Morphological Variation and Community Science in Orthoptera

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Abstract
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Amy Byerly

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Advisor: Erica Larson, Ph.D.
Abstract

Patterns of morphological divergence across species’ ranges provide insight into local adaptation and speciation. Here, we compare phenotypic divergence among 4,221 crickets from 337 populations of two related species of field cricket, *Gryllus firmus* and *G. pennsylvanicus* and their hybrids. We find that these species differ across their geographic range in key morphological traits, such as body size and ovipositor length, and we directly compare phenotype with genotype for a subset of crickets demonstrating nuclear genetic introgression, phenotypic intermediacy of hybrids, and essentially unidirectional mitochondrial introgression. We discuss how these morphological traits relate to life history differences between the species. Our comparisons across geographic areas support prior research suggesting cryptic variation within *G. firmus* that may represent different species. Overall, our study highlights how variable morphology can be across wide ranging species, and the importance of studying reproductive barriers in more than one or two transects of a hybrid zone.
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Chapter One: Geographic variation in phenotypic divergence between two hybridizing field cricket species

Introduction

Phenotypic divergence can provide insight into evolutionary processes acting across different scales of biological organization. Within a single species, phenotypic divergence can reflect differences between environments, between population histories, or a combination of these factors (Gavrilets et al., 2001; Uyeda et al., 2009; Runemark et al., 2010; Oneal & Knowles, 2013; Jenck et al., 2020). Phenotypic divergence can signal the possible early stages of species differentiation (Wolf et al., 2008; González et al., 2011; Skoglund et al., 2015) and in closely related species, can shed light on local adaptation and patterns of increasing divergence (Britch & Cain, 2001; Shaw & Mullen, 2011). Most studies of species divergence have limited replication across the ranges of a species pair and the specific traits that maintain reproductive barriers between species are not always clear (Harrison & Larson, 2016). Geographically comprehensive surveys of phenotypic divergence are much harder (Jiménez & Ornelas, 2015; Wang et al., 2017; Polly & Wójcik, 2019; Moran et al., 2020), but critical if we are to understand the origin and maintenance of species boundaries.

The relationship between divergent phenotypic characteristics and reproductive barriers is most easily studied in places where the ranges of closely related species
overlap and heterospecific individuals mate and produce offspring (Barton & Hewitt, 1985; Harrison, 1990). In the resulting hybrid zone, as the different species co-exist, compete, and interbreed, phenotypic characteristics may be more variable among individuals and compared to the pure allopatric populations that lie outside the hybrid zone (Hollander et al., 2018; Sottas et al., 2018). By comparing this phenotypic variation both between conspecific allopatric and sympatric populations, as well as between heterospecific populations, it becomes possible to examine potential causes of phenotypic evolution, speciation, and how those mechanisms lead to the reproductive barriers that maintain species boundaries (Shaw & Mullen, 2011).

Here, we examine the phenotypic divergence between two closely related and geographically widespread species of North American field crickets, *Gryllus pennsylvanicus* and *G. firmus*, whose common ancestry dates to roughly 200,000 years ago (Willett et al., 1997; Maroja et al., 2009a). The more northern, inland species, *G. pennsylvanicus*, is broadly distributed throughout the United States, while the more southern, coastal species, *G. firmus*, is restricted to the east coast and west into Texas (Alexander, 1968; Harrison & Arnold, 1982; Weissman & Gray, 2019). These species form a hybrid zone along the eastern front of the Appalachian Mountains (Harrison & Arnold, 1982) and where they co-occur they are isolated by multiple reproductive barriers. The most striking barrier is a one-way incompatibility - *G. firmus* females mated to *G. pennsylvanicus* males lay few eggs that do not hatch (Harrison, 1983; Maroja et al., 2009b; Larson et al., 2012). These two species are also isolated by habitat - *G. firmus* is often found in sandy habitats and has a lighter coloration and longer ovipositors that can presumably lay eggs deeper in sandy soils (Harrison, 1986; Ross & Harrison, 2006).
*Gryllus firmus* is also a larger cricket, though size may vary with the length of the growing season (Masaki, 1961). In some parts of the hybrid zone, *G. firmus* develops faster and emerges earlier in the season, leading to temporal isolation (Harrison, 1985).

These morphological differences have been well characterized in a handful of locations within the hybrid zone (*e.g.*, Connecticut), but whether these morphological traits are consistently different between *G. firmus* and *G. pennsylvanicus* is an open question (Weissman & Gray, 2019). When species differences are studied in only a few locations - it is impossible to distinguish species-specific traits from local adaptation. Morphological traits like lighter color and longer ovipositors may have evolved in specific areas due to habitat selection. Likewise, body size may vary with climate and latitude. Here we conduct the first geographically comprehensive comparison between *G. firmus* and *G. pennsylvanicus* by combining published and unpublished morphological datasets for these two species across their geographic range. Our dataset includes 337 populations of 4,221 crickets, spanning collections four decades. We have three objectives. First, we quantify morphological divergence between species across their geographic range. Second, for populations near the hybrid zone, we test whether traits that distinguish species are correlated with ancestry. Finally, we examine the correlation between morphological traits and environmental variables across these species’ ranges. In doing so, we aim to gain a greater understanding of how population variation and local adaptation contributes to divergence and speciation.
Methods

Cricket collections

We compiled a dataset of 4,221 crickets, the majority being *G. pennsylvanicus*, but also *G. firmus*, and their hybrids, from 337 collecting localities (Fig. 1). Crickets were sampled throughout the United States and Canada, with the largest collections in the northeastern United States and the hybrid zone. Sampling spanned 38 years (1983 to 2022) with collections performed by A.R. Byerly, E.L. Larson, L.S. Maroja, C.L. Ross and R.G. Harrison. In addition to these previously unpublished morphological data, we included data from Ross & Harrison (2002), Larson *et al.* (2013), and Weissman and Gray (2019). Weissman and Gray (2019) was the most geographically widespread dataset. We also included morphological data from a newly described cricket species, *G. thinos* (Weissman & Gray, 2019). *Gryllus thinos* is in the closest sister clade to *G. pennsylvanicus* and *G. firmus* (Gray *et al.*, 2020). We included *G. thinos* to compare morphological variation within *G. firmus* with a closely related species that occupies the same habitat but is classified as a separate species.

We categorized each collecting location as allopatric or sympatric based on past sampling of the field cricket hybrid zone (Harrison & Arnold, 1982; Willett *et al.*, 1997; Maroja *et al.*, 2009a). Populations in and near the hybrid zone often have individuals that are pure *G. firmus* or pure *G. pennsylvanicus*, but they also have many backcross and recent generation hybrids (Harrison & Bogdanowicz, 1997; Maroja *et al.*, 2009a; Larson *et al.*, 2013a, 2014). Because of this, we considered any collecting locations that were near the hybrid zone as “sympatric” unless individuals within the population had been previously genotyped with species diagnostic markers and determined to be allopatric.
(Larson et al., 2013a, 2014). We also assigned each collecting location to a geographic region (labeled in Fig. 1). These regions, identified using climatological data (Karl & Koss, 1984), were as follows: central (CTR: IL, IN, KY, MO, OH, TN, WV); east north central (ENC: IA, MI, MN, WI); northeast (NE: CT, DE, ME, MD, MA, NH, NJ, NY, PA, RI, VT); northwest (NW: ID, OR, WA); south (SO: AR, KS, LA, MS, OK, TX); southeast (SE: AL, FL, GA, NC, SC, VA); southwest (SW: AZ, CO, NM, UT); west (WE: CA, NV); west north central (WNC: MT, NE, ND, SD, WY).

In all cases, crickets were collected by hand and maintained in plastic containers with food (cat and rabbit food), water vials, and shelter prior to freezing. Most samples were collected as adults, but in some cases, crickets were collected as late instar nymphs. Nymphs were allowed to mature to adult stage in the laboratory before freezing. Most collections were done in August-September, but some crickets were collected in late July or early October.

Figure 1: Map of North American cricket collecting locations. Allopatric populations of *G. firmus* are in yellow, *G. pennsylvanicus* are in teal, *G. thinos* populations are in purple, and sympatric *G. firmus* and *G. pennsylvanicus* populations are
in red. The size of the circle corresponds to the sample size for each location. A. Entire range of collection locations in the United States and Canada. B. Enlarged area of densely sampled locations in northeast, central, and southeast United States.

**Morphological measurements**

We focused only on traits that were measured using the same methods across different studies. Crickets were measured for body size, approximated by either body length, femur length, and/or pronotum width. Body length was measured from the head to the longest point of the abdomen, straightening the body when necessary. Pronotum width was measured at the widest part of the pronotum. Femur length was measured from the proximal to distal end of the femur. Female ovipositor length was measured from the point of attachment on the abdomen to the distal end of the ovipositor. Because ovipositor length varies isometrically with body size (Fig. A1), we also calculated relative ovipositor length as the length of the ovipositor divided by pronotum width or femur length, depending on sample availability. We obtained all measurements using Vernier calipers and recorded values to the nearest 0.1 mm.

For a subset of samples where wings were available (31 allopatric crickets and 437 sympatric crickets), we measured male tegmina color using a USB4000 spectrophotometer with an Ocean Optics PX-2 pulsed xenon lamp and SpectraSuite v2.0 software. We mounted a probe on a metal stand at a 90° angle 0.7 mm from the surface of the tegmina. For each male, we recorded and averaged spectral reflectance for three points near the center of the tegmina. We recorded spectral measurements as the percentage of reflected light relative to a Spectralon white standard, restricted our analyses to wavelengths of 300–700 nm, and used a segmental classification method to estimate brightness, chroma, and hue using the CLR v1.1 (Montgomery 2008).
calculated total brightness (B) as R300–700, the summed reflectance from 300 nm to 700 nm. We also divided our reflectance data into four bins of 100 nm each, calculated the total brightness for each bin (Br=600–700, By=500–600, Bg=400–500, and Bb=300–400), and then calculated chroma: √((BrBg)^2+(ByBb)^2 and hue: arctan((ByBb)/Br)/((BrBg)/B).

Molecular markers

A subset of the crickets in our data were previously genotyped for mitochondrial DNA haplotype (Harrison et al., 1987; Harrison & Bogdanowicz, 1997; Willett et al., 1997; Maroja et al., 2009a; Larson et al., 2013b) and/or 110 SNPs from nuclear genes with elevated divergence between G. pennsylvanicus and G. firmus (Larson et al., 2013a, 2014). Mitochondrial DNA haplotype was determined by sequencing cytochrome oxidase I, the adjacent tRNA, and a portion of cytochrome oxidase II (Willett et al., 1997). SNPs were identified from transcriptomes of male accessory glands from two focal populations (Andrés et al., 2013), were genotyped using Sequenom MassARRAY platform, and were used to calculate a hybrid index, which is defined as the proportion of alleles that were inherited from G. firmus (hybrid index = 1) and G. pennsylvanicus (hybrid index = 0) (Larson et al., 2013a, 2014). We then compared how these morphological traits were related to species identity, estimated with genotyping data.

Environmental predictors of species distributions

We tested the relationship between phenotype (ovipositor length and pronotum width) and environmental variables that we predicted would be important in determining species range or local adaptation at two scales: 1) across the species ranges and 2) at an intermediate scale in a well-characterized region of the hybrid zone (Connecticut). Across
the species ranges, we used only allopatric crickets that were most clearly differentiated by morphology, and at the intermediate scale, we used both allopatric and sympatric crickets. We identified 10 environmental variables that might be good predictors of species’ distributions. Elevation, precipitation, and temperature data were collected from the PRISM Climate group website (https://prism.oregonstate.edu/). Elevation was calculated from an 800m digital elevation model of the continental US. For each site, we collected minimum and maximum temperature and precipitation variables for the year in which each cricket was collected. PRISM data were not available for sites in Canada. Soil data were collected from the USDA STATSGO2 soil survey (US sites: https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/geo/?cid=nrcs142p2_053629) and the Soil Landscapes of Canada database (Canada sites: https://sis.agr.gc.ca/cansis/ndb/slc/v3.2/index.html). For a subset of sites in the Northeastern US we used soil data from ISRIC SoilGrids (Poggio et al., 2021) due to the smaller spatial scale. These data were accessed and compiled using the R package soilDB v2.6.14. We used the following variables: average percent sand, average percent clay, and average percent organic matter. We excluded average soil percent silt due to the high intercorrelation of with other soil variables. We used model selection tests that included these 10 environmental variables to find the combination of variables that best explains morphological variation. We ranked competing models using Akaike Information Criterion (AIC) and we reported the models with the highest goodness-of-fit.

Data analysis

All analyses were conducted in R v4.1.2 (R Core Team, 2020). To manipulate data, we used the R packages dplyr v1.0.6 and tidyverse v1.3.1. To plot our data, we used
the R packages ggplot2 v3.3.5 and ggpubr v0.4.0 and to make our maps we used Maps v3.3.0. For statistical analyses, we used commands from the R packages MASS v7.3-54 and car v3.0-12. We used the R packages corrplot v0.92 and Hmisc v4.5-0 to determine environmental variable correlation. We used the R packages AICcmodavg v2.3-1 and MnMln 1.43.1 to rank models based on Akaike Information Criterion and test models.

To test for differences in morphological traits between species and regions we used the Kruskal-Wallis Test, followed by a Pairwise Wilcoxon Rank Sum Test (PWRST) to determine differences between multiple groups. We chose these non-parametric tests because our dataset failed the Levene’s Test for homogeneity of variance. We quantified how well morphological traits could classify crickets using a Linear Discriminant Analysis (LDA) on allopatric crickets. For all analyses, we present the unadjusted p-values and indicate in bold the values that were significant following FDR correction (Benjamini & Hochberg, 1995).

**Data accessibility**

All morphological data, collection site information, including GPS coordinates and environmental data and scripts, are published in Dryad.

**Results**

**Estimates of body size**

In total, our dataset comprised 4,221 crickets, with > 1,100 crickets per sex for each morphological trait measured, except for male tegmina color (Table 1). We first evaluated the relationship between three morphological traits that reflect overall body size in crickets: body length, femur length, and pronotum width. We found that body
length measurements varied greatly depending on how crickets responded to being frozen in the lab (see also Weissman & Gray, 2019). Consequently, we chose to exclude body length measurements from our analyses, but still include them in our supplemental datasets. Male and female individuals of both *G. pennsylvanicus* and *G. firmus* had strong positive relationships between femur length and pronotum width (male *G. pennsylvanicus*: $R^2 = 0.53$, $F_{1,233} = 265$, $p < 2.2 \times 10^{-16}$ and male *G. firmus*: $R^2 = 0.76$, $F_{1,117} = 363.1$, $p < 2.2 \times 10^{-16}$, Appendix Fig. 1A; female *G. pennsylvanicus*: $R^2 = 0.53$, $F_{1,192} = 21$, $p < 2.2 \times 10^{-16}$ and female *G. firmus*: $R^2 = 0.74$, $F_{1,89} = 254.7$, $p < 2.2 \times 10^{-16}$, Appendix Fig. 1B). Therefore, we used pronotum width as our estimate for overall body-size to maximize the number of individuals we could compare across datasets. In female individuals, pronotum width and ovipositor length were also positively related in both species (*G. pennsylvanicus*: $R^2 = 0.44$, $F_{1,214} = 165.7$, $p < 2.2 \times 10^{-16}$ and *G. firmus*: $R^2 = 0.26$, $F_{1,87} = 30.48$, $p = 3.44 \times 10^{-7}$, Appendix Fig. 1C). In comparisons with *G. thinos*, we used femur length to estimate body size to maximize the numbers of individuals in those comparisons.

**Table 1**: Summary of sample sizes for morphological measurements by sex, population type, and region.

<table>
<thead>
<tr>
<th></th>
<th>Pronotum Width</th>
<th>Femur Length</th>
<th>Ovipositor Length</th>
<th>Ovipositor Pronotum Ratio</th>
<th>Ovipositor Femur Ratio</th>
<th>Tegmina Color</th>
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<td>Females</td>
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<tr>
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<td>1134</td>
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<tr>
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</tr>
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<td>5</td>
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<td>167</td>
<td>85</td>
<td>132</td>
<td>1480</td>
<td>108</td>
</tr>
</tbody>
</table>
Morphological differences between species

There were significant differences among allopatric *G. pennsylvanicus*, *G. firmus*, *G. thinos*, and sympatric populations (*e.g.*, *G. firmus*, *G. pennsylvanicus* and hybrids) in male body size (Kruskal-Wallis, $\chi^2 = 35.79$, df = 3, $p = 8.29 \times 10^{-8}$), female body size (Kruskal-Wallis, $\chi^2 = 51.89$, df = 3, $p = 3.16 \times 10^{-11}$), female ovipositor length (Kruskal-Wallis, $\chi^2 = 1277.2$, df = 3, $p < 2.2 \times 10^{-16}$), and relative ovipositor length (Kruskal-Wallis, $\chi^2 = 82.10$, df = 3, $p < 2.2 \times 10^{-16}$). When comparing allopatric *G. pennsylvanicus* and *G. firmus*, male pronotum ($p = 2.1 \times 10^{-5}$, Fig. 2A), female pronotum ($p = 1.4 \times 10^{-11}$, Fig. 2B), ovipositor length ($p < 2.2 \times 10^{-16}$, Appendix Fig. 2A), and relative ovipositor length ($p = 2.8 \times 10^{-16}$, Fig. 2C) were all significantly different. However, for each of these traits there was still considerable overlap between allopatric species. Ovipositor length had the most striking differences between species (Appendix Fig. 2A), even when controlling for body size (Fig. 2C).
For males, tegmina color alone classified most individuals from allopatric populations as either *G. pennsylvanicus* or *G. firmus* (LDA, misclassification rate 3%). One of the 24 *G. pennsylvanicus* males was misclassified as *G. firmus* and 0 of the 7 *G. firmus* males were misclassified as *G. pennsylvanicus*. When looking at male body size alone the misclassification rate was much higher at 23% with 56 of the 268 *G. pennsylvanicus* males misclassified and 27 of the 90 *G. firmus* males misclassified. There was not enough overlap in body size and tegmina color data to perform these analyses using both variables. For females, body size and relative ovipositor length classified most individuals from allopatric populations as either *G. pennsylvanicus* or *G. firmus* (LDA, misclassification rate 12%). Fifteen of the 189 *G. pennsylvanicus* were misclassified as *G. firmus* and 17 of the 90 *G. firmus* were misclassified as *G. pennsylvanicus*.

Crickets from areas near the hybrid zone, which we refer to as sympatric, had considerable overlap with those from allopatric populations. Sympatric crickets were not different from *G. firmus* for male body size, but they were on average larger than *G. pennsylvanicus* (*G. pennsylvanicus*: p = 6.0x10^{-6}, *G. firmus*: p = 0.16, Fig. 2A), but were still different from both allopatric species for female body size (*G. pennsylvanicus*: p = 9.4x10^{-7}, *G. firmus*: p = 0.00032, Fig. 2B), female ovipositor length (*G. pennsylvanicus*: p < 2.0x10^{-16}, *G. firmus*: p < 2.0x10^{-16}, Appendix Fig. 2A), and female relative ovipositor length (*G. pennsylvanicus*: p = 4.6x10^{-8}, *G. firmus*: p = 1.0x10^{-9}, Fig. 2C). This suggests that while these sympatric populations may have individuals that are more *G. firmus*-like or *G. pennsylvanicus*-like, they still have intermediate morphology compared to allopatric populations.
Figure 2: Allopatric populations of *G. firmus* and *G. pennsylvanicus* differ in overall body size and ovipositor length. A. Male pronotum width by species. B. Female pronotum width by species C. Relative ovipositor length (ovipositor length/pronotum width). Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups (PWRST with corrected p-values < 0.05).

**Intraspecific variation in key morphological traits**

We then tested how these traits varied across different geographic regions of each species. We found differences among regions of *G. pennsylvanicus* for male pronotum width (Kruskal-Wallis, $\chi^2 = 56.11$, df = 6, $p = 2.76 \times 10^{-10}$), female pronotum (Kruskal-Wallis, $\chi^2 = 63.44$, df = 6, $p = 8.9 \times 10^{-12}$), ovipositor length (Kruskal-Wallis, $\chi^2 = 185.72$, df = 6, $p < 2.2 \times 10^{-16}$), and relative ovipositor length (Kruskal-Wallis, $\chi^2 = 33.6$, df = 6, $p = 8.03 \times 10^{-6}$). Male and female *G. pennsylvanicus* were largest in the southern and midcentral US (SE, SO, SW, CTR, Fig3A, C) and they had the smallest body size in the northern west (WNC, NW). There were differences among regions in *G. firmus* pronotum width (Kruskal-Wallis, males, $\chi^2 = 9.27$, df = 2, $p = 0.01$; females, $\chi^2 = 9.15$, df = 2, $p = 0.01$), in ovipositor length (Kruskal-Wallis, $\chi^2 = 78.65$, df = 2, $p < 2.2 \times 10^{-16}$), and relative ovipositor lengths (Kruskal-Wallis, $\chi^2 = 54.49$, df = 2, $p = 1.47 \times 10^{-12}$). Male and female *G. firmus* were larger in the south than in the northeast, while *G. firmus* in the south were not significantly different from crickets in either the northeast or the southeast (Fig 3B,
D). In *G. pennsylvanicus*, ovipositor length varied by region. Eastern populations (NE, SE) had the shortest ovipositors, and the central US (CTR) had the longest ovipositors - although there was very limited sample size for this region (Fig S2B, Fig 3E). There was considerable variation in ovipositor length among *G. firmus* populations; southern *G. firmus* females had significantly shorter relative ovipositors than *G. firmus* in the southeast, who in turn had shorter relative ovipositors than *G. firmus* in the northeast (Fig S2C, Fig 3F). However, *G. firmus* in the southeast actually had very similar absolute ovipositor lengths to northeastern *G. firmus* - but had larger body sizes, whereas southern *G. firmus* simply had shorter ovipositors (Fig S2C, Table A1).
Figure 3: Cricket body size and relative ovipositor length varies by geographic region. A. Male pronotum width by species and region. B. Female pronotum width by species and region. C. Relative ovipositor length by species and region. Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups within each species (PWRST with corrected p-values < 0.05).

Recent work from Weissman and Gray (2019) documented cryptic variation in southern G. firmus populations, so we took a closer look at these crickets, separating crickets collected in Florida from those collected in Texas. We also included the recently
described sister species, *G. thinos*, which is sympatric with Texas *G. firmus* (Weissman & Gray, 2019). We found that male (Kruskal-Wallis, $\chi^2 = 29.26$, df = 3, $p = 1.98 \times 10^{-6}$) and female (Kruskal-Wallis, $\chi^2 = 24.88$, DF = 3, $p = 1.63 \times 10^{-5}$) body size and ovipositor length (ovipositor length: Kruskal-Wallis, $\chi^2 = 101.39$, df = 3, $p < 2.2 \times 10^{-16}$; relative ovipositor: Kruskal-Wallis, $\chi^2 = 89.57$, df = 3, $p < 2.2 \times 10^{-16}$) differ among these groups (Figs 4, S2). Compared to northeastern *G. firmus*, Florida *G. firmus* were much larger (Figs 4A, B), but had only slightly larger ovipositor lengths (Fig S2C), giving them shorter relative ovipositors (Fig 4C). Texas *G. firmus* did not differ in overall body size from northeastern *G. firmus*, but had even shorter relative ovipositor lengths (S2D, Fig 4C, Table A3). The magnitude of the morphological differences among Florida, Texas, and northeastern *G. firmus* is similar to differences between *G. firmus* and the recently described *G. thinos*. Gray et al. (2020) found that *G. firmus* in Texas and Florida are distinct groups, with Texas *G. firmus* sister to *G. pennsylvanicus* and Florida *G. firmus* sister to both *G. pennsylvanicus* and Texas *G. firmus*. Altogether, the morphological differences and the phylogenetic relationships support the findings by Weissman and Gray (2019) that Texas *G. firmus* may be a cryptic species.
Figure 4: Morphological variation in *G. firmus* consistent with proposed cryptic species. A. Male femur length, B. Female femur length and C. Relative ovipositor length (ovipositor length/femur length). There is considerable morphological variation among northeastern, Florida and Texas *G. firmus*, which is similar to the magnitude of morphological divergence observed in the sister species *G. thinos*. This combined with genetic divergence suggests there may be cryptic species in what is currently considered *G. firmus*. Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups (PWRST with corrected p-values < 0.05).

**Morphology in sympatric populations**

For the subset of crickets that were from the hybrid zone or nearby (sympatric populations), and were also genotyped with molecular markers, we looked at the relationship between admixture and morphological traits. We found that each trait had a similar transition from *G. pennsylvanicus* to *G. firmus*, with highly admixed individuals having intermediate phenotypes (Fig. 5). We found male pronotum (R² = 0.18, F₁,279 = 62.97, p = 5.14x10⁻¹⁴), male tegmina color (R² = 0.32, F₁,133 = 61.71, p < 1.19x10⁻¹²), female pronotum (R² = 0.27, F₁,275 = 103.8, p < 2.2x10⁻¹⁶), and relative ovipositor length (R² = 0.47, F₁,270 = 239.5, p < 2.2x10⁻¹⁶) all had strong correlation with the hybrid index. Because the SNPs used to calculate the hybrid index are concentrated on the X chromosome (54 out of 110, (Maroja *et al.*, 2015; Gainey *et al.*, 2018)), females (XX) were more likely to be classified with an intermediate hybrid index than males (X0).
Overall, morphological traits were also correlated with mtDNA haplotypes - crickets that had *G. pennsylvanicus* mtDNA tended to be smaller (males: Kruskal-Wallis, \( \chi^2 = 43.14, \) df = 1, \( p = 5.11 \times 10^{-11} \); females: Kruskal-Wallis, \( \chi^2 = 44.86, \) df = 1, \( p = 2.11 \times 10^{-11} \)), darker (Kruskal-Wallis, \( \chi^2 = 33.75, \) df = 1, \( p = 6.27 \times 10^{-9} \)) crickets with shorter ovipositors (Kruskal-Wallis, \( \chi^2 = 37.67, \) df = 1, \( p = 8.40 \times 10^{-10} \)) (Fig. 6). We found that crickets with *G. firmus* ancestry at nuclear markers (hybrid index = 1) often had *G. pennsylvanicus* mtDNA haplotypes (Fig. 7), indicating asymmetric introgression of the mtDNA.

![Figure 5](#)

**Figure 5:** Crickets with more hybrid background have intermediate morphological traits. The relationship between the hybrid index (*G. pennsylvanicus* = 0 and *G. firmus* =
1) and A. male pronotum width, B. female pronotum width, C. relative ovipositor length, and D. male tegmina color.

Figure 6: Morphological traits tended to correspond to mtDNA haplotypes. A. male pronotum width, B. female pronotum width, C. relative ovipositor length and D. male tegmina color. Boxplots indicate the mean values of each trait, quartiles, the range of the data (whiskers), and outliers. Individual data points are overlaid as scatterplots.

Figure 7: Mitochondrial DNA introgression is largely asymmetric. Crickets with \textit{G. firmus} ancestry at nuclear markers (hybrid index = 1) often had \textit{G. pennsylvanicus} mtDNA haplotypes.

Environmental predictors of morphology

In allopatric populations throughout broad ranges, we found latitude, elevation, average soil percent clay, minimum and maximum temperature created the best model for ovipositor length. Latitude, longitude, soil percent sand, and minimum temperature created the best model for pronotum width (Table 2). Average soil percent clay, as well
as higher minimum and maximum temperatures, were positively associated with longer ovipositor lengths and higher minimum temperatures were positively associated with larger body size, characteristics of *G. firmus* (Appendix Fig. 3). In the subset of Connecticut sympatric and allopatric populations, minimum and maximum temperatures, as well as soil percent organic matter, created the best model with positive associations for all three variables and ovipositor length (*Table 2, Appendix Fig. 3*).
Table 2: Results of linear regression and AIC to test the relationship between environmental variables and morphological traits in female crickets.

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**Pronotum All Allopatric Populations**

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Ovipositor CT Sympatric & Allopatric Populations
<p>| Variable                      | Coef | Std. Error | t value | Pr(&gt;|t|) | Df | 95% CI | 99% CI |
|-------------------------------|------|------------|---------|----------|----|--------|--------|
| (Intercept)                   | 2936.5 | 766.87     | 16.3818 | 0.1501   | 109.132 | &lt; 2.00E-16*** |
| Precipitation*                | 0.716 | 2935.7     | 768.78  | -        | -   | -      | -      |
| Human Footprint               | 0.624 | 2935.8     | 768.8   | -        | -   | -      | -      |
| Avg Soil % Sand               | 0.561 | 2935.9     | 768.8   | -        | -   | -      | -      |
| Latitude                      | 0.345 | 2936.1     | 768.83  | -        | -   | -      | -      |
| Avg Soil % Clay               | 0.206 | 2936.2     | 768.85  | -        | -   | -      | -      |
| Elevation                     | 0.011 | 2936.4     | 768.87  | -        | -   | -      | -      |
| Longitude                     | 0.008 | 2936.4     | 768.87  | -        | -   | -      | -      |
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</table>
Discussion

Cryptic diversity in a wide-ranging species

The hybrid zone between the field crickets *G. firmus* and *G. pennsylvanicus* has been a model for understanding speciation (Harrison & Rand, 1989; Harrison & Larson, 2014). The field cricket hybrid zone stretches from the northeastern US as far south as Virginia, and likely further into the southeast. Divergence in morphology, nuclear and mitochondrial DNA, and reproductive barriers have been carefully studied in several major regions of the hybrid zone (Harrison, 1985; Rand & Harrison, 1989; Ross & Harrison, 2002; Maroja et al., 2009a; b; Larson et al., 2012, 2014). Yet even in this well-studied system there is geographic diversity across the ranges of these species that complicates their relationships.

Our results confirm that allopatric populations of these two species, defined by genetic markers (Harrison & Arnold, 1982; Willett et al., 1997; Broughton & Harrison, 2003; Maroja et al., 2009a), can be largely differentiated by a combination of body size, male tegmina color, and female ovipositor length (Fig. 2). At the same time, there is regional variation in these traits within each species (Fig. 3). These differences may be due to local adaptation of life history traits such as egg diapause and development time (discussed below). But in some cases, they may also indicate cryptic diversity in field crickets.

In a recent revision of North American field crickets, Weissman & Gray (2019) proposed that there was cryptic diversity in southern populations of *G. firmus*, particularly in Texas. Importantly, our phenotypic comparisons confirm that Texas and
Florida *G. firmus* are morphologically distinct from northeastern *G. firmus* (Fig. 4). In a recent nuclear phylogeny, Texas and Florida *G. firmus*-like crickets also formed distinct clusters within the larger *G. pennsylvanicus* group (Weissman & Gray, 2019; Gray et al., 2020). Unfortunately, we do not have a phylogeny that includes both Texas and Florida *G. firmus* and northeastern *G. firmus*, so the relationships among these groups are still unclear. The combination of distinct morphology and phylogenetic relationships suggests these might be cryptic species of *Gryllus*, but this will not be resolved without further genotyping and/or evaluations of reproductive compatibility among these populations.

**Intermediate phenotypes in hybrid zone crickets**

The morphological traits that best distinguish species in allopatry can also be used to distinguish these species in or near the hybrid zone. In this study, we used a conservative approach to defining allopatric and sympatric populations. Allopatric populations were those well outside of where the two species co-occur or populations that have been genotyped with species-diagnostic markers. We found that in sympatry, crickets that were mostly *G. firmus* or mostly *G. pennsylvanicus* at nuclear markers (Larson et al., 2013a, 2014) had morphological traits that are also *G. firmus*-like or *G. pennsylvanicus*-like. Both male and female body size, male tegmina color, and relative ovipositor length had clinal variation from *G. pennsylvanicus*-like to *G. firmus*-like, with highly admixed individuals having intermediate phenotypes (Fig. 5). Male tegmina color stood out as having the fewest individuals with intermediate hybrid index values (Fig 5 D), but that is most likely because the SNPs used to calculate the hybrid index were predominately X-linked, so male XO crickets were rarely heterozygous at those SNPs.
and had overall lower hybrid indices (Larson et al., 2014; Maroja et al., 2015; Gainey et al., 2018).

The relationship between morphology and mitochondrial haplotype was less clear for populations near or in the hybrid zone. Crickets that were mostly *G. firmus* at nuclear markers often had *G. pennsylvanicus* mtDNA (Fig. 7). This pattern fits with what we expect based on the one-way prezygotic incompatibility between *G. firmus* females and *G. pennsylvanicus* males (Harrison, 1983; Maroja et al., 2009b; Larson et al., 2012). All F1 hybrids are produced from crosses with *G. pennsylvanicus* mothers, thus *G. pennsylvanicus* mtDNA will be more likely to introgress into *G. firmus*. Even rare instances of hybridization might lead to mtDNA introgression, like the mtDNA capture observed in many mammal species (Melo-Ferreira et al., 2005; Good et al., 2008).

**Adaptations to soil type**

Ovipositor length is one of the most striking morphological differences between *G. firmus* and *G. pennsylvanicus*. Female crickets use their ovipositors to lay their eggs in the soil and ovipositor length has been hypothesized to relate to the soil type and/or the depth of egg laying. The depth of egg laying may be a particularly critical life-history trait in *G. pennsylvanicus* and *G. firmus* because these species overwinter as eggs, as opposed to most field crickets that overwinter as early instar nymphs (Alexander, 1968; Harrison & Bogdanowicz, 1995). For eggs to be viable, they must withstand low winter temperatures and freeze/thaw cycles (Ross & Harrison, 2006). Throughout its range, *G. firmus* is most often found on sandy coastal soils (Harrison & Arnold, 1982; Weissman & Gray, 2019) and tends to have a longer ovipositor than *G. pennsylvanicus* (Figs 3, A2).
This may be an adaptation to laying eggs deeper in sandy substrates. In some parts of the hybrid zone, such as Connecticut, the association with different soil types is striking. The two species have been found on micro habitat patches of loam (G. pennsylvanicus) and sandy (G. firmus) soils in Connecticut (Harrison, 1986; Harrison & Rand, 1989; Rand & Harrison, 1989) and interactions between the two species occur across these habitat patch boundaries on the scale of only hundreds of meters (Ross & Harrison, 2002; Larson et al., 2014).

Despite what appears to be strong habitat associations, the relationship between soil type and ovipositor length is complicated. Ovipositor length does not necessarily determine egg-laying depth, instead females may wield long ovipositors at different angles (Réale & Roff, 2002). It is also not clear exactly how the association between ovipositor length and soil type is maintained. Females of both species prefer to lay eggs in loamy soil and there is no difference in overwintering egg viability in different soil types (Ross & Harrison, 2006). Finally, these associations are clearly established only in a small part of the species’ ranges - Connecticut (Rand & Harrison, 1989; Ross & Harrison, 2002; Larson et al., 2013b). Even where soil associations appear to be the strongest, the transition from sandy to loamy soils is more gradual and less distinct than we might expect based on the patchiness of G. firmus and G. pennsylvanicus populations (Ross & Harrison, 2002; Larson et al., 2014). Here we find that both across the broad ranges of these species, and at an intermediate scale in the Connecticut hybrid zone, there is no strong association between ovipositor length and sandy soils. In fact, we tend to see crickets with longer ovipositors on clay soils (Table 2). This might be due to the different
methods used to quantify soil type (soil survey data versus on-site soil sampling), but altogether this suggests that habitat associations in these species are variable and should be investigated further.

**Body size, climate, and life cycle**

In insects, seasonality and the length of the growing season are critical to the rate of development and adult body size (Masaki, 1961; Tauber & Tauber, 1981). This is particularly true for hemimetabolous insects, which often go through many nymphal stages and have long development times before reaching their full size and sexual maturity (Kivelä et al., 2011). Insects at higher latitudes have shorter growing seasons and as a result may develop more quickly or reach an overall smaller body size (Masaki, 1967; Parsons & Joern, 2014). This pattern of smaller body sizes at higher latitudes is sometimes referred to as the converse of Bergman’s rule, which states that individuals have larger body sizes in colder climes (Masaki, 1967; Mousseau, 1997). We see this pattern most clearly in *G. pennsylvanicus*, where we found the populations with the smallest body size tended to be farther north (WNC and NW, **Fig. 2**). Indeed, we found that crickets at higher latitudes had on average smaller body size and that there was a significant relationship between body size and latitude (**Table 2**).

We may not expect a direct relationship between body size and latitude if the length of the growing season allows for multiple generations per year. Insects can shift from continuous development in the south to univoltine (one generation per year) in the north (Masaki, 1961, 1967). As a result, there may be regions where body size is smaller than expected based on latitude in order to accommodate multiple generations per year.
We did not find this pattern in our results; but we may not have had the resolution of latitudinal samples to see a sawtooth pattern in body size. However, there is some evidence that development time in *G. firmus* varies with latitude. In Virginia, *G. firmus* emerge earlier in the season than *G. pennsylvanicus* - leading to temporal isolation in that part of the hybrid zone, but in Connecticut, the two emerge simultaneously (Harrison, 1985). In Florida, *G. firmus* is reported to have multiple generations per year (Walker, personal observation, reported in Weissman & Gray, 2019), where throughout its range it otherwise appears to have a single generation per year (Walker, 1980). Notably, despite having many generations per year, Florida *G. firmus* are considerably larger than northern populations. It is unclear whether there is a continuous shift in life cycle across the range of *G. firmus*, or if Florida *G. firmus* have a distinct life history from other *G. firmus*.

**Conclusions**

In studies of speciation and to understand the effects of local selection, it is critical to quantify morphological and genetic variation across the geographic range of widespread species. The field cricket hybrid zone is an example of how important the larger geographic context can be. In some regions of the field cricket hybrid zone, *G. pennsylvanicus* and *G. firmus* have a patchy distribution and *G. firmus* crickets are found on sandy soils (Rand & Harrison, 1989; Ross & Harrison, 2002). But the strong soil association breaks down in other regions of the hybrid zone (Larson *et al.*, 2013b) and across their geographic range, suggesting the soil association may be a result of local adaptation or colonization history (Hauffe & Searle, 1993; Gompert *et al.*, 2010). Our
results provide a foundation for future geographically expansive studies that compare genetic divergence and the role of specific traits in reproductive barriers to better understand local adaptation and speciation in this system. More broadly, this is an example of how critical it is to move studies of speciation beyond the comparison of a few focal populations. Geographically expansive studies of phenotypic and genetic divergence will also be important for understanding how species distributions and hybrid zones shift over time and in a changing climate (Britch & Cain, 2001; Taylor et al., 2015).
Chapter Two: The role of community science in Orthoptera research

Introduction

Before the modern era, scientific discovery was commonly made by people who were not scientists by profession (Brenna 2011, Miller-Rushing et al. 2012). In the middle of the nineteenth century, that began to change. Science became highly academic with a greater “gatekeeping” of knowledge. Experimentation and fieldwork became increasingly expensive to conduct. Because of this, much of the knowledge gained during that time has been effectively withheld from non-scientists in expensive, difficult to obtain, and challenging to read scientific journals. The advent of the internet is changing the inaccessibility of science. People around the world are now instantly connected with each other and scientific articles are more readily available to the public with the introduction of open access journals. The recent boom in smartphone applications brought inexpensive tools to non-scientists. We are now seeing a renaissance of scientific discovery and exploration by people without a formal science education. These “citizen” or “community” scientists are filling gaps in the modern approach to scientific inquiry. Here we provide an overview of community science and focus on how acoustic surveys are used to study Orthoptera biodiversity. We highlight the importance of community science, particularly in Orthoptera, best practices for acoustic-based research, and concerns related to community science.
**Importance of Community Science**

Community science is the participation of people who are not professional scientists in scientific inquiry. There are typically two main avenues for community science. In the first, scientists guide the project, usually using an established protocol, with varying degrees of input from local volunteers and organizations. The second is the less structured collection of data, generated largely by individuals, that is then recorded and shared through apps, such as iNaturalist or social media (Paiero et al. 2020, Skejo et al. 2020b, Kasalo et al. 2021a, 2021b, Trewick 2021). These internet-based forums provide anyone with a smartphone or computer the ability to add to a collective database that is accessible by scientists and nonscientists everywhere.

Community science fills several gaps left by the modern academic approach to scientific inquiry. Community science initiatives are usually oriented locally, providing fine scale data, but in aggregate, cover entire regions (Theobald et al. 2015), which allows these projects to cover a larger geographic area and gather much more data than a small group of scientists (Pocock et al. 2015, Kaláb et al. 2021). Organized initiatives led by passionate amateur scientists have long been recognized as valuable in tracking changes in populations over time (Pocock et al. 2015). Locals can record data year-round, which can be difficult and costly for scientists who are often based further afield (Kaláb et al. 2021). Local knowledge of the area is also often invaluable to scientists conducting fieldwork (Penone et al. 2013, Medin and Bang 2014).

The scale and speed in which anthropogenic change is affecting biodiversity requires the collection of as much data as possible as quickly as possible. Scientists alone
are unable to generate the amount of data required to inform policy decisions (Theobald et al. 2015). Community science initiatives have been successful in monitoring conservation efforts (Barlow et al. 2015, Kallimanis et al. 2017), sighting species thought to be extinct (Woller and Hill 2015, Buzzetti et al. 2021), discovering new species (Kasalo et al. 2021b, Trewick 2021), locating occurrences of range expansion (Beckmann 2017, Paiero et al. 2020, Kaláb et al. 2021), and invasive species (Okayasu et al. 2020, Ahnelt et al. 2021, Kasalo et al. 2021a). In some taxa, the majority of newly discovered species are first described by people who are not professional scientists (Fontaine et al. 2021).

Community science plays an important role in diversity, equity, and inclusion. Involving indigenous peoples in research based on their native lands brings immense value to the quality of the work through differing perspectives and context, but also, perhaps more importantly, to the consideration and preservation of their cultures (Medin and Bang 2014). Volunteers can help in ways unrelated to fieldwork, such as transcribing/digitizing field notebooks, which creates opportunities for those who cannot engage in fieldwork (Woller and Hill 2015). Community science can also increase accessibility, including the opportunity for publication, within underprivileged or underrepresented groups (Skejo et al. 2020b).

A scientifically literate voting populace is necessary to get matters such as climate change and public health issues addressed by elected officials. Current models of human learning contend that learning and understanding most effectively occur through an active process of exploration and inquiry, not the passive teaching of facts that occurs in most
classrooms (Wieman 2007). Since classroom instruction is the only exposure most people have to science, it is difficult for them to grasp the importance of scientific inquiry. But, if they are given opportunities (through community science initiatives) to actually “do” science, the general public is able to connect with science and understand the impact it has on their daily lives. Community science personalizes science for participants, which becomes important when scientific ideas counter long-held beliefs. When individuals can witness first-hand the validity of scientific information, they can be less resistant to changing their thinking (Wieman 2007). We encountered an instance of this in our own fieldwork, when a participant, who did not believe that climate change was real, began asking questions about his own observation of declining honeybee numbers and possible causes for it. These were questions he had been curious about for some time, but had not had the access to ask before, an occurrence that is common at the intersection of science and the general population (Fontaine et al. 2021).

Importance of Orthoptera as Study Systems

Because of their short life cycles and, in some species, specialization in habitat, food source, and egg-laying, insects are the “canaries in the coal mine” for climate change (Riede 1998, Jeliazkov et al. 2016, Beckmann 2017). Due to their sensitivity to climate change and ubiquity, Orthoptera are particularly important organisms for climate change research (Fartmann et al. 2012, Löffler et al. 2019). Because male Orthoptera sing to attract mates, studies regarding species richness, abundance, and emergence times are relatively simple in Orthoptera, as acoustic data can be recorded from the roadside.
The study of acoustic signaling of or near threatened species allows for effective monitoring for conservation purposes, while reducing disruption and preserving fragile habitats (Moran et al. 2014, McNeil and Grozinger 2020). New technologies in acoustic monitoring allow for large-scale monitoring of singing insects which provides an easier, less time-consuming way to estimate such metrics as species abundance and richness. Community scientists can sustainably crowd-source this vital information in a way that scientists are not able to use a traditional approach.

Nearly 85% of the world population owns a smartphone (Turner 2018). Every smartphone has audio and video recording, GPS, and internet capabilities, placing these tools for data collection, storage, and transmission at the fingertips of most people on the planet. Highly accurate new tools, such as TADARIDA (a Toolbox for Animal Detection in Acoustic Recordings Integrating Discriminant Analysis) and AI, make using the vast quantities of acoustic and photographic data generated by community scientists useful on a massive scale (Bas 2016, Kasalo et al. 2021b). In the case of acoustical monitoring, data for many different species across taxa can be captured and analyzed from a single recording, a practice that could further utilize existing recordings, increase the rate of new data collection, decrease costs, and encourage collaboration (Jeliazkov et al. 2016, Newson et al. 2017). Smartphone technology also allows us to easily record data that is outside normal human sensory range, which provides a means to detect species that might otherwise go unnoticed (Moran et al. 2014).
Table 3: Published Orthoptera research that has included a community science element.

<table>
<thead>
<tr>
<th>Type</th>
<th>Country</th>
<th>Species Name</th>
<th>Number of Participants</th>
<th>Involvement Type</th>
<th>Question Type(s)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structured</td>
<td>France</td>
<td>11 species of bush crickets (Tettigoniidae family)</td>
<td>10 individuals</td>
<td>Roadside acoustic data collection</td>
<td>Species richness; species abundance; environmental factors</td>
<td>Penone et al., 2013; Jeliazkov et al. 2016</td>
</tr>
<tr>
<td>Structured</td>
<td>Germany</td>
<td>Oak bush-cricket (Meconematinae family)</td>
<td>~8 individuals</td>
<td>Photograph collection; social media</td>
<td>Range expansion</td>
<td>Ahnelt et al. 2021</td>
</tr>
<tr>
<td>Structured</td>
<td>United Kingdom</td>
<td>Bush Crickets (Tettigoniidae family)</td>
<td>unknown</td>
<td>Placement of static acoustic sensors</td>
<td>Species richness</td>
<td>Newson et al. 2017</td>
</tr>
<tr>
<td>Structured</td>
<td>Japan</td>
<td>Pink-winged grasshopper (Pyrgomorphidae family)</td>
<td>unknown</td>
<td>Field specimen collection</td>
<td>Invasive species</td>
<td>Okayasu 2020</td>
</tr>
<tr>
<td>Structured</td>
<td>United States</td>
<td>Camel crickets (Rhaphidophoridae family)</td>
<td>unknown</td>
<td>Photograph collection; specimen</td>
<td>invasive species</td>
<td>Epps et al. 2014</td>
</tr>
<tr>
<td>Status</td>
<td>Country</td>
<td>Species Description</td>
<td>Numbers</td>
<td>Data Collection Methodologies</td>
<td>Record Type</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>---------------------------------------------------------------</td>
<td>-----------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Structured</td>
<td>United States</td>
<td>Grasshopper (Acrididae family)</td>
<td>unknown</td>
<td>Transcription of field journals</td>
<td>Rare species record</td>
<td>Woller and Hill 2015</td>
</tr>
<tr>
<td>Unstructured</td>
<td>Australia</td>
<td>Pygmy Grasshoppers (Tettigidae family)</td>
<td>8 individuals</td>
<td>Photograph collection; social media</td>
<td>Rare species record</td>
<td>Skejo et al. 2020a</td>
</tr>
<tr>
<td>Unstructured</td>
<td>Canada</td>
<td>Red-headed bush cricket and restless bush cricket (Gryllidae family)</td>
<td>~15 individuals</td>
<td>Photograph collection; social media</td>
<td>Range expansion</td>
<td>Paiero et al. 2020</td>
</tr>
<tr>
<td>Unstructured</td>
<td>United Kingdom</td>
<td>Conocephalus discolor and Metrioptera roeselii</td>
<td>2000+ people</td>
<td>Photograph collection</td>
<td>Range expansion; environmental factors</td>
<td>Beckmann et al. 2015</td>
</tr>
<tr>
<td>Unstructured</td>
<td>United States</td>
<td>Acrididae and Romaleidae families</td>
<td>unknown</td>
<td>Photograph collection; social media</td>
<td>Species richness; species abundance</td>
<td>Harman et al. 2022</td>
</tr>
<tr>
<td>Unstructured</td>
<td>United States</td>
<td>Japanese burrowing cricket (Gryllidae family)</td>
<td>unknown</td>
<td>Photograph collection; social media</td>
<td>Invasive species; range expansion</td>
<td>Bowles 2018</td>
</tr>
<tr>
<td>Location</td>
<td>Country</td>
<td>Species Description</td>
<td>Sample Size</td>
<td>Identification Method</td>
<td>Identification Method</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>----------------------------------------------------------</td>
<td>-------------</td>
<td>-------------------------------------------</td>
<td>------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Unstructured</td>
<td>New Zealand</td>
<td>ground weta (Anostostomatidae family)</td>
<td>unknown</td>
<td>Photograph collection; social media</td>
<td>New species identification</td>
<td>Trewick 2021</td>
</tr>
<tr>
<td>Unstructured</td>
<td>Madagascar</td>
<td>Southern Devils pygmy grasshopper (Tetrigidae family)</td>
<td>4 individuals</td>
<td>Photograph collection; social media</td>
<td>New species identification</td>
<td>Skejo et al. 2020b</td>
</tr>
<tr>
<td>Unknown</td>
<td>Czech Republic</td>
<td>Bush Crickets (Tettigoniidae family)</td>
<td>unknown</td>
<td>Photograph and acoustic collection; social media</td>
<td>Range expansion</td>
<td>Kalab et al. 2021</td>
</tr>
</tbody>
</table>
A literature search on Google Scholar yielded only 14 studies that mention using “citizen science” or “community science” in Orthoptera research (Table 3). Many of these studies did not indicate how many non-professionals participated; however, of the studies that did, most were small groups of less than 15 people. We see a fairly equal split between studies that are structured around a protocol created by professional biologists and those that are not (i.e., from iNaturalist, social media), with 43% of studies being structured, 50% unstructured, and the remaining 7% unclear. Studies involved questions of species richness, species abundance, novel/threatened species identification, range changes/expansion, invasive species, and environmental factors impacting species. In most studies, participants helped in collecting photographic and/or acoustic data (Figure 8).

![Figure 8](image)

**Figure 8: General categories of study questions grouped by study structure type.** Some studies investigated multiple question categories.
Best Practices for Community Science in General

The validity of data collected by volunteers is critical to its usefulness in scientific study, so measures to ensure accuracy and precision must be implemented (Pocock et al. 2015). Non-scientists in a European Union study were generally less complete and covered a smaller geographic area when compared to scientists at the same site. However, when the observations of the larger number of volunteers were compared in aggregate with the relatively fewer observations from scientists, the results are largely comparable (Kallimanis et al. 2017). Projects with larger sample sizes can also reduce the statistical effect of variation collection method (Theobald et al. 2015).

As geographic scale and longevity of projects increase, so does participation (Theobald et al. 2015). One under-utilized avenue that provides opportunity for both of these criteria is the integration of research programs in grade school and secondary education (Okayasu et al. 2020). Projects with larger temporal and spatial scales, publicly available data (i.e. on a project website), and/or taxonomic training are more likely to be used in scientific publication than academically sponsored work, whereas data collection method training and government partnered projects correlate to a decreased publication rate (Theobald et al. 2015).

Theobald et al. (2015) estimate that community science initiatives contribute between $667 million and $2.5 billion in labor and materials, yet only 12% of projects resulted in scientific publication. This demonstrates that there is a lot of public engagement, but scientists are not making effective use of it (Theobald et al. 2015). Particularly in the field of systematics, many biologists are resistant to using data
generated by community scientists, delaying necessary conservation efforts for undiscovered species (Kasalo et al. 2021b). Evidence of invasive species in new territories that is stored on iNaturalist has been unnoticed by scientists for years (Kasalo et al. 2021a), which delays possible action towards curbing further expansion.

The use of social media and online newspapers has been successful in generating targeted engagement (Ahnelt et al. 2021). Just as businesses use these relatively inexpensive avenues to advertise to potential customers, it is reasonable to use those same channels to engage volunteers in community science initiatives. Considering the great financial return that volunteer person-hours provide, allocating a portion of grant funding for that purpose could be a sound investment.

**Best Practices for Community Science in Orthoptera Research**

Despite Orthoptera’s known sensitivity to climate change, ubiquity, and ease of data collection, there are very few organized, long-term community science programs that focus on this taxa (Burton 2003, Fartmann et al. 2012, Newson et al. 2017, Löffler et al. 2019). With that in mind, we propose some best practices for creating effective community science programs in Orthoptera research.

Organized studies appear to be better suited for long-term studies of species abundance, but less organized studies work well for studies of species richness (Penone et al. 2013). Detailed records of environmental data allow for the correlation of Orthoptera richness, abundance, and morphological changes with environmental factors, such as temperature, precipitation, and land use (Byerly et al. n.d., Larson et al. 2013, Jeliazkov
et al. 2016). This use of environmental data can also be applied to historical records through the use of databases, such as the PRISM Climate Group website and Last of the Wild (Byerly et al. n.d., Fontaine et al. 2021).

Clear, concise protocols are necessary for organized studies (Matteson et al. 2012, Penone et al. 2013). For acoustics, this means instructions on how to record sound, recommended recording distance, and length of recording. If possible, automating the process by means of a smartphone app can reduce error. Zilli et al. (2014) designed and deployed a smartphone app that used acoustical data to identify specific species in real-time. When designing apps for use by non-scientists, mimicking the design of existing, popular apps (i.e. Shazam) can increase user uptake and engagement (Moran et al. 2014).

In organized studies, workshops, online tutorials, fieldnotes, and/or video demonstrations should be used to provide training to volunteers (Barlow et al. 2015). In the case of collecting acoustic data, example audio recordings of the subject specie(s) are helpful to participants. In studies that require volunteers to make identifications, it is helpful to include an “unsure” column to reduce guessing when participants are uncertain (Barlow et al. 2015).

**Concerns Regarding Community Science**

Despite its growing popularity and many benefits, several concerns have emerged regarding the methods used in community science initiatives. Many naturalists are concerned that the overuse of technology in community science will be a distraction for people attempting to interact with their environment. They fear that the overuse of
smartphone microphones, cameras, and apps will keep people from using their own senses (Moran et al. 2014). The same concern exists for artificial intelligence applications, which can be a double-edged sword in that they allow for the processing of massive amounts of data, but also reduce the need for public involvement (Kasalo et al. 2021b). Locals, naturalists, and professional scientists have concerns regarding the damage that numerous, untrained visitors can do to fragile ecosystems (Moran et al. 2014). This concern is so great that, in some cases, the locations of species are withheld from the public to protect them (Moran et al. 2014). Another concern is that community science initiatives over-represent terrestrial, particularly vertebrates, and freshwater species. Often, charismatic species, such as plants, birds, and butterflies, appeal to the interests and accessibility of the general public in a way that many marine, invertebrate, and fungus species do not. However, this bias is also seen in traditional scientific research (Theobald et al. 2015).

Conclusion

Community science projects are quickly increasing in number but are drastically under-utilized in scientific literature (Theobald et al. 2015). In Orthoptera, projects using acoustic data recorded by community scientists can help answer questions related to species abundance, species richness, emergence time, and changes in range and distribution due to anthropogenic change (Penone et al. 2013); however we were only able to locate 14 published studies which specifically mentioned the use of community science in their methods. Community science is growing in popularity and provides many
benefits, including increasing scientific knowledge and engaging the general public, enhanced conservation, and providing much-needed work hours to advance research goals. However, these benefits can be outweighed by damage to fragile ecosystems and threatened wildlife, if participants are not properly trained. Thus, it appears that community science, as with the natural world it surveys, requires balance to be sustainable. Because they are easily identified through mating song, Orthoptera species provide excellent study systems for achieving all of these goals from distances that can help protect vulnerable habitat.
References


Burton, J.F. 2003. The apparent influence of climatic change on recent changes of range by European insects (Lepidoptera, Orthoptera). repository.naturalis.nl.


Kaláb, O., Pyszko, P. & Kočárek, P. 2021. Estimation of the Recent Expansion Rate of Ruspolia nitidula (Orthoptera) on a Regional and Landscape Scale. *Insects* **12**.


Appendix A

Appendix Table 1: P-values for PWRST post hoc contrasts of allopatric *G. pennsylvanicus* populations by region. P-values marked with *** lost significance after correction.

<table>
<thead>
<tr>
<th>Male Pronotum (Fig. 3A)</th>
<th>CTR</th>
<th>NE</th>
<th>NW</th>
<th>SE</th>
<th>SO</th>
<th>SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>0.545</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NW</td>
<td>0.071</td>
<td><strong>0.006</strong></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SE</td>
<td>0.586</td>
<td><strong>0.002</strong></td>
<td><strong>1.40E-04</strong></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SO</td>
<td>0.545</td>
<td><strong>0.020</strong></td>
<td><strong>6.52E-04</strong></td>
<td>0.453</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>SW</td>
<td>0.186</td>
<td><strong>5.52E-06</strong></td>
<td><strong>4.49E-06</strong></td>
<td>0.077</td>
<td>0.515</td>
<td>NA</td>
</tr>
<tr>
<td>WNC</td>
<td>0.127</td>
<td><strong>0.023</strong></td>
<td>0.183</td>
<td><strong>9.04E-06</strong></td>
<td><strong>0.001</strong></td>
<td><strong>3.89E-08</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Female Pronotum (Fig. 3C)</th>
<th>CTR</th>
<th>NE</th>
<th>NW</th>
<th>SE</th>
<th>SO</th>
<th>SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>0.068</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>NW</td>
<td><strong>0.009</strong></td>
<td><strong>0.027</strong></td>
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<td>NA</td>
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<tr>
<td>SE</td>
<td>0.874</td>
<td><strong>2.81E-06</strong></td>
<td><strong>2.04E-08</strong></td>
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<td>NA</td>
<td>NA</td>
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<tr>
<td>SO</td>
<td>0.933</td>
<td><strong>0.024</strong></td>
<td><strong>0.003</strong></td>
<td>0.664</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>SW</td>
<td>0.544</td>
<td><strong>3.34E-06</strong></td>
<td><strong>2.89E-07</strong></td>
<td>0.114</td>
<td>0.605</td>
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<tr>
<td>WNC</td>
<td>0.080</td>
<td>0.756</td>
<td>0.070</td>
<td><strong>6.79E-06</strong></td>
<td><strong>0.031</strong></td>
<td><strong>6.56E-06</strong></td>
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<table>
<thead>
<tr>
<th>Relative ovipositor (Fig. 3E)</th>
<th>CTR</th>
<th>NE</th>
<th>NW</th>
<th>SE</th>
<th>SO</th>
<th>SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td><strong>0.001</strong></td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>NW</td>
<td><strong>0.005</strong></td>
<td><strong>0.003</strong></td>
<td>NA</td>
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</table>
### Ovipositor length (Appendix Fig. 2B)

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>NE</th>
<th>NW</th>
<th>SE</th>
<th>SO</th>
<th>SW</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>0.001</td>
<td>NA</td>
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<tr>
<td>NW</td>
<td>0.002</td>
<td>1.9E-05</td>
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<tr>
<td>SE</td>
<td>0.003</td>
<td>3.8E-15</td>
<td>0.102</td>
<td>NA</td>
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<tr>
<td>SO</td>
<td>0.461</td>
<td>2.8E-05</td>
<td>0.006</td>
<td>0.009</td>
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<td>NA</td>
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<tr>
<td>SW</td>
<td>0.013</td>
<td>2.45E-16</td>
<td>3.11E-05</td>
<td>0.001</td>
<td>0.166</td>
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<tr>
<td>WNC</td>
<td>0.007</td>
<td>3.7E-09</td>
<td>0.974</td>
<td>0.069</td>
<td>0.011</td>
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### Male Pronotum (Fig. 3A)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>0.002</td>
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<tr>
<td>SO</td>
<td>0.253</td>
<td>0.092</td>
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### Female Pronotum (Fig. 3C)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>0.006</td>
<td>NA</td>
</tr>
<tr>
<td>SO</td>
<td>***0.049</td>
<td>0.449</td>
</tr>
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</table>

**Appendix Table 2:** P-values for PWRST post hoc contrasts of allopatric *G. firmus* populations by region. P-values marked with *** lost significance after correction.
Relative ovipositor (Fig. 3E)

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>7.5E-05</td>
<td>NA</td>
</tr>
<tr>
<td>SO</td>
<td>1.1E-11</td>
<td>8.7E-4</td>
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</table>

Ovipositor length (Appendix Fig. 2B)

<table>
<thead>
<tr>
<th></th>
<th>NE</th>
<th>SE</th>
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</thead>
<tbody>
<tr>
<td>SE</td>
<td>0.1</td>
<td>NA</td>
</tr>
<tr>
<td>SO</td>
<td>2.0E-16</td>
<td>2.0E-10</td>
</tr>
</tbody>
</table>

**Appendix Table 3**: P-values for PWRST post hoc contrasts of *G. thinos* populations and *G. firmus* populations in the Northeast, Florida, and Texas.

### Male *G. firmus/thinos* Femur by Region (Fig 4A)

<table>
<thead>
<tr>
<th></th>
<th>NE <em>G. firmus</em></th>
<th>FL <em>G. firmus</em></th>
<th>TX <em>G. firmus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>FL firmus</td>
<td>2.6E-06</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>TX firmus</td>
<td>0.809</td>
<td>2.2E-05</td>
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<tr>
<td>TX thinos</td>
<td>0.004</td>
<td>6.1E-06</td>
<td>0.009</td>
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### Female *G. firmus/thinos* Femur by Region (Fig 4B)

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<td>TX thinos</td>
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### Female *G. firmus/thinos* Ovi/Femur Ratio by Region (Fig 4C)

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<td>TX firmus</td>
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<td>3.24E-04</td>
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Appendix Figure 1: Relationship among phenotypic characteristics for allopatric populations. (a) Male Gryllus firmus (yellow) and G. pennsylvanicus (teal) linear models show positive relationships in femur length and pronotum width (G. firmus: $R^2 = 0.76, F_{1,117} = 363.1, p\text{-value} < 2.2e-16$; G. pennsylvanicus: $R^2 = 0.53, F_{1,233} = 265.0, p\text{-value} < 2.2e-16$). Female linear models show positive relationships in both (b) femur length and pronotum width (G. firmus: $R^2 = 0.74, F_{1,89} = 254.7, p\text{-value} < 2.2e-16$; G. pennsylvanicus: $R^2 = 0.53, F_{1,192} = 217.0, p\text{-value} < 2.2e-16$) and (c) ovipositor length and pronotum width (G. firmus: $R^2 = 0.26, F_{1,87} = 30.48, p\text{-value} = 3.44e-07$; G. pennsylvanicus: $R^2 = 0.44, F_{1,214} = 165.7, p\text{-value} < 2.2e-16$).
Appendix Figure 2: Ovipositor length differences between species and among populations of each species. A. Ovipositor length differences between *G. firmus*, *G. pennsylvanicus* and sympatric populations (*G. firmus* vs *G. pennsylvanicus* p < 2.0E-16; *G. firmus* vs sympatric p <2.0E-16; *G. pennsylvanicus* vs sympatric p <2.0E-16). B. Ovipositor length differences among populations of *G. pennsylvanicus*. Post hoc p-values are presented in Appendix Table 1. C. Ovipositor length differences among populations of *G. firmus* and *G. thinos*. Post hoc p-values are presented in Appendix Table 3. Boxplots indicate the mean values of each trait, quartiles, and the range of the data (whiskers). Individual data points are overlaid as scatterplots. Letters indicate the significant differences among groups (PWRST with corrected p-values < 0.05).
Appendix Figure 3: Scatterplots of significant AIC environmental variables. For all female allopatric populations: ovipositor length vs. latitude (a.), elevation (b.), average soil percent clay (c.), minimum temperature (d.), and maximum temperature (e.). For all female allopatric populations: pronotum width vs. latitude (f.), longitude (g.), average soil % sand (h.), and minimum temperature (i.). For female allopatric and sympatric
Connecticut populations: ovipositor length vs. minimum temperature (j.), maximum temperature (k.), and average soil % organic matter (l.).