

Ecological drivers of body size evolution and sexual size dimorphism in short-horned grasshoppers (Orthoptera: Acrididae)

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Abstract

Sexual size dimorphism (SSD) is widespread and variable in nature. Although female-biased SSD predominates among insects, the proximate ecological and evolutionary factors promoting this phenomenon remain largely unstudied. Here, we employ modern phylogenetic comparative methods on eight subfamilies of Iberian grasshoppers (85 species) to examine the validity of different models of evolution of body size and SSD and explore how they are shaped by a suite of ecological variables (habitat specialization, substrate use, altitude) and/or constrained by different evolutionary pressures (female fecundity, strength of sexual selection, length of the breeding season). Body size disparity primarily accumulated late in the history of the group and did not follow a Brownian motion pattern, indicating the existence of directional evolution for this trait. We found support for the converse of Rensch's rule (i.e. females are proportionally bigger than males in large species) across all taxa but not within the two most speciose subfamilies (Gomphocerinae and Oedipodinae), which showed an isometric pattern. Our results do not provide support for the fecundity or sexual selection hypotheses, and we did not find evidence for significant effects of habitat use. Contrary to that expected, we found that species with narrower reproductive window are less dimorphic in size than those that exhibit a longer breeding cycle, suggesting that male protandry cannot solely account for the evolution of female-biased SSD in Orthoptera. Our study highlights the need to consider alternatives to the classical evolutionary hypotheses when trying to explain why in certain insect groups males remain small.

Introduction

Body size is a key trait in any organism, as it influences fitness through its effects on reproduction and survival (Fairbairn *et al.*, 2007). Body size can respond to different evolutionary forces in a sex-specific manner and, as a result, this trait often differs between males and females in many taxa (Darwin, 1871). Although male-biased sexual size dimorphism (SSD) is the common rule among endotherms (mammals and birds), female-biased SSD predominates among insects (e.g. Elgar, 1991; Hochkirch & Gröning, 2008; Cheng & Kuntner,

2014; Bidau *et al.*, 2016). In those species in which females are larger than males, it is assumed that natural selection on female body size (*via* increased fecundity) overrides sexual selection (through competition advantages during mate acquisition) on male body size. However, many other ecological pressures (e.g. habitat, substrate use, length of life cycle) can determine body size evolution in one or both sexes and contribute to shape observed patterns of SSD (Blanckenhorn *et al.*, 2007a; Fairbairn *et al.*, 2007; Fairbairn, 2013).

Body size variation among related species often follows evolutionary patterns that are remarkably consistent across taxa. According to Rensch's rule, SSD increases with body size when males are the larger sex and decreases with size when females are larger (Rensch, 1950; Abouheif & Fairbairn, 1997; Fairbairn, 1997). In contrast, if selection pressures on females are the

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main driver of SSD evolution, then SSD should increase with average body size in female-biased SSD species (the converse of Rensch's rule; Webb & Freckleton 2007). Finally, SSD can remain isometric when body size changes in males and females at the same rate, a plausible scenario when multiple evolutionary forces (e.g. sexual selection and fecundity selection) act synergically with no overall general trend. Another broad-scale pattern of body size variation is the Bergmann's rule, which posits the existence of a positive relationship between body size and latitude and altitude, with smaller individuals being found at lower latitudes/altitudes where temperatures are generally higher (Mayr, 1956). However, ectotherms often follow the converse of Bergmann's rule with larger individuals and species at lower latitudes and altitudes (reviewed in Shelomi, 2012). The most likely explanation for this inverted cline is the interaction between the length of the growing season (which decreases as latitude and altitude increase) and the time available to complete development (Mousseau, 1997). Slowly developing insects may compensate for a short season by decreasing development time, that is, by reaching the adult stage at a smaller size (e.g. reducing the number of larval instars; Blanckenhorn & Demont, 2004; Esperk *et al.*, 2007). However, some species do not exhibit protandry and both sexes reach adulthood at about the same time but at different sizes (Blanckenhorn *et al.*, 2007b).

In Orthoptera, females are usually larger than males. Hochkirch & Gröning (2008) reported that virtually all of the 1106 Caelifera species analysed showed female-biased SSD (see also Bidau *et al.*, 2016 for a review). From the female perspective, it seems to be clear that a large body size may confer an advantage in terms of increased fecundity (e.g. Cueva del Castillo & Núñez-Farfán, 1999; Whitman, 2008). In addition, males may also benefit from mating with large, fecund females (Gwynne, 1981; Kvarnemo & Simmons, 1999). Conversely, small males may benefit from early maturity (protandry), greater agility or lower predation rates (see Whitman, 2008 and references therein; see also Blanckenhorn, 2000). When the evolution of male and female body size follows divergent evolutionary trajectories, it can lead to a decoupling of male and female size evolution. However, absolute decoupling is rather unlikely because genetic correlations between males and females will tend to constrain independent size evolution of both sexes. Body size decoupling has been suggested as the main cause for the existence of extremely female-biased SSD in spiders, a taxonomic group that has dominated studies on arthropods in this respect (Hormiga *et al.*, 2000; Kuntner & Coddington, 2009; Kuntner & Elgar, 2014). On the contrary, there is a paucity of interspecific studies on SSD in Orthoptera even though they are fairly abundant, easy to collect, and have large geographic distributions, which makes them an ideal model system to address these questions.

In this study, we employ phylogenetic comparative methods to examine the evolution of body size in short-horned grasshoppers (Orthoptera: Caelifera: Acrididae) and test how this trait co-varies with SSD through evolutionary time. To this end, we constructed a phylogeny comprising a representative sample ($n = 85$ taxa) of all extant species present in the Iberian Peninsula (Presa *et al.*, 2007), including slant-faced grasshoppers (subfamily Gomphocerinae, 48 spp.), band-winged grasshoppers (Oedipodinae, 19 spp.), spur-throated grasshoppers (Catantopinae, 6 spp.) and other minority groups (e.g. silent slant-faced grasshoppers, Acridinae). Specifically, we first assessed the tempo and mode of evolution of body size and SSD, which allowed us to infer whether neutral or selective forces drove the evolution of these traits. Second, we examined patterns of body size evolution, including altitudinal clines of body size (Bergmann's rule) and allometric scalings of male and female body size (Rensch's rule). Finally, we analysed the proximate ecological factors (habitat specialization, substrate type, altitude) and evolutionary constraints (female fecundity, strength of sexual selection, length of the breeding season) that may underlie the evolution of male and female body size in these large-bodied insects. A brief summary of the different hypotheses examined in this work along with their main predictions is presented in Table 1. Our study constitutes the first to provide a comprehensive view about the factors promoting body size evolution and size dimorphism at the inter-specific level in Orthoptera.

Materials and methods

Sampling

Grasshoppers were collected during several field campaigns carried out throughout the Iberian Peninsula (see, e.g. Ortego *et al.*, 2010, 2015). Specimens were identified using current identification keys for Palearctic gomphocerine species (Harz, 1975; Pascual, 1978; Pardo *et al.*, 1991; Llucià-Pomares, 2002) and preserved in 96% ethanol. Our sample ($n = 85$ taxa) accounted for three quarters of all extant Acrididae species present in the Iberian Peninsula (83% and 66% of all Gomphocerinae and Oedipodinae species, respectively; Presa *et al.*, 2007). Thus, our sample is representative of the natural variation in this region (580 000 km²), including eight of the nine families into which Iberian short-horned grasshoppers have been grouped (Presa *et al.*, 2007). More than half of the species (56%) included in this study are endemic to the Iberian Peninsula or have a distribution restricted to Iberia, France and North Africa.

Molecular data

Genomic DNA was extracted from the hind femur of the specimens using a salt-extraction protocol (Aljanabi

Table 1 Hypotheses tested in this study for elucidating the possible influence of different evolutionary forces and ecological factors in explaining the evolution of body size and sexual size dimorphism (SSD) in short-horned grasshoppers.

(i) Hypothesis and rationale	(ii) Trait	(iii) Prediction
Fecundity selection Fecundity selection favours the evolution of large female body size	Ovariole number	Ovariole number correlates with female body size
Sexual selection Sexual selection favours the evolution of large male body size	Relative length of the stridulatory file	The length of the stridulatory file predicts the level of SSD
Intrasexual competition for resources Selection for larger male size is expected to be greater in species with a narrow ecological niche (specialist species)	Degree of habitat specialization (PDI)	Higher levels of SSD in generalist species
Substrate type ('gravity' hypothesis) Selective advantages for small individuals in vertical habitats	Substrate type (ground; plant)	Higher levels of SSD in plant-perching species
Seasonality (protandry) A shorter growing season should select for earlier maturation and smaller body size	Length of the breeding season	Smaller body sizes (and higher SSD) in species with a short reproductive window
Altitudinal cline (Bergmann's rule) Shorter growing seasons at higher elevations limit the body size organisms can achieve	Altitude (low; medium; high)	Smaller body sizes (and higher SSD) at higher altitudes
Isometric scaling of male and female size (converse of Rensch's rule) Selection pressures on females are the main driver of SSD evolution in female-biased species	Female vs. male body size	SSD increases with average body size ($b < 1$)

♂ Martínez, 1997). Four mitochondrial gene fragments – (1) cytochrome c oxidase subunit 1 (COI), (2) NADH dehydrogenase subunit 5 (ND5), (3) 12S rRNA (12S) and (4) a fragment containing parts of 16S rRNA (16S) – were amplified by polymerase chain reaction and sequenced. Two nuclear genes were also tested (elongation factor 1 α EF-1 and 28S ribosome unit), but these were discarded because their analysis yielded uninformative topologies with poor resolution (see also Song *et al.*, 2015). For some taxa, we failed to obtain reliable sequences, so we complemented our data set with additional sequences retrieved from GenBank. We mainly relied on sequences from two previously published phylogenies: Contreras & Chapco (2006) and Nattier *et al.* (2011).

Phylogenetic analyses

Sequences were aligned in MAFFT online version 7 (<http://mafft.cbrc.jp/alignment/server/>; Katoh & Standley, 2013) using a L-INS-i strategy. The alignments of the ribosomal genes (12S, 16S) contained highly unequal distribution of indels and thus were edited by hand to eliminate divergent regions of the alignment and poorly aligned positions. Protein-coding genes (COI, ND5) were checked for stop codons and their correct translation to amino acids in Geneious 8.1.7. The sequences of the four genes (12S, 16S, COI and ND5) were trimmed to 380, 469, 568 and 635 base pairs (bp), respectively, to reduce the proportion of missing data. We used Sequencematrix 1.7.8. (Vaidya *et al.*, 2011) to concatenate single alignment fragments, resulting in a concatenated matrix for a total length of 2055 bp. We were not able to obtain reliable sequences from all four

markers for some taxa. However, we opted for adding taxa with missing data as this generally increases phylogenetic accuracy (see Hughes & Vogler, 2004). The number of sequences per locus obtained was as follows: 79 for COI, 67 for ND5, 80 for 12S and 78 for 16S. *Pyrrogomorpha conica* (Acridoidea) was included as out-group in all phylogenetic analyses (Chapco & Contreras, 2011).

We performed phylogenetic inference and assessed the support of the clades following two methods: maximum likelihood (ML) and Bayesian inference (BI). We calculated the best-fit models of nucleotide substitution for each of the four genes according to the weighted Akaike Information Criterion (AIC) using jModelTest 0.1.1 (Posada, 2008). The TIM2+I+ Γ substitution model was selected for 12S, GTR+I+ Γ for 16S, TrN+I+ Γ model for ND5 and TPM3uf+I+ Γ was selected for COI. Maximum-likelihood analyses were conducted using GARLI version 2.0 (Zwickl, 2006) and PHYML (Guindon & Gascuel, 2006). A ML bootstrapping procedure was run in GARLI with two search replicates and 1000 bootstrap replicates. The best-fit substitution model for each partition (gene) was assigned by setting the rate matrix, nucleotide state frequencies and proportion of invariable sites. We selected the best (optimal) tree and obtained support for the clades from a majority-rule (50%) consensus tree computed in PAUP* version 4 (Swofford, 2002).

Bayesian analyses were conducted using MrBayes 3.2 (Ronquist *et al.*, 2012) applying a nucleotide substitution model specific to each partition (gene): HKY+I+ Γ for 12S and COI, and GTR +I+ Γ for 16S and ND5. We performed two independent runs with four simultaneous Markov chain Monte Carlo (MCMC) chains,

running for a total of 10 million generations and sampled every 1000th generation. The first 25% of samples were discarded as burn-in and a consensus tree from the remaining 7501 trees was built using the 'sumt' command before visualization in FigTree v.1.3.1 (Rambaut, 2009) (see Supporting Information). A second Bayesian analysis was run with BEAST 1.8.0 (Drummond *et al.*, 2012) to estimate the relative time of divergence of the studied taxa. Runs were carried out under an uncorrelated lognormal relaxed-clock model and applying a Yule process as tree prior. Two calibration points were used to impose age constraints on some nodes of the tree allowing us to translate the relative divergence times into absolute ones. We employed as a first calibration point the split between Gomphocerinae and Oedipodinae, estimated to have occurred ~100 Mya ago. This estimate is based on dated ancient cockroach fossils (Gaunt & Miles, 2002; Fries *et al.*, 2007). As a second calibration point, we used the divergence between *Sphingonotus azureescens* (mainland species) and *S. guanchus* (endemic to La Gomera Island, Canary Islands), whose estimated age is around 3.5 Mya (Husemann *et al.*, 2014). *Sphingonotus guanchus* was only included in the BEAST analysis for calibration purposes. Substitution parameters were based on analyses previously conducted in jModeltest. We performed two independent runs of 100×10^5 generations, sampled every 1000 generations. We then used Tracer 1.4.1. (Rambaut & Drummond 2009) to examine convergence, determine the effective sample sizes (EES) for each parameter and compute the mean and 95% highest posterior density (HPD) interval for divergence times. We confirmed EES > 200 was achieved for all parameters after the analyses. Tree and log files (9500 trees after a 5% burn-in) of the two runs were combined with LogCombiner 1.4.7 (Drummond *et al.*, 2012), and the maximum clade credibility (MCC) tree was compiled with TreeAnnotator 1.4.7. The obtained phylogenies were robust and largely consistent with previous studies (Nattier *et al.*, 2011).

Morphological data

Adult size was characterized from the length of the left hind femur. We used femur length as an indicator of body size as the total length of females varies substantially with the oviposition cycle (Chapman & Joern 1990; Hochkirch & Gröning, 2008). Femur length was strongly correlated with structural body length excluding the abdomen (i.e. head + thorax) in both sexes (males: $R^2 = 0.70$, $P < 0.001$; females: $R^2 = 0.65$, $P < 0.001$). Femur length scales isometrically with body length ($\beta_{\text{males}} = 0.964$, $\beta_{\text{females}} = 1.048$) and thus constitutes a good proxy for adult body size (see also Laiolo *et al.*, 2013; Anichini *et al.*, 2016; Bidau *et al.*, 2016). Hind legs were carefully removed from the body of adults in the laboratory under a ZEISS stereomicroscope

(SteREO Discovery V.8; Carl Zeiss Microscopy GmbH, Germany) and photographed using ZEISS image analysis software (ZEN2). Measurements of femur length were made on a total of 720 individuals, 365 males and 355 females (4–5 individuals of each sex per species). Because SSD is practically always female sex-biased in most orthopteroids, we quantified sexual size dimorphism as the ratio of female to male femur length (the simplest SSD estimator; see Lovich & Gibbons, 1992). In addition, we quantified the relative length of the stridulatory file (i.e. length of the stridulatory file/femur length*100) for Gomphocerinae species. The stridulatory file consists of a row of pegs located on the inner side of the femur of each hind leg (e.g. Jago, 1971). Gomphocerine grasshoppers produce acoustic signals (songs) by rubbing this structure against the forewings. Calling songs are used to search for conspecific mates and, thus, the evolution of the stridulatory apparatus is expected to be subject to sexual selection (von Helversen & von Helversen, 1994; Mayer *et al.*, 2010; Nattier *et al.*, 2011). Males with larger sound-generating organs are able to produce low-frequency sound, which is associated with larger male body size (see Anichini *et al.*, 2016 and references therein) and under directional selection due to female mate preference (Klappert & Reinhold, 2003). For example, in a comparative study of 58 bushcricket species, Montealegre-Z (2009) showed that the length of the stridulatory file correlated positively with male body size and pulse duration. In this sense, there is evidence that grasshopper females are able to judge a male's condition and health from the acoustic signals he produces (e.g. Stange & Ronacher, 2012). Hence, we used the relative length of the stridulatory file as a proxy for the strength of precopulatory sexual selection in this subfamily.

Life history and ecological data

Fecundity

Given that large females can generally allocate more resources and energy to reproduction resulting in more offspring and/or higher-quality offspring, fecundity selection usually favours larger body size in females (reviewed in Pincheira-Donoso & Hunt, 2017). We tested for fecundity selection using mean ovariole number (which reflects the number of eggs produced in a discrete time interval) as an index of a species' potential fecundity. Ovariole number is a strong determinant of fecundity and therefore fitness because it sets the upper limit for reproductive potential (i.e. females with more ovarioles can produce more eggs in a discrete time interval) (Bellinger & Pienkowski, 1985; Stauffer & Whitman, 1997; Taylor & Whitman, 2010). This parameter was extracted from different sources (Ingrisch & Köhler, 1998; Reinhardt *et al.*, 2005; Schultner *et al.*, 2012) for a subset of species ($n = 20$; see Supporting Information).

Substrate use

The substrate or structure on which a species rests can have major implications for the evolution of body size and SSD. For example, Moya-Laraño *et al.* (2002) proposed the so-called gravity hypothesis to explain patterns of SSD in spiders whereby species building webs high in the vegetation are predicted to show greater SSD than those that build lower down. However, Brandt & Andrade (2007) proposed that the prevalence of female-biased SSD in species that occupy elevated substrates may be explained by selective advantages for small males in vertical habitats and for large males of low-dwelling species to run faster on the ground. In this sense, a previous study has shown that life form (substrate type use) correlated with individual size in grasshoppers from Inner Mongolia (Yan & Chen, 1997); larger species were typically terricoles, whereas the smaller ones were typically planticoles. Here, we tested if ground-dwelling grasshoppers exhibit a lower level of SSD than those species that perch on plants after correcting for phylogeny. To that end, each species was assigned to one of the two categories (ground vs. plant-perching) based on the literature (e.g. Defaut & Morichon, 2015), personal observations and a survey of photographs available in open-access online repositories (<http://www.biodiversidadvirtual.org>; <http://www.pyrgus.de>; <http://www.orthoptera.ch>).

Length of the breeding season

Season length has been postulated as another important factor in driving body size evolution (see, e.g. Gotthard, 2001). Individuals can become larger by lengthening the growth period but at the expense of a high cost: they may die before reproducing. In contrast, for example in ephemeral habitats, an individual can rush through its development to reach adulthood faster and reproduce (Roff, 1980; Blanckenhorn & Demont, 2004). Thus, protandry (faster development of males with respect to females) should be favoured by sexual selection in highly seasonal habitats (Morbey & Ydenberg, 2001; Esperk *et al.*, 2007; Lehmann & Lehmann, 2008). In this sense, the length of the breeding season in conjunction with ambient temperature has been postulated as the main cause for the existence of altitudinal phenotypic clines in many ectothermic species with short generation times (Masaki, 1967; Chown & Klok, 2003). In Orthopterans, several studies have reported a reduction in body size with altitude (e.g. Berner & Blanckenhorn, 2006; Bidau & Martí, 2007a; Eweleit & Reinhold, 2014; but see also Sanabria-Urbán *et al.*, 2015). Accordingly, we would expect small adult size in species with a short lifespan and/or species inhabiting higher altitudes (supposedly more seasonal habitats) because a shorter growing season should select for earlier maturation and smaller body size. To test such a hypothesis, we compiled information on the length (in months) of the life cycle of each species from the

available literature (e.g. Llucià-Pomares, 2002) and our own field observations. The length of the breeding season oscillated between 2 and 12 months, that is, from species only present as adults during the summer period to those present all year round. Species that have adults present year round likely have more than one generation each year (e.g. bivoltine species) and thus a period of sexual diapause. Because it might compromise the reliability of our results, we repeated our analyses after excluding such species (five excluded species). In addition, a subset of species was classified into three categories (low altitude, medium altitude, high altitude) according to the altitudinal range in which these species can be found (e.g. Pardo & Gómez, 1995; Llucià-Pomares, 2002; Olmo-Vidal 2006; authors, *pers. obs.*). Those species ($n = 16$) with a broad altitudinal range (e.g. from 0 to 2000 m; see Supporting Information) were discarded from this analysis.

Habitat specialization

The level of ecological specialization of an organism, that is, its variance in performance across a range of environmental conditions or resources, has implications in terms of population density and local competition (e.g. Devictor *et al.*, 2010; Parent *et al.*, 2013), two factors often associated with the extent of sexual dimorphism. Selection for larger male size is expected to be greater in species with a narrow ecological niche (i.e. specialist species) and/or limited dispersal ability due to strong intrasexual competition for resources. Accordingly, we predict higher levels of SSD in generalist species. To obtain a measure of ecological specialization, we calculated the so-called 'Paired Difference Index' (PDI) (Poisot *et al.*, 2011, 2012) from a species-habitat matrix in which we rated the level of association of each species (from complete generalist, 0, to complete specialist, 3) with the nine most common habitats in which these species can be found (see Supporting Information). The PDI is a robust specialization index which takes into account not only the number of resources used by a species, but also the strength of the association between the species and its resources (Poisot *et al.*, 2012). Scores of species-habitat association were obtained directly from the literature (research articles, monographs and field guides) and our own personal observations (Table S3). PDI values were computed using the *BIPARTITE* package in R (Dormann *et al.*, 2016).

Phylogenetic comparative analyses

For each studied variable (male size, female size, SSD, length of the breeding season, length of the stridulatory file, ovariole number, PDI), we assessed the amount of phylogenetic signal, a measure of how similar closely related species are to one another for a given trait. To assess phylogenetic signal, we used Pagel's lambda (λ ; Pagel, 1999) and Blomberg's *K* (Blomberg *et al.*, 2003)

computed using the ‘*phylosig*’ function in the R package *PHYTOOLS* (Revell, 2011). To visualize substrate (binary variable; ground: 0; plant-perching: 1) variation among species on the phylogenetic tree, we used maximum-likelihood reconstruction in *MESQUITE* v. 3.04 (Maddison & Maddison, 2015). We also reconstructed ancestral states for our focal trait, SSD, in *MESQUITE*.

We tested for departure from a null model of constant rate of diversification using the γ statistic as implemented in *ape* (Paradis *et al.*, 2014). A significantly negative value of γ indicates a decelerating rate of cladogenesis through time. The γ statistic is biased by incomplete taxon sampling, because the number of divergence events tends to be increasingly underestimated towards the present (favouring negative values for the γ). Therefore, we corrected for undersampling using the Markov chain constant-rates (MCCR) test (Pybus & Harvey, 2000) as implemented in the *LASER* package (Rabosky, 2006). Recent *MEDUSA* analyses performed by Song *et al.* (2015) indicate that the lineage leading up to Acrididae has undergone a significant increase in diversification rate with little or no extinction. Thus, values of γ are unlikely to be biased by extinction rates.

We investigated the mode of male and female body size evolution by comparing fits of these traits to four different models of evolution using the Akaike information criterion (Akaike weights, AICw, and size-corrected Akaike values, AICc): (i) pure Brownian Motion (null) model (BM), (ii) Ornstein-Uhlenbeck (OU), (iii) ‘early-burst’ (EB) and (iv) time-variant rate (TVR) model. Under BM, traits evolve along a random walk whereby each change is independent of the previous change (morphological drift; Felsenstein, 1985). The OU model describes a random walk with a single stationary peak, such that trait values have a tendency to return to an optimal value (θ) (Hansen, 1997; Butler & King, 2004). Under an EB model, the net rate of evolution slows exponentially through time as the radiation proceeds (Blomberg *et al.*, 2003), whereas the TVR model is similar to an EB model but also allows an exponential increase of evolutionary rates through time (Pagel, 1999). The TVR model can be used to evaluate the nonconstant rate of evolution through time using the path-length scaling parameter Pagel’s delta, δ . This parameter detects differential rates of evolution over time (i.e. $\delta = 1$ means gradual evolution). If $\delta < 1$, shorter paths contribute disproportionately to trait evolution (decelerating evolution), whereas if $\delta > 1$ is the signature of accelerating evolution as time progresses (see Hernández *et al.*, 2013). Specifically, we expected female size to show a trend towards larger sizes (i.e. directional selection for increased female size), whereas males would likely be maintained at optimal values (i.e. directional selection for the maintenance of small male size according to an OU model). From these models, we calculated the evolutionary rate (σ^2) for each

sex to determine whether body size evolution of males was faster or slower than body size evolution of females. Evolutionary models were run using the R package *GEIGER* (Harmon *et al.*, 2008). Additionally, we applied two complementary methods: the morphological diversity index (MDI, Harmon *et al.*, 2003) and the node-height test (Freckleton & Harvey, 2006) to analyse patterns of evolution. Both methods test for departure from Brownian motion but differ in the approach used to test for this departure. First, we calculated disparity through time (DTT) plots using the ‘*dt*’ function in the *GEIGER* package. DTT analyses compare phenotypic diversity simulated under a Brownian Motion model with observed phenotypic diversity among and within subclades relative to total disparity at all time steps in a time-calibrated phylogeny. Low (i.e. negative) values of relative disparity indicate that most morphological disparity originated early in the history of the group (early divergence), whereas high (positive) values indicate that most morphological disparity originated more recently compared to a random walk pattern (recent phenotypic divergence). Values near 0 indicate that evolution has followed BM. The MDI was calculated as the sum of the areas between the curve describing the morphological disparity of the trait and the curve describing the disparity under the null hypothesis of BM (1 000 simulations). Finally, we used the node-height test (Freckleton & Harvey, 2006) to test whether grasshopper body size evolution has slowed over time. We computed the absolute value of standardized independent contrasts for body size on our MCC tree and correlated them with the height of the node at which they are generated. A significant negative relationship between absolute contrast value and node age implies that rates of body size evolution slow down over time according to a niche-filling model (‘early-burst’ of trait evolution).

Ecological correlates of body size and SSD

To explore the association between SSD-body size and our continuous ecological (habitat specialization, breeding season length) and reproductive (fecundity, length of the stridulatory file) variables, we used phylogenetic generalized least squares (PGLS_i). Maximum-likelihood estimates of the branch length parameters delta (a measure of disparity of rates of evolution through time, see above), lambda (a measure of phylogenetic signal) and kappa (which contrasts punctuational vs. gradual trait evolution, see Hernández *et al.*, 2013) were obtained to optimize the error structure of the residuals in each comparison as recommended by Revell (2010). PGLS regression analyses were performed using the R package *CAPER* (Orme, 2013) and graphically visualized by means of phylogenetically independent contrasts (PIC) computed using the PDAP:PDTREE module in *MESQUITE* (Midford *et al.*, 2005). To test the influence of

categorical variables (substrate, altitude class) on our focal traits independently from the phylogeny, we employed *phyANOVA* (10 000 simulations) as implemented in the R package *PHYTOOLS* (Revell, 2011).

We tested for greater evolutionary divergence in male size compared with female size (Rensch's rule test) by regressing log-transformed male body size against log female body size using phylogenetic major axis regressions (PRMA; Revell, 2011), a method that accounts for the shared evolutionary history of species. This analysis was performed at two taxonomic levels, across our entire phylogeny and within the two largest subfamilies (Gomphocerinae, Oedipodinae) because Rensch's rule was originally proposed for 'closely related species' (Rensch, 1950). We tested if the slope (β) of the regression of body size of males on females was larger than 1 (as predicted by Rensch's rule), smaller than 1 (converse of Rensch' rule) or not different from 1 (i.e. $\beta = 1$; isometric pattern) (see Ceballos *et al.*, 2013 for more details about the possible scenarios for the relationship between SSD and body size of males and females). Statistical significance of the allometric pattern was determined based on the 95% confidence intervals (CI) of β using the *SMATR* R package (Warton *et al.*, 2012).

Results

Phylogenetic signal

Both male and female body size exhibited a strong phylogenetic signal (male body size: $\lambda = 0.955$, $P < 0.01$; $K = 0.267$, $P = 0.016$; female body size: $\lambda = 0.956$, $P < 0.001$; $K = 0.213$, $P = 0.03$), which indicates that the body size of related species is more similar than expected under Brownian Motion. Accordingly, we also found a strong phylogenetic signal for SSD ($\lambda = 0.904$, $P = 0.03$; $K = 0.225$, $P < 0.01$; Fig. 1). The relative length of the stridulatory file showed a moderate phylogenetic signal ($\lambda = 0.589$, $P = 0.09$; $K = 0.114$, $P = 0.02$), whereas the level of ecological specialization (PDI) ($\lambda \sim 0$, $P = 1$; $K = 0.06$, $P = 0.36$) and the length of the breeding season ($\lambda = 0.627$, $P = 0.01$; $K = 0.107$, $P = 0.08$; Fig. 1) did not show phylogenetic inertia. Ovariole number showed a strong phylogenetic signal ($\lambda \sim 1$, $P < 0.001$; $K = 1.987$, $P < 0.001$). Substrate type (ground vs. plant-perching) seems to be a conservative trait in short-horned grasshoppers; ground-species are predominant in the Oedipodinae subfamily, whereas plant-perching species are more abundant within the Gomphocerinae subfamily (see Fig. 1).

Tempo and mode of body size evolution

The rate of diversification accelerated with time ($\gamma = 1.68$) indicating that rapid diversification occurred late in the evolutionary history of the group. When comparing alternative models of evolution across the entire

data set, the OU model (Brownian Motion with selective constraint) exhibited the best fit for the evolution of male body size, whereas the OU and the TVR models were equally supported for female body size ($\Delta\text{AICc} < 2$) (Table 2). When restricting our analyses to the Gomphocerinae subfamily, we found that the best-supported model for the evolution of both male and female body size was TVR (Table 2), suggesting that trait evolution is nonconstant through time. In Oedipodinae, the BM model provided a better fit than the other models for the evolution of male body size, whereas BM and the two nonconstant models (EB and TVR) were similarly supported ($\Delta\text{AICc} < 2$) in females (Table 2). The comparison of evolutionary rates between sexes indicated that body size evolved at a similar pace (Table 2).

Maximum-likelihood estimates of δ computed for all taxa and for each of the two subfamilies were high (δ values: all clades; male body size: 2.45, female body size: 3.00; *Gomphocerinae*; male body size: 2.53, female body size: 3.00; *Oedipodinae*; male body size: 3.00, female body size: 3.00) suggesting that longer paths (i.e. later evolution of the trait in the phylogeny) contribute disproportionately to trait evolution ('late-burst').

DTT plots showed that phenotypic disparity within lineages is greater than expected under a BM model late in the diversification of Acrididae (Fig. 2). We obtained positive MDI statistics, indicating that the proportion of total morphological disparity within clades was less than expected by a Brownian Motion model for all taxa and each of the two subfamilies (average MDI values: all clades; male body size: 0.138, female body size: 0.101; *Gomphocerinae*; male body size: 0.342, female body size: 0.212; *Oedipodinae*; male body size: 0.088, female body size: 0.357).

Concordant with late shifts in the acceleration of the net diversification rate, the node-height test resulted in a positive but nonsignificant relationship between the absolute values of standardized length contrasts and node age in both sexes (*males*: $t = 1.01$, d.f. = 83, $P = 0.32$; *females*: $t = 0.72$, d.f. = 83, $P = 0.47$) across all taxa. For the Gomphocerinae subset, we found a positive and significant relationship between the absolute value of independent contrasts and the height of the node from which they were generated (*males*: $t = 2.46$, d.f. = 45, $P = 0.02$; *females*: $t = 3.11$, d.f. = 45, $P = 0.003$), indicating that body size evolution has increased through time. When restricting our analyses to the Oedipodinae subfamily, the node-height test yielded a negative and nonsignificant relationship for the evolution of male and female body size (*male body size*: $t = -0.60$, d.f. = 18, $P = 0.55$; *female body size*: $t = -1.02$, d.f. = 18, $P = 0.32$).

Ecological correlates of body size and SSD

We found a negative significant association between SSD and female body size (PGLS; estimate:

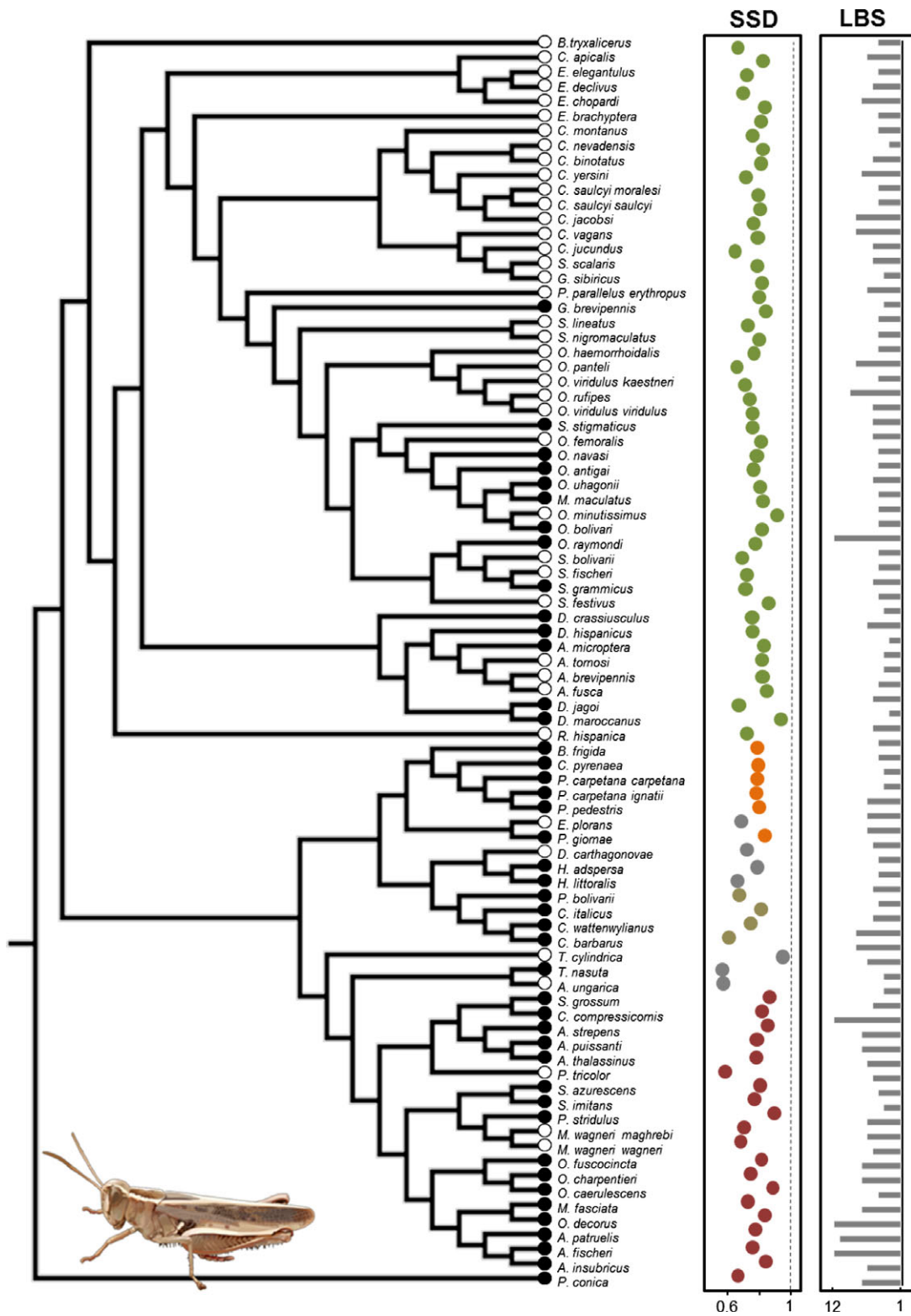


Fig. 1 Variation in degree of sexual size dimorphism (SSD) and length of the breeding season (LBS, in months) among the studied Acrididae species (in the SSD scatterplot species belonging to the same subfamily are indicated with the same colour). Colour of dots next to tips in the tree denotes the main substrate used by each species (black dots: ground; white dots: plant).

Table 2 Relative support for alternative evolutionary models of male and female body size in short-horned grasshoppers. BM: Brownian Motion; OU: Ornstein-Uhlenbeck; EB: early-burst; TVR: time-variant rate model. σ^2 denotes the estimated reproductive rate for each sex. AICc, corrected Akaike's information criterion (AIC) value; AICw, AICc weight. The best-fit model/s is/are highlighted in bold type.

	Sex	BM			OU			EB			TVR		
		AICc	AICw	σ^2	AICc	AICw	σ^2	AICc	AICw	σ^2	AICc	AICw	σ^2
All taxa	Male	-152.94	0.135	0.240	-156.01	0.627	0.264	-150.79	0.191	0.240	-153.63	0.191	0.159
	Female	-134.22	0.109	0.299	-136.82	0.398	0.332	-132.07	0.037	0.299	-137.08	0.455	0.191
Gomphocerinae	Male	-102.20	0.097	0.323	-102.24	0.099	0.334	-99.92	0.031	0.323	-106.33	0.771	0.220
	Female	-91.85	0.002	0.400	-92.15	0.002	0.413	-89.57	0.001	0.400	-104.56	0.995	0.233
Oedipodinae	Male	-33.43	0.603	0.003	-30.73	0.156	0.003	-30.74	0.144	0.001	-30.76	0.095	0.004
	Female	-32.33	0.439	0.001	-29.62	0.113	0.001	-31.38	0.274	0.002	-30.46	0.172	0.006

-0.444 ± 0.102 , $t = -4.34$, $P < 0.001$) but not such association between SSD and male body size (PGLS; $t = 1.05$, $P = 0.30$) across taxa. A similar result was found for the Gomphocerinae subset (PGLS, *female size*: estimate: -0.458 ± 0.101 , $t = 4.492$, $P < 0.001$; *male size*: $t = 0.52$, $P = 0.60$). In contrast, in Oedipodinae, we obtained the opposite pattern: a significant association between SSD and male body size (PGLS; estimate: 0.666 ± 0.138 , $t = 4.82$, $P < 0.001$) but not between SSD and female body size (PGLS; $t = 0.054$, $P = 0.95$). Overall, the emerging picture was that both sexes tend to be progressively more similar as size increases. When

size decreases within one lineage through evolutionary time, then male size decrease disproportionately with respect to female body size.

When testing for fecundity selection, we failed to find a significant relationship between ovariole number (a good proxy for the reproductive potential of a given species) and female body size (PGLS; $n = 20$, $t = 0.067$, $P = 0.947$). When only considering gomphocerine grasshoppers, no significant association was found between the size-corrected length of the stridulatory file and SSD (PGLS; $n = 48$, $t = 0.12$, $P = 0.90$) (Fig. 3). Short-lived species did not show either a greater degree

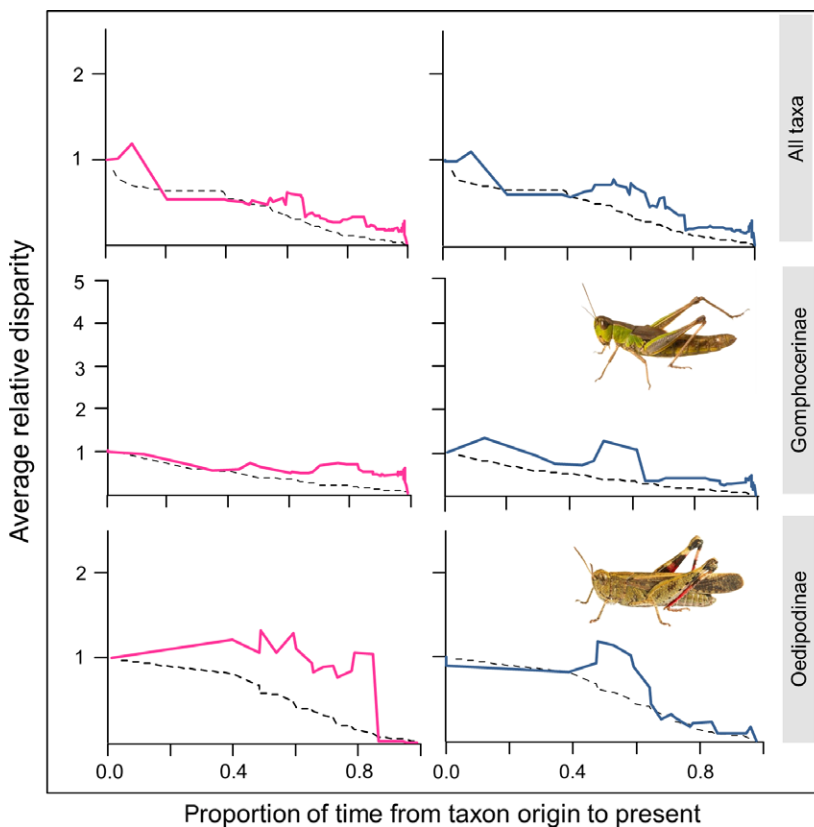


Fig. 2 Relative disparity plots for short-horned grasshoppers compared with expected disparity based on phylogenetic simulations. The continuous line shows the actual pattern of phenotypic disparity (graphs are colour-coded according to sex; pink colour: female body size, blue colour: male body size), and the dotted line represents the median result of Brownian model simulations (1000 simulations). Time is relative to phylogenetic depth from the base of the phylogeny on the left to the terminal tips on the right.

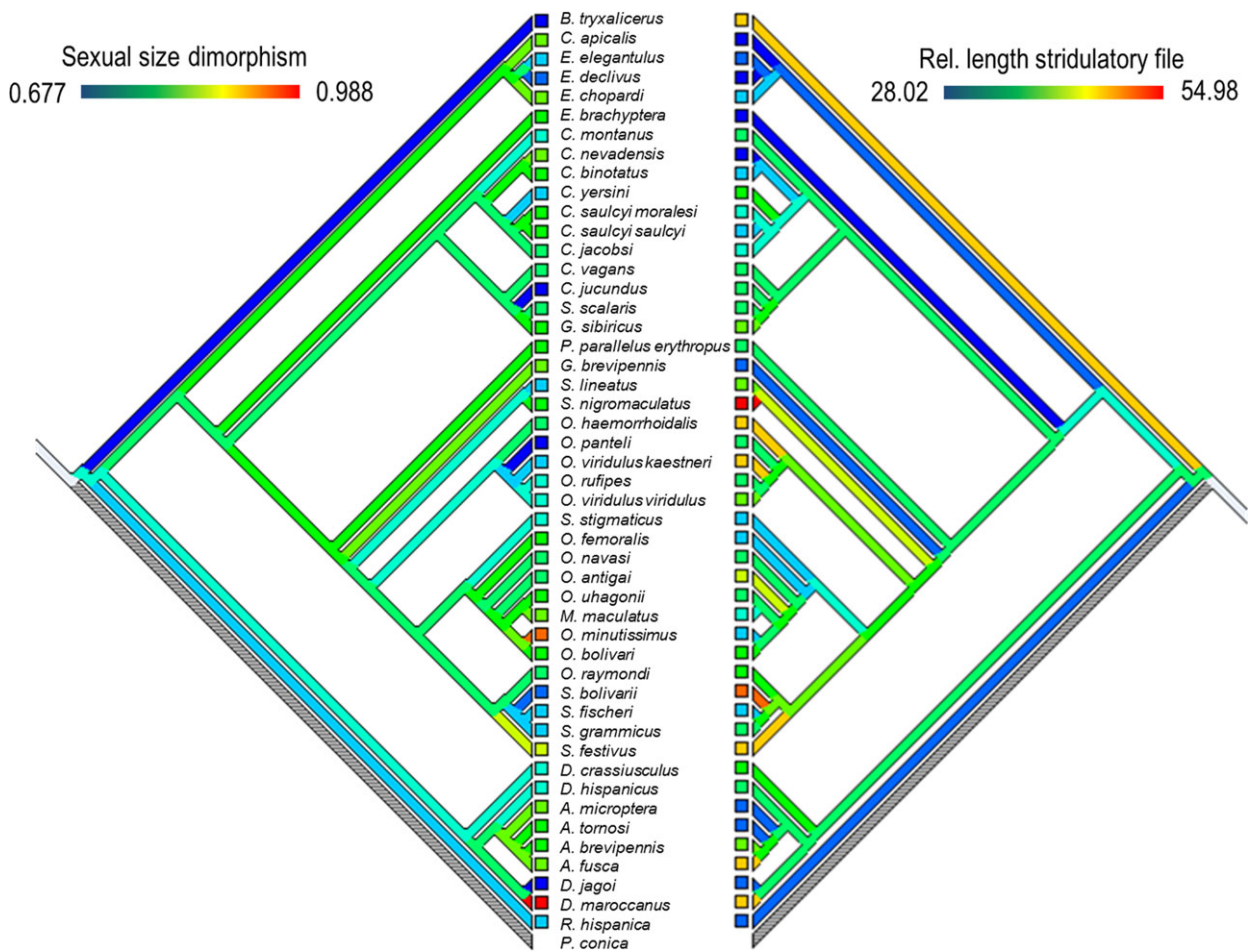


Fig. 3 Reconstructed evolution of sexual size dimorphism (sexual size dimorphism ratio, with lower values indicating more dimorphic species and higher values indicating less dimorphic species, so 1 denotes monomorphism) and relative (size-corrected) length of the stridulatory file (length of the stridulatory file/femur length*100) in the grasshopper subfamily Gomphocerinae. Colours denote size classes.

of development of the stridulatory organ as expected if the strength of sexual selection is higher in species with a shorter breeding period (PGLS; $n = 48$, $t = -0.59$, $P = 0.55$). The degree of female-biased SSD was phylogenetically correlated with the length of the breeding season; species with a long phenology are more dimorphic in size (i.e. females > males) than those with a short reproductive window (PGLS; estimate: -0.010 ± 0.004 , $n = 85$, $t = -2.16$, $P = 0.035$; Fig. 1, see also Fig. S1 for a representation of this relationship using data noncorrected for phylogeny, OLS regression). Such a relationship became more significant when excluding year-round species (length of the breeding season = 11–12 months) from the data set (PGLS; estimate: -0.011 ± 0.004 , $n = 80$, $t = -2.34$, $P = 0.021$; Fig. 4). Although we found that habitat specialist species (high PDI values) have a shorter breeding cycle than more generalist species (PGLS, estimate: -6.596 ± 1.545 , $n = 85$, $t = -4.27$, $P < 0.001$), there

was no significant relationship between the level of SSD and the degree of ecological specialization (PDI index) (PGLS; $t = -1.16$, $P = 0.25$).

Regarding categorical variables, we did not find significant differences between ground and plant-perching species in terms of SSD (PhyloANOVA; $F_{1,83} = 2.72$, $P = 0.51$), male body size ($F_{1,83} = 0.69$, $P = 0.75$) or female body size ($F_{1,83} = 0.02$, $P = 0.96$) after controlling for the shared evolutionary history of species. Lastly, there was a trend towards low altitude species being larger than those inhabiting medium- and high-altitudes, but differences were not statistically significant after correcting for phylogeny (PhyloANOVA; female body size: $F_{2,66} = 8.51$, $P = 0.13$; male body size: $F_{2,66} = 6.38$, $P = 0.21$) (Fig. 5). The length of the breeding season, but not the level of SSD, differed significantly among altitude categories (breeding season length: $F_{2,66} = 4.22$, $P = 0.018$; SSD: $F_{2,66} = 3.23$, $P = 0.45$), being shorter at higher altitudes (mean \pm

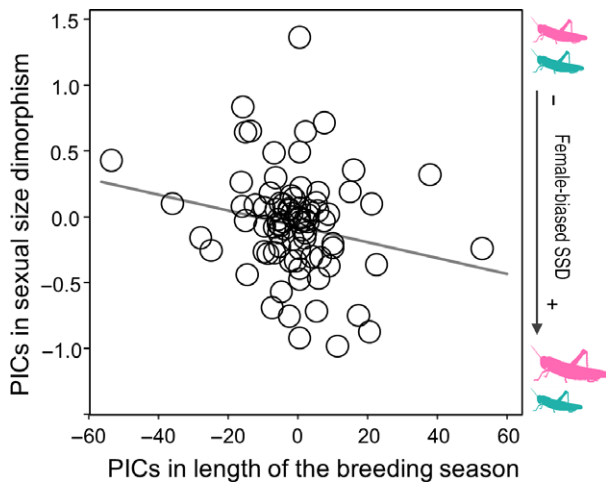


Fig. 4 Relationship between sexual size dimorphism (SSD) and the length of the breeding season represented in the form of standardized phylogenetic independent contrasts (PICs). Year-round species (length of the breeding season = 11–12 months) were excluded from this analysis. More negative values for the SSD index indicate a higher degree of female-biased dimorphism, whereas the difference in size between both sexes decreases (towards sexual size monomorphism) in the opposite direction. Thus, females became disproportionately larger than males as the length of the breeding season increases.

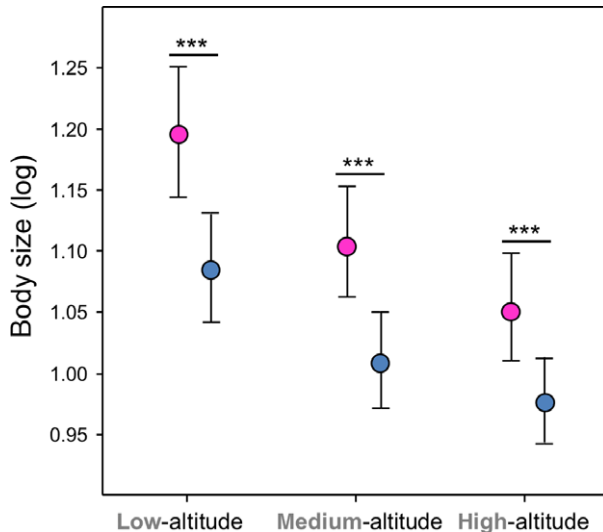


Fig. 5 Differences in average male (blue dots) and female (pink dots) body size between grasshopper species inhabiting low (<800 m a.s.l., $n = 18$), medium (800–1500 m, $n = 25$) and high-altitudes (> 1500 m, $n = 26$). Statistical significance determined by paired t -tests is indicated ($***P < 0.001$).

S.D. breeding season length; low altitude: 5.83 ± 1.01 , medium altitude: 5.36 ± 2.46 , high altitude: 4.07 ± 2.70 months).

Allometry of SSD: Do Acrididae grasshoppers conform to Rensch's rule?

The degree of SSD was similar across all taxa (all taxa: 0.801 ± 0.076) and when considering the two most speciose subfamilies separately (Gomphocerinae: 0.808 ± 0.062 ; Oedipodinae: 0.817 ± 0.076). Our results supported the existence of a pattern consistent with the converse to Rensch's rule as the relationship of male body size with female body size across all taxa had a slope less than one (PRMA; $\beta = 0.895$, CI: 0.804–0.940). However, we found an isometric pattern (i.e. the slope did not differ significantly from 1) when data were analysed separately for Gomphocerinae (PRMA; $\beta = 0.898$, CI: 0.869–1.106) and Oedipodinae (PRMA; $\beta = 0.866$, CI: 0.846–1.422).

Discussion

Evolution of body size in a recent radiation

The very short branches deep in our phylogeny suggest recent divergence and thus rapid speciation in some lineages. This is in agreement with the findings of Song *et al.* (2015), who suggested that Acrididae may have undergone an explosive adaptive radiation during the Cenozoic when global climate became temperate and grasses evolved and became dominant. The evolution of a new niche space (grasslands) may have powered the radiation of graminivorous species, especially strong fliers like band-winged grasshoppers (Oedipodinae) (Song *et al.*, 2015). Later, climatic oscillations during the Pleistocene led to thermophilic species (as most Gomphocerinae are) being restricted to southern refuges during glacial stadials. This probably resulted in divergent evolution of allopatric populations by geographic isolation (Taberlet *et al.*, 1998; Hewitt, 1999; Mayer *et al.*, 2010). This plausible scenario matches with the notion raised by Schluter (2000) who noted that 'a continuous spread to new environments is the dominant trend of adaptive radiation'. Although the term 'adaptive radiation' is frequently used to describe a slowdown in diversification and morphological evolution after an initial phase of rapid adaptation to vacant ecological niches ('niche filling'), recent studies stress that the definition of adaptive radiation should not be conditioned by the existence of early-bursts, which indeed seem to be uncommon across the tree of life (Harmon *et al.*, 2010; Pincheira-Donoso *et al.*, 2015). Rather, an adaptive radiation should be defined as the process in which a single lineage diversifies into a variety of species, occurring at a fast net rate, irrespective of the timing (Harmon *et al.*, 2010). In this study, model comparison, maximum-likelihood values for the δ parameter (which tests for acceleration vs. deceleration) and node-height tests provide no significant support for an 'early-burst' followed by a slowdown in

morphological evolution in this taxonomic group. Instead, we found the opposite pattern; the high values of δ indicate recent, high rates of phenotypic divergence, whereas the results of the node-height tests indicate that it increased as the number of taxa increased. This pattern suggests that most divergence seems to be concentrated later in the evolutionary history of this group (i.e. recent and rapid diversification; see also Boucher *et al.*, 2012; Edwards *et al.*, 2015).

Body size evolution in Iberian Acrididae is inconsistent with a Brownian Motion process, indicating that selection and not drift underlies body size evolution in this group. This finding is consistent with the notion that directional selection almost universally favours larger and heavier individuals and adds to the growing pile of evidence showing that both natural and sexual selection are the major evolutionary forces driving this process in most organisms (e.g. Heim *et al.*, 2014; Baker *et al.*, 2015; see also Blanckenhorn, 2000 for a review). Our results show that body size variation is best explained by a time-dependent model (TVR model: Pagel, 1997, 1999), which indicates an accelerated rate of body size evolution over time. However, across all subfamilies the OU model provided a better fit to the data suggesting a process of stabilizing selection in which variation of body size revolves around stationary optimal values. That is, deviant body sizes are 'polished' towards an optimum value, which was estimated to be around 9.79 and 13.49 mm for male and female femur length, respectively. The pattern described for the entire family reflects the existence of scale-dependent processes that act differentially across stages of the diversification process (see Ceballos *et al.*, 2013). Taken together, our results evidence that even in taxonomic groups showing limited morphological and ecological disparity, natural selection seems to play a more important role than genetic drift in driving the radiation process.

Ecological correlates of body size and SSD

Although the predominance of female-biased SSD in Acrididae suggests that fecundity selection may be the most important selective force acting on this family, we failed to find support for this hypothesis as ovariole number and female body size were not correlated. However, this specific analysis was performed using a reduced data set ($n = 20$), and thus, our results should be interpreted cautiously. Alternatively, it is also likely that fecundity selection acts primarily at intraspecific level, preventing us to detect its effect through our analyses. Regarding sexual selection, we failed to find a significant correlation between the relative length of the stridulatory file (our proxy to measure the strength of sexual selection) and the level of SSD. This may indicate that sexual selection is not driving SSD in Gomphocerinae or, alternatively, that this trait does not

accurately reflect the strength of this selective force at interspecific level due to its strong genetic component (Saldamando *et al.*, 2005). The size-corrected length of the stridulatory apparatus showed a nonphylogenetic signal, supporting the view that in gomphocerine grasshoppers the value of acoustic characters as an indicator of phylogeny is very limited despite the fact that they have long been used to resolve taxonomic uncertainties at the species level (Ragge, 1987; Ragge & Reynolds, 1988).

Substrate use (ground or plant-perching) may be expected to affect body size as different functional demands between the two types are expected to generate different selective peaks. However, we did not find evidence for the gravity hypothesis; ground-dwelling species did not show a significantly larger size than plant-perching species as expected if climbing ability selects for reduced body size (Moya-Laraño *et al.*, 2002). Thus, we failed to detect strong selection for increased female size (fecundity selection) or for the maintenance of small male size (agility hypothesis) in these insects. Regarding the other ecological variable, habitat specialization (PDI index), we did not find a higher level of SSD in generalist species in which selection for large male body size should be smaller in comparison with species with specific habitat requirements and whose populations may be affected by higher intrasexual competition. A plausible explanation for this result is that male–male competition for food resources may not be intense enough to boost the evolution of male body size as most species depend on food resources that are rarely limited (e.g. gramineous). This finding thus reinforces the view that both inter- and intrasexual competition for food is unlikely in small herbivorous organisms and that these play a subsidiary role in the evolution of SSD (Fairbairn *et al.*, 2007).

Larger-bodied and slower developing arthropods like Orthoptera are expected to be more affected by seasonal limitations than faster developing insects. Shorter breeding season lengths should promote life history adaptations leading to smaller body size to facilitate the completion of the life cycle within the reduced time available for development. When the developmental time window is short, individuals reach maturity at smaller sizes and develop faster. When the length of the breeding season is longer, more time is available to reach the reproductive stage at a larger size (Berner & Blanckenhorn, 2006). Contrary to our initial expectation, we found a lower level of SSD in species with a short phenology. SSD was more pronounced in species with longer breeding seasons, an effect that seemed to be caused by a larger difference in size between sexes. It suggests that selection pressure for large body size in males may be stronger in ephemeral or more seasonal environments (i.e. in species with a short reproductive window). Alternatively, this result may be due to the fact that in most grasshopper species the females are

more variable in size than males (e.g. female size variation is exceptionally high in *Calliptamus* species), and that unfavourable environmental conditions may compromise body size (Teder & Tammaru, 2005). This indicates that time constraints do not seem to impose limits for the evolution of male body size (rather the contrary, it seems to be favoured) and that female body size is more sensitive to environment than male size. On the other hand, our results are consistent with the converse Bergmann's rule; grasshopper body size tended to decrease with elevation but the differences were not statistically significant after correcting for phylogeny. This pattern is normally explained by the existence of gradients of precipitation and sun exposure, which are likely indicators of other ecological factors that exert control on body growth, such as resource availability and conditions for effective thermoregulation (Laiolo *et al.*, 2013). Although most evidence comes from single-species studies (Blanckenhorn *et al.*, 2006; Bidau & Martí, 2007b, 2008), our study is one of the first to test for altitudinal clines in body size at the interspecific level (i.e. across species) in Orthoptera.

Rensch's rule

We found evidence that SSD and body size in short-horned grasshoppers fitted a converse Rensch's rule: females are proportionally bigger than males in large species. This result is in agreement with previous studies carried out on a smaller scale (Bidau *et al.*, 2013; Laiolo *et al.*, 2013) and reinforces the view that Rensch's rule is infrequent in taxonomic groups exhibiting female-biased SSD. Likewise, when performing our analyses within subfamilies, we did not find a pattern consistent with Rensch's rule: sizes of males and females scaled isometrically. A plausible explanation for these results is that if females are more sensitive to environmental conditions than males, they could achieve a better body size development under more benign conditions leading to an increase in SSD (Shine, 1990; Stilwell *et al.*, 2010). Thereby, Rensch's rule and its converse would mirror sex-specific environmental sensibility, and thus, these patterns may be considered subproducts of body size variations in relation to ecological conditions. Thereby, our study supports the idea that the so-called Rensch's rule probably does not deserve the attribute 'rule' at least in arthropods, wherein support for this pattern remains rather mixed (reviewed in Blanckenhorn *et al.*, 2007a).

Conclusions

Different and complex evolutionary pressures can affect body size evolution in Orthoptera. Fecundity, sexual selection and predictable, long breeding season environments are thought to select for larger size, whereas time constraints, predators and unpredictable and

poor-resource habitats are thought to select for small body size. Here, we found no support for either the fecundity or the sexual selection hypothesis, the two primary adaptive forces traditionally invoked to explain SSD. Nor did we find an effect of substrate – ground vs. plant – on body size evolution, a factor (agility) that has been suggested to explain why males of certain insect groups remain small. Our results also reinforce the idea that Rensch's rule is probably not a rule at all but a limited pattern only found in a few taxonomic groups and more frequently, at the intraspecific level (e.g. De Lisle & Rowe, 2013; Liao *et al.*, 2013; Bidau *et al.*, 2016). Finally, and contrary to expected, we found a higher level of SSD in species with a long reproductive window, which is counter to the idea that SSD is favoured in short-season habitats due to the fact that males have no time to fully develop (resulting in small adult sizes). These findings support laboratory studies at the intraspecific level showing that under poor conditions female Orthoptera are more strongly affected than males, reducing SSD (Teder & Tammaru, 2005). We conclude that it is unlikely that protandry constitutes the main factor determining the existence of female-biased SSD in this insect radiation.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Information on body size (male and female femur length, mm), duration and length (in months) of the breeding season, substrate and altitude range for Iberian short-horned grasshoppers (Acrididae).

Table S2 Habitat requirements and 'Paired Difference Index' (PDI) values computed for the 85 grasshopper species included in this study.

Table S3 Association of each grasshopper species with the nine most common habitats in which these can be found, where 0 = species not recorded in the habitat, 1 = minor association, 2 = moderate association, and 3 = strong association between the species and the habitat.

Figure S1 Ordinary least square (OLS) regression of sexual size dimorphism on the length of the breeding season for the grasshopper species analysed in this paper.

Figure S2 The Bayesian maximum clade credibility (MCC) tree.

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