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Insight into the Evolution of Anuran Foot Flag Displays: A Comparative Study of Color and Kinematics

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Understanding how complex animal displays evolve is a major goal of evolutionary organismal biology. Here, we study this topic by comparing convergently evolved gestural displays in two unrelated species of frog (Bornean Rock Frog, *Staurois parvus*, and Kottigehara Dancing Frog, *Micrixalus kottigeharensis*). This behavior, known as a foot flag, is produced when a male 'waves' his hindlimb at another male during bouts of competition for access to mates. We assess patterns of variation in the color of frog feet and the kinematics of the display itself to help pinpoint similarities and differences of the visual signal elements. We find clear species differences in the color of foot webbing, which is broadcast to receivers during specific phases of the display. Analyses of foot-trajectory duration and geometry also reveal clear species differences in display speed and shape—*S. parvus* generates a faster and more circular visual signal, while *M. kottigeharensis* generates a much slower and more elliptical one. These data are consistent with the notion that color, speed, and shape likely encode species identity. However, we also found that foot flag speed shows significant among-individual variation, particularly the phase of the display in which foot webbings are visible. This result is consistent with the idea that frogs alter temporal signal components, which may showcase individual condition, quality, or motivation. Overall, our comparative study helps elucidate the variability of foot flagging behavior in a manner that informs how we understand the design principles that underlie its function as a signal in intraspecific communication.

A core goal of evolutionary biology is to understand how phenotypic adaptation occurs in the natural world. One of the most fruitful lines of work on this topic focuses on convergent evolution, which refers to the independent emergence of similar traits in unrelated lineages (Morris, 2003, 2008; Losos, 2011). Many interpret convergence as robust evidence of adaptation, considering that it reflects an instance in which selection arrives at the same solution to enhance fitness in the face of similar environmental problems (Endler, 1986; Harvey and Pagel, 1991; Schlüter, 2000; Elias-Costa and Faivovich, 2019; Fischer et al., 2019). The tree of life is replete with such examples, ranging from the repeated emergence of carbon capturing mechanisms in plants (Heyduk et al., 2019) to camera eyes in animals (Land and Nilsson, 2012). This allows biologists to use convergence as a “natural experiment” to probe various facets of phenotypic adaptation, including its genetic, developmental, and functional bases.

Behavior often evolves convergently in unrelated taxa, given that it is a common target of selection (Trillmich and Trillmich, 1984; Ord et al., 2013). This point is especially true with respect to communication behavior (Alexander, 1962; Marler, 1967; Morton, 1975; Ord et al., 2013), which evolves according to several well-described principles that are derived through an animal’s physiological and environmental context (Wiley, 2006; Dangles et al., 2009). For example, the

design of display behavior is shaped by content-based and efficacy-based selection pressures (Guilford and Dawkins, 1991; Hebert and Papaj, 2005). Content-based selection refers to the evolution of signal message or information, and thus is linked to factors associated with the individual performing the signal. On the other hand, efficacy-based selection refers to how efficiently a signal transmits through the environment, and therefore how effectively a receiver perceives the signal. Importantly, individual components of a complex display can each evolve along their own trajectory in response to these two selection pressures, and such effects are sometimes observed through patterns of display variation (Podolak, 2001; Seddon, 2005; Derryberry et al., 2009; Miles et al., 2018a). Display components, for instance, are often distinguished by high levels of among-individual variability (Gerhardt, 1991; Gomez et al., 2009, 2011). These components are likely set by neuro-motor capability and energetic constraints, and thus likely function by indicating a signaler’s current condition or health status. By contrast, highly invariant components of a display, showing little variation, are thought to be relatively fixed indicators of genetic condition, and thus likely function in species recognition (Searcy and Andersson, 1986; Gerhardt, 1991). Accordingly, by combining all these principles, we expect to see the following: when display behavior convergently evolves in response to similar content- and efficacy-based

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selection pressures, common signatures of display variability will arise.

We investigate this idea by studying the convergent evolution of gestural displays, which are displays performed through body movement. This mode of communication is surprisingly common in the animal kingdom, emerging in just about every major vertebrate and invertebrate lineage. Studies show that gestural displays evolve in response to several factors related to a species' physical and social environment (Endler, 1992; Ord et al., 2002; Miles et al., 2017; Miles and Fuxjager, 2018). Even taxa that rely primarily on acoustic or visual signals incorporate gestural routines into their display repertoire, which often enhances the processes of sound production and/or visual conspicuity (Narins et al., 2003; Cooper and Goller, 2004; Soma and Garamszegi, 2015; Miles et al., 2017; Miles and Fuxjager, 2018). In some cases, specific gestures can serve as a functional foundation of a given display, evolving adaptively *before* subsequent signal "add-ons," such as gaudy morphological ornaments, are incorporated into the system (Miles and Fuxjager, 2019). Yet, despite the clear prevalence and importance of gesture to animal communication, we know little about how elaborate gestural signals are designed and function in intraspecies communication. One major gap in this area is our understanding of how different elements of a movement program evolve to influence a receiver's behavior. Addressing this issue can be challenging, because it typically requires testing receiver responses to different gestural displays (a logistical hurdle for many reasons). A potential first step is to study patterns of variation in convergently evolved gestural displays. Indeed, this approach can point to putative dynamic and static components of a display, which in turn hints at the components' functional significance.

We focus our work on gestural displays in frogs. Of course, most frogs use acoustic signals as their predominant mode of communication (Gerhardt and Huber, 2002), and studies show that different features of these calls can often function as dynamic (e.g., gross-temporal parameters) and static (e.g., spectral call parameters) signal properties that are shaped over evolutionary time by receiver responsiveness. For example, in some taxa, static call parameters like frequency correlate with body size and show clear differences from various congeners and heterospecifics (Ryan, 1988; Gingras et al., 2013), and thus can encode species identity (e.g., Hödl, 1977). By contrast, in a host of other taxa, call duration (Wells and Schwartz, 2007) and call complexity are highly variable among males of a given species and can alter the male's attractiveness to females (Ryan and Keddy-Hector, 1992). However, several anuran species have evolved other signal modalities that are produced in combination with or without vocalization (Hödl and Amézquita, 2001), which can also function as static and dynamic signals. For instance, the spectral properties of colorful anuran vocal sacs inflating while calling was suggested to act as a dynamic condition-dependent cue informing about male quality (Gomez et al., 2009). Gesture is a prime example for vocalization-independent signals, as some anuran taxa have evolved the ability to produce foot flag displays. Males slowly lift their rear leg off the ground and extend it out from the body in an arch. The frog then retracts its leg back toward the body, ending the display. Aesthetically, this signal looks like a slow, deliberate wave from one individual to another. Sometimes foot flags

reveal colored interdigital foot webbings, adding to the conspicuousness of the visual signal. The display occurs predominantly in stream-dwelling species, and likely arises in response to strong sexual selection by mediating male-male agonistic interactions for mates (Preininger et al., 2009, 2013a). In some species, foot flagging displays are the predominant display mode during male encounters, facilitating detection and recognition in noisy environments, whereas calls function as alerting and attention-altering signals (Grafe and Wanger, 2007; Grafe et al., 2012). Importantly, foot flagging behavior has emerged in only a handful of species across the anuran phylogeny, which are separated by millions of years of evolution (Hödl and Amézquita, 2001). This display is also thought to be rooted in the same neuro-motor mechanisms among these taxa, as suggested by comparative studies that illustrate how convergence in this display is marked by convergence in underlying physiological mechanisms (Mangiameli et al., 2016; Anderson et al., 2021). One factor common to most foot flagging species is that they inhabit especially noisy environments (e.g., torrents, waterfalls); thus, it is thought that this display evolves to promote short-range visual communication, when acoustic cues may otherwise be unreliable. Altogether, this means that foot flagging is an example of behavioral convergence, given that its emergence "solves" the same environmental problem in social communication among unrelated species.

Here we study the design of the foot flag by comparing signaling behavior in two unrelated species—Kottigehara Dancing Frogs (*Micrixalus kottigeharensis*) and Bornean Rock Frogs (*Staurois parvus*). We expect that each species has evolved similar patterns of variation in the kinematics and signal design of their respective foot flag displays. More specifically, we predict that *M. kottigeharensis* and *S. parvus* evolved similar movement programs and display components, such as arch components and arch durations, to successfully signal in acoustically loud environments (efficacy-based selection pressure). We predict differences in foot flag display to occur between species with regard to webbing coloration and foot flag geometry (i.e., shape from the path of the foot during the display) as these are likely under content-based selection and may serve a species recognition purpose. Additionally, we predict that foot flag durations and foot flag kinematics are variable components as these can be indicators of an individual's neuro-motor capability and are under energetic constraints.

MATERIALS AND METHODS

Study species.—The study protocol adhered to the Animal Behavior Society guidelines for the use of animals in research and all necessary permits were obtained for the field study and approved by the responsible authorities. Visual displays of males of *S. parvus* were collected in a bio-secure container facility at the Vienna Zoo. The Vienna Zoo conducts a breeding program for *S. parvus* with individuals originating from the Ulu Temburong National Park, Brunei Darussalam in Borneo. Several generations are housed in a semi-natural breeding terrarium at temperature of 23–25°C and relative humidity of 70–90% (Preininger et al., 2012). Adult *M. kottigeharensis* were recorded in the natural stream habitat in Agumbe Hobli, Karnataka, India.

Table 1. Successive components of a foot flagging signal.

Foot flagging component	Description
Lift	Lifting the foot from the ground with closed toes (webbings are not visible) and folded leg.
Extension	Extending the knee and vertical stretching of the leg until it reaches full extension. Toes are spread (webbings are visible).
Arch	Down- and backwards movement of the leg in an arc motion. Webbings are visible and displayed in different directions.
Pull-in	The leg, foot, and knee retract inward to the body until toes are closed (webbings are partially visible).
Lower	The fully pulled-in and folded leg with closed toes is lowered to the ground (webbings are not visible).

Reflectance measurements.—Reflectance spectra of foot webbings of *S. parvus* ($n = 16$) and *M. kottigeharensis* ($n = 13$) were obtained with a spectrometer (JAZ series; Ocean Optics, Dunedin, FL, USA) between 300 and 700 nm. The spectrometer had an integrated pulsed xenon light source (Jaz-PX) with a spectral response of 190–1,100 nm. The reflectance data were collected for 300–700 nm and expressed in percentage of reflectance relative to a white standard (WS-1 Diffuse Reflectance Standard, Ocean Optics). We used a custom-made probe holder to keep the reflection probe at a distance of 5 mm and an angle of 45° to the skin surface in order to reduce specular reflection and prevent stray light from entering. Three reflectance measurements per individual were averaged. All reflection measurements were taken on handheld, non-anesthetized frogs immediately after capture to shorten handling time and disturbance. Black rubber gloves were worn to avoid light reflection from the human skin in measurements of both species.

For each frog, coloration parameters were extracted from the reflectance spectra using Avicol software v6 (Gomez, 2006). To determine color differences of foot webbings, we compared brightness values that correspond to the total reflectance, calculated as the surface area under the spectral curve. We omitted the color parameter hue as the reflectance spectra of our study species lack distinct peaks at specific wavelengths.

Behavioral observation and recording.—We collected video recordings of foot flags from adult males of *S. parvus* ($n = 20$, with 224 foot flags) and adult males of *M. kottigeharensis* ($n = 14$, with 128 foot flags) using a Sony RX 10 II slow-motion camera at 250 fps. For the duration analyses, we included all foot flag recordings with a clearly visible foot and distinct beginning and end of the behavior (*S. parvus* $n = 16$, with 170 foot flags; *M. kottigeharensis* $n = 13$, with 33 foot flags). However, for the geometry analyses, we only used recordings in which an individual was facing directly toward or away from the camera (*S. parvus*, $n = 7$ individuals and $n = 8$ foot flags, with one male having two foot flags; *M. kottigeharensis*, $n = 4$ individuals with one foot flag per male). This was determined by ensuring a perpendicular angle of the frog's snout–vent length (SVL) relative to the camera's perspective. By ensuring that we captured foot flags from this viewpoint,

we standardized measures of foot trajectories across space and time along the x- and y-axes. After video documentation, all focal individuals were captured and SVL (± 0.1 mm), body mass (± 0.01 g), and reflectance spectra of foot webbings were measured.

After preliminary inspection of the foot flag videos, we noticed similar patterns in the sequence of leg movement for the foot flag display. We categorized the foot flag into five components: lift, extension, arch, pull-in, and lower (Table 1). This allowed us to have standardized criteria for separating different display components and for comparing foot flags between the two species.

Quantification of foot flag geometry.—We additionally examined the differences in the foot flag trajectories between *S. parvus* and *M. kottigeharensis* by analyzing the shape of the foot flags using Kinovea software. In order to execute this, eye-width length of an individual was used to calibrate the tracking of each foot flag. In order to acquire these measurements, we performed a linear regression on a large dataset ($n = 51$) of known eye width to SVL measurements from multiple individuals of *S. parvus*. We assessed the correlation between eye width and SVL and develop an equation to calculate missing measurements of eye width in our dataset ($t = 4.42$, $P < 0.001$, $R^2 = 0.285$). We then checked if the proportion of the eye width to SVL was the same between *S. parvus* and *M. kottigeharensis* by using a Welch's two-sample *t*-test, so that we could use the same eye-width equation to extrapolate values for both species. We found that the proportions were similar ($t = 0.390$ and $P = 0.721$) and that the variance for each species was low (*S. parvus* < 0.001 and *M. kottigeharensis* $= 0.002$). We then imputed missing SVL and eye widths for *S. parvus* ($n = 16$, mean SVL = 20.61, mean eye width = 7.618) and *M. kottigeharensis* ($n = 13$, mean SVL = 21.27, mean eye width = 7.704). We additionally calculated percent error of our equation by randomly taking out five individuals of *S. parvus*, re-running the linear regression, using the new equation to estimate the eye width of the removed individuals, and comparing that calculated values to the known eye widths. Our equation had a percent error of 3.350%.

We then analyzed the foot flag coordinates with the 'Momocs' package in R Studio (Bonhomme et al., 2014). We first ran an elliptical Fourier analysis (EFA) on the x and y coordinates of the foot trajectory to quantify the display's shape (i.e., geometry of the path of movement). An EFA is a standard heuristic, widely used in ecological and evolutionary studies to quantify object shape (Chitwood and Sinha, 2016; Rubin et al., 2018; Telesca et al., 2018), and to our knowledge, the current study uses EFA for the first time to measure movement shape. Generally, an EFA reduces a shape to a series of simple sine (x coordinate) and cosine (y coordinate) components, standardizing shape comparisons across groups. It then combines the components to form a set of interlinked ellipses or harmonics, which move about one another through time. While a larger harmonic order means that a shape can be more accurately drawn, a large harmonic order can also decrease the amount of shape information that is necessary to compare between shapes (Caple et al., 2017). Thus, we calibrated our EFA to 11 harmonics, which accounts for 99% of the total harmonic power of the foot flag geometry.

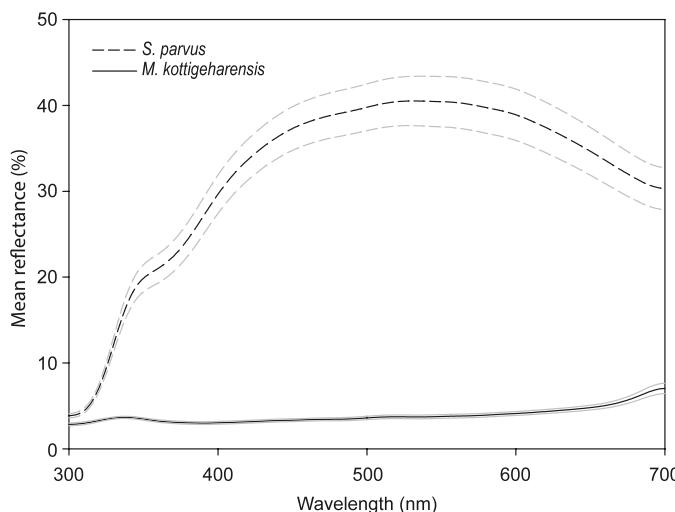


Fig. 1. Reflectance spectra of the foot webbings of *Staurois parvus* ($n = 16$, dashed line) and *Micrixalus kottigeharensis* ($n = 13$, solid line). Gray lines represent respective standard errors.

We next performed a principal component analysis (PCA) on the matrix of coefficients from the EFA to quantify shape variation in the foot flag itself (Chitwood and Sinha, 2016; Rubin et al., 2018; Telesca et al., 2018). This approach reduced the array of EFA data into 12 principal components (PCs), with the first nine PCs accounting for 99% of variation in the display's 2D geometry. See results for examples for the PC loadings. Using a multivariate analysis of variance (MANOVA) on these first nine PCs, we tested for differences in the amount of separation in PC space between foot flag geometries of *S. parvus* and *M. kottigeharensis*. We also ran a set of Welch's two-sample *t*-tests on each PC score to examine whether any single PC differed between the two species. We ran final Welch's two-sample *t*-tests on the x displacement, y displacement, and overall path length of the foot flags, as well as the x displacement, y displacement, and path lengths for each foot flag component to further assess shape differences between *S. parvus* and *M. kottigeharensis*.

Finally, we performed a linear discriminant analysis (LDA) to reduce our PCs into two axes and identify the linear combination of geometrical features that best distinguish foot flags between *S. parvus* and *M. kottigeharensis*. An additional Welch's two-sample *t*-test uncovered the degree to which the foot flags from the two species were identified and separated from one another. The LDA also identified which shape variables are best for distinguishing foot flags from both species. Finally, we implemented a standard "leave-one-out" cross-validation model to assess the accuracy of our LDA. This method randomly removed one foot flag shape from the dataset and created an LDA with the remaining foot flags. The model then recursively assigned the removed shape as either *S. parvus* or *M. kottigeharensis* using the derived algorithm. The model ran through all possible combinations of foot flag shapes and output a final percent assignment accuracy value.

Duration of foot flag.—Foot flagging videos for the duration analyses were processed in Kinovea (<https://www.kinovea.org>), which allowed precise frame by frame breakdown of the duration of the visual behavior. We excluded videos when

components of foot flag display were missing. Several *M. kottigeharensis* foot flags were removed from the analysis because the frog failed to complete the pull-in and lower components during the recording period of the slow-motion video.

We compared the total duration of foot flags as well as the duration of each foot flag component between *S. parvus* ($n = 16$) and *M. kottigeharensis* ($n = 13$) using linear mixed models (LMMs). LMMs allow for repeated measurements of the same individual to be fitted in the model as random variables, thus controlling for differing number of individuals per species and foot flags per individuals. The durations of foot flag components were entered as dependent variables, with species as a predictor variable. We entered the individual-male identities nested within species and foot flag identities nested within individual male as random variables to correct for multiple males per species and non-independent foot flags per individual.

Similarly, we compared durations among individuals of each species. The durations of foot flag components were again entered as dependent variables, with individual male as a predictor variable. We entered the foot flag identities as a nested random variable within individual male, to correct for multiple non-independent foot flags per individual.

Foot flagging and foot color.—Finally, we tested whether individual variation in foot-webbing brightness predicts variation in duration components of the foot flag or male body weight and size (SVL). This allowed us to explore whether males that have whiter foot webbings showcase this information for a longer period of time during their display, and whether brightness might serve as a cue for an individual's size. To conduct these analyses, we ran Pearson's correlations between male foot-webbing brightness and measures of foot flag durations and body size (weight and SVL). When males produced more than one foot flag, we computed an average duration value for this analysis. Additionally, we ran a Levene's test for equality of variances to qualitatively assess the variation in foot color between *S. parvus* and *M. kottigeharensis*.

RESULTS

Foot color.—We first obtained reflectance measures of both species to verify that the color of their foot webbing was different. Consistent with the appearance of a bright white foot, we found high levels of reflectance across the entire light spectrum in the foot webbings of *S. parvus*. By contrast, *M. kottigeharensis* showed low levels of reflectance, indicating that the color of its foot webbing is almost translucent and thus mostly reflecting the color of the black background. This difference bears out statistically, as levels of brightness being significantly greater in *S. parvus* (Mann-Whitney *U* test = 91.0, $N_1 + N_2 = 29$, $P < 0.001$; Fig. 1). Also noteworthy is that variation in brightness of foot webbing was significantly greater in *S. parvus* compared to *M. kottigeharensis* ($F_{1,27} = 38.86$, $P < 0.001$).

Geometry of the foot flag display.—Using EFA analyses on trajectories of foot movement during foot flag displays, we next compared the geometry of each species' foot flag. The first step of this process involved running a PCA on the shape of the foot flag display. We found that the entire PC space characterizing the foot flag geometry differed significantly

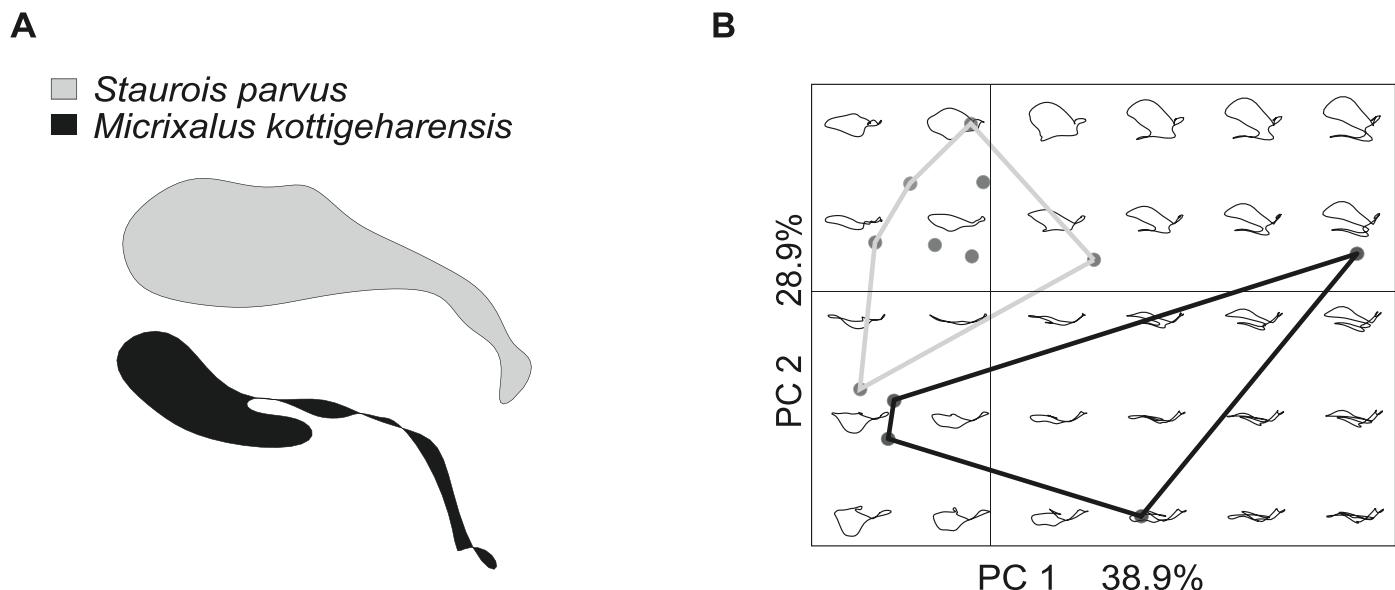


Fig. 2. Foot movements of *Staurois parvus* (gray) and *Micrixalus kottigeharensis* (black) differ during respective foot flagging displays. Visual mean shape differences of the foot flag trajectory (A) and *S. parvus* and *M. kottigeharensis* occupy different areas in principal component (PC) space (B). Points represent individual foot flag trajectories. Shape approximations of PC loadings are plotted in the background.

between the species, denoting a difference in the mean foot flag shape between *S. parvus* and *M. kottigeharensis* (MANOVA: Wilks' lambda = 0.006, $F_{1,9} = 35.99$, $P = 0.027$; Fig. 2A). To help visualize this effect, we plotted the first two PCs against each other, which collectively account for 67.8% of the variation that describes the shape of the foot flag (Fig. 2B). Otherwise, when we broke down the PCA, we uncovered a total of nine separate PCs that describe different aspects of the display's shape (Table 2). Only PC2 differed significantly among species ($t = -2.699$, $df = 4.586$, $P = 0.047$), and it defines the general circularity of the foot trajectory (Fig. 3B). Thus, *S. parvus* has higher PC2 values than *M. kottigeharensis*, which denotes a more circular foot flag.

We verified this shape analysis with an LDA. This approach helps pinpoint factors associated with the geometry of the foot flag that best predict the species generating it. Accordingly, our LDA was successful in identifying and differentiating foot flags from *S. parvus* and *M. kottigeharensis* solely on shape information ($t = -49.15$, $df = 8.970$, $P <$

0.001; Fig. 3A). The LDA also output the percentage of each PC used to discriminate between species. Higher percentages reflect a more robust effect in the model, and thus greater discriminatory power. Accordingly, PC1, PC2, PC4, and PC5 were the most important in terms of determining whether the foot flag in question is attributed to either *S. parvus* or *M. kottigeharensis* (Fig. 3B). Finally, we implemented a leave-one-out cross-validation procedure to examine the accuracy of our LDA. We found that our LDA categorized the correct species from a foot flag shape 75% of the time.

Properties of foot movement.—We next explored species differences in the foot flag display (Table 3). We started by looking at total duration of the behavior, finding that it differed significantly between taxa ($F_{1,201} = 22.08$, $P < 0.001$). *Micrixalus kottigeharensis* produced displays that were longer in duration compared to *S. parvus*. This effect was driven by changes in duration to specific components of the display itself, including duration of the arch ($F_{1,201} = 5.589$, $P = 0.019$), pull-in ($F_{1,201} =$

Table 2. Comparisons of principal component (PC) scores, or shape information, between *Staurois parvus* and *Micrixalus kottigeharensis*. Values represent estimated means \pm standard error (SE), t statistics, degrees of freedom (df), P -values from Welch's two sample t -tests, and percent contribution of each PC (from linear discriminant analysis) to the discrimination between foot flags of *S. parvus* and *M. kottigeharensis*. Significant P -values are bolded.

Principal component	Principal component score					
	<i>M. kottigeharensis</i> Mean \pm 1 SE	<i>S. parvus</i> Mean \pm 1 SE	t statistic	df	P -value	% Contribution to discrimination
1	0.248 \pm 0.321	-0.110 \pm 0.063	1.040	3.330	0.368	1.408
2	-0.371 \pm 0.177	0.165 \pm 0.083	-2.699	4.586	0.047	2.056
3	0.046 \pm 0.181	-0.020 \pm 0.038	0.323	3.383	0.766	0.206
4	0.069 \pm 0.099	-0.030 \pm 0.050	0.899	5.478	0.406	0.425
5	0.069 \pm 0.053	-0.031 \pm 0.046	1.321	8.741	0.220	0.417
6	-0.034 \pm 0.050	0.015 \pm 0.036	-0.780	7.907	0.458	0.210
7	0.018 \pm 0.067	-0.008 \pm 0.027	0.254	4.815	0.810	0.076
8	-0.020 \pm 0.016	0.009 \pm 0.030	-0.643	9.290	0.536	0.110
9	-0.021 \pm 0.024	0.009 \pm 0.022	0.867	9.581	0.407	0.128

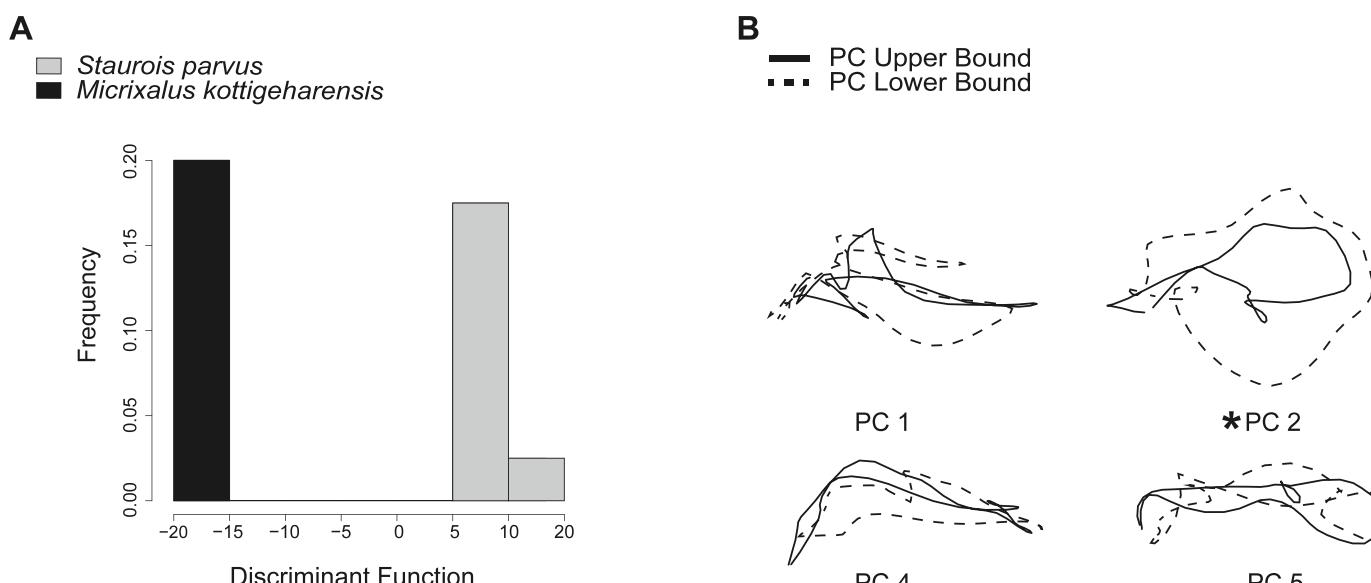


Fig. 3. Foot flagging trajectory shapes separate *Staurois parvus* (gray) and *Micrixalus kottigeharensis* (black). Scores of linear discriminant analysis (LDA) based on trajectory shapes (A). Particular principal components (PC) of shape information that best distinguish between *S. parvus* and *M. kottigeharensis* (B). The solid line and dotted line for each PC represent the upper and lower extremes of the PC axis, respectively. The overlap of the two lines show what shape information is loaded into each PC. The asterisk denotes significant differences ($P < 0.05$).

44.32, $P < 0.001$), and lower ($F_{1,201} = 8.056$, $P = 0.005$) components, which were longer in *M. kottigeharensis* than *S. parvus*. However, the durations of the lift ($F_{1,201} = 2.829$, $P = 0.094$) and extension ($F_{1,201} = 0.145$, $P = 0.704$) components were statistically indistinguishable between species.

In a subset of individuals, we also explored difference in path length of the foot flag (Table 4). Overall, *M. kottigeharensis* traced a longer path with the foot while displaying than *S. parvus* ($t = 9.232$, $df = 7.066$, $P < 0.001$). This effect was attributed mainly to a significantly longer path etched during the extension component of the foot flag by *M. kottigeharensis* ($t = 6.411$, $df = 3.674$, $P = 0.004$). Notably, during this phase of the display, *M. kottigeharensis* produces several back-and-forth jerking motions that look like it is pumping its leg as it slowly extends outward above the head (Fig. 4A). *Staurois parvus* on the other hand produces smooth and steady leg movements during the knee extension phase (Fig. 4B). Path lengths associated with other components of the display were indistinguishable between the species (Lift: $t = 0.751$, $df = 4.362$, $P = 0.491$; Arch: $t = -0.071$, $df = 7.879$, $P = 0.946$; Pull-in: $t = 0.222$, $df = 4.207$, $P = 0.835$; Lower: $t = 0.543$, $df = 6.134$, $P = 0.606$).

Mirroring the path length results, we found that total vertical ($t = 6.367$, $df = 5.368$, $P = 0.001$) and horizontal

displacement ($t = 5.425$, $df = 10.98$, $P < 0.001$) of the foot was significantly greater in *M. kottigeharensis*. Again, this main effect is attributed to greater vertical ($t = 4.733$, $df = 3.802$, $P = 0.010$) and horizontal displacement ($t = 5.879$, $df = 3.473$, $P = 0.006$), specifically during the extension component of the foot flag of *M. kottigeharensis*, likely tracing back to its jerking movements. The measures of displacement are similar for all other components of the behavior (Table 4).

Among-male comparisons.—In both species, we looked to see whether individual males differed with respect to the duration of their foot flag display and its constitutive components. We found evidence of significant differences among males for extension components (*S. parvus*: $F_{15,154} = 14.033$, $P < 0.001$; *M. kottigeharensis*: $F_{12,20} = 4.040$, $P = 0.003$; Table 5) and arch components (*S. parvus*: $F_{15,154} = 5.101$, $P < 0.001$; *M. kottigeharensis*: $F_{12,20} = 4.961$, $P = 0.001$; Table 5) in both species. Note that these are the phases of foot flag when the webbings are visible to the receiver. With respect to the duration of the pull-in and lower components of the display, only *S. parvus* showed evidence of among-male differences (Pull-in: $F_{15,154} = 6.258$, $P < 0.001$; Lower: $F_{15,154} = 3.305$, $P < 0.001$; Table 5). It is important to note that the variation in

Table 3. Comparison of durations of foot flagging signal components of *Staurois parvus* and *Micrixalus kottigeharensis*. Values represent estimated means, standard errors (SE), F statistics, degrees of freedom (df), and P -values of linear mixed models (LMM). Significant P -values are bolded.

Foot flagging component	Duration (ms)						
	<i>S. parvus</i>		<i>M. kottigeharensis</i>		F statistic	df	P -value
		Mean \pm 1 SE		Mean \pm 1 SE			
Lift		96 \pm 10		120 \pm 15	2.829	1, 201	0.094
Extension		715 \pm 48		744 \pm 60	0.145	1, 201	0.704
Arch		523 \pm 59		744 \pm 72	5.589	1, 201	0.019
Pull-in		59 \pm 31		381 \pm 37	44.32	1, 201	<0.001
Lower		63 \pm 5		86 \pm 7	8.056	1, 201	0.005
Full display		1454 \pm 83		2069 \pm 101	22.08	1, 201	<0.001

Table 4. Comparison of distance measurements for foot flagging signal components between *Staurois parvus* and *Micrixalus kottigeharensis*. Values represent distance measurements (x-axis, y-axis, and total path length), estimated means and standard error (SE), t statistic, degrees of freedom (df), and P-value from Welch's two sample t-tests. Significant P-values are bolded.

Foot flag component	Distance measurements	Distance (mm)		t statistic	df	P-value
		<i>M. kottigeharensis</i> Mean \pm 1 SE	<i>S. parvus</i> Mean \pm 1 SE			
Lift	x-axis	4.750 \pm 1.156	2.600 \pm 0.438	1.686	4.367	0.161
	y-axis	6.780 \pm 1.444	6.720 \pm 0.748	0.033	4.320	0.975
	Total path length	9.117 \pm 1.860	7.626 \pm 0.892	0.751	4.362	0.491
Extension	x-axis	58.40 \pm 6.650	17.82 \pm 2.012	5.879	3.473	0.006
	y-axis	33.28 \pm 3.945	13.43 \pm 1.512	4.733	3.802	0.010
	Total path length	72.66 \pm 7.010	25.33 \pm 2.621	6.411	3.674	0.004
Arch	x-axis	17.73 \pm 3.814	15.79 \pm 2.977	0.385	7.512	0.711
	y-axis	12.50 \pm 2.891	15.34 \pm 2.045	-0.706	7.659	0.481
	Total path length	24.75 \pm 5.173	25.24 \pm 4.020	-0.071	7.829	0.946
Pull-in	x-axis	6.60 \pm 1.863	10.30 \pm 2.006	-1.425	8.485	0.190
	y-axis	11.50 \pm 2.322	7.470 \pm 0.921	1.617	3.964	0.182
	Total path length	14.86 \pm 2.998	14.14 \pm 1.488	0.222	4.207	0.835
Lower	x-axis	2.480 \pm 0.421	2.290 \pm 0.410	0.327	7.959	0.752
	y-axis	5.180 \pm 0.875	4.110 \pm 0.508	1.079	4.706	0.333
	Total path length	6.215 \pm 0.980	5.562 \pm 0.698	0.543	6.134	0.606
Full display	x-axis	88.27 \pm 5.420	47.86 \pm 3.412	6.367	5.368	0.001
	y-axis	67.67 \pm 2.144	46.18 \pm 2.151	5.425	10.98	<0.001
	Total path length	126.1 \pm 4.386	73.58 \pm 3.616	9.232	7.066	<0.001

foot flagging display durations for adult males of either species cannot be explained by body size (*S. parvus*: $r_s = 0.338$, $P = 0.200$; *M. kottigeharensis*: $r_s = -0.538$, $P = 0.058$) or weight (*S. parvus*: $r_s = 0.142$, $P = 0.599$; *M. kottigeharensis*: $r_s = -0.509$, $P = 0.538$).

Finally, we tested whether male variation in the color of foot webbing was associated with such variation in display durations (Table 6). Neither species showed evidence of an association between the brightness of a male's foot webbings and the duration of his full display (*S. parvus*: $r_s = -0.121$, $P = 0.656$; *M. kottigeharensis*: $r_s = 0.143$, $P = 0.642$). After looking more closely at the specific components of the foot flag where webbings are visible, we again found no evidence of a correlation between web brightness and extension duration (*S. parvus*: $r_s = -0.144$, $P = 0.594$; *M. kottigeharensis*: $r_s = 0.291$, $P = 0.334$; Table 6) and arch duration (*S. parvus*: $r_s = -0.032$, $P = 0.905$; *M. kottigeharensis*: $r_s = 0.082$, $P = 0.789$; Table 6). Additionally, we found no relationship between individual variation in brightness and body weight (*S. parvus*: $r_s = 0.255$, $P = 0.341$; *M. kottigeharensis*: $r_s = -0.148$, $P = 0.630$; Table 6) or body size (*S. parvus*: $r_s = -0.182$, $P = 0.499$; *M. kottigeharensis*: $r_s = 0.148$, $P = 0.629$; Table 6) in either species.

DISCUSSION

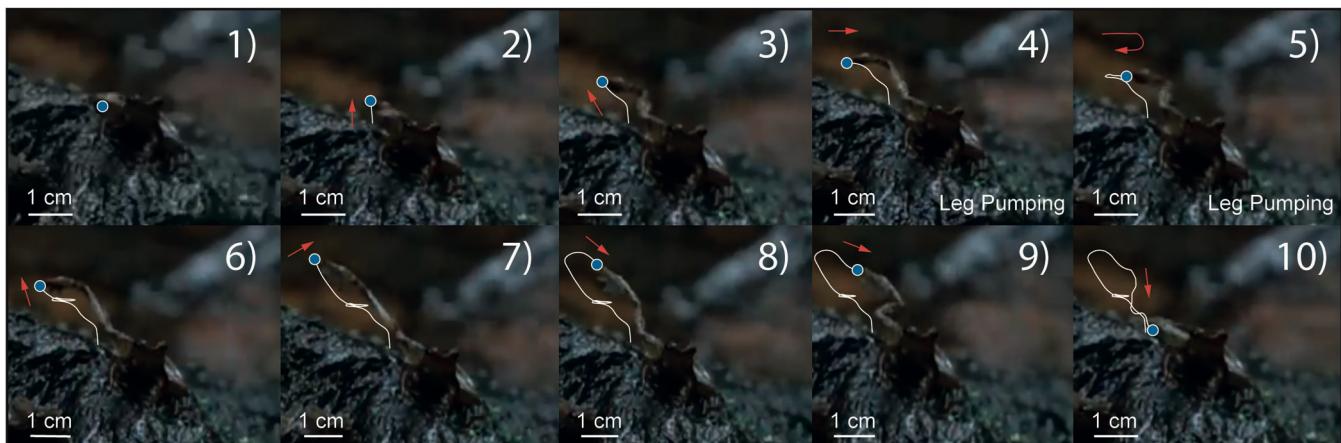
Here, we compare convergently evolved foot flag displays in two anuran taxa *M. kottigeharensis* (Kottigehara Dancing Frog) and *S. parvus* (Bornean Rock Frog). The design of these displays likely occurred in response to similar content- and efficacy-based selection pressures, thereby allowing us to assess patterns of variation in this display and their putative functional significance.

Foot color.—Foot coloration is perhaps the most straightforward component of the signal to distinguish. We see clear species differences in the color trait: *S. parvus* has bright

white foot webbings, whereas *M. kottigeharensis* has darker, translucent gray webbings with a reflective sheen. The foot webbings of *S. parvus* pose a 13 times stronger contrast against their visual background than those of *M. kottigeharensis* (Preininger et al., 2013b). There is certainly evidence of among-individual variation in web coloration, at least with respect to *S. parvus*, whereas *M. kottigeharensis* shows little brightness variation in the color of its webbings. This difference in variation between taxa may reflect differences in the degree to which selection influences foot color, with greater variation possibly signifying more robust effects of sexual selection (Ryan and Keddy-Hector, 1992; Pomiankowski and Møller, 1995; Rodríguez and Greenfield, 2003; Hosken and Stockley, 2004; Johnston et al., 2013).

At the same time, foot-webbing brightness fails to predict corresponding temporal variation of foot movement during the display, particularly during the components in which individuals expose their webbings (extension and arch components). Hence, males with brighter webbing do not present them for longer or shorter periods to conspecific rivals. Furthermore, foot brightness does not predict differences in body size or weight, which likely plays an important role in deciding the outcome of agonistic disputes or might influence female receivers. We therefore hypothesize that foot brightness functions as invariant color signal, likely encoding species identity (Searcy and Andersson, 1986; Maan and Cummings, 2008; Klomp et al., 2017; but see Maynard Smith, 1991). Further support for this idea comes from two lines of evidence. First, in *S. parvus*, signal brightness increases from metamorphosis until adulthood and thus is likely related to age and/or sexual maturity (Stangel et al., 2015). Second, populations of each species in this study occur in sympatry with other foot flagging congeners that have different web coloration—*M. kottigeharensis* lives alongside *M. niluvasei* which has only partly webbed feet with black webs and whitish toes, whereas some

A *Micrixalus kottigeharensis*



B *Staurois parvus*

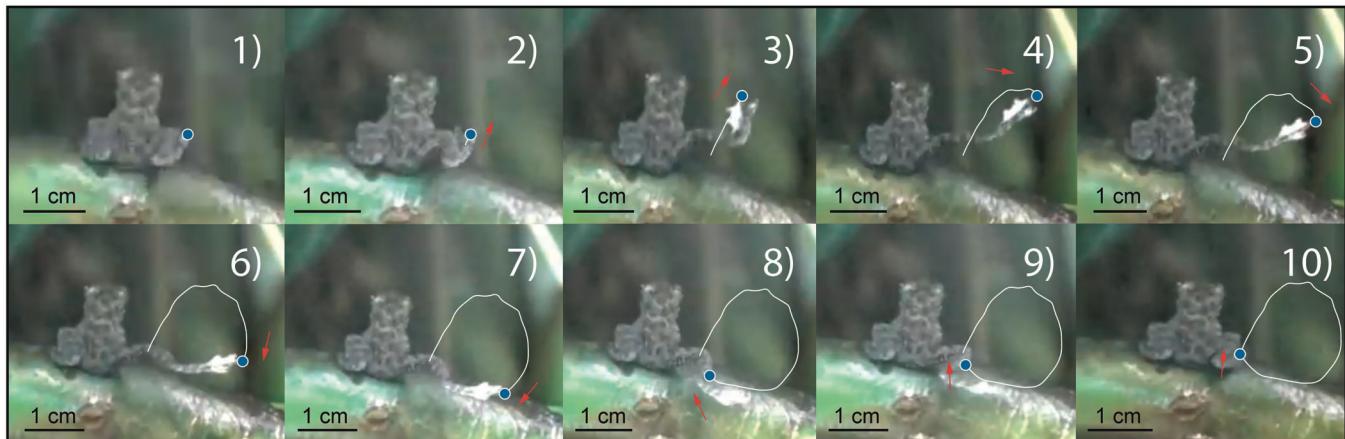


Fig. 4. Frame-by-frame stills that show *Micrixalus kottigeharensis* (A) and *Staurois parvus* (B) performing a foot flag, while tracking foot trajectory (white line and blue dot) through space and time (1–10 stills). The red arrows indicate the direction of foot movement. Note that *M. kottigeharensis* faces directly toward the camera, where *S. parvus* faces directly away from the camera. Both points of view produce the same 2D shape of the foot flag signal as it is traced in the air (see Methods); thus, we provide both views to illustrate this point, even though we collected data from each species from both of these ‘front and back’ positions.

populations of *S. parvus* live alongside *S. guttatus* which has bluish webbings (Stangel et al., 2015; M. J. Fuxjager and D. Preininger, pers. obs.).

Why did white foot webbing evolve in *S. parvus* and less conspicuous gray foot webbing in *M. kottigeharensis*? We

suspect that color evolution plays out this way to balance signal efficacy in a context of sympatric life with congeners. This idea is first based on the notion that signal color enhances signal conspicuousness by sharpening its contrast to an animal’s environmental backdrop (Endler, 1991, 1992,

Table 5. Among-male variation of foot flagging component durations. During the components “Extension” and “Arch” foot webbings are visible. Linear mixed model (LMM) results are shown. Significant *P*-values are bolded. * Bonferroni corrected pairwise comparison shows no significant differences.

Foot flag component	Duration (ms)						
	<i>S. parvus</i>			<i>M. kottigeharensis</i>			
	<i>F</i> statistic	df	<i>P</i> -value		<i>F</i> statistic	df	<i>P</i> -value
Lift	1.527	15, 154	0.102		1.771	12, 20	0.125
Extension	14.03	15, 154	0.001		4.040	12, 20	0.003
Arch	5.101	15, 154	0.001		4.961	12, 20	0.001
Pull-in	6.258	15, 154	0.001		1.429	12, 20	0.232
Lower	3.305	15, 154	0.001		2.342	12, 20	0.045*
Full display	16.58	15, 154	0.001		2.773	12, 20	0.021*

Table 6. Within-species correlation of male web brightness with the duration of the full display and the signal components: extension, arch, and with body weight and snout-vent length (SVL).

Foot flag component	<i>S. parvus</i> web brightness		<i>M. kottigeharensis</i> web brightness	
	<i>r</i> _s	P-value	<i>r</i> _s	P-value
Extension (ms)	−0.144	0.594	0.291	0.334
Arch (ms)	−0.032	0.905	0.082	0.789
Full display (ms)	−0.121	0.656	0.143	0.642
Weight (g)	0.255	0.341	−0.148	0.630
SVL (cm)	−0.182	0.499	0.148	0.629

1993). Thus, the white foot webbing of *S. parvus* likely creates a strong achromatic contrast with the black granite substrate on which individuals display. Foot color in *M. kottigeharensis* is more challenging to explain, as males display against an orange-gold riverbed; thus, one possibility is that this species' grayish webbings provide sufficient contrast in this setting. Another possibility is that gray foot webbing is an efficacious contrast to arise in response to selection, given a host of other factors such as the receiver's sensory biology and/or developmental constraints on color evolution. Further, natural selection by predation (Endler, 1983; Stuart-Fox et al., 2003) or other factors may be in opposition to white foot webbing (or other colorations than gray). None of these ideas are mutually exclusive, and this issue can only be solved through further investigation of the biology of 24 *Micrixalus* frog species of the Western Ghats of India.

Foot flag geometry.—The overall shape of the foot flag differs markedly among the species. In *S. parvus*, the foot traces a more circular path with more or less equal vertical and horizontal displacement. By contrast, the foot of *M. kottigeharensis* traces a more oval path with a far greater horizontal displacement. Our PCA further supports this finding, as the primary PC that differs between the taxa is the one that reflects circularity in foot flag geometry. Equally important is the finding that our LDA can reliably use geometry to categorize species. These results again point to the idea that the overall "shape" of the gestural display can encode important information about species identity. Anecdotal observations of sister taxa living sympatrically with the two focal species assessed herein provide further support to this idea, given that these other species appear to produce foot flags that are markedly different in their geometric composition. Selection may therefore drive the evolution of geometry in part to appropriately visually recognize conspecifics during male–male encounters.

Unfortunately, our sample size is not large enough to test whether differences in display geometry predict aspects of body size or foot color. Within each species, we do see individual variation in the shape of the foot flag. It is therefore not unreasonable to suspect that, in addition to mediating species recognition, display geometry reveals information about the signaler's quality or condition (Hill, 2015). In fact, we might expect among-individual variation, given that such variable elements of a signal are often closely tied to neuro-motor performance and energetic constraints (Gerhardt and Bee, 2006; Reichert and Gerhardt, 2012). Production of the foot flag may require superior motor command, particularly at the levels of the spinal cord where

coordination of the display program likely rests (Tresch et al., 1999; Poppele and Bosco, 2003). Assessing this idea requires a deeper exploration into the physiological control of foot flagging.

Foot movement.—Leg movement parameters during the foot flag show significant differences between the species. First, durations of the arch, pull-in, and lower components of the foot flag are markedly longer in *M. kottigeharensis* than in *S. parvus*. The path length of these same components, however, are indistinguishable between the taxa, which implies that *M. kottigeharensis* moves its leg more slowly through the air, as it traces the oval path. In this sense, speed is a key difference in the display.

The discussion above does not address the extended knee phase of the display. Our data suggest that species do not differ in terms of the time it takes to complete the knee extension component of the display. However, this is not likely the case, as *M. kottigeharensis* heavily jerks its leg back-and-forth and up-and-down as it completes the knee extension, whereas *S. parvus* does not (rather, it produces smooth leg movement during knee extension). The jerky movements suggest that *M. kottigeharensis* takes more time to complete this component of the display routine; yet, the durations are actually indistinguishable across species, demonstrating that *S. parvus* performs its knee extension as slower and continuous movement. This is also supported by our findings that vertical and horizontal path length are greater in knee extensions of *M. kottigeharensis*, as this effect is largely driven by the jerking movements described above. In fact, the effects of jerking movement were so strong, they also drove the significant species difference in path length for the entire foot flag. Hence, we observed distinct species differences during the extension component and similarities during the arch component. Additionally, we found high among-individual variation of duration in both the extension and arch component in *S. parvus* as well as in *M. kottigeharensis*. As mentioned in the section foot color, these duration differences between conspecific males could not be explained by web brightness. Consequently, we suggest that signal speed may provide information about motivational state, physiological condition, or fighting ability, which then can be used by opponents to determine their response in an agonistic interaction.

Principles of foot flag design.—Our data demonstrate highly variable and invariant elements of the foot flag, while simultaneously suggesting that these elements have also convergently evolved. Patterns of variation, for example, suggest that color is likely a fixed component of the foot flag. It shows clear species differences (even among sister taxa) and does not correlate with other elements of the foot flag display. In this way, we hypothesize that foot color encodes species identity and sexual maturity but does not necessarily convey further information about signaler quality.

Foot flag geometry is more difficult to categorize in this manner. It shows clear species differences, but our sample size is not large enough to test whether variation in shape predicts aspects of body size or other components of the foot flag. We therefore conclude that geometry may encode species identity as the geometries are quite different between species, but we also recognize and leave room for the possibility that geometry corresponds to signaler quality.

This idea is supported by substantial among-individual variation that we have found with regard to the hormonal control of foot flag display geometry in *S. parvus* (Anderson et al., in press), yet receiver responses to signal variations need to be tested to draw further conclusions.

Patterns of variability suggest that foot speed—which we surmise from measures of duration and path length—has the capacity to encode species and individual attributes. *Staurois parvus* clearly perform shorter foot flags than *M. kottigeharensis*, whereas both taxa also show evidence of among-individual variation in the duration parameters associated with various components of the foot flag. The latter are hallmark signatures of dynamic signal elements (Gerhardt, 1991)—thus, we propose that kinematic foot flag properties are similar to dynamic aspects of call and/or call-note duration in anurans (Gerhardt and Huber, 2002). However, to unravel each component's functional significance in terms of dynamic and static expression of signal properties, within-individual variation and how it influences receivers also need to be assessed.

For the observed variations, we also expect that neuro-motor limits and energetic constraints set the performance boundaries that define how the foot is moved during the display, at least with respect to speed (Miles et al., 2018b; Tobiansky et al., 2020). Others speculate that the ability to approach these limits during behavioral performance offers a route to showcase one's motor skill, which in turn provides honest information about a signaler's quality, condition, and/or developmental integrity (Dunn and Cockburn, 1999; Bortosky and Mathis, 2016). Building on this idea further, past research shows that a more "skillfully" produced gestural display—namely, complex displays produced at greater speed—either attracts potential mates or repels potential rivals (Byers et al., 2010; Barske et al., 2011; Schuppe and Fuxjager, 2018).

Interestingly, the strongest signatures of signal variation are in foot speed during the extension and arch components of the foot flag, which is precisely when the signaler exposes its foot webbing. This part of the signal may therefore be the most salient to receivers, as it represents the moment at which the signaler expresses multiple elements (foot color, display shape, and leg speed) that could potentially influence receivers. Importantly, we show that temporal integration among such display components is similar between the two unrelated species. This result implies that synergy in visual information is a major aspect of convergently evolved foot flagging behavior.

Despite our emphasis on convergent properties of the foot flag display, we must recognize that our data also uncover several differences. Understanding why differences do emerge is challenging. One possibility is that neutral processes underlie divergence in components of the display, particularly those that have less functional importance (for example, horizontal movements; see above). By contrast, such differences may adaptively evolve. Although foot flagging frogs inhabit noisy environments that represent reproduction niches in species-rich habitats, these microhabitats impose their own unique suites of constraints. For example, both study species signal in perennial stream habitats, but *Micrixalus* spp. limit reproduction in these streams to periods of two to three months at the end of monsoon rains, when streams provide stable water conditions for eggs and tadpoles. Frogs in *Staurois*, on the other

hand, experience relatively continuous (all year long) breeding conditions, even after heavy rains that occur frequently. Such differences in breeding season may influence the intensity of sexual selection (Andersson, 1994; Jones, 2009), which in turn impacts the design of the foot flag. Likewise, as we indicate above, both focal species in this study occur in sympatry with sister taxa that foot flag. In each case, the species may occupy and display in different microhabitats that differentially shape how the foot flag evolves to ensure optimal signaling (Leal and Fleishman, 2002). Such possibilities are not mutually exclusive, and several other explanations exist. Future work that explores the evolutionary pressures that shape foot flag design is therefore necessary to address these issues.

DATA ACCESSIBILITY

Supplemental material is available at <https://www.ichthyologyandherpetology.org/h2020160>. Supplemental Video S1: Slow motion video of foot flagging behavior of *Staurois parvus*. Supplemental Video S2: Slow motion video of foot flagging behavior of *Micrixalus kottigeharensis*.

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