

Local climate determines intra- and interspecific variation in sexual size dimorphism in mountain grasshopper communities

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Abstract

The climate is often evoked to explain broad-scale clines of body size, yet its involvement in the processes that generate size inequality in the two sexes (sexual size dimorphism) remains elusive. Here we analyze climatic clines of sexual size dimorphism along a wide elevation gradient (1) among grasshopper species in a phylogenetically controlled scenario and (2) within species differing in distribution and cold tolerance, in order to highlight patterns generated at different time scales, mainly evolutionary (among species or higher taxa) and ontogenetic or micro-evolutionary (within species). At the interspecific level, grasshoppers were slightly smaller and less dimorphic at high elevations. These clines were associated with gradients of precipitation and sun exposure, which are likely indicators of other factors that directly exert selective pressures, such as resource availability and conditions for effective thermoregulation. Within species, we found a positive effect of temperature and a negative effect of elevation on body size, especially on condition-dependent measures of body size (total body length rather than hind femur length) and in species inhabiting the highest elevations. In spite of a certain degree of species-specific variation, females tended to adjust their body size more often than males, suggesting that body size in females can evolve faster among species, and can be more plastic or dependent on nutritional conditions within species living in adverse climates. Natural selection on female body size may therefore prevail over sexual selection on male body size in alpine environments, and abiotic factors may trigger consistent phenotypic patterns across taxonomic scales.

Key-words: Bergman rule, body size, geographic variation, life histories, Rensch's rule

Introduction

The underlying mechanisms and patterns of variation of sexual size dimorphism (e.g. the inequality of body sizes between the two sexes; hereafter SSD) reflect key aspects of evolutionary adaptation and diversification of organisms (Shine, 1989). Adaptive SSD is normally attributed to sex differences in the response to different sources of selection, involving the relationships between body size and fecundity (natural/fecundity selection), survival (natural/viability selection), or mating success (sexual selection) (Fairbairn, 1997; Szekely *et al.*, 2004; Cox & Calsbeek, 2009). In most animal species body size is a major constraint on female fecundity, resulting in larger females than males (Honek, 2003; Fairbairn *et al.*, 2007). The value of male body size increases, however, when it confers an advantage in competition for matings and territories (Trivers, 1972), or in allocation to reproductive reserves, such as nuptial gifts or ejaculate size (Thornhill, 1976; Wiklund & Forsber, 1991). In plants, females often trade off reproduction with growth and show hypoallometry with respect to males, which instead grow continuously (Obeso, 2002). Non-adaptive or epigenetic explanations for SSD are also possible, if phylogenetic constraints impede changes in response to changing environments, or if sexes differ in plasticity for body size (Cheverud *et al.*, 1985; Fairbairn, 1990). For instance, SSD may be magnified by condition dependence, if resource availability and foraging efficiency affect the relative rates of allocation to size in females and males (Bonduriansky, 2007).

Broad-scale patterns of body size variation are common in nature, and are typically embodied in Bergmann or converse Bergmann clines, which describe size variation through latitude or elevation (larger at higher latitudes and elevations or vice versa, respectively), and in Rensch's rule, which postulates greater body size divergence in males compared to females as body size increases (converse Rensch's rule assumes the opposite; Bergmann, 1847; Rensch, 1950; Abouheif & Fairbairn, 1997). Clines in body size are mainly explained by variation in the climate, which acts directly via an effect on metabolic rate or indirectly through seasonal resource availability (Ashton, 2004). Temperature and precipitation, for instance, affect growth and developmental rates in ectotherms, evaporative heat loss in homeotherms, and activity in both groups (Kingsolver *et al.*, 2007). Biotic factors, such as competition and predation, can also play a role, especially in homeotherms (McNab, 1971). Clines of

SSD are not rare, but have been investigated less profusely than those in body size (Blanckenhorn & Demont, 2004; Young, 2005, Blanckenhorn *et al.*, 2006), and even less attention has been paid to their environmental determinants. The few studies that focused on spatial (Mahoney *et al.*, 2001; Stillwell *et al.*, 2007) and temporal (reviewed in Badyaev, 2002) drivers of SSD variation in animals have indeed pointed to the importance of climate in determining the direction and magnitude of SSD. They also showed that plasticity in growth and development schedules is the most common proximate mechanism of SSD changes within species, in contrast with the prevailing evolutionary explanation of SSD variation among species (Teder & Tammaru, 2005; Stillwell *et al.*, 2010).

A few studies on SSD have explicitly focused on inter- and intra-specific variation at a time (Nishikawa & Maly, 1996), and some evidence can be derived from studies on body size variation (see Shelomi, 2012). Nevertheless, combining analyses of inter- and intra-specific variation of SSD within the same evolutionary and climatic scenarios may serve to contrast the effects of the climate on different underlying mechanisms, mainly evolutionary or macro-evolutionary (among species or higher taxa) and ontogenetic or micro-evolutionary (within species or populations) (Simmons, 2002). Indeed, most of published comparative analyses have scrutinized intra-specific patterns using latitude as a proxy for the climate, or predate contemporary methods of controlling for species evolutionary history when addressing macro-evolution. In this study we explore the effect of climatic variation on male and female body size within and among species of mountain grasshoppers along a 1500 m elevation range. Elevational gradients allow more precise quantifications of climate influence than latitudinal ones since variation in the climate is sharper than along latitudes, and does not covary with day length and light availability (Hodkinson, 2005). Moreover, the barrier effect of many mountain chains creates striking climatic divides over very short linear distances, thus permitting the study of intra-specific variation within close local populations. In this study, we address elevation and climatic clines of body size and SSD (1) across grasshopper species after controlling for species phylogeny, and (2) within species widely differing in their elevational range and cold tolerance. We consider morphological proxies that account for condition dependence and structural size, and climatic predictors with an ecological relevance for ectotherms, such as temperature and sun exposure

(controlling for growth and activity), rainfall (local productivity), and within year variation in temperature (seasonality) (Telfer & Hassall, 1999; Chown & Klok, 2003; Stillwell *et al.*, 2007).

Grasshoppers have historically served as ideal models for studying variation of life history traits along climate gradients (Dearn, 1977; Willott & Hassall, 1998; Tatar *et al.*, 1997; Walters & Hassall, 2006), thus clear hypotheses can be formulated on the direction and magnitude of SSD variation. Growing season length at higher elevations limits the body size grasshoppers can achieve; therefore we expect small adult sizes with short growing seasons, low temperatures and sun exposure at both the inter- and intra-specific levels (Berner *et al.*, 2004; Berner & Blanckenhorn, 2006 and 2007). At the intraspecific level, we also expect wider shifts in condition-dependent traits compared to structural morphological characteristics, since the former more readily match nutritional conditions and local resource availability (Chown & Gaston, 2009). Moreover, protandry (faster development of males with respect to females) should result from sexual selection on males to mature at smaller sizes, in order to maximize matings under adverse climates, such as cold conditions and high seasonality (Morbey & Ydenberg, 2001; Esperk *et al.*, 2007; Lehmann & Lehmann, 2008). Ultimately, alpine species should be affected by abiotic factors proportionally more than species occupying milder thermal niches (Hodkinson, 2005; Berner & Blanckenhorn, 2007).

Methods

Study background

The study was carried out in the Cantabrian Mountains, a southern European mountain ridge steeply rising from the north Atlantic Spanish coast to over 2600 m above sea level. Because of their location and orientation, perpendicular to the prevailing north-west oceanic winds, the Cantabrian Mountains host the natural boundary of the Mediterranean region, the wet Atlantic region of the north, and the alpine climate of the highlands. Sharp climatic contrasts occur at a local scale due to the steep and rugged topography of the area (Duckworth *et al.*, 2000).

In July-November 2011-2012 we collected adult grasshoppers in natural or semi-natural grasslands from 56 sites (Fig. 1). The geographic position and elevation of each site was recorded by

means of Garmin Navigators. Sampling sites varied in elevation from 900 to 2400 m a.s.l., covered \approx 1 ha, were located from 0.6 to 104 km apart from each other in 15 different ‘massifs’ (i.e. groups of close, connected mountains rising as independent units). During each field day, we randomly located one to five sampling sites along 10-21 km long transects ascending 700-1450 m. We always walked up to mountaintops to cover the entire upland gradient and maximize climatic differences. Lowlands (<900 m) were excluded from sampling to reduce the potential confounding effect of habitat and human-driven transformations on the patterns of trait variation and species distribution. Open habitats of lowlands strikingly differ from those at higher elevations, since hay meadows with tall vegetation, urbanized areas or plantations prevail over pastures and rocky outcrops.

A total of 1386 grasshoppers from nine univoltine species belonging to the subfamilies Catantopinae and Gomphocerinae were collected alive by hand or sweep net, stored in 50 ml centrifuge tubes and frozen upon return to the laboratory, after spending \approx 6-8 hrs without food. Four of the collected species have a wide Palearctic or western Palearctic distribution (*Myrmeleotettix maculatus*, *Stenobothrus nigromaculatus*, *Stenobothrus stigmaticus*, *Chorthippus parallelus*), one is geographically limited to western Mediterranean regions (*Chorthippus binotatus*) and four are endemic to the Iberian Peninsula: *Chorthippus yersini*, widely distributed in Spain, *Omocestus kaestneri* and *Podisma carpetana*, endemics of northern and central mountain chains, and *Chorthippus cazurroi*, originally known from only two Cantabrian massifs but found in five during the course of this study (Harz, 1975; Reynolds, 1987; Ragge & Reynolds, 1988; Lluçia-Pomares, 2002). These species represent 98% of all individuals sampled in the upland Acrididae community (Appendix S1). Seventeen individuals belonging to five other Acrididae (*Oedipoda caeruleascens*, *Gomphocerus rufus*, *Euchorthippus* spp., *Calliptamus barbarus*, *Omocestus haemorroidalis*) were caught but excluded from analyses because of low sample size, and because they were sampled at the border of their elevational or geographical distribution.

C. yersini was first identified in the field by the characteristics of its calling song, to distinguish it from other members of the *C. biguttulus* group inhabiting lowlands (*C. brunneus* and *C. jacobsi*). The acoustic identification was then confirmed at the stereomicroscope by analyzing the biometric

relationships between forewing veins and the number of stridulatory pegs (Ragge & Reinolds, 1988; Bailey *et al.*, 2012). In order to further control for species identity, we selected 41 individuals of *C. cazurroi*, 38 *C. yersini*, nine *S. stigmaticus*, five *M. maculatus*, three *S. nigromaculatus* spanning their study distribution, and one individual per species of *P. carpetana*, *O. kaestneri*, *C. binotatus* and *C. parallelus* for mtDNA analyses. DNA was extracted from the hind femur using the Qiagen Dneasy kit according to the manufacturer's instructions. A region of the mitochondrial cytochrome oxidase subunit I (COI) was amplified using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). Polymerase chain reactions (PCR) were performed in a 10 µl volume containing 5.0 µl of Gotaq Green Master Mix (Promega), 1.5 µl of DNA (around 50 ng of template) and 0.5 µl of each primer (10 mM), following conditions used by Vedenina and Mugue (2011). Sequencing of the PCR products was performed for both strands using LCO1490 and HCO2198 primers and following the procedures and conditions described in Illera *et al.* (2008)

Overall, we measured body size (see below) in 270 females and 135 males of *C. cazurroi*, 162 females and 171 males of *C. parallelus*, 190 females and 136 males of *C. yersini*, 95 females and 33 males of *S. stigmaticus*, 61 females and 52 males of *O. kaestneri*, 24 females and 6 males of *P. carpetana*, 14 females and 6 males of *M. maculatus*, 13 females and 4 males of *S. nigromaculatus* and 10 females and 4 males of *C. binotatus*. Interspecific comparisons were performed taking into account the nine species, while variation at the intraspecific level was analyzed in the species with the largest sample: *C. cazurroi*, *C. yersini* and *C. parallelus*. These species markedly differ in their distribution along the study elevation range: *C. yersini* was found across all elevations although became scarcer towards the mountaintops, *C. cazurroi* and *C. parallelus* were relatively more abundant at elevations > 1800 and < 1500 m a.s.l., respectively (Fig. 2, Appendix S2).

Morphologic indicators of body size within and among species

By means of a stereoLEICA M125 fitted with an ocular micrometer we measured three different proxies for body size: (1) length of the left hind femur, (2) mid-dorsal pronotal length, and (3) total body length (from the head to the last abdominal tergite, excluding supra-anal and sub-genital plates)

(Duke & Crossley, 1975; Butlin *et al.*, 1987; Willott & Hassall, 1998). In a few individuals the left hind femur was missing and the right one was therefore measured.

Patterns of SSD variation among species were studied by using hind femur length as a sole indicator of body size, since regression slopes between the different measures did not differ from 1 (i.e., isometry) when considering their 97.5% confidence intervals, as tested by major axis regressions (RMAs) performed on \log_{10} transformed average measures per species. As opposed to interspecific comparisons, where variation among species is generally large enough to involve all traits, SSD within species can vary among traits because of functional/condition-dependent variation (LaBarbera, 1989; Blanckenhorn *et al.*, 2006; Stillwell *et al.*, 2010). In grasshoppers, for instance, morphological measures of body size may also indicate individual status (e.g., body length; Dearn, 1977), or allocation in muscular tissue for dispersal (e.g., femur or pronotal length; San Martín y Gómez & Van Dyck, 2012). Here we analyzed the relationship between traits, and among traits and dry body weight (indicating individual nutritional status, Yang & Joern, 1994) in individuals of *C. yersini*, *C. parallelus* and *C. cazurroi*. Pronotum and hind femur showed hypoallometry to total body length in all *Chorthippus* species, but scaled isometrically to each other in *C. yersini* and *C. cazurroi* (Appendix S3). For comparisons with body mass, samples from 24 individuals of *C. yersini*, 22 *C. parallelus* and 21 *C. cazurroi*, were dried for 24 hrs at 70°C after measurements, and then weighed by means of a Mettler Toledo MS105DU microbalance (accuracy 0.01 mg). In all species, total body length scaled isometrically with the cube root of body mass (mass increases to the cube of its linear dimensions), while pronotal and hind femur length increase at a significantly slower rate (Appendix S3), thus suggesting that total body length better tracks variation in individual nutritional condition than the other traits. In light of the above findings, in intra-specific analyses we took into account both total body length (as a proxy of condition-dependent body size) and hind femur length (as a proxy of structural body size) as morphological measures.

Climatic data

To approximate the local growth conditions of sampled grasshoppers we estimated a suite of climatic variables in buffers of 300 m radii around the centre of sampling sites, taking into account that lifetime dispersal ranges from ≈ 14 m (*Podisma*) to 100 m (macropterous *Chorthippus*) and rare long distance dispersal occurs within 1 km (Barton & Hewitt, 1982; Telfer & Hassall, 1999; Bridle & Butlin 2002; Bailey *et al.*, 2003). Within each buffer we calculated the mean annual temperature and average temperature range (difference between average maximum and minimum annual temperatures, an index of seasonality), and accumulated precipitation from the digital layers of the Climate Atlas of the Iberian Peninsula, which were built with a resolution of 200 m by modeling 15 years of meteorological data from the local stations of the Spanish National Meteorological Institute (Ninyerola, 2005). Sun exposure was also obtained from the same Atlas, but was extrapolated from digital elevation models of slope, exposure and shadiness developed from satellite data and corrected for the relative position of the Earth –Sun axis. This parameter therefore represents a measure of the potential radiation input reaching the soil in standard and uniform atmospheric conditions (Ninyerola, 2005).

Three out of four climatic factors varied with elevation. Mean annual temperature and accumulated precipitation decreased upwards ($R_p = -0.97$, $N=56$ sites, $P<0.001$ and $R_p = -0.27$, $P=0.043$, respectively) and temperature range increased ($R_p = 0.32$, $P=0.016$), while sun exposure did not vary ($R_p = -0.12$, $P=0.36$). Therefore, uplands are characterized by colder and drier conditions and higher seasonality than lowlands.

Phylogenetic analysis

Sequences were aligned by eye using BioEdit version 7.0.9 (Hall, 1999). The phylogenetic relationship among the nine study species was inferred using a Bayesian Inference (BI) implemented in the software Mr.Bayes version 3.1 (Huelsenbeck & Ronquist, 2001). The most adequate substitution model for the BI was inferred with the software jModeltest version 2.1 (Darriba *et al.*, 2012). Mr. Bayes was run for 10,000,000 generations with trees sampled every 1000 generations using the default temperature ($t = 2.0$) to switch among chains. Trees obtained during the burn-in generation

period were discarded and the remaining trees were used to estimate the posterior probabilities and a 50% majority rule consensus tree.

Statistical analysis

Interspecific variation in SSD

We considered a sample of nine species as statistically adequate for comparative analyses (see also Cheverud et al. 1985; Cheverud et al. 1989; Laiolo & Rolando 2003), also taking into account that these mountains are characterized by a relatively species-poor grasshopper community (Appendix S1). We tested whether SSD was associated with elevation and climatic variables by means of phylogenetic generalized least squares regressions (PGLSs). This method allows analyzing the covariance in continuous traits and variables across taxa controlling for phylogeny. We derived species phylogeny from a fragment of 575 base pairs of the mitochondrial gene COI (see above), resulting in the phylogenetic tree shown in Fig. 3 (sequences have been deposited in the NCBI gene bank database and accession numbers are provided in Appendix S4). The magnitude of the phylogenetic signal (λ) was estimated by maximum likelihood. The ratio of the average hind femur length of females (the larger sex) to males of each species was used as a measure of SSD (Lovich & Gibbons, 1992).

As predictors, we considered the average elevation, accumulated precipitation, sun exposure, mean annual temperature and temperature range of the localities where the individuals were caught. The use of average values in comparative studies may lead to biased results if sampling covers an incomplete set of biological and environmental conditions. However, we feel confident of the effectiveness of our sampling design, in which pair-wise morphological and climatic data were obtained (i.e., from the same sites, and not from geographically independent sources), and sampling represents a complete set of sizes and climates within our study area. The geographic distribution, thus the climatic environment, of grasshopper species was fairly well-defined by extensive sampling, as demonstrated by the extension of the known distribution of some species, and by comparisons with other studies, which substantiate that low sample sizes represent actual rarity in the study region (Ragge & Reynolds, 1988; Isern-Vallerdu & Pedrocchi, 1994; Jauregui *et al.*, 2008).

Variation in the SSD of C. cazurroi, C. parallelus and C. yersini

First, we analyzed whether body size (hind femur and total body length) varied between sexes and with elevation by means of generalized linear mixed models (GLMMs). The interaction of elevation \times sex was tested to appreciate SSD variation with elevation. Latitude and longitude were entered as covariates to control for the non-independence of data from close populations, and massif identity as a random factor to control for pseudoreplication.

We then tested whether the two proxies for body size responded differently to climatic variation in the two sexes by running GLMMs in which accumulated precipitation, sun exposure, mean annual temperature, temperature range and their interactions with sex were entered as fixed factors, and the massif was entered as a random factor. A Gaussian distribution of errors was used in all models since male and female measurements in the three species were normally distributed (Kolmogorov Smirnov test: $0.06 < d < 0.11$, all $P > 0.1$). Type II models were run, testing each main effect after controlling for the other determinants. We used an information criterion to extract those climate variables that best explained body size variability since a large number of parameters was considered (five climate variables and their interactions with sex, quadratic terms for non-linear trends), as opposite to GLMMs testing for elevation clines or interspecific analyses, in which a null hypothesis testing criterion was preferred because of fewer parameters and interactions (Anderson *et al.*, 2000). Hence, for climate clines we tested a set of models that was most appropriate and plausible a priori and that included variables significantly affecting traits (listed in Appendix S5), using an iterative forward variable-selection process and ranking models on the basis of their Akaike's Information Criterion (AIC) values (Buckland *et al.*, 1997). Models separated by less than four AIC points from the model with the lowest AIC were considered as equally probable (Burnham & Anderson 2002).

All statistical analyses were performed with R 2.15.2 (R Development Core Team 2009), with the packages *lmodel2* (RMAs), *caper* (PGLSs) and *lme4* (GLMMs). Quadratic relationships were tested by means of orthogonal polynomials (*poly*) and predicted values for PGLSs (Appendix S6) were estimated with the function *predict*. Regression slopes are expressed as *b* coefficients, thus their

absolute value depends on the units of measurement of the respective variables. Elevation, latitude and longitude are measured in m (a.s.l. or UTM), precipitations in mm, temperatures in °C, sun exposure in $\text{kJ m}^{-2} \text{ day}^{-1}$ and morphological measurements in mm. Unless specified otherwise, P -values are for two-tailed tests.

Results

Interspecific variation in SSD

The slope of the regression of male on female size (both \log_{10} transformed) was significantly lower than 1.0 ($b=0.76 \pm 0.04\text{SE}$; $t=5.23$, $P=0.0012$, $N=9$ species) and the amount of phylogenetic signal (λ) was significantly different from zero ($P=0.038$). This suggests that females are proportionally bigger than males in large species (converse Rensch's rule).

There were almost significant tendencies of SSD (ratio female/male hind femur length) to decrease with elevation, and of female and male body sizes to decrease following a quadratic trend (Tab. 1a; Fig. 4; Appendix S6). When replacing elevation with climatic variables, SSD significantly increased with precipitation and sun exposure (Tab.1b; Fig. 4). The elevation cline in SSD disappeared when precipitation and sunshine were entered in the models (elevation: $t=0.92$, $P=0.408$; precipitation: $t=3.01$, $P=0.039$; sunshine: $t=2.79$, $P=0.049$), indicating that variation along elevation was primarily driven by climatic factors. Variation in SSD with the climate seems to be due to a sex effect on the slope of the precipitation cline: the increase in female size with precipitation is more pronounced than that in male size (comparison among slopes: $t=2.12$, $P=0.033$, one-tailed t test) (Tab.1b). Mean annual temperature and temperature range had a poorer effect on dimorphism ($t=1.8$, $P=0.13$ and $t=2.0$, $P=0.10$, respectively). The variation in body size driven by the climate and elevation was not strongly conditioned by species phylogeny but λ was almost significantly different from zero in all regressions ($0.054 < P < 0.14$).

Variation in the SSD of *C. cazurroi*, *C. parallelus* and *C. yersini*

When considering hind femur length as a proxy for body size, only *C. cazurroi* showed an elevation cline that significantly varied with the sex: female femur became proportionally shorter than in males towards mountaintops (Tab. 2). No clear elevation cline was found in *C. parallelus* and *C. yersini* size (Tab. 2). When taking into account total body length, however, *C. cazurroi* and *C. parallelus* showed a significant sex effect on variation along elevation. The sex \times elevation interaction was not fully significant in *C. yersini* ($P=0.06$; Tab. 2) but when removing non-significant geographic covariates (latitude and longitude) from the model the effect turned significant (sex \times elevation: $b \pm SE=0.0009 \pm 0.0004$, $t=2.04$, $P=0.042$; elevation: $b \pm SE=0.0056 \pm 0.003$ $t=1.64$, $P=0.10$; sex: $b \pm SE=-6.93 \pm 0.74$ $t=5.49$, $P<0.001$). As highlighted by Figure 5 and by the direction (sign) of the sex effect and its interaction with elevation (Tab. 2), *C. cazurroi* and *C. yersini* decreased dimorphism with elevation, although in the former species body size declined in both sexes, while in the latter only females decreased in length (the elevation effect was significant only in its interaction with sex). Conversely, dimorphism increased with elevation in *C. parallelus*, and males became proportionally shorter than females (the elevation effect was significant only in its interaction with sex) (Tab. 2; Fig. 5).

When replacing geographic coordinates and elevation with climatic variables, mean annual temperature was included in the best models explaining variation in total body length of all species, and in the best models explaining variation in hind femur length of *C. cazurroi* and *C. parallelus*. Therefore, temperature was the climatic variable that best predicted intraspecific variability in body size in our study system. A significant sex effect in conditioning climate clines occurred in *C. cazurroi* (hind femur length) and *C. parallelus* (total body length) and a marginally significant effect was found in *C. yersini* (total body length, $P=0.056$), although the interaction of sex with climate parameters (temperature and/or precipitation) was consistently included in the best models predicting variation of one or both measures of body size in all the three species (Tab. 3). In general, the size of the largest sex (female) appeared to be more sensitive to increasing temperatures than male size in *C. cazurroi* and *C. yersini*, while male size was more sensitive in *C. parallelus* (as highlighted from interaction signs in Table 3). Precipitation had a positive effect on body size in *C. parallelus* and a

negative effect in *C. cazurroi*, sun exposure positively affected *C. yersini* total body length (Tab. 3; Appendix S5).

Discussion

At the interspecific level we found that both females and males slightly diminished in size with elevation, in line with a converse Bergman cline. This pattern was associated with gradients of precipitation and sun exposure, which are likely indicators of other factors that directly exert selective pressures, such as resource availability and conditions for effective thermoregulation. Variation of male and female size was not isometric, and fitted a converse Rensch's rule: female size changed more than male size in the largest species, and responded more quickly to shifts in environmental conditions. At the intraspecific level, the positive effect of temperature (in all species) and the negative effect of elevation on body size (in mid-high elevation species) pointed to the importance of thermal conditions, especially for condition-dependent body size. Sex-specific patterns of climatic sensitivity and species-specific patterns of SSD variation emerged, possibly resulting from inter-specific differences in life-histories and mating strategies.

Interspecific variation in SSD

Grasshopper body size decreased with elevation as in a wide range of insect species (reviewed in Roff, 2002 and Hodkinson, 2005). Such patterns are normally explained by cooler temperatures that lead to delayed hatching and rapid growth at high elevations (Dearn, 1977; Orr, 1996; Tatar *et al.*, 1997; Lehmann & Lehmann, 2008). In our study system, however, the temperature effect was stronger within species than among species, possibly because within species this factor more easily impinges on developmental plasticity, or drives selection on developmental times, than it affects higher-level evolutionary processes (Thompson, 1999; Kingsolver *et al.*, 2004; Fairbairn, 2005). Among species, the largest species inhabited wet and sun-exposed sites. Precipitation likely improves resource quality, quantity and predictability, thus permitting large and energy-demanding species to settle (or to evolve) (Yang & Joern, 1994), while solar radiation increases activity rate and in turn food assimilation and

ingestion (Chappell & Whitman, 1990). Remarkably, large grasshoppers are more efficient in modulating heat gain and loss when exposed to sunshine (Digby, 1954).

In our study system females are the largest sex, a fact that indicates that natural selection for female fecundity is strong. This selection often results in female size changing proportionally more than male size when resource availability improves in unpredictable habitats (Teder & Tammaru, 2005; Bunce *et al.*, 2003; Blanckenhorn *et al.*, 2007), as we found in our study, in which the most dimorphic species inhabited wet (more productive) environments. In these ecological settings, directional selection on female size might be detected by strong, positive relationships between female growth and plant availability/nutritional content (Joern & Behmer, 1997) or local precipitation regimes (Davidowitz, 2008).

Intraspecific variation in SSD

In all three species we found a slight tendency for size to decrease with elevation in one or both sexes, often in association with variation in mean annual temperatures. In grasshopper studies, mean annual temperature is often used as an indicator of the time over which conditions are suitable for growth (Roff & Mousseau, 2005), so that warmer conditions entail delayed maturation, faster growth, or both phenomena, with significant effects on body size (Dearn, 1977; Telfer & Hassall, 1999). We also found that patterns of body size and SSD varied among traits, with total body length clines being significantly steeper than those of femur length across environmental gradients. The isometric relationship of total body length with body mass, and thus with nutritional conditions, suggests that this variable could change more readily than other structural measures in response to diet quality. This finding is in line with evidence obtained in other species in which condition dependence accentuate SSD because of sex differences in phenotypic plasticity within species (Davidowitz *et al.*, 2004; Fairbairn, 2005; Stillwell *et al.*, 2007). The fact that female total body length varies more along gradients than that of males in mid-high elevation species also supports the idea that female body size in insects is more plastic than male body size when measured by body mass or isometric measures (Stillwell *et al.*, 2010). Therefore, although we addressed ultimate (environmental) causes and not the

proximate mechanisms, these findings appear to suggest that intra-specific variation may be at least partly due to plasticity, because most of the observed variation entailed a condition-dependent trait (Dearn, 1977), and occurred over very small spatial scales as compared to other studies that detected substantial variation in life histories along environmental gradients (e.g. Dearn, 1977; Telfer & Hassall, 1999). These considerations do not exclude, however, that sex or population differences in plasticity could be adaptive, or result from selection on developmental times, and thus evolve as a result of local selection pressures (Thompson, 1999; Stillwell *et al.*, 2010).

Within the study pool of species, *C. cazurroi* showed the most dramatic elevation- and climate-driven variation: changes in body size and SSD involved both condition dependent and structural measures of body size, clines were steeper and relationships with climatic variables the most complex. *C. cazurroi* is the highest elevation *Chorthippus* grasshopper in our study system, thus encountering colder climatic conditions more than other species and, supposedly, stronger phenotypic selection (Etterson, 2007). Harsh climates indeed impose more significant resource limitations and time constraints on high elevation insects than on those that occupy mid-low elevations, causing more evident trade-offs between size and reproduction (Hodkinson, 2005; Berner & Blanckenhorn, 2007).

SSD variation did differ among species: *C. cazurroi* and *C. yersini* showed less dimorphism in body length with elevation while *C. parallelus* showed an increase. The latter species was the only one that showed a pattern of variation according to our expectations of male accelerated development and protandry at high elevations (Lehmann & Lehmann, 2008; Berner & Blanckenhorn, 2006). Nevertheless, the patterns of SSD variation in *C. cazurroi* and *C. yersini*, entailing greater changes in female than in male size, did occur in other grasshopper species along climate gradients. Females of *Chorthippus brunneus*, a species phylogenetically close to *C. yersini*, produce supernumerary instars when raised at high temperature and high quality diet (Hassal & Grayson, 1987). This allows females to become considerably larger than males in milder conditions, similarly to what was observed in *C. cazurroi* and *C. yersini*. Notably, *C. parallelus* is one of the few *Chorthippus* of which the female is unable to change the number of instars during development when exposed to varying heat supplies (Schädler & Witsack, 1999). This implies that this species may respond less readily to shifting thermal

conditions as compared to its congeners, a fact that may constrain its distribution to the mildest niche and impede an upward expansion as species showing a greater flexibility of responses (Hodkinson, 2005).

The stasis in male size and sharp changes in female size in *C. cazurroi* and *C. yersini* may also (or alternatively) be dictated by a mating system involving a transfer of nutrients from males to females during mating, a behaviour observed in *C. brunneus* (Butlin *et al.*, 1987) and other insects living in unpredictable environments (Leimar *et al.*, 1994). In these conditions the value of large sizes increases for males, resulting in a drop in SSD.

Concluding remarks

In a study that covers a gradient that includes mountaintops, we show that abiotic agents regulate allometry for SSD across taxonomic scales. At the interspecific level, productive and sunny environments appear to engender selection for larger female body sizes; at the intraspecific level, female plasticity or natural selection during development likely leads to better adjustments of body size to the prevailing local conditions, especially in species occupying mid-high elevations (Esperk *et al.*, 2007). Thus, among species and often within species, SSD variation in relation to elevation and climate is more tightly associated with females changing their size than with males doing so, contrary to the hypothesis that male body size should vary, or evolutionarily diverge, more than female size (e.g. Rensch's rule; Fairbairn, 1997; Blanckenhorn *et al.*, 2006). This may be related to the fact that the fitness of female grasshoppers as in many insects, strongly depends upon size and thus upon food (Blanckenhorn *et al.*, 2007), whose availability is in turn regulated by climate, especially in harsh and unpredictable mountain environments (Hodkinson, 2005).

Although the mechanisms that generate elevation clines in body size need to be identified in the future by detailed experimental approaches, our study shows that local abiotic factors are able to trigger consistent phenotypic clines, which in grasshoppers fit patterns converse to Bergman and Rensch's rules across taxonomic scales. It also shows that natural selection on female body size may

prevail over sexual selection on male body size in alpine environments, and create clines of decreasing sexual dimorphism with elevation as those observed in homeotherms (Badyaev, 1997).

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Figure Legend

Fig.1. Location of the 56 sampling sites in the Cantabrian Mountains along the elevational gradient (scale darker towards mountaintops). Annual accumulated precipitation (scale darkest in the wettest areas) and mean temperature (scale darkest in the warmest areas) are also shown.

Fig.2. Relative abundance of *Chorthippus cazurroi*, *C. yersini* and *C. parallelus* in five elevational bands along a gradient rising 1500 m. Abundances are standardized for sampling effort, as detailed in Appendix S2.

Fig.3. Bayesian consensus tree (575 bp) based on mitochondrial (COI) sequences of nine grasshopper species, and based on the HKY + I model of evolution. Numbers above branches show Bayesian posterior probabilities.

Fig. 4. Elevation (left) and precipitation (right) clines of sexual size dimorphism (female/male hind femur length) in the nine study species.

Fig.5. Elevation clines for total body length of females and males of *Chorthippus cazurroi*, *C. yersini* and *C. parallelus*.

Table 1. Relationships between sexual size dimorphism, female and male body size *versus* elevation (a) and climatic variables (b) in the nine study species, as tested by phylogenetic generalized least squares regressions. Dimorphism is expressed by female/male hind femur length, body size as \log_{10} (hind femur length).

| <i>a) Relationships with elevation</i> | | | |
|--|------------------|------|--------|
| Sexual size dimorphism ($R^2=0.47$, $F_{2,6}=5.35$, $P=0.046$) | $b \pm SE$ | t | P |
| Intercept | 1.37±0.006 | 21.3 | <0.001 |
| Elevation | -0.00009±0.00003 | 2.31 | 0.060 |
| Female body size ($R^2=0.78$, $F_{3,5}=8.7$, $P=0.019$) | $b \pm SE$ | t | P |
| Intercept | 1.04±0.022 | 45.4 | <0.001 |
| Elevation | -0.100±0.033 | 2.91 | 0.033 |
| Elevation ² | -0.065±0.029 | 2.27 | 0.072 |
| Male body size ($R^2=0.74$, $F_{3,5}=7.3$, $P=0.028$) | $b \pm SE$ | t | P |
| Intercept | 0.95±0.018 | 52.6 | <0.001 |
| Elevation | -0.075±0.027 | 2.78 | 0.038 |
| Elevation ² | -0.049±0.024 | 2.09 | 0.090 |
| <i>b) Relationships with climatic variables</i> | | | |
| Sexual size dimorphism ($R^2=0.81$, $F_{3,5}=10.4$, $P=0.013$) | $b \pm SE$ | t | P |
| Intercept | -0.84±0.046 | 1.81 | 0.13 |
| Accumulated annual precipitation | 0.0008±0.0001 | 4.24 | 0.008 |
| Sun exposure | 0.0005±0.0001 | 3.70 | 0.014 |
| Female body size ($R^2=0.89$, $F_{3,5}=20.5$, $P=0.003$) | $b \pm SE$ | t | P |
| Intercept | -1.93±0.47 | 4.13 | 0.009 |
| Accumulated annual precipitation | 0.0009±0.0001 | 5.22 | 0.003 |
| Sun exposure | 0.0009±0.0001 | 5.94 | 0.002 |
| Male body size ($R^2=0.87$, $F_{3,5}=16.9$, $P=0.004$) | $b \pm SE$ | t | P |
| Intercept | -1.29±0.39 | 3.29 | 0.021 |
| Accumulated annual precipitation | 0.0006±0.0001 | 4.46 | 0.006 |
| Sun exposure | 0.0007±0.0001 | 5.58 | 0.003 |

Table 2. Results of generalized linear mixed models (GLMMs) testing for sexual dimorphism in elevation clines of body size in *Chorthippus cazurroi*, *C. yersini* and *C. parallelus*. Body size was expressed in terms of hind femur and total body length. The massif was entered as a random factor and latitude and longitude as covariates to control for the spatial autocorrelation of data.

| <i>Chorthippus cazurroi</i> : Hind femur length | | | |
|---|--------------------|----------|----------|
| | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | -527±98 | 5.36 | <0.001 |
| Sex (male vs. female) | -2.94 ± 0.44 | 6.69 | <0.001 |
| Elevation | -0.0008 ± 0.0001 | 4.87 | <0.001 |
| Sex × Elevation | 0.0005±0.0002 | 2.11 | 0.035 |
| Longitude | -0.00001±0.000001 | 7.82 | <0.001 |
| Latitude | 0.00001±0.000002 | 5.97 | <0.001 |
| <i>Chorthippus cazurroi</i> : Total body length | | | |
| | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 155 ±177 | 0.871 | 0.38 |
| Sex (male vs. female) | -8.35±1.31 | 6.34 | <0.001 |
| Elevation | 0.0033±0.0004 | 7.77 | <0.001 |
| Sex × Elevation | 0.0015±0.0006 | 2.27 | 0.024 |
| Longitude | -0.00002±0.000009 | 1.73 | 0.084 |
| Latitude | -0.00003±0.00004 | 0.69 | 0.49 |
| <i>Chorthippus yersini</i> : Hind femur length | | | |
| | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 2.74±0.18 | 1.48 | 0.88 |
| Sex (male vs. female) | -3.23±0.27 | 11.54 | <0.001 |
| Elevation | -0.0003±0.0001 | 1.84 | 0.06 |
| Sex × Elevation | 0.0002±0.0001 | 1.42 | 0.16 |
| Longitude | -0.000004±0.000001 | 2.9 | 0.004 |
| Latitude | 0.000002±0.000003 | 0.62 | 0.53 |
| <i>Chorthippus yersini</i> : Total body length | | | |
| | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 114±79 | 1.44 | 0.15 |
| Sex (male vs. female) | -6.79±0.74 | 9.13 | <0.001 |
| Elevation | -0.0008±0.00002 | 1.93 | 0.05 |
| Sex × Elevation | 0.0008±0.0004 | 1.85 | 0.06 |
| Longitude | 0.00001±0.000007 | 1.79 | 0.09 |
| Latitude | -0.00002±0.00001 | 1.12 | 0.22 |
| <i>Chorthippus parallelus</i> : Hind femur length | | | |
| | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | -72 ±43 | 1.73 | 0.077 |
| Sex (male vs. female) | -1.72±0.24 | 6.99 | <0.001 |
| Elevation | 0.00002±0.00001 | 0.11 | 0.9 |
| Sex × Elevation | -0.0002±0.0001 | 1.41 | 0.15 |
| Longitude | 0.000002±0.000003 | 0.43 | 0.66 |
| Latitude | 0.00002±0.000008 | 2.04 | 0.042 |
| <i>Chorthippus parallelus</i> : Total body length | | | |
| | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 45±9 | 0.5 | 0.61 |
| Sex (male vs. female) | -4.51±0.62 | 7.22 | <0.001 |
| Elevation | 0.0006±0.0004 | 1.45 | 0.15 |
| Sex × Elevation | -0.0009±0.0004 | 2.07 | 0.039 |
| Longitude | 0.00003±0.000007 | 4.07 | <0.001 |
| Latitude | -0.000007±0.00001 | 0.38 | 0.7 |

Table 3. Results of generalized linear mixed models (GLMMs) testing for sexual dimorphism in climatic clines of body size in *Chorthippus cazurroi*, *C. yersini* and *C. parallelus*. Body size was expressed in terms of hind femur and total body length. Only models with the lowest AIC (separated by less than 4 points from the model with the lowest AIC) are shown. See Appendix S5 for the complete list of model tested.

| <i>Chorthippus cazurroi</i> : Hind femur length | | | |
|---|-----------------|----------|----------|
| Model 1. AIC=581 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 7.81± 0.27 | 28.2 | <0.001 |
| Sex (male vs. female) | -1.31± 0.23 | 5.56 | <0.001 |
| Mean annual temperature | 0.49± 0.04 | 12.7 | <0.001 |
| Sex × Mean annual temperature | -0.14± 0.047 | 3.02 | 0.003 |
| Accumulated precipitation | -6.14± 1.08 | 5.67 | <0.001 |
| (Accumulated precipitation) ² | 4.07± 0.57 | 7.18 | <0.001 |
| <i>Chorthippus cazurroi</i> : Total body length | | | |
| Model 1. AIC=1383 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 15.8± 0.43 | 34.8 | <0.001 |
| Sex (male vs. female) | -5.39± 0.16 | 33.2 | <0.001 |
| Mean annual temperature | 0.67± 0.09 | 7.62 | <0.001 |
| Accumulated precipitation | -7.68± 2.61 | 2.94 | 0.003 |
| (Accumulated precipitation) ² | -2.22± 1.97 | 1.13 | 0.26 |
| Sex × Accumulated precipitation | 5.25± 3.18 | 1.65 | 0.10 |
| Sex × (Accumulated precipitation) ² | 0.049± 3.14 | 0.16 | 0.87 |
| <i>Chorthippus yersini</i> : Hind femur length | | | |
| Model 1. AIC=589 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 12.47±0.06 | 205 | <0.001 |
| Sex (male vs. female) | -2.84± 0.07 | 42.2 | <0.001 |
| <i>Chorthippus yersini</i> : Total body length | | | |
| Model 1. AIC=1226 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 17.35± 1.20 | 14.5 | <0.001 |
| Sex (male vs. female) | -5.40 ± 0.18 | 30.6 | <0.001 |
| Sun exposure | 0.0021 ± 0.0006 | 3.54 | <0.001 |
| Model 2. AIC=1228 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 21.36± 0.48 | 44.4 | <0.001 |
| Sex (male vs. female) | -5.45 ± 0.17 | 30.6 | <0.001 |
| Mean annual temperature | 0.029 ± 0.07 | 0.41 | 0.69 |
| Model 3. AIC=1229 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 20.81± 0.55 | 37.51 | <0.001 |
| Sex (male vs. female) | -4.14 ± 0.70 | 5.87 | <0.001 |
| Mean annual temperature | 0.11 ± 0.08 | 1.38 | 0.16 |
| Sex × Mean annual temperature | -0.21 ± 0.11 | 1.91 | 0.056 |
| <i>Chorthippus parallelus</i> : Hind femur length | | | |
| Model 1. AIC=489 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 10.8± 0.23 | 47.55 | <0.001 |
| Sex (male vs. female) | -2.07± 0.053 | 38.76 | <0.001 |
| Mean annual temperature | 0.092± 0.028 | 3.23 | 0.014 |
| Model 2. AIC=492 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |
| Intercept | 11.5± 0.13 | 84.55 | <0.001 |
| Sex (male vs. female) | -2.08± 0.054 | 38.45 | <0.001 |
| <i>Chorthippus parallelus</i> : Total body length | | | |
| Model 1. AIC=1010 | <i>b</i> ± SE | <i>t</i> | <i>P</i> |

| | | | |
|--|-------------|-------|--------|
| Intercept | 22.3± 0.79 | 27.93 | <0.001 |
| Sex (male vs. female) | -7.37± 0.72 | 10.13 | <0.001 |
| Mean annual temperature | 0.28± 0.09 | 2.81 | 0.005 |
| Sex × Mean annual temperature | 0.23± 0.10 | 2.22 | 0.027 |
| Accumulated precipitation | 6.39± 3.86 | 1.65 | 0.099 |
| (Accumulated precipitation) ² | 0.14± 2.89 | 0.052 | 0.96 |

Fig. 1

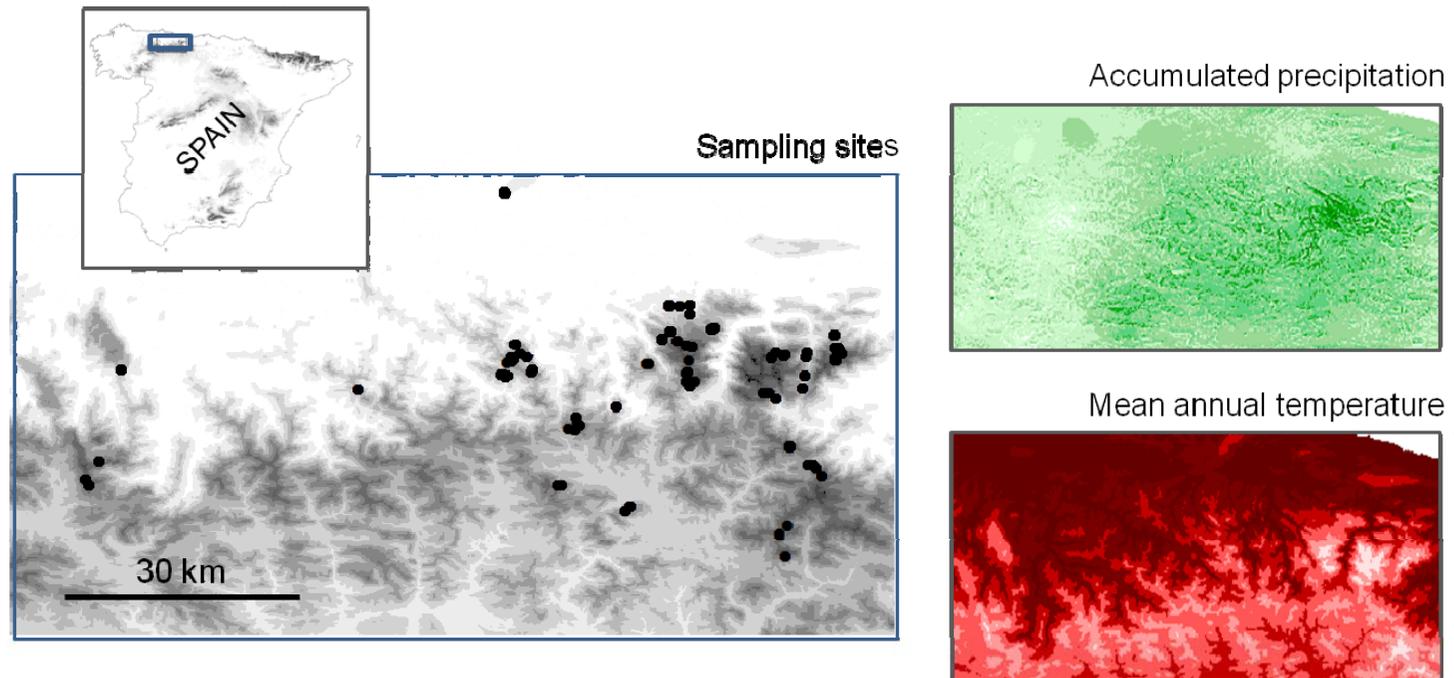


Fig.2

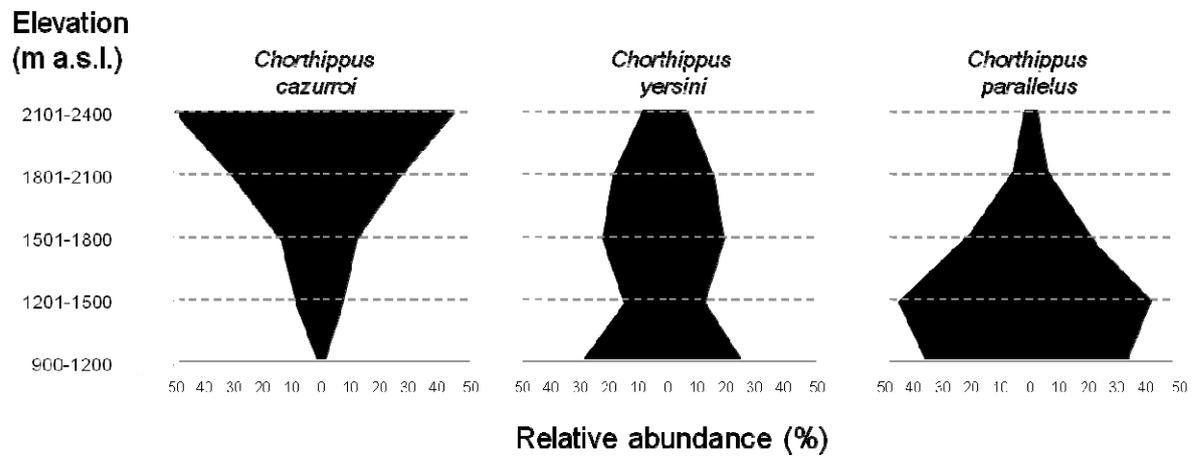


Fig. 3

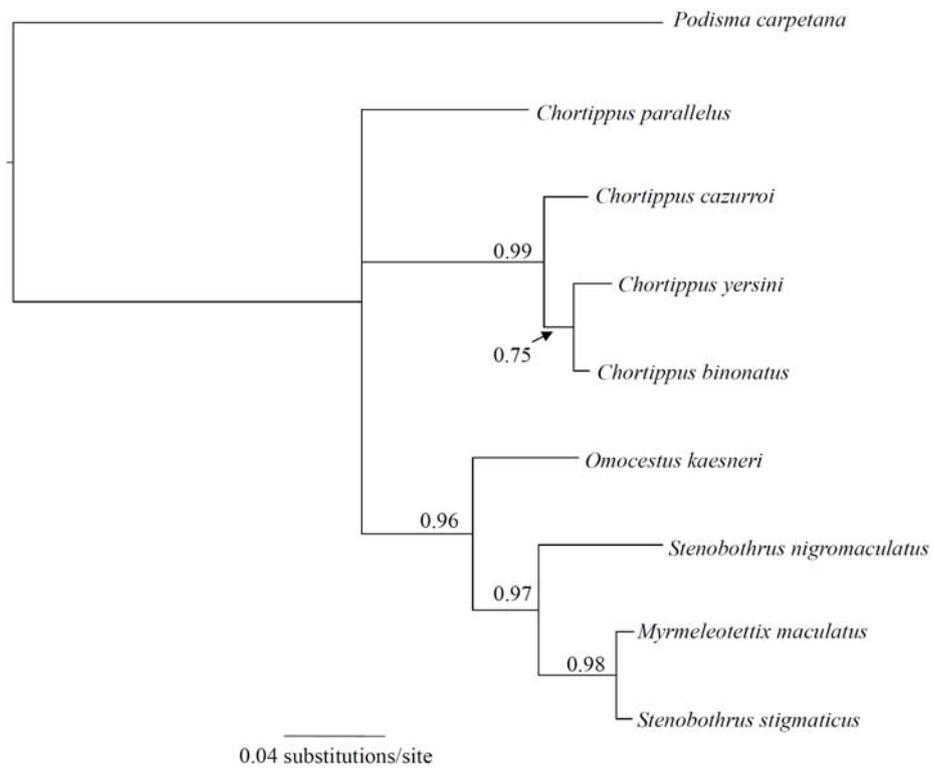


Fig. 4.

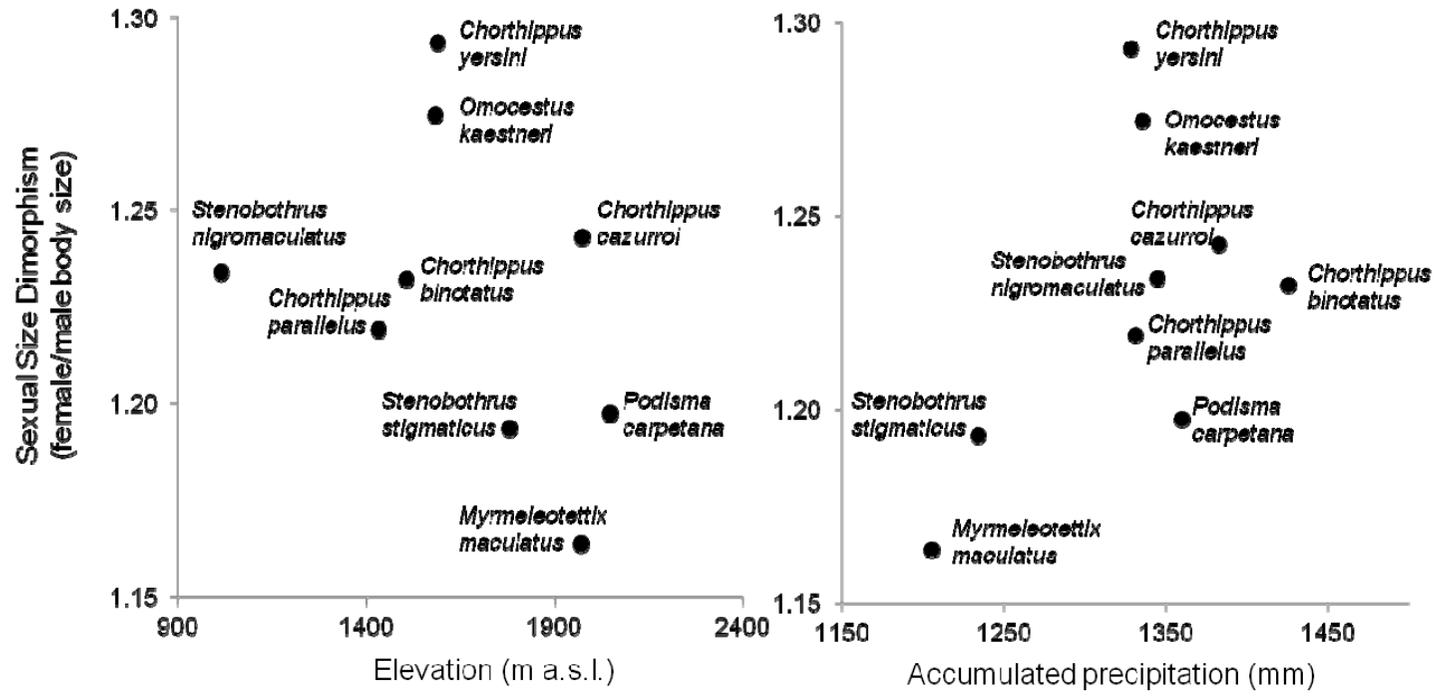


Fig. 5

