legs, (2) monomorphic Central/SE Costa Rican populations with red dorsum and red or black legs and (3) polymorphic Panamanian populations. The first group of populations corresponds to the NW genetic group and the two other groups correspond to the SE genetic group.

We calculated bio-acoustic differences in calls between populations with Euclidean distances from z-transformed averages of all measured call parameters. We used Mantel's tests (Smouse et al. 1986) in Arlequin (Schneider et al. 2000) to estimate the correlations and partial correlations between geographic distance, bio-acoustic distance (Euclidean distances) and genetic distance (K2P) between populations. One thousand permutations determined the statistical significance of the correlation coefficients.

A cluster analysis (Linkage rule: Unweighted pair group average; distance measure: Euclidean distance) was used to look for possible geographic pattern in call differences. Additionally, other linkage rules (e.g. complete linkage, unweighted pair group

**Table 2:** Population mean and SD, within and among coefficient of variation (CV<sub>w</sub>, CV<sub>a</sub>) and ANOVA results testing for differences in call parameters among 21 populations of *Dendrobates pumilio*

	Mean	SD	CV <sub>w</sub> mean	CV <sub>w</sub> SD	CV <sub>a</sub>	CV <sub>a</sub> /CV <sub>w</sub>	F	p
Call rate (1/s)	6.94	1.02	7.9	2.7	13.1	1.65	21.6	<0.001
Call duration (ms)	64.0	13.8	11.2	3.5	19.4	1.74	20.9	<0.001
Duty cycle (ms/s)	426.8	61.0	10.0	2.8	10.7	1.07	10.1	<0.001
Pulse rate (1/ms)	0.27	0.05	13.3	6.3	14.5	1.09	7.8	<0.001
Dom. Freq. (kHz)	4.45	0.41	5.6	1.54	8.04	1.44	15.8	<0.001
Body size (SVL, cm)	19.7	2.01	3.42	1.48	9.89	2.89	68.7	<0.001
Weight (g)	0.65	0.20	11.7	3.95	29.6	2.53	53.9	<0.001

centroid) were also employed in combination with other distance measures (e.g. Squared Euclidean distances, City block Manhattan Distances) available in STATISTICA. Apart from single linkage and Chebychev Distance Matrix, all combinations of linkage rule/distance measures yielded very similar results to our original method. A chi-squared test was employed to test the null hypothesis that the classification of calls from the two bio-acoustic clusters to the NW and SE genetic group is arbitrary.

Correlation coefficients were implemented to find possible relationships between call parameters, weight and size of the frogs, and the distance from the most NW population (=Upala; Fig. 1). As we found several variables to be correlated with each other, we continued with a multiple regression analysis to find out which variables explained how much variation there was in calls among populations.

Further, a nested analysis (populations nested in genetic groups) of covariance was conducted to compare the differences in calls between two genetic groups and those among populations. Two different models for every call parameter were calculated: (1) A nested ANOVA without co-factor and (2) a nested ANCOVA with size as co-factor. In an analysis of covariance within-population differences of co-factors are checked for. By checking for the within-population variances of co-factors, the model should improve and the F-statistics increase as the differences among the groups would become more clear-cut. However, in case of interaction taking place between cofactors and populations, the model would worsen and F-statistics decline.

Populations of *D. pumilio* are variable in a multidimensional space and some of the variables measured were correlated with one another. To reduce multidimensional variance, we used a Principal Component Analysis (PCA) including all call parameters, size and weight. PCA combines correlated variables to factor axes in a lower dimensional space onto which the original variables are projected.

## Results

All call parameters varied between populations (Table 2, Fig. 2). Pulse rate and call duration showed the highest variation within and among populations. Dominant frequency was the least variable parameter. Call rate and call duration varied most among populations relative to variability within a population (Table 2).

Variation in calls among populations is visualized in Fig 2. Some call parameters showed a rough clinal

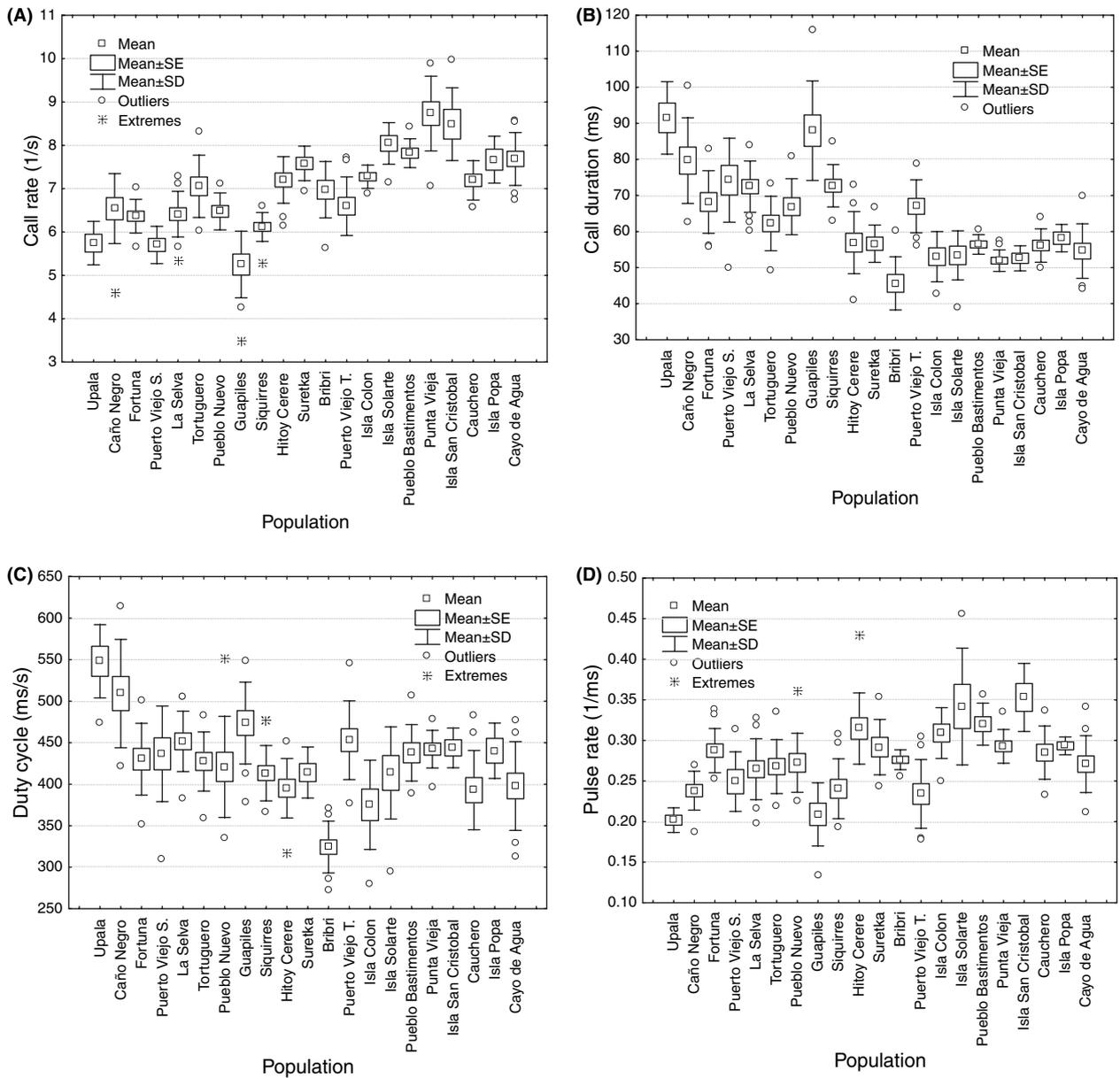
pattern: call rate, pulse rate and dominant frequency increased from NW to SE, while call duration and duty cycle decreased from NW to SE (Fig. 2a–e; see also Table 4). However, the clinal pattern was usually restricted to geographic sub-regions: call rate and dominant frequency were clinal in the SE but not in the NW, while call duration and duty cycle were clinal in the NW but not in the SE. Some populations showed extreme values in some call parameters in relation to other populations close-by: for example Guapiles showed a very low call rate and very long call duration while Bribri showed an extremely low-duty cycle in comparison with all other populations.

Frog populations in and around the Bocas Archipelago showed several remarkable patterns of call variation. Firstly, the populations Pueblo Bastimentos and Punta Vieja differed markedly in dominant frequency, in spite of being on the same island. Secondly, the population on Popa Island and the population Punta Vieja (Bastimentos Island) had very high dominant frequency. Thirdly, island and mainland populations in Panama showed very similar call duration values.

Bio-acoustic differences (calculated as Euclidean distances) were significantly correlated with geographic and genetic distances between population pairs (Table 3). However, the correlation between bio-acoustic and genetic distance disappeared when the data were controlled for geographic distance (partial correlation). The correlation and partial correlation between genetic and geographic distance were higher than between bio-acoustic-genetic and bio-acoustic-geographic distance (Table 3).

The cluster analysis identified two main bio-acoustic clusters: one (1) principally containing populations from northern Costa Rica, and another (2) that for the most part contained populations from South Costa Rica and Panama (Fig. 3). The border between these groups seems to lie somewhere between Pueblo Nuevo/Siquirres and Hitoy Cerere (Fig. 1).

The previously observed geographic/genetic pattern (Hagemann & Pröhl 2007) was not fully in accordance with the bio-acoustic clusters found here: two populations of the northern bio-acoustic cluster A (Siquirres, Puerto Viejo T.) fell into the SE genetic group, while two populations of the (southern) bio-acoustic cluster B (Tortuguero, Pueblo Nuevo) could be categorized in the NW genetic group (Fig. 1; Fig. 3). Nevertheless, significantly more populations of Cluster A were assigned to the northern geographic/genetic group, and significantly more



**Fig. 2:** Variation in call parameters and body size across the geographic range from north-west Costa Rica, through central and south-east Costa Rica to the Bocas del Toro Archipelago in Panama: A, call rate; B, call duration; C, duty cycle; D, pulse rate; E, dominant frequency; F, body size (SVL).

populations of cluster B were assigned to the southern geographic/genetic group than vice versa ( $\chi^2 = 7.4$ ,  $df = 1$ ,  $p < 0.01$ ).

All call parameters except for duty cycle were correlated with the size (snout-vent length) of the frogs. Call duration was positively correlated with size, whereas call rate, pulse rate and dominant frequency were negatively correlated with size (product moment correlation: call rate:  $r = -0.72$ ,  $p <$

$0.00049$ ; call duration:  $r = 0.50$ ,  $p = 0.029$ ; duty cycle:  $r = 0.09$ ,  $p = 0.72$ ; pulse rate:  $r = -0.67$ ,  $p = 0.0017$ ; dominant frequency  $r = -0.81$ ;  $p < 0.0001$ ;  $n = 167$  frogs). The weight of the frogs was highly positively correlated with size ( $r = 0.87$ ,  $p < 0.0001$ ) and therefore its influence on the frogs' calls was not further analysed. We also detected a correlation between distance from Upala and size: ( $r = -0.49$ ,  $p = 0.0001$ ). Nevertheless, frogs in southern Costa

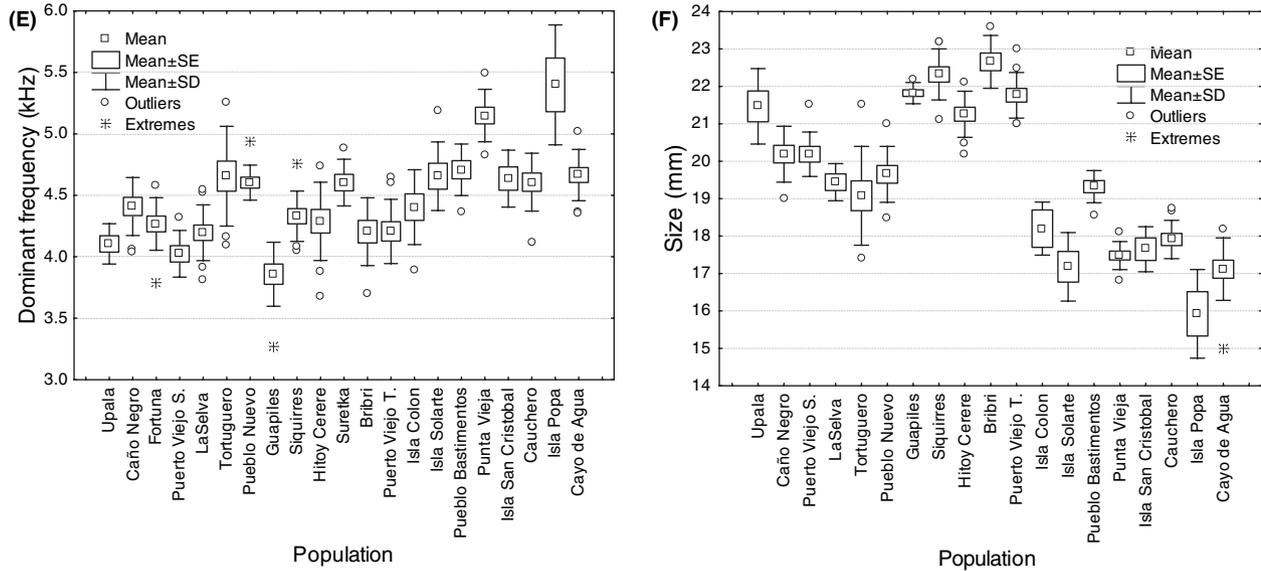


Fig. 2: (Continued)

**Table 3:** Correlations (above diagonal) and partial correlations (below diagonal) of bio-acoustic, geographic and genetic distances between populations of *Dendrobates pumilio*.  $\alpha$ -Values were Bonferroni adjusted ( $\alpha = 0.005/6 = 0.0083$ ) for multiple testing with the same data set. Significant correlation coefficients ( $p < 0.0083$ ) are written in bold

Correlation	Bio-acoustic D	Geographic D	Genetic D
Bio-acoustic D	–	<b>0.59</b>	<b>0.35</b>
Geographic D	<b>0.51</b>	–	<b>0.69</b>
Genetic D	–0.09	<b>0.64</b>	–

Rica deviate considerably from the cline, being larger than frogs in NW Costa Rica and in Panama (Fig. 2f).

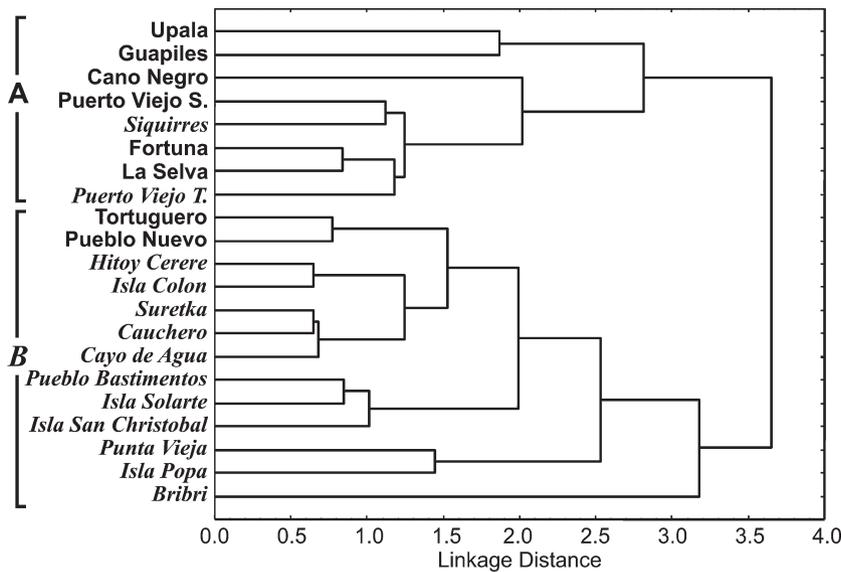
The results of a multiple regression on population mean call parameters with size and distance to Upala as independent variables are shown in Table 4. Distance to Upala significantly influenced four call parameters (all except dominant frequency). The only variable that greatly influenced population mean dominant frequency was the size of the frogs. Size also correlated significantly, but to a lower degree with call rate.

We conducted a nested analysis of co-variance to test for differences between the genetic groups. Firstly, we calculated a nested ANOVA (populations nested in genetic groups) without co-factor. In a subsequent analysis, we used size (Table 5) as co-factor. In an analysis of covariance within-populations differences in co-factors are controlled for.

All call parameters were more distinct between genetic groups than between populations. The difference between both genetic groups was most pronounced in call rate and call duration. All effects were highly significant (Table 5). With size as co-factor, the models did not improve: instead, F-values decreased, indicating that population and body size jointly influence call variation.

A PCA recovered most variance in calls and morphology with two factor axes (88%; Fig. 4). Call rate, call duration, pulse rate and dominant frequency loaded most heavily on the first principal component, while duty cycle loaded most heavily on the second principal component. Variation within the NW genetic group was mostly explained by the first principal component, while a combination of both principal components contributed to the variance in the SE genetic group. The PCA plot revealed a smooth transition in the bio-acoustic/morphological trait combination between both genetic groups.

Colour polymorphism in the Panamanian populations was not associated with higher variability in call parameters relative to the monomorphic Costa Rican populations (Fig. 5). In fact, the variability in call duration, duty cycle and pulse rate was considerably lower across the polymorphic Panamanian populations than across the monomorphic Costa Rican populations. Call rate was slightly less variable and dominant frequency slightly more variable in the Panamanian populations. Yet, variation in colours



**Fig. 3:** Neighbour joining tree of male calls from different populations of *Dendrobates pumilio*. Sample localities in normal type belong to the NW genetic group, sample localities in *italics* to the SE genetic group.

**Table 4:** Multiple regression results (partial correlation and whole model) with call parameters as dependent variables and distance to Upala and size as independent variables. Significant results ( $p < 0.05$ ) are written in bold

	$r_{\text{partial}}$	$p$	$R^2_{\text{model}}$	$F_{2,16}$	$p$
Call rate			<b>0.73</b>	<b>21.65</b>	<b>&lt;0.00003</b>
Distance to Upala	<b>0.66</b>	<b>0.028</b>			
Size	<b>-0.49</b>	<b>0.036</b>			
Call duration			<b>0.74</b>	<b>22.53</b>	<b>&lt;0.00003</b>
Distance to Upala	<b>-0.70</b>	<b>0.00005</b>			
Size	-0.05	0.85			
Duty cycle			<b>0.49</b>	<b>7.78</b>	<b>0.0043</b>
Distance to Upala	<b>-0.69</b>	<b>0.0012</b>			
Size	-0.44	0.0660			
Pulse rate			<b>0.61</b>	<b>12.26</b>	<b>&lt;0.00006</b>
Distance to Upala	<b>0.53</b>	<b>0.022</b>			
Size	-0.42	0.083			
Dominant Frequency			<b>0.68</b>	<b>17.08</b>	<b>=0.00001</b>
Distance to Upala	0.28	0.259			
Size	<b>-0.69</b>	<b>0.0014</b>			

seems to be disconnected or even negatively associated with bio-acoustic variation.

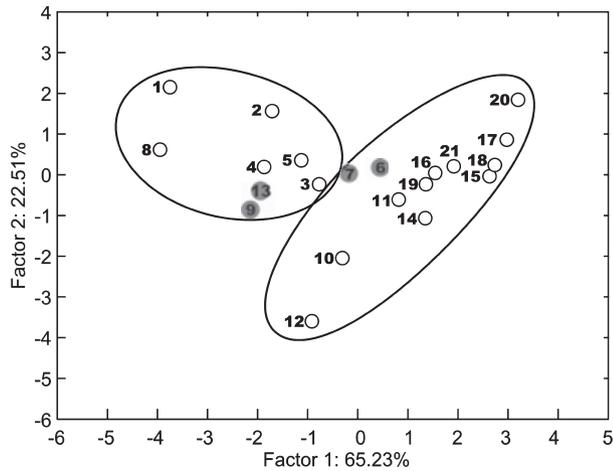
**Discussion**

This study demonstrated that populations of the strawberry poison frog vary in their advertisement calls. Most variation among populations was found in call rate and call duration, whereas pulse rate was the most variable parameter within populations.

**Table 5:** Results of nested analysis of (co-)variance. Call parameters are the dependent variables; genetic group and population (nested in geographic group) are the independent categorical variables. Size is used as co-variable in model 2

Call parameters	1. Nested ANOVA		2. Covariable: size	
	Effect of genetic group	Effect of population	Effect of genetic group	Effect of population
Call rate (1/s)	F = 183.9 $p < 0.0001$	F = 14.10 $p < 0.0001$	F = 136.6 $p < 0.0001$	F = 13.35 $p < 0.0001$
Call duration (ms)	F = 242.9 $p < 0.0001$	F = 11.37 $p < 0.0001$	F = 194.04 $p < 0.0001$	F = 10.74 $p < 0.0001$
Duty cycle (ms/s)	F = 69.08 $p < 0.0001$	F = 8.06 $p < 0.0001$	F = 59.19 $p < 0.0001$	F = 6.96 $p < 0.0001$
Pulse rate (1/ms)	F = 51.48 $p < 0.0001$	F = 6.57 $p < 0.0001$	F = 36.45 $p < 0.0001$	F = 5.96 $p < 0.0001$
Dom. Freq. (kHz)	F = 43.91 $p < 0.0001$	F = 12.94 $p < 0.0001$	F = 30.97 $p < 0.0001$	F = 12.45 $p < 0.0001$

Most of the differences in the advertisement call are explained by a geographic cline: call rate, pulse rate and dominance frequency increased from North Costa Rica to Panama while call duration and duty cycle decreased towards the south. However, the cline is most obvious in sub-regions: call rate was more clinal in the SE while call duration was more clinal in the NW. Except for dominant frequency, which was mostly influenced by the body size of the frogs, call properties were mostly influenced by distance from the most NW population. Yet, body size



**Fig. 4:** Principal Component Analysis plot based on the population means of call parameters, size and weight. Most variation among frogs of different populations is associated with the first principal component. Numbers represent populations as described in Fig. 1. Encircled areas join bio-acoustic groups as found in the cluster analysis (Fig. 3); for populations highlighted in grey, the affiliations to the bio-acoustic group and to the genetic group do not coincide.

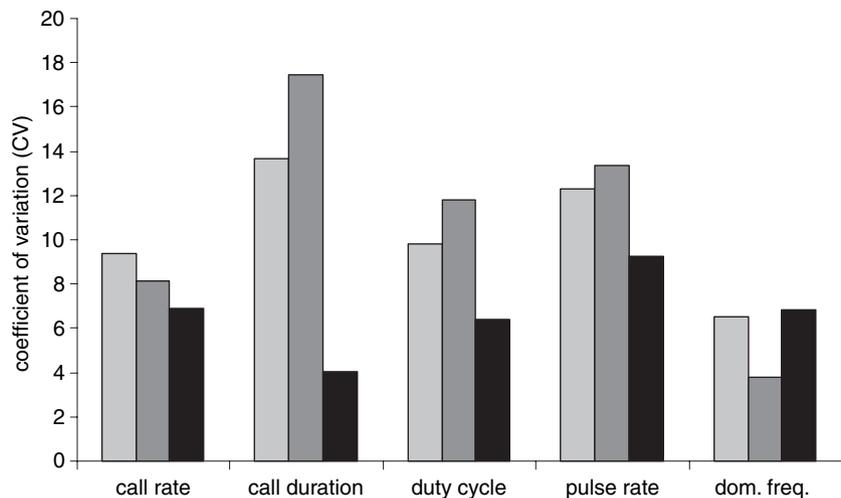
and locality jointly account for much geographic variation in call properties. A cluster analysis extracted two main bio-acoustic groups, which roughly correspond to two genetic groups: one group in the north of Costa Rica, the second one in south Costa Rica and Panama. All call parameters differed significantly between the two genetic groups. However, visualizing variation among populations in a PCA plot revealed no strict bio-acoustic separation between genetic groups. Interestingly, most call parameters were less variable among colour poly-

morphic Panamanian populations than monomorphic Costa Rican populations.

A recent analysis of phylogenetic relationship among almost the same strawberry frog populations (Hagemann & Pröhl 2007) has demonstrated a clear genetic pattern with a division between Guápiles (NW group) and Siquirres (SE group). In the bio-acoustic grouping, some populations from the SE genetic group appear in the NW bio-acoustic group, and some populations from the NW genetic group appear in the SE bio-acoustic group. Together with the results of the Mantel's test, this is an indication that genetic divergence is not a very reliable predictor of bio-acoustic divergence in *D. pumilio*.

Several other studies have examined the relationship between genetic divergence and bio-acoustic divergence in anurans. In túngara frogs, the correlation between genetic and bio-acoustic distances decreased or disappeared after correcting for geographic distance (Ryan et al. 1996; Pröhl et al. 2006). In *Hyla leucophyllata*, the same pattern, i.e. concordance between call structure and geography but not genetic differentiation, was observed (Lougheed et al. 2006). In *Leptodactylus fuscus*, no correlation among geography, calls and genetic patterns could be found (Heyer & Reid 2003). A lack of correlation between genetic and sexual signal dissimilarity seems to be a common phenomenon as it was also observed in other taxa (*Drosophila*: Gleason & Ritchie 1998; birds: Lougheed & Handford 1992; Soha et al. 2004). It may, however, simply reflect the failure of certain genetic markers to accurately measure the gene flow among populations. Our suggestion is that sexual signals and their underlying genes evolve much faster than genes usually used

**Fig. 5:** Among population coefficients of variation for three geographic groups of *Dendrobates pumilio* which differ in their degree of colour polymorphism: light grey bars = North Costa Rica (phenotypically monomorph), dark grey bars = central/south Costa Rica (monomorph), black bars = Panama (polymorph). The first group of populations belongs to the north-western genetic group, the remaining population groups to the south-eastern genetic group.



in population genetic or phylogenetic studies. The emerging pattern that changes in behaviour (e.g. signals or signal preferences) precede genetic changes is now widely recognized (Dall 1997; Gleason & Ritchie 1998). Differences in local selection pressures on body size, on signal transmission efficiency, sexual selection or competition in calling assemblages may be potential causes (Wollermann & Wiley 2002). To date, there is little evidence that the environment affects signal structure in frogs (review: Gerhardt & Huber 2002, but see Castellano & Giacoma 1998 and Ryan et al. 1990). This might be due to the fact that only few environmental variables have been measured, or that analyses have failed to detect interactions among variables. Strawberry poison frogs occur in a variety of habitats (e.g. primary forest, secondary forest, forest edge, cocoa or banana plantations). These probably vary in selection pressures on call parameters because of microclimate, habitat structure or predation pressure. For example, the calls from Pueblo Bastimentos and Punta Vieja were rather different in call rate and dominant frequency. The localities are on the same island only 7 km apart, but the habitats are quite different (S. Hagemann, pers. obs.). Systematic sampling of environmental parameters over longer time periods together with bio-acoustic and morphological analyses has not been carried out for any frog species. Only such a rigorous analysis can disentangle the dependence of morphology and calls from a potential large set of environmental factors.

Differences in local selection pressures are probably also responsible for the colour polymorphism observed in Panama, this not being consistent with geographic call variation or genetic divergence (Hagemann & Pröhl 2007). Populations of the Bocas del Toro Archipelago are genetically very similar as are their calls. This is not surprising given the geographic proximity of populations (and the relatively young age of the islands: 6.000 to 12.000 years; Anderson & Handley 2002) in contrast to the Costa Rican populations. We are not aware of any other study that compared variation pattern of two or several signal modalities, such as calls and colours, within the same taxa. However, intuitively, it seems likely that selection pressures on calls and colours are not the same.

Most of the variation in call characters in *D. pumilio* followed a clinal pattern at least in sub-regions, i.e. the pattern of variation is different between the NW and SE group (Figs. 2 and 4). The fact that the relationship between geography and call variation is not random is also supported by the results of the

Mantel tests. A clinal pattern in phenotypic traits would be expected in the presence of morphological or environmental gradients which in our case might be different between both genetic groups. Clinal variation in call characters is not uncommon in anurans. Several call parameters including dominant frequency and duration also show a geographic cline in túngara frogs (Ryan et al. 1996), but it has not been analysed if body size plays a role in call variation. In cricket frogs, *A. crepitans*, clinal variation in several call parameters was observed, but could not be linked to pleiotropic effects of body size or any other morphological trait (Ryan & Wilczynski 1991).

In our study, body size and geography influenced call characters. Especially, the negative correlation between spectral call components and body size is a well-known relationship in many anuran species (Castellano & Giacoma 1998; Bee et al. 1999; Murphy 1999; Castellano et al. 2002), which makes it difficult to disentangle whether selection acts on call frequency or body size. Besides, the general tendency in *D. pumilio* to decrease in size towards the south, central and southern Costa Rican populations is larger than North Costa Rican populations (Fig. 3f), i.e. a large proportion of variation in size is not clinal.

Interestingly, in *D. granuliferus*, which occurs on the Pacific slope of SE Costa Rica and adjacent south-western Panama, shows opposite geographic trends than those observed in *D. pumilio*: individuals in southern populations are larger, produce longer calls at a slower rate and lower dominant frequency than in the north (Meyer 1996). The fact that *D. granuliferus* from populations with small individuals show a different colour pattern is reminiscent of the colour polymorphism in the tiny Panamanian *D. pumilio*; this intriguing pattern yearns for an evolutionary explanation.

*Dendrobates pumilio* possess relatively simple calls. Many studies have shown that female frogs prefer certain call parameters such as complex calls, calls of long duration, high call rate or low frequency calls (Halliday & Tejedo 1995). Our previous and ongoing studies do not provide much evidence that call characteristics are important for sexual selection in *D. pumilio*. Instead, as in many other species (Halliday & Tejedo 1995; Friedl & Klump 2005), calling activity of individual frogs is most important for female attraction, i.e. males that call the most also have the highest mating success (Pröhl & Hödl 1999; Pröhl 2003; Ivonne Meuche, Institute of Zoology, University of Veterinary Medicine, Hannover, Germany, unpubl. data). This result can be explained by

the simple fact that males that do not call cannot be found by fertile females which may have little time for mate searching. Neither body size nor weight seems to significantly affect calling activity in *D. pumilio* (Pröhl 2003).

Even if females of *D. pumilio* do not discriminate among males of their population based on certain call characteristics, they might be able to distinguish between calls from their own and foreign populations as has been shown in túngara frogs (Pröhl et al. 2006). Populations from North Costa Rica and Panama are rather different, and these differences in the advertisement call could lead to pre-zygotic isolation. Intriguingly, the calls of the population Guápiles deviate substantially from the cline by differing from neighbour populations in several call parameters (longer calls and lower dominant frequency in contrast to the next populations La Selva, Pueblo Nuevo and Siquirres). The border between both genetic groups lies somewhere between Guapiles and Siquirres. The divergence in calls between Guapiles and Siquirres might be an example of reproductive character displacement, provided that both groups have been or still are in contact, an unknown fact to date. Thus, playback experiments and further genetic analysis (including measuring introgression) would present an exciting continuation of this study.

Moreover, populations as close as Pueblo Bastimentos and Punta Vieja differ in calls as well as coloration (frogs from Pueblo Bastimentos show high colour polymorphism within a population, and can be pale green, orange, red or yellow, while frogs from Punta Vieja are bright red). An interaction between visual (Summers et al. 1999) and acoustic sexual communication might have been important in the divergence of sym- or parapatrically distributed phenotypic morphs found in many dendrobatid species (Walls 1994) and might promote fast speciation (Panhuis et al. 2001). Addressing this idea in future studies would contribute greatly to understanding the interface between behaviour and speciation.

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