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Spatial and temporal dynamics of exuberant colour polymorphism in the southern cricket frog

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ABSTRACT

Intraspecific variation in colour pattern is widespread across multitudinous amphibian species. In some species, many distinct colour patterns are maintained within populations, a phenomenon referred to as exuberant colour polymorphism. The underlying causes of exuberant colour pattern polymorphism are poorly understood but are likely explained by selection, rather than neutral processes like genetic drift. Nevertheless, empirical data are needed to understand the selective drivers of this phenomenon, but such data are currently lacking for most polymorphic species. We studied frequency, spatial, and linkage dynamics of colour pattern across nine populations of the southern cricket frog (*Acris gryllus*) from southeastern Georgia, USA. Using 233 individuals, we combined direct field observations with examination of natural history specimens to look at colour pattern characteristics as they relate to space, time, and sex over a 30-year time frame. We found evidence of spatial and temporal variation in colour pattern across populations. We also discovered associations among colour pattern traits and between two colour pattern traits and sex. Our results suggest that the exuberant colour polymorphism of *A. gryllus* may involve correlations between traits and be caused by spatial and/or temporal variation in selection. However, similar studies in other species are necessary to allow us to discriminate among different drivers of colour pattern in exuberantly polymorphic frogs. Collectively, such systems offer important opportunities for understanding the evolution of colour and phenotypic diversity.

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Introduction

Colour polymorphism, which can be defined as the occurrence of two or more discrete colour patterns within a population at the same time, is a ubiquitous form of phenotypic diversity in nature (Gray and McKinnon 2007). However, the maintenance of colour polymorphism in nature implies the presence of selection or some other non-random

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evolutionary force that maintains variation, as neutral colour pattern variation should fix on one trait value as the result of genetic drift (Ford 1930; Haldane 1930; Fisher 1930a, 1930b). The evolutionary diversity of colour polymorphism can be acted upon by several types of selection. First, sexual selection can act upon colour pattern to drive presence of multiple morphs (Gray and McKinnon 2007). These morphs are often sex-linked in expression and can lead to the evolution of assortative mating and reproductive incompatibility (Jiggins et al. 2001; Puebla et al. 2007; Seehausen et al. 2008; Corl et al. 2010). Second, both colour polymorphism and geographic variation in colour polymorphism are common in mimicry systems, where natural selection can drive evolution of multiple mimetic morphs that are conspicuous in colouration (Kunte 2009; Nelson 2010; Cox and Davis Rabosky 2013; Davis Rabosky et al. 2016b; Cox et al. 2018). Finally, disruptive natural selection can drive the evolution of multiple cryptic or inconspicuous morphs (Nevo 1973; King 1987; Bond 2007; Fitzpatrick et al. 2009; Farallo et al. 2012). In this case, different morphs are cryptic in different microhabitats (Nevo 1973; Farallo et al. 2012), favouring the evolution of multiple morphs (Mather 1955; Bond 2007). In extreme cases, individual species can evolve so many colour pattern morphs that they become dubbed as exuberantly polymorphic (Franks and Oxford 2009; Croucher et al. 2011). However, relatively little is known about either the patterns or causes of spatial and temporal variation in colour pattern in many systems with exuberant colour polymorphism.

One specific type of selection that can be important for maintaining spatial and temporal heterogeneity is negative frequency-dependent selection, also known as apostatic selection or rare-morph advantage (Endler 1988). This type of selection can arise from increased predation on the most common morph, which facilitates an increase in the frequency of a rarer morph until the latter becomes the most common and begins to attract increased predation (Allen 1988; Fitzpatrick et al. 2009). A related form of selection occurs when the predator is neophobic or has dietary wariness, which causes it to avoid novel prey items (Franks and Oxford 2009, 2011). Dietary wariness may be considered a special case of negative frequency-dependent selection, as the newest and rarest morph has a fitness advantage over a longer-established, more common morph (Franks and Oxford 2009, 2011). Negative frequency-dependent selection thus induces temporal variation in selection and natural oscillations in frequency of morphs as the identity of the rarest morph shifts (Takahashi and Hori 1994; Gillespie and Oxford 1998; Cox and Davis Rabosky 2013). In turn, metapopulation structure that produces asynchrony of frequency-dependent oscillations among sub-populations can cause spatial heterogeneity in strength and form of selection (Joron and Mallet 1998; Cox and Davis Rabosky 2013; Holmes et al. 2017). However, the presence of spatial and temporal variation in polymorphism, which is a common outcome for negative frequency-dependent selection, is not well known across most species with colour polymorphism.

Anuran amphibians are a taxonomic group that can exhibit exuberant colour polymorphism (Hoffman and Blouin 2000). In particular, cryptic, leaf-litter dwelling anurans are often polymorphic in colour pattern (Resnick and Jameson 1963; Milstead et al. 1974; Wente and Phillips 2003) and can have over a dozen morphs within a single population that are comprised of multifarious combinations of individual traits such as lines or masks on the head, stripes or crosses on the back, and blotches and stripes on the legs (Lynch et al. 1966; Savage and Emerson 1970; Woolbright and Stewart 2008; Streicher et al. 2009,

2014). These polymorphic traits can remain fixed throughout development and may have a Mendelian genetic basis (Pyburn 1961; O'Neill and Beard 2010). Because of this astounding diversity, the spatial, temporal, and linkage dynamics of colour pattern in anurans with exuberant colour polymorphism remain an important area for research.

We studied variation in colour trait frequency and trait linkage in the polymorphic southern cricket frog (*Acris gryllus*: Hylidae: Anura). All three species in the genus *Acris* have polymorphism in the colour of the vertebral stripe, which can be grey/brown, red, or green within a single population. Previous research has documented substantial spatial and temporal heterogeneity in the frequency of colour morphs of the vertebral stripe within populations, which has been linked to both predation and climatic clines (Nevo 1973; Milstead et al. 1974; Gray 1983; Gorman 1986). The vertebral stripe morphs have simple Mendelian inheritance, with green dominant to red and grey (Pyburn 1961), although there is some evidence that the expression of the vertebral stripe can vary temporally and could even change during the lifetime of a frog (Pyburn 1961; Gray 1972). However, all previous research has focused on the vertebral stripe alone, ignoring other colour traits that are polymorphic. We conducted a study of southern cricket frogs using natural history collections and direct field observations to explore spatial and temporal heterogeneity of colour polymorphism and associations between body size, sex, and colour pattern traits.

Materials and methods

Study of specimens in a natural history collection

We used 90 formalin-fixed, alcohol-preserved specimens of *Acris gryllus* from the Georgia Southern University – Savannah Science Museum Herpetology Collection to characterise the spatial distribution and putative linkages of colour pattern traits (Table S1). Although cricket frogs are distributed widely throughout the southeastern United States, we focused our study on southeastern Georgia because we were interested in local, rather than regional, patterns of colour polymorphism. We used specimens from seven discrete localities that were collected at different timepoints over a 30-year span; individuals were sourced from Bryan County (1967, N = 12), Effingham County (1957, N = 19), Evans County (1977, N = 13), Screven County (1957, N = 22), Tattnall County (1946, N = 13), Ware County (1972, N = 11), and two populations in Long County (Long1, 1973, N = 10 and Long2, 1962, N = 12). Using these preserved specimens, we characterised sex, body size, and seven colour pattern traits for each individual. We determined sex by noting the colour of the underside of the throat, which is darkened by the presence of a vocal sac in males (Powell et al. 2016). We measured body size as snout-vent length (SVL) to the nearest mm using a ruler. We note that colour does not persist in preservative, and so for this study, we focused on colour pattern traits that are visible in preserved specimens. We determined colour pattern traits (Figure 1) as the presence or absence of a light line under the eye, a light line inside of the hindlimb, circular hindlimb blotches (as opposed to a line), a dark patch behind the eye, a dark patch behind the forelimb, three blotches on the upper hindlimb (as opposed to four blotches), and three blotches on the lower hindlimb (as opposed to four, five, or six blotches). Although there are many colour pattern components that we could have assessed in cricket frogs, we focused on patterns that are easily



Figure 1. Morphological traits that were scored from specimens in a natural history collection. a) upper hindlimb blotches, b) lower hindlimb blotches, c) dark patch behind eye, d) light line under eye, e) dark patch behind the forelimb. The light line inside of the hindlimb is not pictured.

diagnosed in preserved specimens and that are polymorphic in other frog species (Lynch et al. 1966; Hoffman and Blouin 2000; O'Neill and Beard 2010).

Field study of dorsal colour polymorphism

While we could not determine colour in museum specimens, we characterised spatial and temporal variation in the colour of the polymorphic vertebral stripe in live wild animals from two different field sites (Table S2). Site A was a hardwood forest situated around a Carolina bay, while Site B was a hardwood forest around a shallow wetland created by impounding a small stream. We collected data from 61 individuals at Site A and 82 individuals from Site B. Both sites contained bald cypress (*Taxodium distichum*), water oak (*Quercus nigra*), tulip poplar (*Liriodendron tulipifera*), black cherry (*Prunus serotina*), sweetgum (*Liquidambar styraciflua*), and red maple (*Acer rubrum*), with an understory of sassafras (*Sassafras albidum*), Carolina cherry laurel (*Prunus caroliniana*), and American beautyberry (*Callicarpa americana*). Site A was sampled in April 2016 and April 2017, while Site B was sampled in May 2016, October 2016, and April 2017. During each sampling trip, we surveyed for frogs for approximately 2 hours with two to four volunteers. Cricket frogs were captured by hand or by net and then immediately placed in a clean plastic bag. For each frog, we determined sex, body size, and dorsal vertebral stripe morph. Sex and SVL were recorded using the same methods as for preserved specimens. We categorised the dorsal vertebral stripe of each individual as either the green or brown morph. Although

other scientists have described red dorsal stripe morphs, we combined both brown and red morphs into a single category because distinguishing red from brown dorsal stripes can be difficult in field conditions, and there is some evidence that individuals can vary in expression of either brown or red dorsal stripes during their lifetime (Gray 1972).

Statistical methods

We combined contingency analyses, nominal logistic analyses, linear models, distance-based matrix comparisons, and multivariate statistics to characterise patterns of colour polymorphism in cricket frogs. We used contingency analyses to test for an association between trait frequencies and population or sex. We used nominal logistic regression to test for a relationship between vertebral stripe frequency and season, sampling time, year, and population. We employed linear models to test for a relationship between categorical independent variables and continuous dependent variables. For linear models, we examined data distributions for normality prior to analyses and transformed variables as necessary. Contingency analyses, nominal logistic regression, and linear models were conducted in JMP v. 14.0 (SAS Software Inc., Cary, NC, USA). We also calculated Euclidean distances between populations based on 1) frequencies of polymorphic traits in natural history specimens (Table S3), 2) geographic distances (in metres) using GPS coordinates (Table S4) and the R package 'geodist' (Padgham and Sumner 2020), and 3) time 'distance' based upon difference between dates of collection in days (Table S5). Using the resulting distance matrices, we then tested for isolation by distance and isolation by time by assessing the correlation between either geographic or time distance and phenotypic distance using the R package 'ape' (Paradis et al. 2004) with 9999 permutations. Finally, we employed logistical principal components analysis as an ordination method that is appropriate for binary variables. We used the R package 'logisticPCA' to perform cross-validation and conduct logistic PCA analyses (Landgraf 2015; Landgraf and Lee 2015). Briefly, we used the cross-validation procedure described in the documentation for the 'logisticPCA' package to determine the correct number of natural parameters (m). We then used $m = 5$ to represent colour polymorphism in two-dimensional space, allowing us to plot principal components loadings for each trait and principal component scores for each individual in each population.

Results

Spatial variation of morph frequencies in natural history collections

Based on the preserved specimens we sampled, populations varied significantly in sex ratios (contingency analysis, $\chi^2 = 84.362$, $P < 0.0001$), with two populations (Long1 and Effingham county) represented only by males, perhaps reflecting collections of calling males during the breeding season. While sexes did not differ significantly in body size (ANOVA, $F_{1,109} = 0.2665$, $P = 0.6067$), populations did vary significantly in body size (ANOVA, $F_{7,105} = 6.2429$, $P < 0.0001$). Across all populations, males had a higher frequency of dark patches behind the eyes (contingency analysis, $\chi^2 = 7.881$, $P = 0.0050$), and females a higher frequency of light lines under the eye (contingency analysis, $\chi^2 = 7.817$, $P = 0.0052$), but sexes did not differ in frequencies of any other traits (contingency analyses, all P 's > 0.16).

We found that the frequencies of most colour traits varied significantly among populations (Figure 2); these traits included the dark patch behind the eye (contingency analysis, $\chi^2 = 49.271$, $P < 0.0001$), the light line under the eye (contingency analysis, $\chi^2 = 20.126$, $P = 0.0053$), the dark patch behind the forelimb (contingency analysis, $\chi^2 = 38.551$, $P < 0.0001$), three blotches on the upper hindlimb (contingency analysis, $\chi^2 = 23.218$, $P = 0.0016$), the presence of three blotches on the lower hindlimb (contingency analysis, $\chi^2 = 38.860$, $P < 0.0001$), and shape of limb blotches (contingency analysis, $\chi^2 = 38.130$, $P < 0.0001$). The frequency of individuals with a light line on the inside of the hindlimb did not vary among populations (contingency analysis, $\chi^2 = 11.018$, $P = 0.0878$). We found that neither geographic (Mantel test, $z = 2082587$, $P = 0.7115$) nor temporal (Mantel test, $z = 127121$, $P = 0.309$) distances between populations were significantly correlated with phenotypic distance.

We found that some polymorphic traits were significantly associated (Table 1). The dark patch behind the forelimb was significantly associated with the dark patch behind the eye, while the presence of three blotches on the upper hindlimb was correlated with the presence of three blotches on the lower hindlimb. We found limited evidence that the presence of three blotches on the lower hindlimb was correlated with the shape of blotches on the hindlimb, and that the light line under the eye was associated with both the dark patch behind the forelimb and light line inside of the hindlimb.

Using logistical principal components analysis on binary polymorphic traits, we found that the first two principal components explained 64.8% of the variation. Principal component loadings revealed three clusters of polymorphic traits (Figure 3), which were three blotches on the upper and lower hindlimbs, dark patches behind the eye and forelimb, and light lines under the eye and inside the hindlimb as well as blotch shape. These groupings, based on principal component loadings, echoed univariate trait associations. However, we found that the colour polymorphism of populations did not cluster or separate in multivariate space (Figure 3).

Spatial and temporal variation in dorsal colour polymorphism in the field

In the field study, body size of cricket frogs did not differ significantly between populations (ANOVA, $F_{3,110} = 0.1632$, $P = 0.6870$) but did vary with sampling period (ANOVA, $F_{3,110} = 106.7089$, $P < 0.0001$), with smaller animals captured in the fall. Sex-ratio differed between populations and was significantly male-biased in site B compared to site A (contingency analysis, $\chi^2 = 6.785$, $P = 0.0092$).

We found evidence for spatial, but not temporal, variation in morph frequency. Dorsal colour morph frequency did not vary among sampling times, whether by individual survey (nominal logistic regression, $\chi^2 = 4.784$, $P = 0.1883$), seasons (nominal logistic regression, $\chi^2 = 3.068$, $P = 0.0799$), or years (nominal logistic regression, $\chi^2 = 1.743$, $P = 0.1867$). Similarly, morph frequency did not differ between the sexes when analysed separately between populations (contingency analyses, both $P_s > 0.35$) nor when combining populations (contingency analysis, $\chi^2 = 0.568$, $P = 0.4509$). However, morph frequency did differ between populations (nominal logistic regression, population, $\chi^2 = 9.899$, $P = 0.0017$, year, $\chi^2 = 0.165$, $P = 0.6844$), with more green morphs in site A compared to site B (Figure 4). In both populations, the brown dorsal stripe was the most common morph.

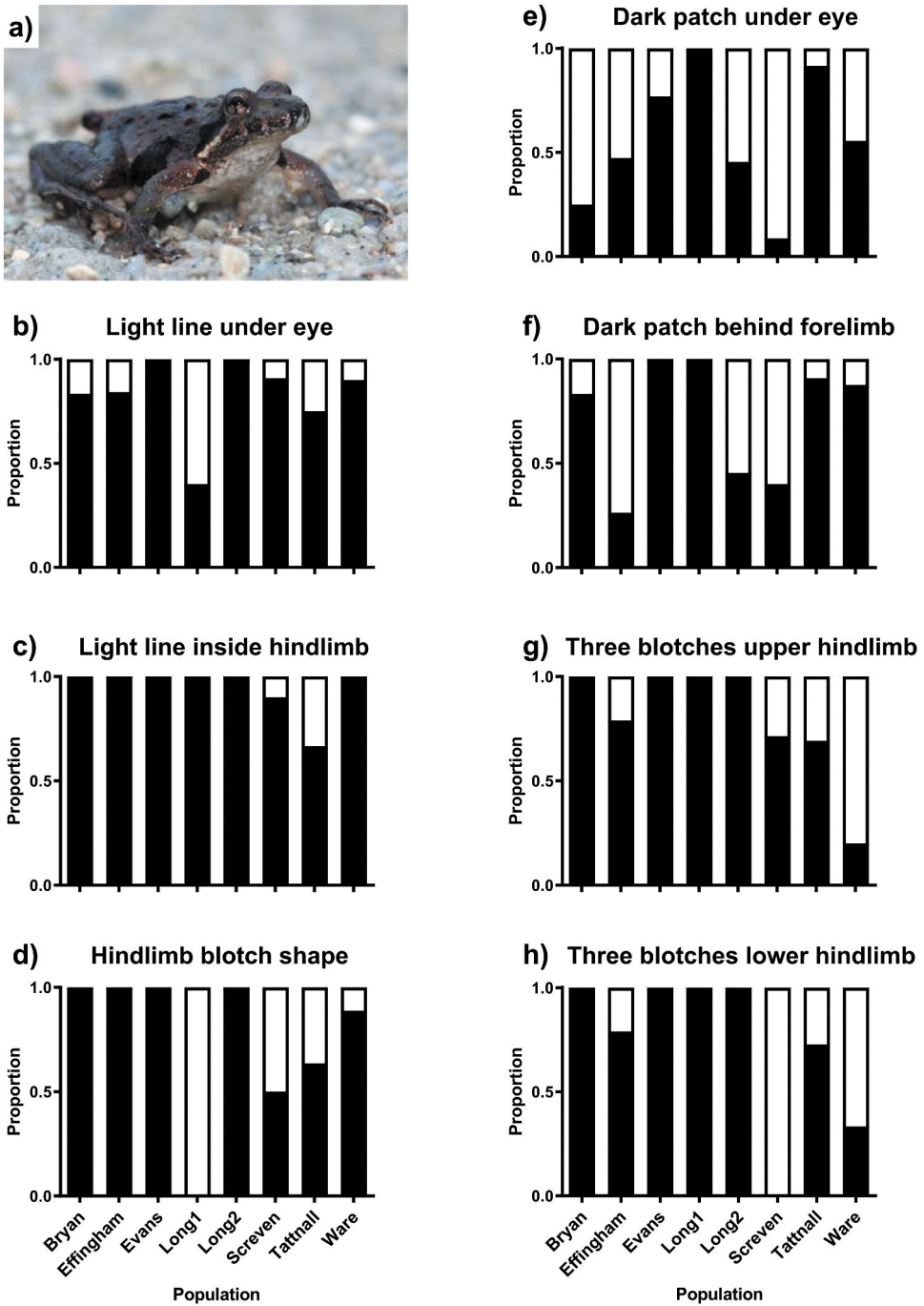


Figure 2. a) The southern cricket frog (*Acris gryllus*). Frequency of individuals with b) a light line under the eye, c) a light line inside of the hindlimb, d) a circular hindlimb blotch, e) dark patch behind the eye, f) dark patch behind the forelimb, g) three blotches on the upper hindlimb, and h) three blotches on the lower hindlimb across 90 museum specimens. All traits varied significantly among populations except for the light line inside of the hindlimb.

Table 1. Associations between polymorphic traits in southern cricket frogs (*Acris gryllus*). R^2 is above the diagonal, and P-value from a contingency analysis is below the diagonal. DPBE = dark patch behind eye, LLUE = light line under eye, DPBF = dark patch behind forelimb, LLIH = light limb inside of hindlimb, 3BUH = presence of three blotches on upper hindlimb, 3BLH = presence of three blotches on lower hindlimb, BISHape = shape of blotch on hindlimbs. Bolded p-values indicate significant differences after a Bonferroni correction for multiple comparisons, while italicised values indicate p-values that are less than an uncorrected $\alpha = 0.05$.

	DPBE	LLUE	DPBF	LLIH	3BUH	3BLH	BISHape
DPBE	-	0.0104	0.1366	0.0015	0.0033	0.0067	0.0269
LLUE	0.2125	-	0.0775	0.0852	0.0086	0.0010	0.0624
DPBF	<0.0001	<i>0.0086</i>	-	0.0005	0.0157	0.0075	0.0393
LLIH	0.6939	<i>0.0361</i>	0.8340	-	0.0489	0.0042	0.0658
3BUH	0.5653	0.5103	0.2489	0.3604	-	0.8199	0.0064
3BLH	0.4153	0.8257	0.4247	0.754	<0.0001	-	0.0635
BISHape	0.0943	0.0741	0.0607	0.2135	0.5079	<i>0.0249</i>	-

Discussion

We found evidence of polymorphism in multiple colour pattern traits of the southern cricket frog. Using natural history specimens, we found that there was variation in the frequency of colour traits among localities, as well as some evidence of associations among colour traits and between sex and colour traits. Because the collection date varied among populations, this result is consistent with either spatial or temporal variation in these colour traits over a 30-year period. Moreover, using field observations, we detected spatial, but not temporal, variation in vertebral stripe frequency, which contrasts with previous research in congeners of southern cricket frogs (Gray 1983; Gorman 1986). However, it is worth noting that the duration of our study would not have allowed us to detect longer-term shifts in morph frequencies. Beyond spatial or temporal variation in morph frequency, we also detected some evidence of sex-bias in two of the polymorphic traits (dark patch behind the eye and light line under the eye). Given that previous studies have not documented sex-bias in morph frequencies in cricket frogs (Pyburn 1961; Nevo 1973), we suspect that the combination of geographic variation in morph frequency and skewed sex-ratios in some populations from the natural history collection could be driving apparent sex-bias. Together, these results suggest that spatial and temporal variation of exuberant colour polymorphism might be underlain by both associations among colour traits and spatiotemporal variation in selection.

There are multiple evolutionary forces that can create spatial and temporal variation in colour pattern. One of the major drivers of spatial variation in colour pattern is spatial heterogeneity in habitat structure or predator populations that leads to local adaptation (Rosenblum 2006; Gray and McKinnon 2007). In this case, colour polymorphism is maintained by gene flow between different populations (Rosenblum 2006; Gray and McKinnon 2007). Given 1) the limited geographic scope of our study (southeastern Georgia), 2) lack of genetic structure among southern cricket frogs in the southeastern US (Gamble et al. 2008), and 3) broad similarity of habitats in the coastal plain of Georgia, we think it is unlikely that our results are driven by greatly divergent selection between populations and gene flow. Spatial patterns of selection on polymorphic traits can also be described as either balancing (when selection favours similar morph frequencies among populations) or diversifying (when selection favours divergent morph frequencies among populations)

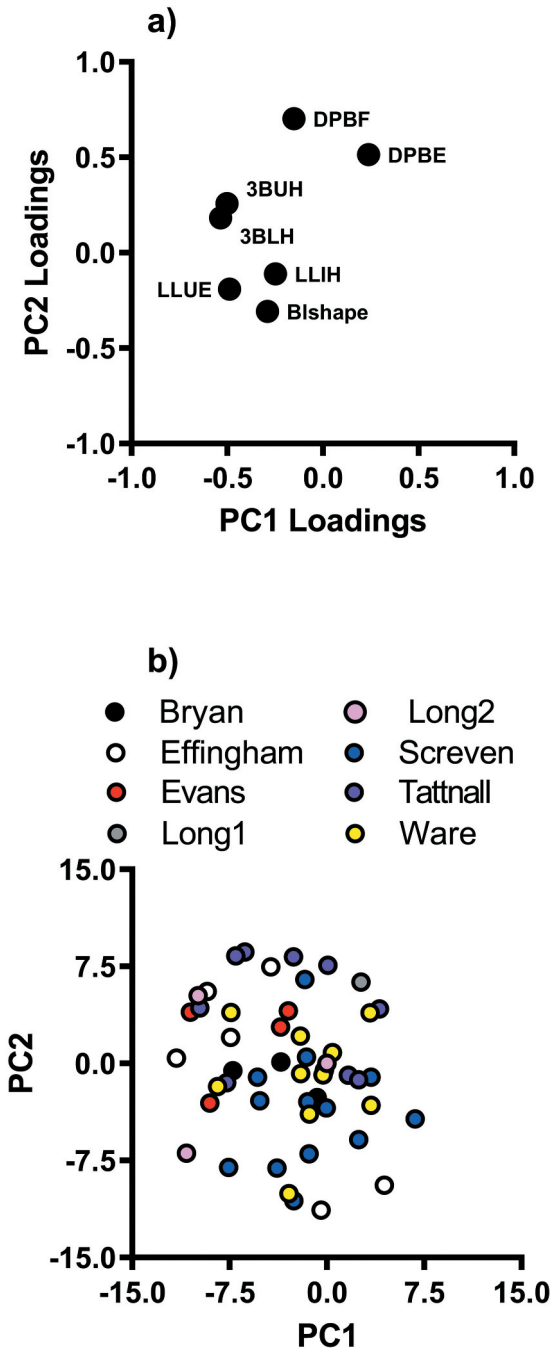


Figure 3. a) Principal components loadings from a logistical principal components analysis for each polymorphic trait measured in *Acris gryllus* using logistical principal components. Points are labelled with each trait, where DPBE = dark patch behind eye, LLUE = light line under eye, DPBF = dark patch behind forelimb, LLIH = light limb inside of hindlimb, 3BUH = presence of three blotches on upper hindlimb, 3BLH = presence of three blotches on lower hindlimb, BISHape = shape of blotch on hindlimbs. b) Principal component scores of principal components 1 and 2, which explained 64.8% of the variance in morph frequency among individuals. Points represent individuals, with colour corresponding to the collecting locality for that individual.

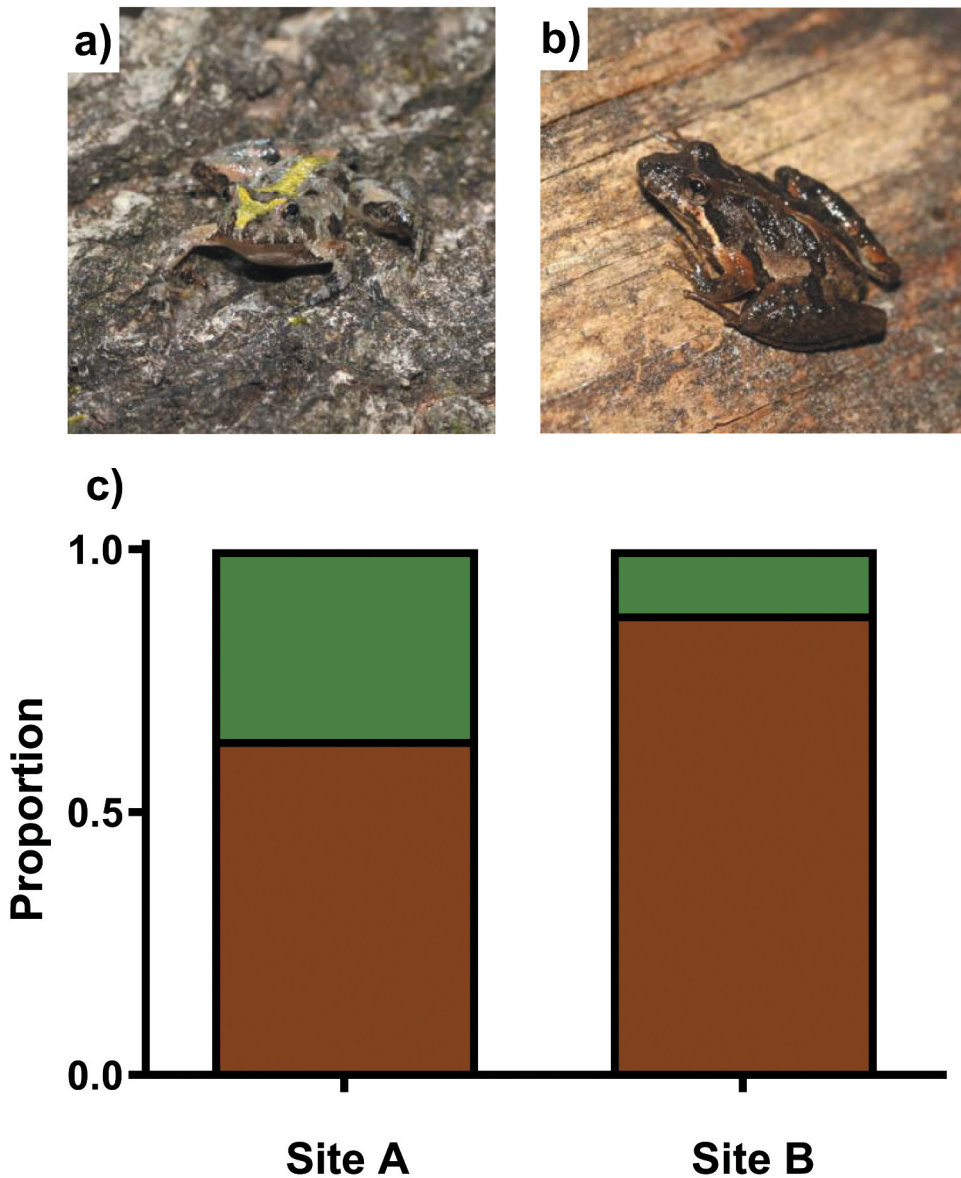


Figure 4. Green (a) and brown (b) dorsal colour morphs of the southern cricket frogs (*Acris gryllus*). c) Frequency of dorsal colour morphs at two study locations in Georgia, USA (N = 61, Site A; N = 82, Site B). Site A had significantly more green morphs than site B. Both sites had proportionally more brown morphs than green morphs.

selection (Hoffman et al. 2006; Abbott et al. 2008). Importantly, negative frequency-dependent selection has been implicated in driving both balancing and diversifying spatial patterns of selection (Croucher et al. 2011; Cox and Davis Rabosky 2013). Negative frequency-dependent selection and neophobia (or dietary wariness) can create temporally-fluctuating selection on individual morphs (Bond 2007; Franks and Oxford 2011; Holmes et al. 2017). Balancing selection is created when there is spatial synchrony

among predator behaviour across the landscape, such that the same morph is the most common and favoured by predators in each population. By contrast, any spatial variation can create asynchrony, such that while the most common morph might be favoured by predators across the geographic range, the identity of the most common morph might vary geographically (Croucher et al. 2011; Cox and Davis Rabosky 2013; Holmes et al. 2017). In the face of metapopulation structure among subpopulations, any spatial asynchrony among subpopulations can be manifested as spatial variation in the position in the negative frequency-dependent cycle (Cox and Davis Rabosky 2013; Holmes et al. 2017). While the same type of selection at one scale (negative frequency dependence) is driving morph frequencies, at the individual population level, survivorship of individual morphs may vary among populations, creating spatial variation in selection on individual morphs among subpopulations (Cox and Davis Rabosky 2013; Holmes et al. 2017). Hence, our findings are consistent with both spatial variation in selection *sensu stricto* and negative frequency dependence.

The patterns of spatial variation that we detected are consistent with selection acting on cryptic colour patterns in populations of *A. gryllus*, which implies a role for colour pattern in avoiding predation. Generally, it is likely that the colour and pattern of cricket frogs (genus *Acris*) serve as both camouflage and disruptive colouration to break up their outline on the complex substrate of the forest floor (Merilaita and Lind 2005; Endler 2006; Schaefer and Stobbe 2006; Stevens and Cuthill 2006; Fraser et al. 2007; Woolbright and Stewart 2008). Traits such as the colour of the vertebral stripe might play a role in background matching of different substrates, avoiding detection by predators (Nevo 1973). However, colour pattern traits likely also play a role in breaking up the outline of frogs, disguising the shape to avoid detection by predators (Merilaita and Lind 2005; Endler 2006; Schaefer and Stobbe 2006; Stevens and Cuthill 2006; Fraser et al. 2007). Additionally, colour and pattern might serve to confuse or dazzle predators as the prey retreat (Stevens 2007; Hämäläinen et al. 2015). The escape behaviour of cricket frogs in response to a large predator follows a common sequence (Blem et al. 1978; Walvoord 2003; McCallum 2011). Upon an attempt to capture, the cricket frog leaps close to a metre away from the predator, often at a different angle than the direction of that the predator is moving (Blem et al. 1978; Walvoord 2003; McCallum 2011). Immediately after landing, the cricket frog often turns its body in readiness of a second leap in a different direction than the first and then freezes (McCallum 2011). The complex dorsal pattern of cricket frogs might facilitate this strategy by confusing the predator during unpredictable movements, while camouflage makes the stationary frog difficult to detect. While this topic requires further research, behaviour is often an important integrated part of antipredator colour traits (Rowe and Halpin 2013), and thus, it is likely that escape behaviour and colour pattern are linked in cricket frogs.

We also found evidence of statistical associations between the expression of some colour traits, but that this correlation is not perfect, as individuals may or may not possess all associated traits at an individual level (Table 1). These types of associations are often evidence of genetic linkage among colour traits, which are common in the context of antipredator-driven colour evolution (Charlesworth and Charlesworth 1975; Murali and Kodandaramaiah 2016; Davis Rabosky et al. 2016a). For example, selection may have driven the evolution of linkage between colour genes in Müllerian mimicry rings in butterflies, causing the creation of so-called supergenes (Kunte et al. 2014). Beyond mimicry, colour traits that enhance decoy colouration are evolutionarily linked in lizards (Murali et al. 2018).

Colour traits can also be linked due to shared genetic bases or pleiotropic interactions of networks that produce the phenotypic traits (Jiggins et al. 2005; Basolo 2006; Cox et al. 2013). For small leaf-litter frogs that are cryptic and polymorphic, trait associations could favour the co-occurrence of traits that enhance crypsis or disruption to avoid predation.

While many frogs possess extraordinary colour diversity within populations of the same species, the term 'exuberant colour polymorphism' has primarily been applied to polymorphic spittle bugs and *Theridion* spiders (Halkka et al. 1970, 2001; Franks and Oxford 2009; Oxford 2009). Within these groups, colour polymorphism is maintained by negative frequency-dependent selection and divergent selection with gene flow, which can favour colour diversity within and among populations (Halkka et al. 1970, 2001; Croucher et al. 2011). Future research should assess how negative frequency dependence or other forms of selection might drive phenotypic diversity in small leaf-litter frogs.

Collectively, our results are consistent with exuberant colour polymorphism in southern cricket frogs involving correlated colour pattern traits and being caused by spatial and/or temporal variation in selection. Southern cricket frogs are only one of many species of cryptically coloured inhabitants of the forest floor and are sympatric with other frog species that share microhabitats and colour pattern elements (Powell et al. 2016), including other cricket frogs (*Acris crepitans* or *A. blanchardi*), chorus frogs (*Pseudacris* spp.), and tree frogs (*Hyla* spp.). In more diverse tropical rainforests, there can be dozens of species of terrestrial frogs belonging to many different families inhabiting the same leaf litter (Toft 1980; Vitt and Caldwell 1994; Caldwell and Vitt 1999; Gottsberger and Gruber 2004). Just as negative frequency-dependent selection can be a major driver of polymorphism within populations, the same selective forces could be responsible for this phenotypic diversity across species. Theoretical research has shown that alternate morphs are favoured when two polymorphic species are sympatric (Franks and Oxford 2017); hence, convergence on cryptic phenotypes in sympatric species would favour the evolution of colour polymorphism due to negative frequency-dependent selection (Franks and Oxford 2009, 2017). In biodiverse areas with many sympatric species, this might generate strong selective pressure that would constantly favour new colour pattern mutations, effectively creating a sort of diversity ratchet. This diversity ratchet would foment diversity within species, but also among species, driving the evolution of extraordinary phenotypic diversity among hidden frogs of the leaf litter.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

Data for this manuscript are included as Supplemental Material.

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