Phylogenetic, geographic and ecological distribution of a green-brown polymorphisms in European Orthopterans*

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Abstract

1. The green-brown polymorphism among polyneopteran insects represents one of the most penetrant color polymorphism in any group of organisms. Yet systematic overviews are lacking. I here present analyses of the phylogenetic, geographic and habitat distribution of the green-brown polymorphisms across the complete European Orthopteran fauna.
2. Overall, 30% of European Orthopterans are green-brown polymorphic (36% when excluding crickets and cave crickets). Polymorphic species are scattered across the entire phylogenetic tree, including roughly equal proportions of Ensifera and Caelifera. Some taxonomic groups, however, include only brown species. Polymorphic species occur more frequently in clades that contain green species than in those without green species. The relative abundance of color morphs in polymorphic species is skewed towards green, and in particular rare/exceptional brown morphs are more common in predominantly green species than rare/exceptional green morphs in predominantly brown species.
3. Polymorphic species are particularly common in moist to mesic grasslands. Alpine and arboreal habitats also host high proportions of polymorphic species, while dry, open, rocky and cave habitats as well as nocturnal lifestyles are dominated by brown species. The proportion of polymorphic species increases from southern to northern latitudes.
4. The results show that the occurrence of the polymorphisms is phylogenetically, geographically and ecologically widespread. The patterns suggest that polymorphic populations might arise from green species by loss-of-function mutations, and the overall distributions is possibly even consistent with mutation-selection balance. This would imply a rather high rate of loss of the functional green pigmentation pathway. However, marked habitat-dependencies also show that coloration is affected by natural selection and/or environmental filtering.

Keywords: balancing selection, environmental filtering, intraspecific diversity, loss-of-function mutations, Orthoptera, phenotypic polymorphisms, Polyneoptera

Introduction

Color polymorphisms have fascinated biologists for a long time. Among the most significant findings, they have spurred the discovery of Medelian inheritance (Mendel 1866), have stimulated models on polygenic inheritance (Fisher 1930), shifting balance theory and isolation-by distance (Wright 1945), have demonstrated rapid adaptation to environmental change (Kettlewell 1958) and the discovery of a shared genetic basis of phenotypic variation across diverse taxa (Hoekstra 2006). Color polymorphisms represent a particularly marked case of intra-specific diversity and have raised the question of how such diversity is maintained. Color polymorphisms are very widespread across a large number of taxa, and each case may have special conditions that create and maintain the polymorphism (Majerus 1998). In some cases, color polymorphisms arise from admixture of divergent populations or shifts from one monomorphic state to another ("transient polymorphisms", Ford 1966). Such polymorphisms represent snapshots in time and may be transient, without the need to invoke any special mechanisms actively favoring the coexistence of discrete color variants. In other cases, color polymorphisms seem to be actively maintained by balancing selection ("balanced polymorphisms", Ford 1966).

Widespread color polymorphisms include melanism in animals (Majerus 1998) and corolla color polymorphisms in flowering plants (Rausher 2008). Melanism occurs across a wide range of species such as among birds, mammals, lizards, lepidopterans and ladybirds. However, the cases are rather spread out and not very penetrant within any large group of organisms. Among birds, for example, only about 3.5% of the species are polymorphic for melanistic variants with highest prevalence of about 33% among owls and nightjars (Galeotti *et al.* 2003; Roulin 2004). Similarly, only about 10% of British macro-lepidopterans show melanistic polymorphisms (Kettlewell 1956). Melanism in lepidopterans and ladybirds comes in many forms and many represent geographical clines rather than the local stable coexistence of discrete variants (Majerus 1998). Corolla polymorphisms, mostly

52 involving colored and white variants, have long been discussed (Darwin 1876), although I am not
53 aware of any systematic review that provides specific estimates on how widespread the
54 polymorphism is (but see Ackerman, Cuevas & Hof 2011). Here I focus on a green-brown color
55 polymorphism in Orthopterans that, I think, competes with melanism and corolla coloration for one
56 of most penetrant color polymorphisms in any groups of organisms.

57 The green-brown polymorphism of grasshoppers represents a long known, but underappreciated,
58 case of a widespread color polymorphism (Rowell 1972; Dearn 1990). The co-occurrence of green
59 and brown morphs within local populations of single species occurs in both major suborders of the
60 Orthoptera, Ensifera and Caelifera, that have separated about 200 Mya (Misof *et al.* 2014).
61 Moreover, the color polymorphisms is not limited to Orthopterans, but is shared more widely among
62 polyneopteran insects (e.g. phasmids, mantises and gladiators) that shared a common ancestor as
63 long as 250 Mya (Misof *et al.* 2014). Grasshoppers have been model systems for the study of
64 polymorphism, including phase polymorphisms (swarming and solitary morphs within the same
65 species, Pener & Yerushalmi 1998), pattern polymorphisms (Nabours 1929; Ahnesjö & Forsman
66 2003) and melanism (Forsman 2011; Peralta-Rincon, Escudero & Edelaar 2017). However,
67 surprisingly few studies have focused on the green-brown polymorphism. Rowell (1972) reports that
68 about 40% of east African Acridid grasshoppers are green-brown polymorphic. In the tropical region
69 the green-brown polymorphism appears more often in seasonal grasslands than in tropical forests
70 and wetlands (Rowell 1972). For temperate regions, it has been found that among British
71 Orthopterans green morphs are more abundant in moist habitats as compared to brown morphs
72 dominating in dry habitats (Gill 1981), illustrating some habitat-dependency of the green-brown
73 polymorphism.

74 Here I present an analysis of the phylogenetic, geographical and ecological occurrence of green-
75 brown polymorphism across the complete European fauna of Orthopterans. Besides documenting
76 the high prevalence of the green-brown polymorphism, the data show some habitat-dependency

and thus environmental filtering or habitat-dependent selection and thus suggest that the green versus brown color and likely the polymorphism itself are naturally selected. Furthermore, the data are consistent with the hypothesis that the brown morph ultimately arises from loss-of-function mutations from a functional green pigmentation pathway. The relative importance of selection versus genetic drift remains unsolved and might vary among species. However, the patterns are potentially consistent with mutation-selection balance if the frequency of loss-of-function mutations is high. The data show evidence that more widespread species are more often polymorphic, but there is no relationship between the green-brown polymorphism and species richness.

Materials and methods

Species compilation

I compiled data for all European Orthopteran species, excluding Cyprus (but including the Greek islands) and excluding the oceanic islands of Madeira, Canaries and Azores. Species were initially derived from the checklist of European species compiled by Heller *et al.* (1998). However, the list is rather incomplete in many regions (in particular for the Iberian peninsula) and species were added from the website Grasshoppers of Europe (Adriaens *et al.* 2019). The geographic scope of Heller *et al.* (1998) is a little wider in the East, such that a few species from the northern Caucasus are included in Heller *et al.* (1998), but not Adriaens *et al.* (2019), and are included here. In all cases, species status was checked with the Orthoptera Species File (Cigliano *et al.* 2019) and all doubtful species and synonyms were excluded. In total, I compiled an exhaustive list of 1086 species.

Taxonomy

Taxonomy follows the Orthoptera Species File (Cigliano *et al.* 2019), including decisions about species status, subgenus, genus, tribe, subfamily, family, superfamily and suborder. In a few cases,

some taxonomic levels were missing. For example, there is no official tribe name for the single genus in the subfamily Troglophilinae. A generic tribe was assigned in this case. In most cases, there was no subdivision into subgenera and in most of the remaining cases, all species of a genus were assigned to subgenera. In very few cases, a genus was split into subgenera, with a few species being unassigned (4 species in *Chorthippus*). These were treated as belonging to a separate (unnamed) subgenus.

Distribution

Distributional information was extracted from Heller *et al.* (1998), with the modification that I separated Greece from the rest of south-eastern Europe: This resulted in 13 distinct regions (1) British Isles and Island, (2) Norway and Sweden, (3) Finland, Estonia, Latvia and northern Russia (north of 58°N), (4) Netherlands, Belgium, Luxemburg and mainland France, (5) Central Europe, including Denmark, Germany, Poland, Czech Republic, Slovakia, western Ukraine and the northern parts of Switzerland and Austria, (6) Eastern Europe including Lithuania, Belarus and the central parts of European Russia (about 50-58°N), (7) Iberian Peninsula including Balearic Islands, (8) Italy, Corsica, Malta and the southern parts of Switzerland and Austria, (9) Balkan states and SE Europe, including Hungary, Romania, Bulgaria and the European part of Turkey, (10) Greece, (11) Eastern Ukraine and southern Russia south of about 50°N, but excluding the Caucasus region, (12) European parts of Kazakhstan and adjacent parts of Russia, (13) northern Caucasus region. The assignment to regions follows Heller *et al.* (1998) except for a few obvious corrections, such as in cases of taxonomic reassignments. Missing species (most of which rather localized) were assigned based on distributional information in the Orthoptera Species File (Cigliano *et al.* 2019). Species from Greece were assigned based on the recent Fauna of Greece (Willemse, Kleukers & Odé 2018). Since Heller *et al.* (1998) combined the entire south-east European region (including Greece), species endemic to Greece were treated as missing from the Balkan region.

Scoring of color morphs

Color morphs were scored based on images from trusted sources (see below) and from own field observations. Green morphs were identified based on areas of clear green color, while all other individuals were scored as brown (see Figure 1 for examples). Note that brown encompasses a range of non-green colors, including gray, black and reddish. Categorization was straightforward in most cases. In a few cases, however, categorization was more difficult. There are three main groups of these: (i) Some species of Pholidopterini show a yellowish underside that might sometimes appear as a greenish tinge. In such cases, only individuals with unambiguously green color were scored as green. (ii) In some species (such as some *Barbitistes* and *Poecilimon*) there is a range from clear green phenotypes with variable black markings to almost entirely black individuals. Only individuals in which there was no green areas left were scored as brown. (iii) In some species (such as species of *Arcyptera*) the body surface is very shiny and color sometimes difficult to judge. However, in most of these cases, it was possible to find unambiguously green and unambiguously brown individuals (hence most of these species are polymorphic). Aside from cases (i) and (ii) in which the green-brown polymorphism might be considered gradual and continuous, variation was almost exclusively discrete. Color morph classification was possible for 949 species (87%).

Polymorphism survey

Polymorphisms were scored from images (and in a few cases verbal descriptions) in field guides and faunas from Catalonia (Olmo Vidal 2006), France (Bellmann & Luquet 2009; Sardet, Roesti & Braud 2015), Switzerland (Baur *et al.* 2006), Austria (Zuna-Kratky *et al.* 2017), Germany (Fischer *et al.* 2016), Central Europe (Bellmann *et al.* 2019), Italy (Iorio *et al.* 2019), Greece (Willemse *et al.* 2018) and eastern Romania (Iorgu & Iorgu 2008). Furthermore, I searched the websites Grasshoppers of Europe (www.grasshoppersofeurope.de, Adriaens *et al.* 2019), Pyrgus (http://www.pyrgus.de,

146 Wagner 2019) and Orthoptera.ch (Roesti & Rutschmann 2019) for further images. These websites
147 are run by experts and provide reliable species identifications.

148 Finally, I searched images on flickr and google based on scientific species names. Flickr provides
149 search results only from the figure caption and these turned out to give very reliable species
150 identification, likely because only people with sufficient expertise would provide scientific species
151 names. Google searches provide fuzzier matching and usually showed some well-identified results
152 followed by a long series of other images. Critical photographs were carefully evaluated by visiting
153 the website, checks of source and location, as well as with my own taxonomic experience on
154 Orthopterans. Finally, I added my own field experience, which covers mostly species from central
155 and western Europe. It might seem useful to search museum collections for the green-brown
156 polymorphisms. However, the green colors are often lost during preservation (e.g. the green
157 pigments appear to be soluble in ethanol) and are subject to fading, such that I consider scoring
158 from museum collections unreliable.

159 Morph abundances were classified into seven categories: monomorphic (100%), dominant (>50%),
160 common (20-50%), regular (10-20%), rare (2-10%), exceptional (<2%), absent (0%). Being based
161 largely on image searches, these categorizations cannot be exact. However, the broad categorization
162 was relatively easy in most cases, with the following exceptions: (i) in species with two very common
163 morphs it is sometimes difficult to judge which one is more common (assigned dominant) and which
164 one is less common (assigned common). Misclassifications between these two categories are
165 therefore expected. (ii) For species with very rare occurrence of one of the morphs and only a few
166 images available, it might well be that exceptional or rare morphs have been overlooked. These
167 were nevertheless categorized as absent, such that the number of polymorphic species is likely
168 underestimated. (iii) The categorization of rare vs. exceptional might seem difficult, but turned out
169 to be relatively easy. The category 'exceptional' was used only when long series of images resulted in
170 only single images of the rare morph. This applied to species like *Phaneroptera falcata* (which is

typically bright green) or *Oedipoda caerulescens* (which is typically brown, grey or black). I consider it more likely that species that show exceptional phenotypes are classified as absent rather than being misassigned as rare. It is possible, that in a few cases, morphs categorized as rare might in fact be rather exceptional.

Further categorizations

Marked age-related polymorphisms were recorded when late nymphal stages showed strikingly different green/brown morphs from adult individuals. Similarly, marked sexual dimorphism was recorded when males and females showed strikingly different color morphs. No attempt was made to identify all cases in which females and males showed different ratios of color morph. I also recorded the occurrence of pied morphs (sensu Dieker *et al.* 2018). Pied morphs are characterized by a pale transverse stripe across the lateral sides of head and pronotum. This can be very marked in some individuals and blurred in others. Records of pied morphs are probably incomplete and give only a cursory overview of where such morphs occur.

Habitat categorization

Habitat preferences were compiled from descriptions in the field guides and faunas mentioned above. I defined the following broad categories: (1) dunes and beaches, (2) pond and river margins (including temporary flooded areas) (3) marshes and reed beds, (4) moist grasslands (including a few tundra species), (5) mesic grasslands, (6) dry grasslands, (7) grasslands with abundant tall herbs, (8) rural areas with herbs, shrubs and open ground, (9) open ground, (10) shrubs and heath, (11) mesic bushland, (12) dry bushland, (13) forest margins, (14) woodlands (including clearings), (15) subalpine and alpine shrubs, (16) subalpine and alpine grasslands, (17) alpine rocky habitats, (18) lowland rocky habitats, (19) stony walls, (20) domestic habitats, and (21) caves. I aimed to assign all species to habitats in which they occur regularly and which can thus be considered typical for a species. Habitat information could be compiled for 670 species (62%).

Habitat use

I categorized relevant aspects of habitat use in five categories: (1) *Subterranean* for species living underground, under logs or stones, or in caves, (2) *ground-dwelling* for species typically locating themselves on the ground, (3) *mixed* for species that partly perch on the ground, partly in the vegetation, and (4) *vegetation-dwelling* for species predominately locating themselves and moving in dense herbal vegetation, low shrubs, or grasses, (5) *bushes and trees* for species typically living in higher vegetation layers. Habitat information could be compiled for 680 species (63%).

Furthermore, I classified the activity pattern into three categories: (1) nocturnal for all species almost exclusively active at night, (2) active during day and night, in particular including species that display at night, but are also active during the day and thus exposed to diurnal predators, (3) diurnal species predominately active during the day. Diurnality categories were mutually exclusive and could be compiled for 673 species (62%). Finally, I categorized seasonal activity of imagoes into (1) spring (April-June), (2) summer (late June-November), and (3) winter (species that overwinter as imagoes). Species active in multiple seasons were assigned to multiple categories. Seasonality information could be compiled for 688 species (63%).

Statistical analyses

Most of the analysis are descriptive summaries of the entire population of European Orthopterans. Being not based on randomized experiments, it is not very useful to apply statistical hypothesis testing, in particular because most of the broad trends are very obvious. There are just three contexts, in which hypothesis testing is useful: (i) I use regression analysis for testing the relationship between the proportion of polymorphic species with species richness, range size and habitat diversity, (ii) I use χ^2 tests the test whether the occurrence of rare morphs is more common in predominantly green or predominately brown populations, (iii) I use χ^2 tests for whether polymorphic species occur more frequently in clades with otherwise only monomorphic green,

otherwise only monomorphic brown or both types of monomorphic species. All data processing and statistical analysis were done in R 2.6.2 (R Core Team 2020).

Results

In total, 949 species (87%) could be reliably assigned a color morph classification. Among classified species, 51% are monomorphic brown, 19% monomorphic green, and 30% polymorphic. Excluding the 165 almost exclusively brown crickets (Grylloidea and Gryllotalpoidea, with on polymorphic exception) and cave crickets (Rhaphidophoroidea, with one polymorphic exception), left 42% of monomorphic brown species, 22% monomorphic green species, and 36% polymorphic species.

Phylogenetic distribution

Both suborders, Ensifera and Caelifera, contain many polymorphic species (29% in Ensifera, 33% in Caelifera, Figure 2). Besides the (almost) exclusively brown crickets (Grylloidea), mole crickets (Gryllotalpoidea), cave crickets (Rhaphidophoroidea), and pygmy mole crickets (Tridactyloidea), all superfamilies include polymorphic species (Figure 2). All subfamilies of the single family Tettigoniidae of Tettigonioidea have polymorphic species (Figure 2) as well as all families of Acridoidea (Figure 2). Only at the level of subfamilies there is a divide with a few smaller subfamilies (besides crickets) being exclusively brown (Calliptaminae [9 species], Pezotettiginae [3 species], Thrinchinae [11 species], Dericorythinae [2 species], Dentridactylinae [1 species]).

Polymorphic species occur more frequently in clades that comprise monomorphic green species than in those with otherwise only monomorphic brown species, a pattern that held at the level of genera and tribes (Figure 3). Clades that contain both, monomorphic brown as well as monomorphic green species, almost exclusively also contain species that are green-brown polymorphic (Figure 3).

This pattern was significant when analyzed at the level of genera (χ^2_2 = 17.5, P = 0.0001) and at the level of tribes (χ^2_2 = 9.6, P = 0.0081).

Relative abundances of color morphs in polymorphic species

Among color-polymorphic species, the rarer morph is common in 32%, regular in 36%, rare in 25%, and exceptional in 6% of the cases (Figure 4). Hence, in the vast majority of cases, the rarer morph seems to be more common than expected if it was based on novel mutations or developmental accidents. The relative abundance of the two morphs varies among color-polymorphic species and seems to be skewed towards cases where green is common and brown is rare (Figure 4). Particularly instructive are those cases in which the rare morph occurs at low frequencies. In 56 out of 73 cases where one of the morphs is rare this is the brown morph (χ^2_1 = 20.8, P < 10^{-5}). In 10 out of 8 cases where one morph occurs exceptionally, this is the brown morph in otherwise green populations (χ^2_1 = 0.22, P = 0.64). While the ratio 10:8 is almost equal, the ratio of purely brown to purely green species (thus the pool of species in which exceptional morphs might arise) is highly skewed towards brown (ratio 2.8:1), suggesting that there is larger flux from green populations to the polymorphic state than from brown to polymorphic.

Geographic distribution of color polymorphisms

The occurrence of brown, green, and polymorphic species follows a geographic pattern. While north and central Europe host about 80% polymorphic species, polymorphic species compass only 40% of the species in southern Europe (Figure 5). The geographic distribution is not simply caused by the dominance of particular genera, since numbers are similar when looking at the proportion of genera and tribes that include polymorphic species. Both monomorphic green and, in particular, brown species are proportionally more abundant in southern Europe.

Distribution of color polymorphisms across habitats

Highest proportions of polymorphic species (about 60%) occur in marshes and moist grasslands (Figure 6). Within grasslands, the proportion of polymorphic species declines from moist to dry grasslands, while the proportion of brown morphs increases. Alpine grass- and shrubland also host relatively high proportions of polymorphic species (45%, Figure 6). Bushlands, wood margins and woodlands host a high proportion of green species and intermediate numbers of polymorphic species. Open and rocky habitats are dominated by brown morphs with relatively low numbers of polymorphic and hardly any monomorphic green species. Patterns are similar when looking at higher levels of taxonomic aggregation.

Color polymorphisms and habitat use

Highest proportions of polymorphic species occur among species that dwell in bushes and trees or in higher vegetation (Figure 7). The proportion of polymorphic species is also high among those species that live in lower vegetation and that regularly visit open ground. Quite to the contrary, predominately ground-dwelling species are usually monomorphic brown with low proportions of polymorphic species. Subterranean species are almost exclusively monomorphic brown. The proportion of polymorphic species is highest among diurnal species, while species that are active day and night and even more species that are exclusively nocturnal show lower proportions of polymorphic species (Figure 7). Polymorphic species occur mostly during spring and summer, while species that overwinter are predominantly brown (Figure 7). Patterns are similar when aggregated at higher taxonomic levels.

Sexual dimorphism and age dependencies

There are a few instances of remarkable age-dependencies. Individuals of the genus *Phaneroptera* are typically bright green as imagoes, but are polymorphic as nymphae, whereas individuals of the

genera *Pezotettix* and *Anacridium* are brown as imagoes, but polymorphic as nymphae. There are only a few cases in which the polymorphism is (almost) completely linked to sex. Males of the genus *Psorodonotus* are usually brown, while females are green, whereas males of the genus *Chrysochraon* are typically bright green, while females are brown. No attempt was made to estimate sex-specific abundances in cases where polymorphisms are present in both sexes.

Species diversity, range sizes, and habitat diversity

There is no relationship between the proportion of polymorphic species and species richness within genera (Figure 8a,b) or at other taxonomic levels. However, there is a marked increase in the proportion of polymorphic species with increasing geographic range size (quantified as the number of geographic areas in which a species occurred) (Figure 8c). Similarly, there is a clear trend for increased polymorphisms in species that occur in multiple habitat categories and can thus be considered more ecologically widespread (Figure 8d).

Discussion

The data illustrate the high prevalence of the green-brown polymorphism in the European orthopteran fauna. This polymorphism is widespread geographically, phylogenetically, and across most habitats. The ratio of monomorphic brown, polymorphic green-brown, and monomorphic green species was about 5:3:2. The bias towards brown species and the dominance of polymorphic over green species might suggest that brown variants tend to be selected for in most habitats. However, the asymmetry is also consistent with a biased loss of green pigmentation. The data also show evidence that within clades, it is more likely to find polymorphic species when there are also green species than in clades that contain only brown species. This is consistent with the hypothesis, that the ability to produce the green pigment is limiting and more easily lost than gained.

Color polymorphisms have been implicated in favoring speciation (West-Eberhard 1986; Gray & McKinnon 2007). The European Orthoptera data does not show an association of taxonomic diversity with intraspecific polymorphisms. This might be a matter of phylogenetic resolution and the lack of a well-resolved and dated phylogeny. However, the current evidence is in no way suggestive of increased speciation in polymorphic orthopterans. Interestingly, polymorphic species tend to be more widespread, both geographically and also ecologically in terms of a broader range of habitats occupied. Causation in these cases might go either way, with either polymorphism favoring successful colonization or heterogeneous selection in widespread species favoring polymorphisms.

A role of biased mutation

Several lines of evidence suggest that the green-brown polymorphism is sourced from loss-of-function mutations of a functional green pigmentation pathway. Frist, the frequency distribution of color morphs within species is skewed towards green. In particular, cases of rare and exceptional morphs are more common in predominantly green populations. Examples of this are represented by species of the genera *Tettigonia*, *Phaneroptera* and *Conocephalus*, that are typically bright green, but show pale yellowish individuals at low frequencies. Second, polymorphic species are relatively more common in groups that contain green species than in clades that contain only brown species. Also, polymorphic species are almost universally present in groups that contain green as well as brown species. Third, breeding data suggest a dominant inheritance of the green variant at least in Gomphocerine grasshoppers (Köhler & Renker 2006; Schielzeth & Dieker submitted). Some species are phenotypically plastic and can facultatively switch between the presence and absence of green colors (see discussion below). However, while this adds another level of complexity, it does not rule out that loss-of-function mutations play an important role. In particular, it is not clear if all individuals of a plastic species are able to switch color. It is possible that phenotypically plastic species consist of a mix of families that are capable of producing green pigments, while others are

limited to the expression of brown color. Only the former are expected to produce color-switching individuals. An obvious, yet untested, prediction of the biased loss-of-function hypothesis is that in polymorphic species there are more non-plastic families that are brown than non-plastic families that are green.

Based on these overall patterns, I suggest that a biased loss of green pigmentation with simultaneous selection for green variants in vegetated habitats creates polymorphic states in mutation-selection balance. Loss-of-function mutations play an important role in evolution (Behe 2010) and have been shown to contribute to the corolla color polymorphism in flowering plants (Whittall *et al.* 2006). This seems to be different from melanism in animals, where different non-synonymous substitutions in a few major genes seem to produce melanistic morphs (Majerus & Mundy 2003; Hoekstra 2006; Manceau *et al.* 2010). Molecular opportunities for loss-of-function mutations likely manifold, thus can arise independently and repeatedly in different groups. The genetic basis of the color polymorphism is thus predicted to be different across different groups of Orthopterans.

The pigment that produces the green color in orthopterans is allegedly biliverdin (Fuzeau-Braesch 1972) and in for *Locusta migratoria* it has recently been shown that β-carotene plays in role in color transitions between green and black (Yang *et al.* 2019). However, it is not known if this applies to all species and biochemical analysis would help elucidating if the pigments are similar across all species. In most groups, the difference between green and brown morphs is in the presence or absence of green pigments. However, in some groups, the pigmentational basis might be different. In some species of *Barbitistes* or *Poecilimon*, for example, green colors appear to be covered by black coloration to a variable degree and it is not clear if green pigments are truly absent in very dark individuals. It is only those species in which green-brown polymorphism might be considered continuous, while in the vast majority of species it is clearly discrete.

Possible selective agents

The geographic distribution and the distribution of the polymorphism across habitats suggests a role for environmental filtering or natural selection in shaping morph ratios. Green morphs (both in a monomorphic state and in the form of polymorphisms) are strongly underrepresented in dry, bare and underground habitats. For open ground species in dry habitats this is likely related to crypsis, since green morphs would be more conspicuous in open, largely non-green habitats. For cave-dwelling, subterraneous and nocturnal species it might not be selection itself that is favoring brown variants, but green pigmentation might simply be lost because it is no longer selected for. Clades that have lost green pigmentation might even find themselves in an evolutionary trap in which a preference for a hidden lifestyle is favored because of loss of crypsis in most vegetated areas.

One of the main candidate mechanisms that favors green variants is likely crypsis in vegetated habitats. Most Orthopterans feed on leaves of grasses, herbs or shrubs (Ingrisch & Köhler 1998) and are exposed to predation from visual predators, such as birds, lizards and predatory (or parasitic) insects (Ramme 1951; Ingrisch & Köhler 1998). Visual predators thus represent potential selective agents, and selection for crypsis on vegetated patches is likely to be a promotor of the green morphs. This will favor the persistence of green pigmentation even if the green pigment is frequently lost by biased mutation.

The widespread distribution of polymorphisms calls for a rather general explanation, likely some in the form of balancing selection. However, the selective agents that might maintain coloration in a polymorphic state within populations are less easy to identify. It seems unlikely that recent admixture creates transient polymorphic states or that a large number of species is in transition from one state to another. There are only few cases of geographic variation in morph ratios that I am aware of. In some species, brown morphs increase in frequency with altitude (Köhler, Samietz & Schielzeth 2017), an case of apparent thermal melanism (Clusella Trullas, van Wyk & Spotila 2007)

380 and thus likely caused by contemporary selection rather than admixture. In other cases, such as in
381 *Chorthippus biguttulus* or *Decticus verrucivorus*, green morphs increase with latitude. This is not a
382 case of thermal melanism, but possibly related to other agents of natural selection or colonization
383 history. I am not aware of any cases in which the geographic distribution would suggest a role of
384 admixture of different populations such as from different glacial refugia.

385 In some species, there are geographic clines in morph ratios (Köhler *et al.* 2017) and these might
386 create polymorphic states in migration-selection balance. Color mediated thermoregulation with
387 thermoregulatory benefits in brown morphs (Köhler & Schielzeth 2020) might favor brown variants
388 in some patches and, if this trades-off with crypsis, green variants in others. While this process might
389 be relevant in some species, it is doubtful how this can serve as a general explanation across
390 hundreds of polymorphic species. A plausible candidate that could impose negative-frequency
391 dependent selection is predation by predators that develop search images for the most abundant
392 prey (Dukas & Kamil 2001; Bond 2007). However, there are no known species-specific predators,
393 such that search image developments in predators (such as birds or lizards) are likely to act on the
394 community levels of Orthopterans. However, no community-level predation study has yet been
395 conducted.

396 The association of green and polymorphic species with habitat and geographic range clearly shows
397 that color is related to environment, suggesting that color may be under environment-specific
398 selection. Yet, aside from habitats such as caves, bare ground and rocks, the distribution of the
399 frequency of color polymorphisms is also consistent with mutation-selection balance. It is thus
400 possible that once polymorphisms arise in green populations, morph ratios follow a random walk,
401 drifting between mutation pressure producing brown and selection favoring green morphs.
402 However, if this was the case, a puzzling question is why the green-brown polymorphism is
403 apparently so prone to loss-of-function mutations, since other functionally important genes are
404 typically under strong purifying selection.

Inheritance versus plasticity

Another important question is whether the color polymorphisms has a genetic basis. We have recently shown that in the Gomphocerine grasshopper *Gomphocerus sibiricus*, the pattern of inheritance seems to be genetically simple with a dominant green allele (Schielzeth & Dieker submitted). Other studies in Gomphocerine grasshoppers also suggest a highly heritable basis (*Pseudochorthippus parallelus*, Köhler & Renker 2006, *Chorthippus dorsatus*, own unpublished data). Outside the Gomphocerinae, there are a number of cases, in which the green-brown polymorphisms is phenotypically plastic such as in *Schistocerca* (Cyrtacanthacridinae), *Acrida* (Acridinae), and *Oedaleus* (Oedipodinae) (Hertz & Imms 1937; Ergene 1950; Ergene 1955; Rowell & Cannis 1971; Tanaka 2004; Tanaka, Harano & Nishide 2012) as well as a central American species of Gomphocerinae (*Rhammatocerus*, Lecoq & Pierozzi 1996). I am not aware of any study on the inheritance of the green-brown polymorphism in Ensifera, but own observations show that the subtropical *Mecopoda elongata* (Mecopodinae) is able to switch between green and brown states during ontogeny. Most ontogenetic changes seem to be associated with molt (own observations on *Acrida ungarica* and *Mecopoda elongata*), though the 'new' color seems to take some days to become fully developed, suggesting that pigments are still deposited in the epidermis after molt.

Other variants

While my main analysis is focused on the discrete green-brown polymorphism, grasshoppers are also remarkable for other color variation. Several open-habitat species vary between pale-grey, almost blackish, reddish and yellowish (Ergene 1952; Edelaar *et al.* 2017; Peralta-Rincon *et al.* 2017; Baños-Villalba, Quevedo & Edelaar 2018). Bright purple or pink variants occur regularly in a number of species (Rowell 1972), but are never dominant in any species of the European fauna. Almost all species are variable in darkness. Most of this variation is probably based on variable amounts and ratios of different melanins (Fuzeau-Braesch 1972), but the variation appears continuous in most

429 cases. However, there are pattern polymorphism in a number of species, such as among Tettrigidae,
430 in which these polymorphisms have a genetic basis (Fisher 1930; Ahnesjö & Forsman 2003; Karlsson
431 *et al.* 2009). Lesser known pattern polymorphisms include pied variants (Dieker *et al.* 2018) among
432 many species of Gomphocerinae (about 41% in my survey) and Oedipodinae (32%) and in one
433 species of Pamphaginae (3%). Pied morphs seem to be absent from Ensifera. Many species, in
434 particular of Gomphocerinae, occur in a dorsal striped variant that also affects the distribution of
435 green coloration (Richards & Waloff 1954; Dearn 1990; Köhler 2006; Köhler *et al.* 2017). However,
436 striped morphs come in very different variants and it is difficult to quantify the homologous striped
437 variants.

438 Conclusions

439 Overall, the results show the phylogenetically, geographically and ecologically widespread
440 occurrence of the green-brown polymorphism in European orthopterans. With a representation in
441 30% out of more than 1,000 species of the European fauna, this represents one of the most
442 penetrant phenotypic polymorphs in any group of organisms. Ecological and geographic patterns
443 suggest an influence of selection and/or environmental filtering on the occurrence of polymorphism.
444 Interestingly, however, the patterns also suggest that biased loss-of-function mutations contributed
445 to the green-brown polymorphism and that polymorphic states are primarily sourced from green
446 rather than brown states.

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Data accessibility

Data will be made freely available upon publication of the manuscript. I intend to provide version-controlled updates of the data as more information becomes available (current version 1.0). R code for the analysis is provided on Github (https://github.com/hschielzeth/OrthopteraPolymorphism) and can be used to visualize new versions of the data in the future.

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633

634

Figure Legends

Figure 1: Examples of polymorphic Orthopteran species from different subfamilies. The panel shows a mix of sexes, but morph identity is not sex-limited in any of the cases shown. The cases of *Tettigonia cantans* and *Bicoloriana bicolors* illustrate cases where faded brown variants occur at low frequency in green populations. *Metrioptera brachyptera* shows a case where green is limited to the dorsal side. *Tetrix ceperoi* shows a case where green morphs occur at relatively low frequency in brown populations. *Arcyptera microptera* illustrates a case where the body surfaces is shiny and colors sometimes more difficult to judge. The species *Mecopoda elongata* does not occur in Europe and is added as another example for the green-brown polymorphism in Ensifera.

Figure 2: Phylogenetic distribution of monomorphic brown (brown), monomorphic green (green) and polymorphic species (purple). Pie charts at each node represent the proportion of species within each clade that represent one of the states. Numbers (a/b) behind tribe names show (a) the number of species with morph information and (b) the total number of species per tribe. White pie charts represent clades with missing data for all species.

Figure 3: Representation of polymorphic species in clades of species that are otherwise either entirely green, entirely brown, or with a mix of brown and green species. Filled bars show that number of clades with more than one species, while shaded bar sections include the number of clades with a single species.

Figure 4: Relative abundance of green and brown morphs across polymorphic species. Truncated bars on the sides represent monomorphic species. Note that the ratio of monomorphic brown to monomorphic green species is about 2.7:1.

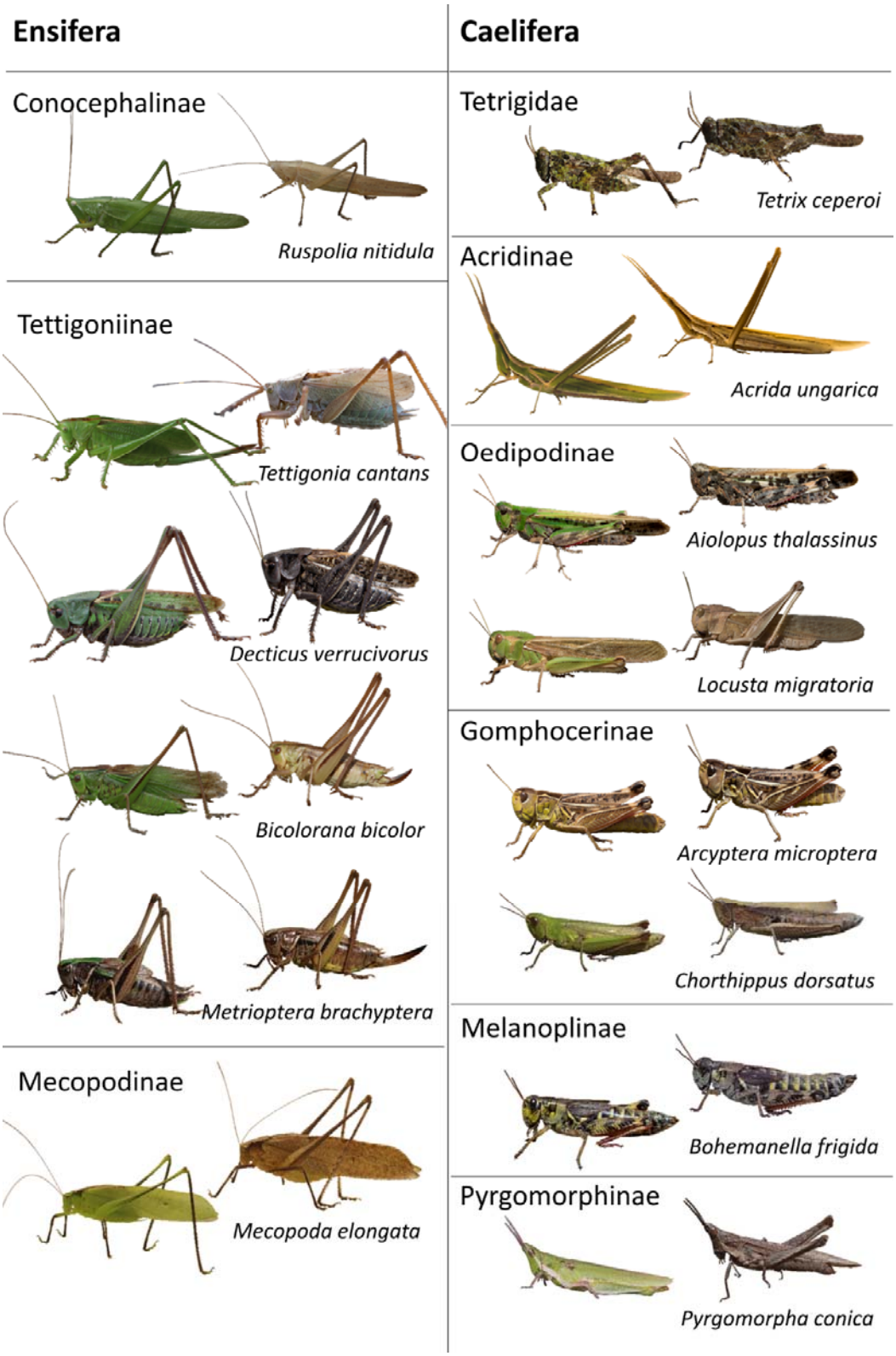
Figure 5: Geographic distribution of monomorphic brown (brown), monomorphic green (green) and polymorphic species (purple). The plot shows the proportion of species of each type in each region.

Figure 6: Habitat distribution of monomorphic brown (brown), monomorphic green (green) and polymorphic species (purple). The plot shows the proportion of species of each type in each habitat.

Figure 7: Habitat use, diurnality and seasonal activity of monomorphic brown (brown), monomorphic green (green) and polymorphic species (purple). The plot shows the proportion of species of each type in each category.

Figure 8: Correlations of species diversity, geographic range size and range of occupied habitats with intra-specific color morph diversity. (a, b) Number of species per genus and proportion of polymorphic species within (sub)genera. Each dot represents one (sub)genus. Points are partly overlaid, slightly jittered along both axes and darker dots show the overlay of multiple genera. Regression lines refer to increasing filtering from (dark to light) all species, minimum 2, 4, and 8 species. None of the regression slopes is significantly different from zero ($P > 0.20$). (c) Proportion of monomorphic brown (brown), monomorphic green (green) and polymorphic species (purple) in relation to the number of geographic regions inhabited by a species. The regression line for the proportion of polymorphic is statistically different from zero ($P < 10^{-5}$). (d) Proportion of monomorphic brown (brown), monomorphic green (green) and polymorphic species (purple) in relation to the number of habitat categories inhabited by a species. The regression line for the proportion of polymorphic is not statistically different from zero ($P = 0.14$).

676 Figure 1



677

Figure 2

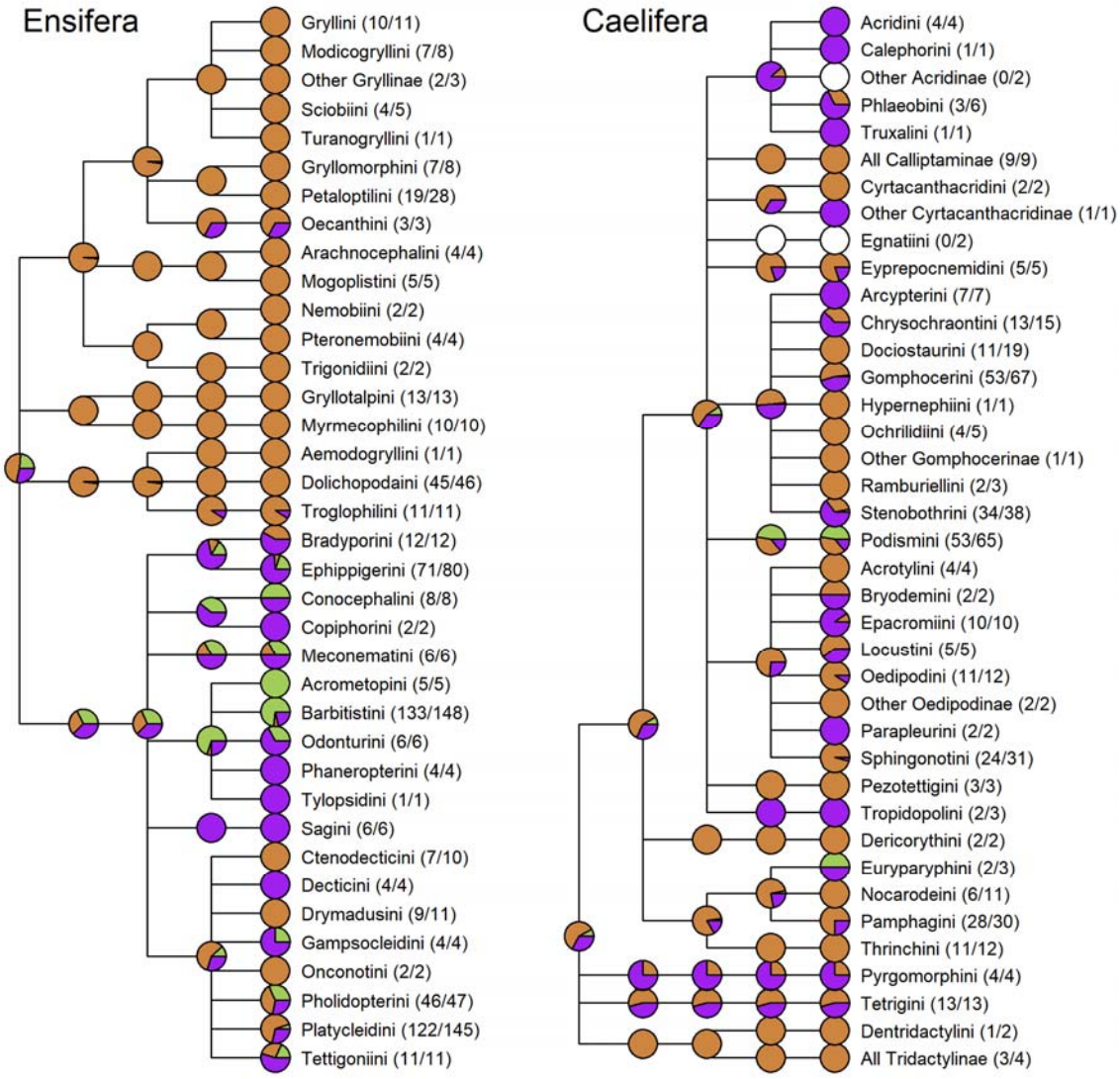

Ensifera
Gryllini (10/11)
Modicogryllini (7/8)
Other Gryllinae (2/3)
Sciobiini (4/5)
Turanogryllini (1/1)
Gryllomorphini (7/8)
Petaloptilini (19/28)
Oecanthini (3/3)
Arachnocephalini (4/4)
Mogoplistini (5/5)
Nemobiini (2/2)
Pteronemobiini (4/4)
Trigonidiini (2/2)
Gryllotalpini (13/13)
Myrmecophilini (10/10)
Aemodogryllini (1/1)
Dolichopodaini (45/46)
Troglophilini (11/11)
Bradyporini (12/12)
Ephippigerini (71/80)
Conocephalini (8/8)
Copiphorini (2/2)
Meconematini (6/6)
Acrometopini (5/5)
Barbitistini (133/148)
Odonturini (6/6)
Phaneropterini (4/4)
Tylopsidini (1/1)
Sagini (6/6)
Ctenodecticini (7/10)
Decticini (4/4)
Drymadusini (9/11)
Gampsocleidini (4/4)
Onconotini (2/2)
Pholidopterini (46/47)
Platycleidini (122/145)
Tettigoniini (11/11)
Caelifera
Acridini (4/4)
Calephorini (1/1)
Other Acridinae (0/2)
Phlaeobini (3/6)
Truxalini (1/1)
All Calliptaminae (9/9)
Cyrtacanthacridini (2/2)
Other Cyrtacanthacridinae (1/1)
Egnatiini (0/2)
Eyprepocnemidini (5/5)
Arcypterini (7/7)
Chrysochraontini (13/15)
Dociostaurini (11/19)
Gomphocerini (53/67)
Hypernephiini (1/1)
Ochrilidiini (4/5)
Other Gomphocerinae (1/1)
Ramburiellini (2/3)
Stenobothrini (34/38)
Podismini (53/65)
Acrotylini (4/4)
Bryodemini (2/2)
Epacromiini (10/10)
Locustini (5/5)
Oedipodini (11/12)
Other Oedipodinae (2/2)
Parapleurini (2/2)
Sphingonotini (24/31)
Pezotettigini (3/3)
Tropidopolini (2/3)
Dericorythini (2/2)
Euryparyphini (2/3)
Nocarodeini (6/11)
Pamphagini (28/30)
Thrinchini (11/12)
Pyrgomorphini (4/4)
Tetrigini (13/13)
Dentridactylini (1/2)
All Tridactylinae (3/4)

Figure 3

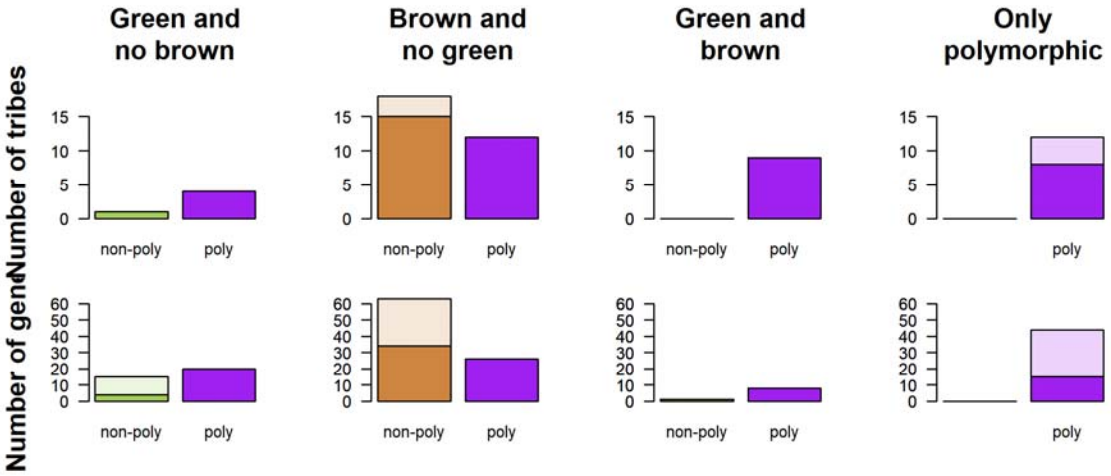
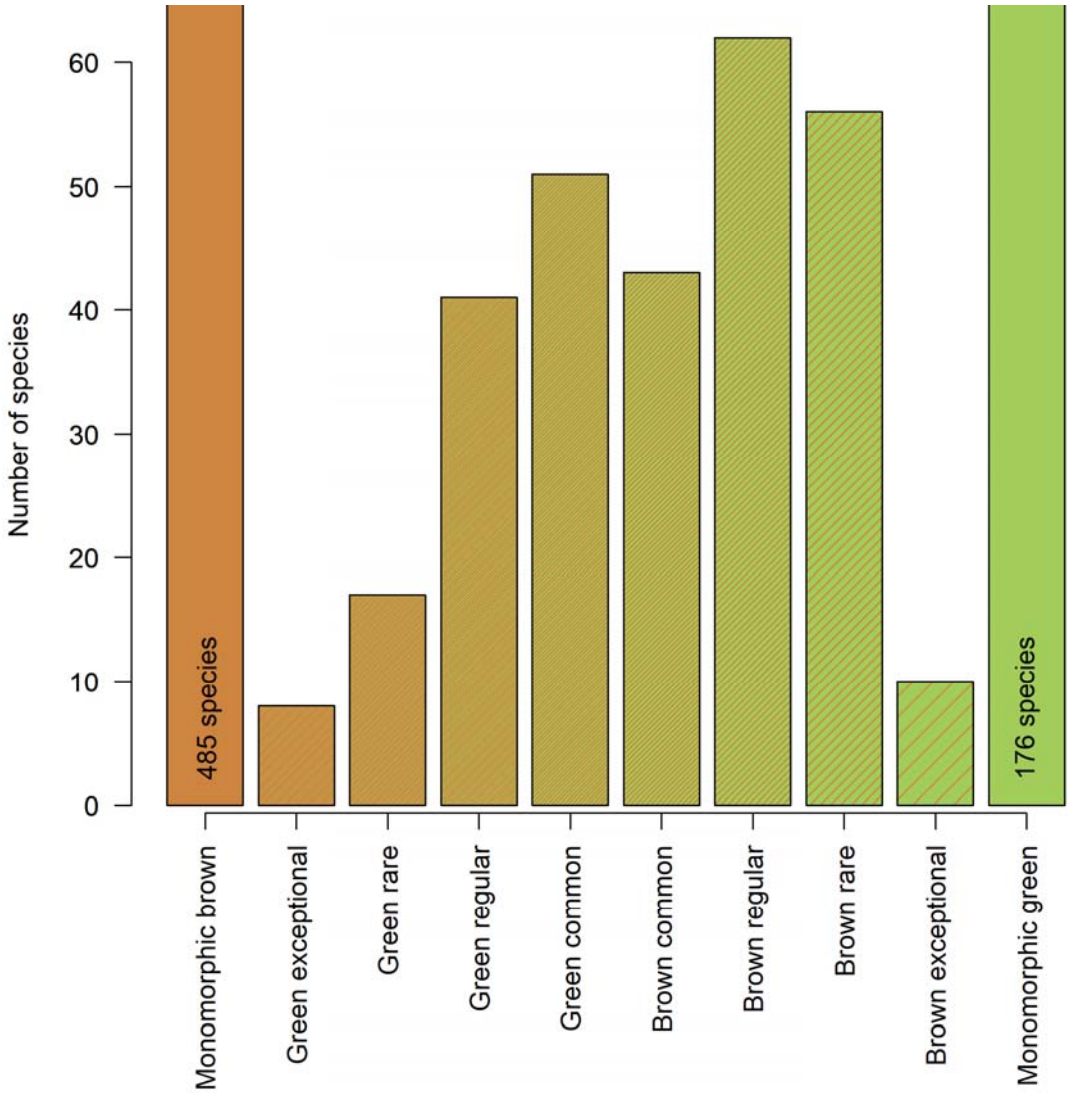
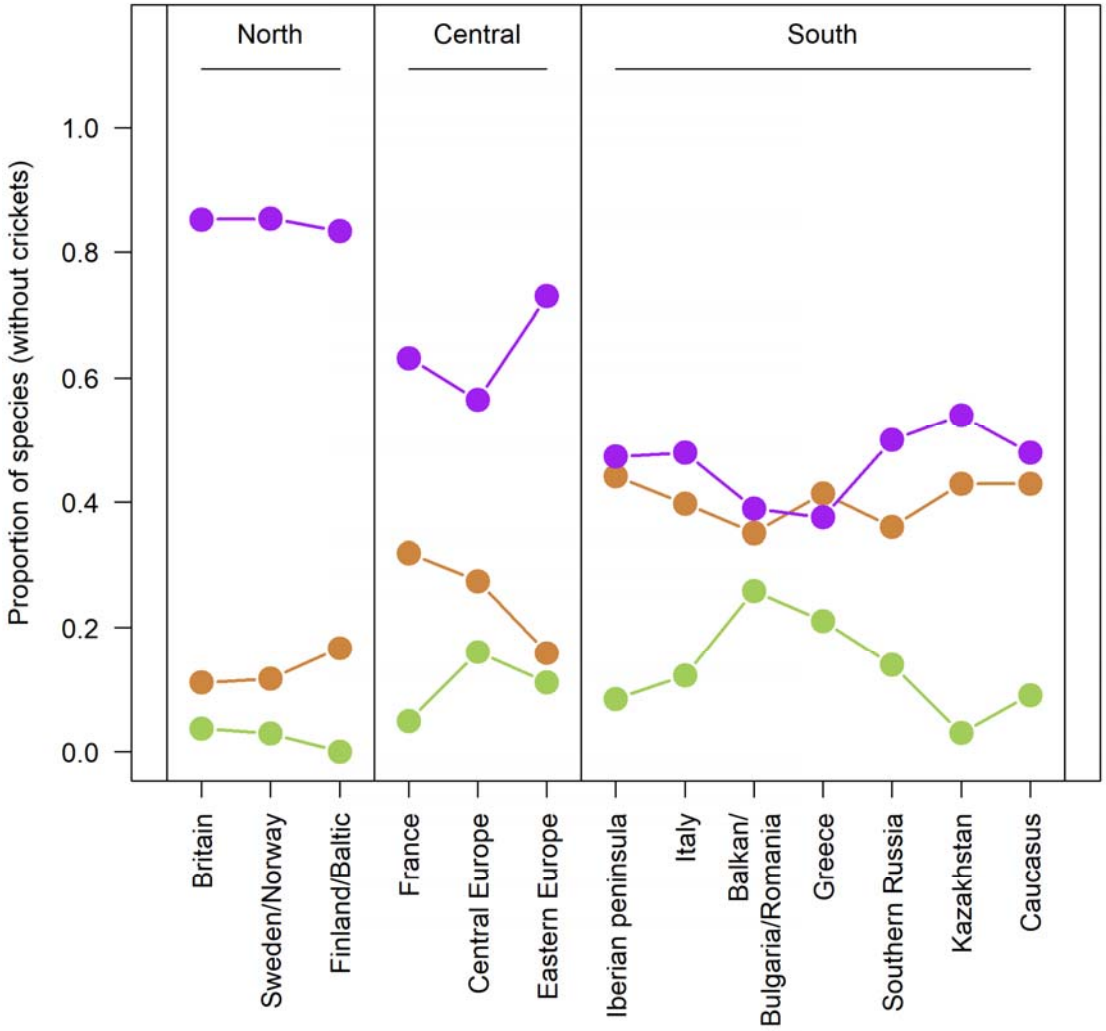

Green and no brown
Brown and no green
Green and brown
Only polymorphic
Number of tribes
Number of gen
non-poly
poly

Figure 4

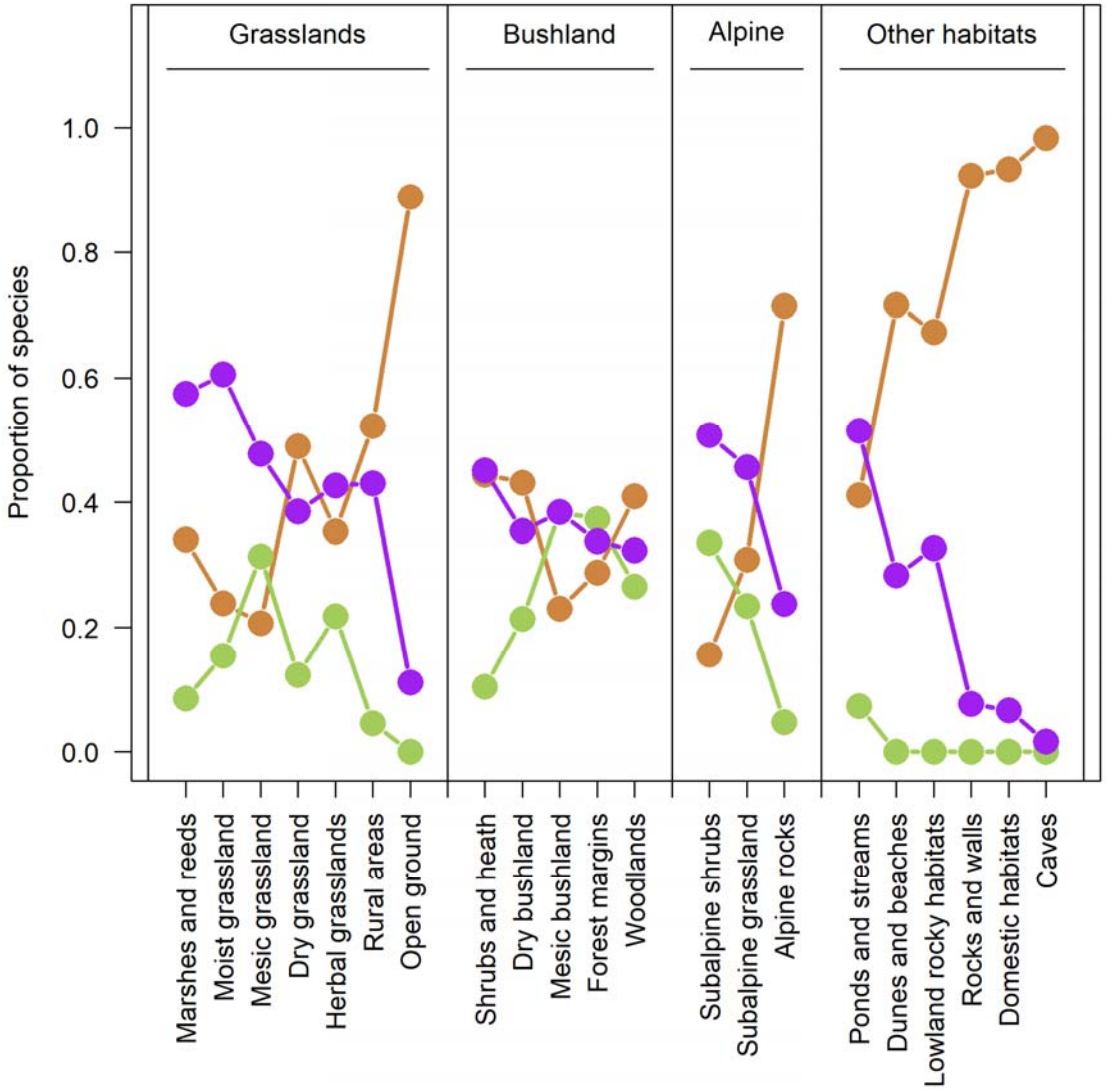

Number of species
0
10
20
30
40
50
60
485 species
176 species
Monomorphic brown
Green exceptional
Green rare
Green regular
Green common
Brown common
Brown regular
Brown rare
Brown exceptional
Monomorphic green

688 Figure 5

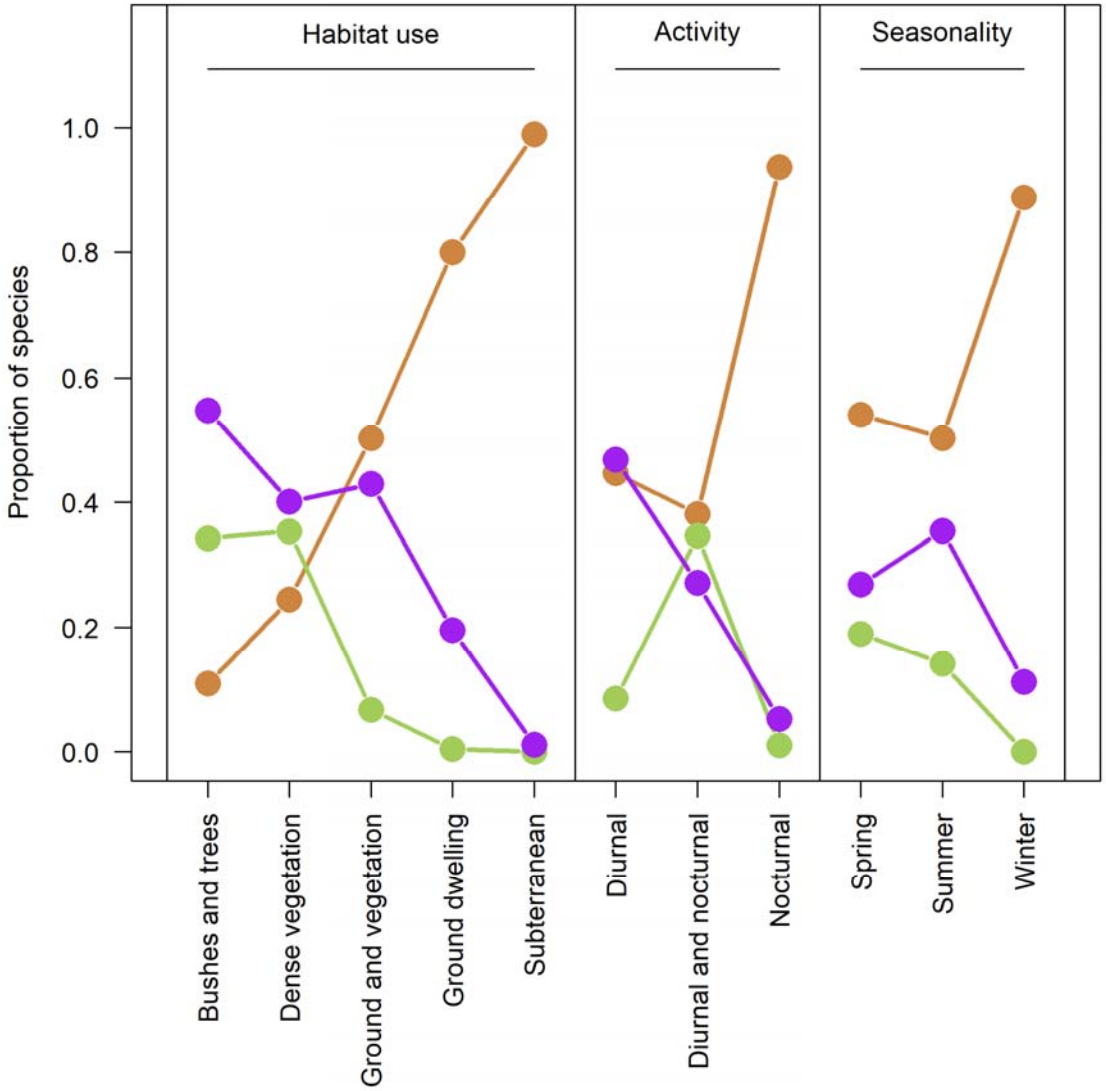


689

Figure 6


Grasslands
Bushland
Alpine
Other habitats
Proportion of species
1.0
0.8
0.6
0.4
0.2
0.0
Marshes and reeds
Moist grassland
Mesic grassland
Dry grassland
Herbal grasslands
Rural areas
Open ground
Shrubs and heath
Dry bushland
Mesic bushland
Forest margins
Woodlands
Subalpine shrubs
Subalpine grassland
Alpine rocks
Ponds and streams
Dunes and beaches
Lowland rocky habitats
Rocks and walls
Domestic habitats
Caves

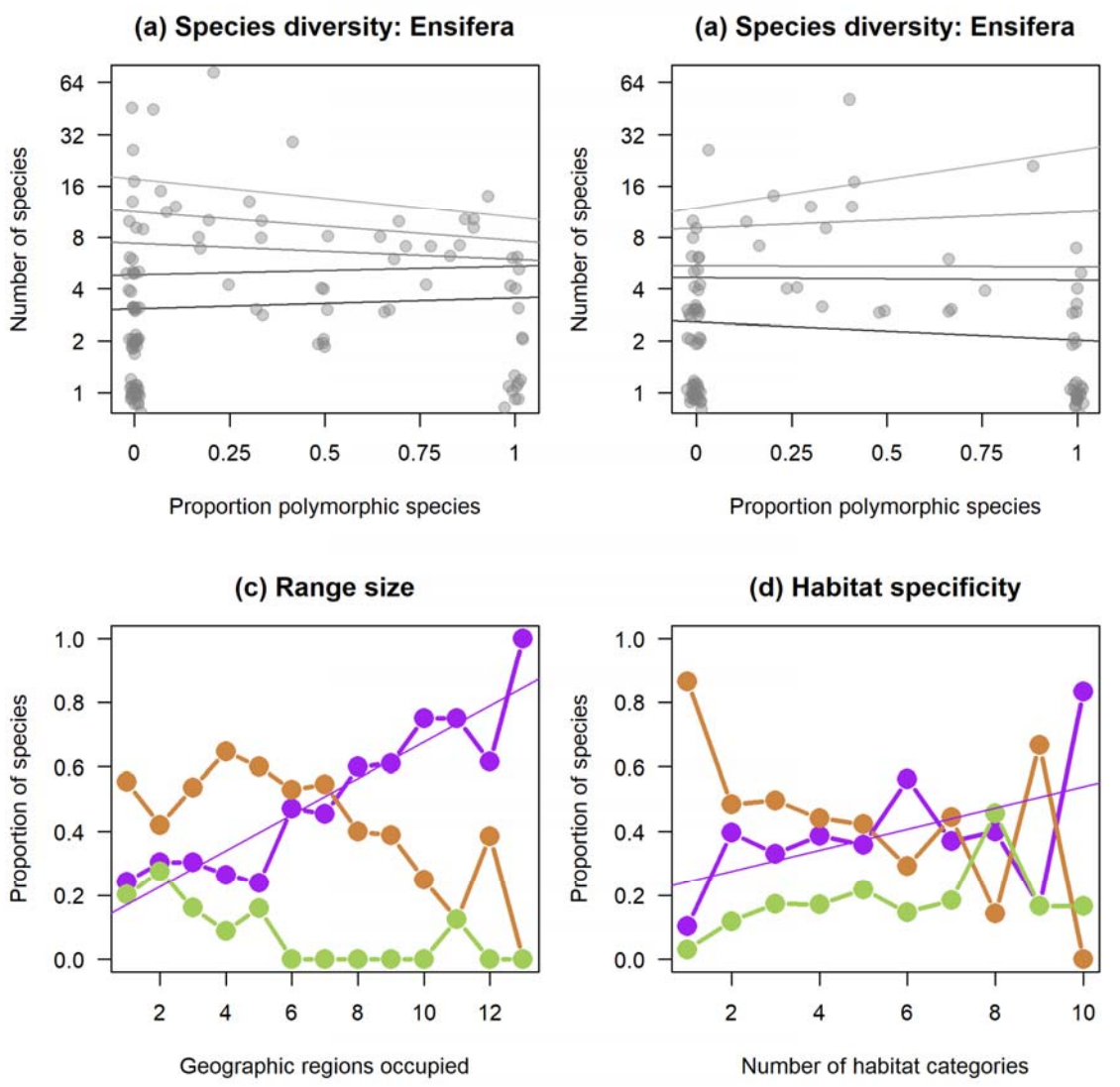
693 Figure 7



694

695

Figure 8


(a) Species diversity: Ensifera
Number of species
64
32
16
8
4
2
1
0
0.25
0.5
0.75
1
Proportion polymorphic species
(a) Species diversity: Ensifera
Number of species
Proportion polymorphic species
(c) Range size
Proportion of species
1.0
0.8
0.6
0.4
0.2
0.0
2
4
6
8
10
12
Geographic regions occupied
(d) Habitat specificity
Proportion of species
Number of habitat categories