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Phenotypic and genetic divergence in reed frogs across a mosaic hybrid zone on São Tomé Island

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Although naturally heterogeneous environments can lead to mosaic hybrid zones, human-induced habitat fragmentation can also lead to environmental heterogeneity and hybridization. Here we quantify phenotypic and molecular divergence across a reed frog mosaic hybrid zone on São Tomé Island as a first step towards understanding the consequences of hybridization across this heterogeneous landscape. The São Tomé giant reed frog (*Hyperolius thomensis*) is strongly tied to cool, wet, forest habitats whereas the distribution of Moller's reed frog (*H. molleri*) spans cool, wet, forests to warm, dry, disturbed habitats. Correspondingly, hybridization is concentrated in the more forested, cool, wet sites relative to non-forested, warmer, drier habitats. Four of six sites with hybrid frogs are artificial water bodies near the forest edge, indicating that both breeding habitat and broader scale environmental variation are probably important for understanding interspecific interactions and the extent of hybridization in this system. Phenotypic variation (body size and ventral coloration) largely tracks genetic and environmental variation across the hybrid zone with larger and more pigmented frogs occurring in forested, cool, wet habitats. Understanding whether human-induced changes in habitat break down reproductive barriers will be essential for conservation management of the less abundant, forest-associated *H. thomensis* in the face of rampant hybridization.

ADDITIONAL KEYWORDS: amphibian – body size – colour – ddRADseq – Gulf of Guinea – human-mediated hybridization.

INTRODUCTION

Hybrid zones are geographical regions in which two distinct lineages converge, mate and produce offspring (Barton & Hewitt, 1985). These regions are often viewed as progressive transitions, resulting in a gradual or abrupt cline of character traits across the transition from one lineage to the other (Endler, 1977). Many empirical systems deviate from this classical model, however, and instead form mosaic hybrid zones that occur in patchy, heterogeneous environments (Harrison, 1986; Harrison & Rand, 1989; Rand & Harrison, 1989; Ross & Harrison, 2002). The heterogeneous environments that lead to mosaic hybrid zones can occur naturally (Rand & Harrison, 1989) or can result from human-induced habitat fragmentation (Kimura & Munehara, 2019). For instance, anthropogenic habitat disturbance impacts hybridization in oak trees (Tovar-Sanchez & Oyama, 2004), anoles (Jezkova *et al.*, 2013) and chipmunks (Frare *et al.*, 2017), by modifying interspecific interactions and/or by bringing ecologically isolated species into contact (reviewed by Grabenstein & Taylor, 2018). This 'disturbance-mediated hybridization' typically occurs only in the disturbed areas, mirroring the often patchy distribution of anthropogenic landscape disturbance (reviewed by Grabenstein & Taylor, 2018). Due to global deforestation, the majority of forest areas now lie within 1 km of the forest edge (Haddad *et al.*, 2015), resulting in sprawling habitat mosaics that may increase secondary contact between species that would otherwise be isolated by habitat. Quantifying how phenotypic and genotypic variation track the environment in these recent contact zones

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is an important first step towards understanding the potential consequences and long-term stability of the hybrid zone across the fragmented landscape.

The island of São Tomé in the Gulf of Guinea is home to two closely related, endemic species of reed frogs: the São Tomé giant reed frog (*Hyperolius thomensis* Bocage, 1886), which occupies closed-canopy habitat (native forest in Fig. 1A), and Moller's reed frog (*H. molleri* Bedriaga, 1892), which occupies open forest, plantation and human-settled habitats throughout São Tomé (secondary forest, shade plantation and non-forested areas in Fig. 1A). The species differ in body size, ventral coloration and advertisement call (Drewes & Wilkinson, 2004; Gilbert & Bell, 2018), and mitochondrial divergence between the species is up to 2.8% (cytochrome *b*; Bell, 2016), yet they hybridize where their ranges meet (Bell *et al.*, 2015a). Consequently, this hybrid zone may present a natural laboratory for investigating the genetic basis and selective pressures underlying the evolution of body size, coloration and advertisement call in reed frogs. In addition, we hypothesize that deforestation is bringing these two species into secondary contact because hybridization appears to coincide with agricultural development at the edge of primary forest habitat (Bell *et al.*, 2015a). Unfortunately, we do not have historical samples available to directly test the association between human disturbance and hybridization; however, we aim to provide baseline data for tracking the position and width of this hybrid zone with respect to habitat fragmentation. Here we: (1) characterize variation in colour and body size

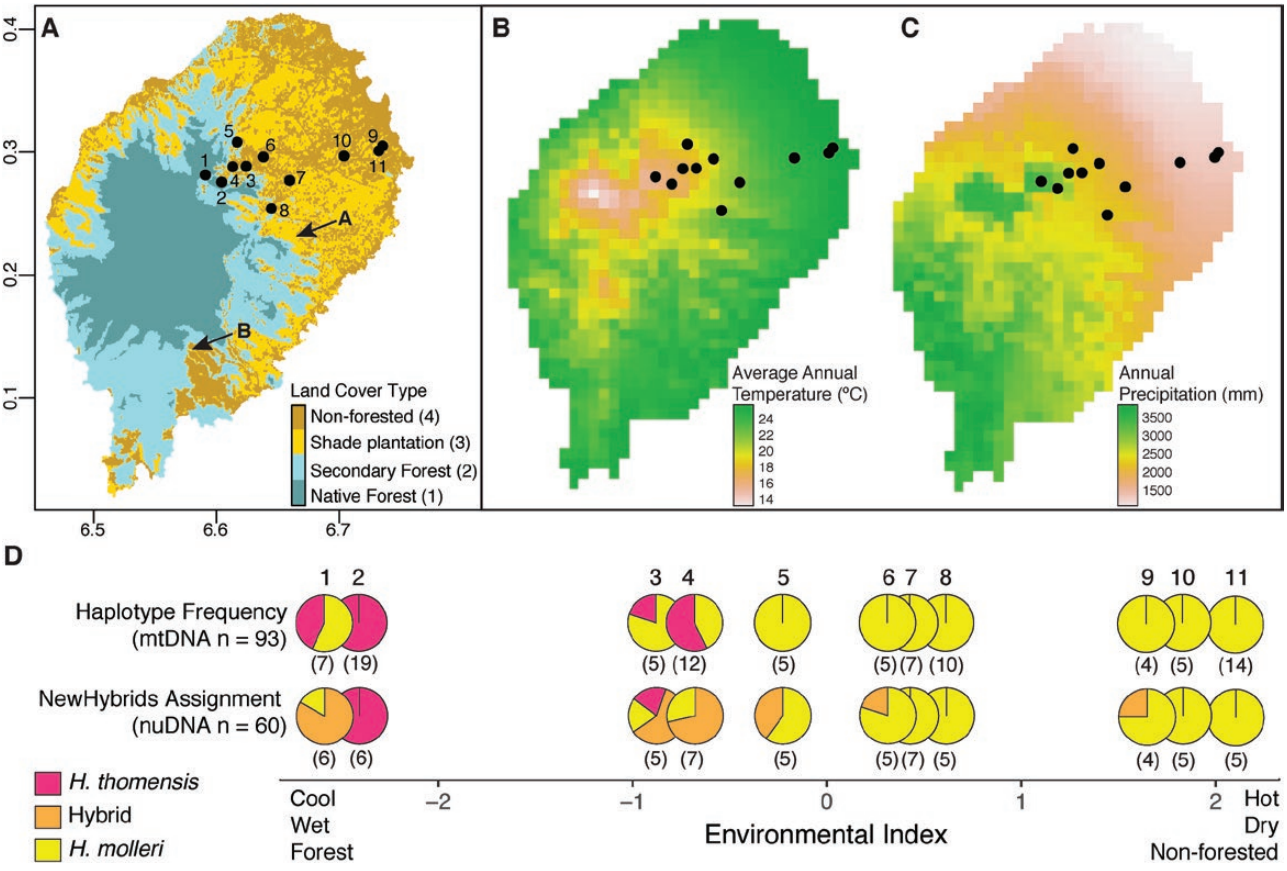

Figure 1. *Hyperolius* sampling localities on São Tomé Island with respect to (A) land cover classification, (B) average annual temperature (°C) and (C) annual precipitation (mm). *Hyperolius thomensis* occurs in native forest while *H. molleri* occurs in secondary forest, shade plantation and non-forested areas across the island. Arrows in A indicate additional localities at which hybridization may be occurring. (D) mtDNA haplotype frequencies and NewHybrids parental and hybrid assignments across the mosaic hybrid zone. Sample sizes for mtDNA and nuDNA at each site are shown in parentheses. Environmental index is PC1 from a principal components analysis of mean annual temperature, annual precipitation and land cover at the sampling localities. 1, Lagoa Amelia (LA); 2, Radio Tower (RT); 3, Terra Batata (TB); 4, Bom Sucesso (BS); 5, Bem Posta (BM); 6, Monte Café (MC); 7, Santy (SA); 8, Java (JA); 9, Quisinda (QI); 10, Caxão Grande (CG); 11, Caxueira (CA).

between the species and their hybrid progeny; (2) develop an environmental index to capture variation in land cover, temperature and precipitation across the hybrid zone; and (3) quantify molecular and phenotypic divergence across the environmental index to assess whether hybridization tracks the landscape mosaic.

MATERIAL AND METHODS

Phenotypic variation in parental and hybrid frogs

We collected mitochondrial DNA (mtDNA) sequences (16S and cytochrome *b*) for 93 frogs from 11 sites on São Tomé (Fig. 1; Table 1), and nuclear DNA (nuDNA) single nucleotide polymorphisms (SNPs) [double digest RAD sequencing (ddRADseq); Peterson *et al.*, 2012] for 60 of these samples in a previous study (Table S1; Bell *et al.*, 2015a). The nuDNA dataset was generated with the STACKS pipeline v.1.13 (Catchen *et al.*, 2011, 2013) and included 3644 SNPs (one per locus, <25% missing data per locus within a species). We collected phenotypic data from all adult specimens in the nuDNA dataset (four sub-adults were not included). We measured 11 morphological traits for these 51 male and five female specimens to the nearest 0.1 mm (details are given in the Supporting Information). We corrected the morphological measurements for differences in allometric scaling (following Thorpe, 1983) and performed a principal components analysis (PCA) to find the best low-dimensional representation of morphological variation in the dataset. To quantify variation in black ventral coloration (darkly pigmented ventral surface), we analysed photographs of the same 56 individuals using ImageJ v.1.49n (Rasband, 1997–2017; details in the Supporting Information). We fitted an ANOVA to the body size (snout–vent length; SVL) and coloration datasets with measurements grouped by parental species and hybrids, and used a Tukey Honest Significant Differences test to calculate adjusted *P* values for group mean comparisons. To assess the relationship between body size and coloration of a given male with his nuDNA genetic composition, we fit a linear regression for the phenotypic measurements and posterior assignment probability to the *H. thomensis* deme derived from STRUCTURE analyses (Pritchard *et al.*, 2000) conducted in a previous study (Table S1; Bell *et al.*, 2015a). Statistical analyses were performed in R v.3.4.3 (R Development Team, 2016). All material examined is accessioned at the California Academy of Sciences (Supporting Information, Table S1).

Environmental variation across sampling sites

We downloaded bioclim environmental layers from the worldclim database (www.worldclim.org/bioclim). For land use (resolution of 0.000833° × 0.000833°, ~91 m^2), we used a layer developed from a combination of visual interpretation of satellite images, field observations of land cover, military maps and expert knowledge (Soares, 2017) that classifies land cover into four categories: Native Forest, Secondary Forest, Shade Plantation and Non-forested. For the 11 sampling sites we extracted values for BIO1 (annual mean temperature in °C), BIO12 (annual precipitation in mm) and land use category (averaged across a 300-m buffer for each site; Table 1). We manipulated the bioclim layers using *rgdal* (Bivand *et al.*, 2014), *sp* (Pebesma & Bivand, 2005) and *raster* (Hijmans & van Etten, 2014) in R v.3.4.3 (R Development Team, 2016). To find the best low-dimensional representation of environmental variation across sites, we performed a PCA and used the first

Table 1. Sampling localities on São Tomé Island, environmental data used to derive the environmental gradient index (EG) and sample sizes for genetic datasets

Locality	Latitude	Longitude	Elevation (m)	Land cover	BIO1 (ºC)	BIO12 (mm)	EG	mtDNA sampled	nuDNA sampled
1 – Lagoa Amelia (LA)	0.2815	6.5908	1424	1.00	16.1	3245	−2.58	7	6
2 – Radio Tower (RT)	0.2757	6.6041	1326	1.00	16.5	3141	−2.41	19	6
3 – Terra Batata (TB)	0.2885	6.6240	1020	2.64	17.8	2522	−0.87	5	5
4 – Bom Sucesso (BS)	0.2882	6.6131	1156	4.00	17.1	2865	−0.72	12	7
5 – Bem Posta (BM)	0.3082	6.6167	848	3.00	18.6	2176	−0.24	5	5
6 – Monte Café (MC)	0.2961	6.6381	758	4.00	19.4	2203	0.32	5	5
7 – Santy (SA)	0.2770	6.6593	376	3.00	21.6	2084	0.38	7	7
8 – Java (JA)	0.2616	6.6512	607	4.00	20.7	2214	0.54	10	5
9 – Quisinda (QI)	0.3011	6.7320	49	3.00	25.0	1381	1.65	4	4
10 – Caxão Grande (CG)	0.2969	6.7038	141	3.99	24.1	1489	1.82	5	5
11 – Caxueira (CA)	0.3023	6.7323	65	4.00	25.0	1381	2.09	14	5

Land cover (averaged across a buffer of 300 m) is classified into four categories: Native Forest (1), Secondary Forest (2), Shade Plantation (3) and Non-forested (4). BIO1 (annual mean temperature, °C), BIO12 (annual precipitation, mm).

principal component to represent the environmental index across the mosaic hybrid zone.

Hybridization across a landscape mosaic

Hyperolius molleri and *H. thomensis* mtDNA haplotypes were identified by creating haplotype networks with TCS v.1.21 (Clement *et al.*, 2000). We used these haplotype assignments to calculate *H. molleri* and *H. thomensis* mtDNA haplotype frequencies at each site. Parental, backcross, F1 or F2 hybrids were previously classified from a NewHybrids (Anderson, 2008) analysis of 386 nuDNA SNPs (minor allele frequency > 0.2; Bell *et al.*, 2015a). For simplicity, we lumped hybrid sub-categories (backcross and F2) in the present study (no individuals were classified as F1 and only two individuals were classified as backcross *H. thomensis*). To visualize hybridization across the landscape mosaic, we plotted mtDNA haplotype frequencies (male, female and juvenile frogs; $N = 93$), parental or hybrid assignments (male, female and juvenile frogs; $N = 60$), body size (adult males; $N = 51$), and ventral coloration (adult males; $N = 51$) along the environmental index. We fit an ANOVA to the environmental variable datasets (temperature, precipitation, land cover, environmental index) with measurements grouped by parental species and hybrids, and used a Tukey Honest Significant Differences test to calculate adjusted P values for group mean comparisons. Statistical analyses were performed in R v.3.4.3 (R Development Team, 2016).

RESULTS

Phenotypic variation in parental and hybrid frogs

The first two principal components captured 92.7% of the morphological variance among males (Supporting Information, Table S2). PC1 (85.89% of the variance) loaded heavily on SVL, indicating that differences in body size were responsible for most of the variance (Table S2). PC2 (6.82% of the variance) loaded heavily on head and foot length measurements (HDW, FTL; Table S2). *Hyperolius thomensis* were clearly distinct from *H. molleri* in the PCA of morphological variation and hybrids were generally intermediate between the parental species (Fig. 2A). Likewise, *H. thomensis* variation in body size (Fig. 2B) and ventral coloration (Fig. 2C) did not overlap with that in *H. molleri*, while hybrid males exhibited intermediate trait values (adjusted $P < 0.001$). Both male body size (adjusted $R^2 = 0.78$, $P < 0.001$) and coloration (adjusted $R^2 = 0.76$, $P < 0.001$) were significantly and strongly correlated with posterior assignment probability to the *H. thomensis* genetic deme. Our small sample size for females ($N = 5$) precluded any statistical analyses; however, the patterns of morphological variation were qualitatively similar to those we detected in males (Fig. S1; Table S3).

Environmental variation across sampling sites

Despite the small spatial scale of our study area, our 11 sites span an ~1400-m elevational gradient and thus temperature, precipitation and land use varied considerably across sites (Fig. 1; Table 1). Most of the total environmental variance was captured in PC1 (82.4%; Supporting Information, Fig. S2) and all three variables contributed roughly equally to PC1 (Table S4); thus, we extracted values from PC1 to represent the environmental index across our sampling sites.

Hybridization across a landscape mosaic

The *H. thomensis* mtDNA haplotype group occurred at high frequency in the forested, cool, wet habitats (LA and RT), at intermediate frequencies in two transitional sites (TB and BS), and was absent in our combined sampling of 50 individuals collected in more non-forested, hot, dry sites (Fig. 1D). Likewise, the NewHybrids assignments based on nuDNA indicated that pure *H. thomensis* occur largely in forested, cool, wet sites (RT), while pure *H. molleri* occur typically in the more non-forested, hotter, drier sites with a few individuals inhabiting forested, cool, wet sites (Fig. 1D). Individuals identified as hybrids occurred in both forested and transitional sites, but the proportion of hybrids was lower in hotter, drier, non-forested sites where most hybrids were classified as *H. molleri* backcrosses (Fig. 1D; Fig. S3, Supporting Information, Table S1). Among the three landscape variables we measured, mean annual temperature and annual precipitation were strongly correlated [variance inflation factor (VIF) > 10] while land cover was not strongly correlated with either climatic variable (VIF < 2). Variation in annual mean temperature (Fig. 3A), annual precipitation (Fig. 3B), land cover (Fig. 3C) and environmental index (Fig. 3D) for *H. thomensis* did not overlap with that in *H. molleri* (adjusted $P < 0.001$). By contrast, hybrids overlapped with *H. thomensis* with respect to both annual mean temperature (Fig. 3A) and annual precipitation (Fig. 3B), but deviated significantly (adjusted $P < 0.001$) from both parental species with respect to variation in land cover (Fig. 3C) and environmental index (Fig. 3D). Phenotypic patterns mirrorred those of the genetic dataset, with larger and more pigmented frogs occurring in forested, cool, wet sites, and smaller and less pigmented frogs occurring in more disturbed, hotter, drier sites (Fig. S3).

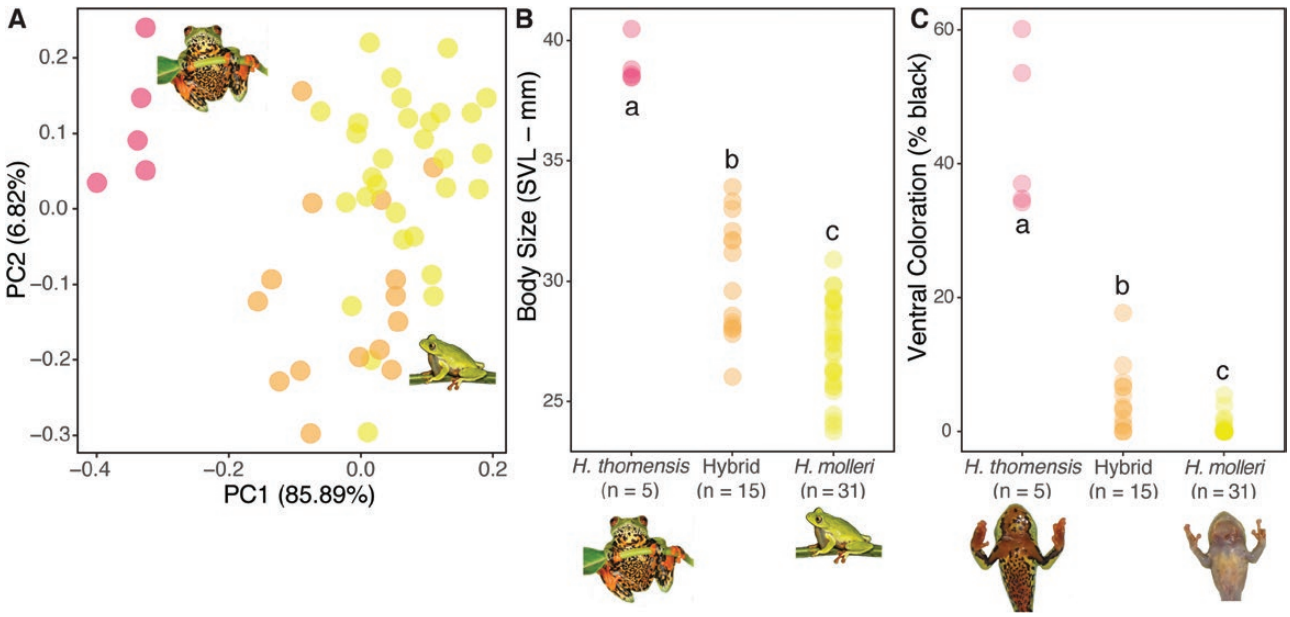

Figure 2. Phenotypic variation in *Hyperolius thomensis*, *H. molleri* and hybrid (F2 and backcross) reed frogs from São Tomé Island. (A) bivariate ordination of the first two components from a principal components analysis (PCA) of adult males. Distribution of body size (B; snout–vent length, mm) and ventral coloration (C; % area black) in adult male *H. thomensis*, *H. molleri* and hybrids. Comparisons significant at an adjusted $P < 0.001$ with a Tukey Honest Significant Difference test are indicated. Photos: A. Stanbridge and D. Lin.

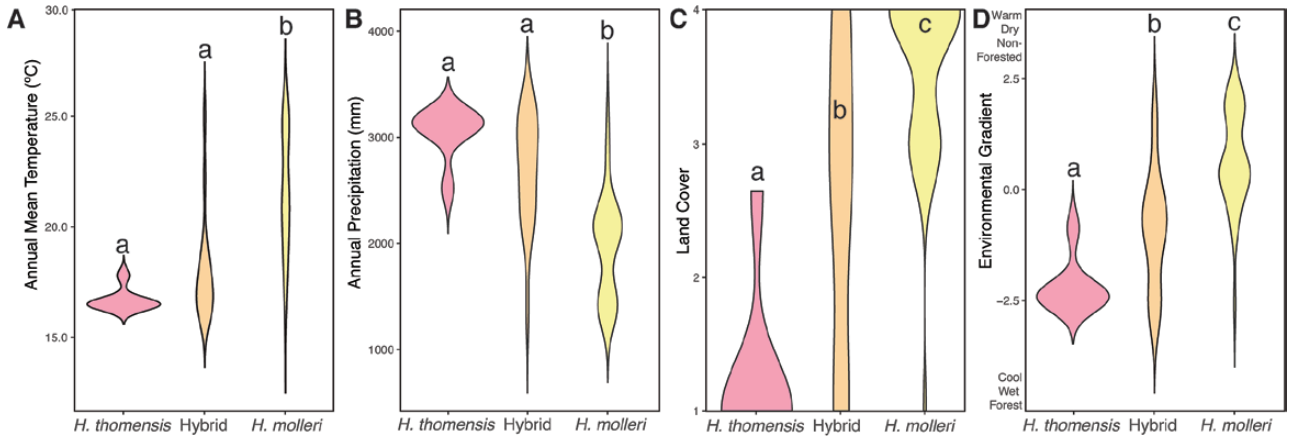

Figure 3. Environmental variation at localities with *Hyperolius thomensis*, *H. molleri* and hybrid (F2 and backcross) reed frogs on São Tomé Island. Distribution of annual mean temperature (A), annual precipitation (B), land cover (C; 1 – native forest, 2 – secondary forest, 3 – shade plantation, 4 – non-forested) and (D) environmental index (PC1 from a principal components analysis of annual mean temperature, annual precipitation and land cover at the sampling localities). Comparisons significant at an adjusted $P < 0.001$ with a Tukey Honest Significant Difference test are indicated.

DISCUSSION

Our results confirm that *H. thomensis* is strongly tied to closed-canopy, cool, wet forest habitats whereas *H. molleri* spans the range of environments sampled in our study. This pattern is similar to that of the well-studied *Drosophila yakuba – D. santomea* hybrid zone on the island (Llopart *et al.*, 2005; Matute *et al.*, 2009). Correspondingly, hybridization between *H. molleri* and *H. thomensis* is concentrated in the more forested, cool, wet sites we sampled. Although the proportion of hybrids at a given site corresponds largely to its environmental index, two sites in our study have similar values along the environmental index and yet one of these sites has a high proportion of hybrids (LA;

four *H. molleri* backcrosses and one F2 hybrid) while the other is composed of pure *H. thomensis* (RT). Both sites are inside native forest and are within 1 km of the forest edge; however, LA is a freshwater wetland (typical breeding habitat for *H. molleri*; Gilbert & Bell, 2018), whereas RT is a water-filled tree hole cavity (typical breeding habitat for *H. thomensis*; Drewes & Wilkinson, 2004). The stark difference in the proportion of hybrids at these two sites suggests that in addition to broad-scale environmental variation (e.g. forested vs. non-forested), the specific breeding habitat (e.g. pond vs. phytotelma) is important for understanding interspecific interactions and the extent of hybridization in this system. In particular, males of *H. molleri* typically call from dense vegetation close to the ground (<1.5 m) near standing water whereas males of *H. thomensis* call from higher perches (1 to >5 m) adjacent to phytotelma in bamboo and trees (Gilbert & Bell, 2018), suggesting that vegetation structure surrounding breeding sites may mediate interspecific interactions. The three other sites with high proportions of hybrids (TB, BS, BP) are shade plantations or open agricultural areas within 2 km of the forest edge in which frogs are breeding in artificial water bodies (e.g. cisterns), suggesting that human-induced changes to land cover, vegetation structure and aquatic breeding habitats promote hybridization. This pattern is similar to that observed in North American treefrogs (*Hyla cinerea* and *Hyla gratiosa*), in which males of the two species typically call from different perch heights. Removing vegetation around breeding sites breaks down this behavioural isolation and leads to high rates of hybridization (Mecham, 1960; Oldham & Gerhardt, 1975). Accordingly, differences in *H. thomensis* and *H. molleri* male calling behaviour may be an important reproductive barrier that is disrupted by practices associated with agriculture.

Phenotypic variation (body size and ventral coloration) largely tracks genetic and environmental variation across the *molleri–thomensis* mosaic hybrid zone. However, variation in both size and coloration among hybrids overlaps with that of pure *H. molleri*, indicating that hybrids cannot be reliably classified without genetic analysis. Adult body size in *H. thomensis*, which is among the largest of the ~150 species in the genus (Portik *et al.*, 2019), does not overlap with that of *H. molleri*, which has a more typical body size for *Hyperolius* (mean male SVL 26.0 ± 4.5 mm; Portik *et al.*, 2019). Likewise, the marbled black and orange ventral coloration of *H. thomensis* is strikingly distinct from that of *H. molleri*, which ranges from white to pale orange with little or no dark pigmentation. Orange and black patterning in frogs is often aposematic and associated with the presence of lipophilic alkaloids in the skin (e.g. Medina *et al.*, 2013; Kang *et al.*, 2017). Many species of *Hyperolius* exhibit colourful dorsal and ventral patterning; however, a study of four brightly coloured reed frog species did not detect typical amphibian defensive compounds (Portik, 2015). Comparisons of biochemical profiles, behavioural traits and predation pressure among *H. thomensis*, *H. molleri* and hybrid frogs may provide insight into the function of bright coloration in reed frogs.

The selective mechanisms underlying body size evolution in *Hyperolius* are largely unknown. However, *H. thomensis* and the next three largest reed frog species (*H. bobirensis* – male SVL 33.3 mm, *H. chlorosteus* – male SVL 35.3 mm, *H. torrentis* – male SVL 35.7 mm; Portik *et al.*, 2019) all occur in dense forest habitats, whereas smaller species in the genus (e.g. *H. ademetzi* – male SVL 18.0 mm, *H. nasutus* – male SVL 18.9 mm, *H. fusciventris* – male SVL 19.4 mm; Portik *et al.*, 2019) tend to occupy more open forest and savannah habitats, suggesting that large body size is advantageous in forest environments. Male body size is tightly linked to the dominant frequency of advertisement calls (larger frogs produce calls with lower frequencies; Hoskin *et al.*, 2009; Gingras *et al.*, 2013; Gilbert & Bell, 2018), which is an important aspect of species recognition and mate choice in most frog species (Blair, 1958). Lower frequency (i.e. longer wavelength) sounds are less distorted than higher frequency (shorter wavelength) sounds when moving through complex environments like dense forests (Marten & Marler, 1977; Ryan, 1988); thus, the lower pitched calls that larger male frogs produce may be more easily detected by females in forest habitats. Variation in body size and advertisement call across the *molleri–thomensis* hybrid zone follows this general trend with larger frogs occurring in dense, forested habitats and producing lower frequency calls and smaller frogs occurring in more disturbed, open habitats and producing higher frequency calls (Gilbert & Bell, 2018). This relationship is consistent with the 'acoustic adaptation hypothesis', which proposes that lower frequency songs occur in more densely vegetated habitats and has primarily been tested in avian systems (reviewed by Boncoraglio & Saino, 2007). Thus, the *molleri–thomensis* hybrid zone may be fruitful for extending the acoustic adaptation hypothesis to anurans and for investigating the relationship between morphological features that affect sound production with respect to environmental variation that attenuates sound transmission (e.g. Derryberry, 2009). Differences in body size between *H. thomensis* and *H. molleri* may also be associated with differentiation in diet. For instance, sympatric reed frog species that differ in body size consume different prey species (Luiselli *et al.*, 2004); thus, future

studies quantifying dietary breadth among parental species and hybrids may reveal differentiation in these aspects of their ecological niches.

Based on the coinciding mosaics of land cover, temperature and precipitation gradients across São Tomé, we predict that hybridization is occurring in the north-western and south-eastern regions of the island. Indeed, Gilbert & Bell (2018) found preliminary evidence of a second contact zone in shade plantation adjacent to native forest on the south-eastern side of the island (arrow A in Fig. 1A). In addition, at the transition between native forest and an oil palm plantation in the south of the island, we previously found several males of *H. thomensis* calling from phytotelma within the forest that were ~300 m from a small stream at the edge of the plantation where large numbers of *H. molleri* were breeding (arrow B in Fig. 1A). Although *H. thomensis* reproduce in anthropogenically modified landscapes (Strauß *et al.*, 2018), they occur in a narrower range of environments than *H. molleri* and are less abundant at shared breeding sites (Gilbert & Bell, 2018); thus, the species may be at risk of extinction by hybridization and introgression if deforestation on São Tomé continues (Rhymer & Simberloff, 1996). Consistently monitoring the extent of hybridization in these areas and investigating how forest fragmentation and artificial breeding sites may break down reproductive barriers between *H. thomensis* and *H. molleri* will be essential for conservation management in the face of rampant hybridization.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Phenotypic data collection and analyses.

Figure S1. Phenotypic variation in female *Hyperolius thomensis*, *H. molleri* and hybrid reed frogs from São Tomé Island. (A) bivariate ordination of the first two components from a principal components analysis (PCA) of adult females. Distribution of body size (B; snout–vent length, mm) and ventral coloration (C; % area black) in adult female *H. thomensis*, *H. molleri* and hybrids.

Figure S2. Bivariate ordination of the first two components from a principal components analysis (PCA) of environmental variation across 11 sampled localities of predominantly *Hyperolius thomensis* (pink), *H. molleri* (yellow) and hybrid frogs (orange).

Figure S3. NewHybrids assignments (A; includes males, females and juveniles), adult male body size (B; snout–vent length, mm) and ventral coloration (C; % area black) across the mosaic hybrid zone. Points are coloured according to NewHybrids parental or hybrid assignments. Environmental index is PC1 from a principal components analysis of mean annual temperature, annual precipitation and land cover at the sampling localities. Numbered sampling localities correspond to those in Figure 1.

Table S1. Sampling localities, voucher information, mtDNA (haplotype) and nuDNA assignments (NewHybrids assignment class, STRUCTURE posterior assignment to *H. thomensis* deme), and phenotypic measurements (SVL, body size; per cent area, ventral coloration).

Table S2. Character loadings for the top five principal components from the principal components analysis of 11 continuously varying morphological measurements (adjusted for ontogenetic composition as described in the Methods) among *Hyperolius thomensis*, *H. molleri* and hybrid males.

Table S3. Character loadings for the top five principal components from the principal components analysis of 11 continuously varying morphological measurements among *Hyperolius thomensis*, *H. molleri* and hybrid females.

Table S4. Character loadings for the principal components analysis of three environmental measurements for 11 sampled localities of *Hyperolius thomensis*, *H. molleri* and hybrid frogs.

SHARED DATA

GenBank accessions for mtDNA (16S: KP137139–KP137228; cytochrome *b*: KP137251–KP137316). Short Reads Archive for the ddRADseq dataset (BioProject ID PR- JNA268025). The SNP dataset is archived in Dryad (Bell *et al*., 2015b: https://doi.org/10.5061/dryad.7s7s7).