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Phenotypic Variation and Sexual Size Dimorphism in *Dichroplus elongatus* (Orthoptera: Acrididae)

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ABSTRACT Patterns of body size evolution are of particular interest because body size can affect virtually all the physiological and life history traits of an organism. Sexual size dimorphism (SSD), a difference in body size between males and females, is a widespread phenomenon in insects. Much of the variation in SSD is genetically based and likely due to differential selection acting on males and females. The importance of environmental variables and evolutionary processes affecting phenotypic variation in both sexes may be useful to gain insights into insect ecology and evolution. *Dichroplus elongatus* Giglio-Tos is a South American grasshopper widely distributed throughout Argentina, Uruguay, most of Chile, and southern Brazil. In this study, we analyzed 122 adult females of *D. elongatus* collected in eight natural populations from central-east Argentina. Females show large body size variation among the analyzed populations and this variation exhibits a strong relationship with fecundity. Our results have shown that larger females were more fecund than smaller ones. We found that ovariole number varied along a latitudinal gradient, with higher ovariole numbers in populations from warmer locations. A considerable female-biased SSD was detected. SSD for three analyzed morphometric traits scaled isometrically. However, SSD for thorax length displayed a considerable variation across the studied area, indicating a larger relative increase in female size than in male size in warmer environmental conditions.

KEY WORDS morphometric trait, fecundity, Orthoptera

In general, it is assumed that variation in body size and life history traits of ectotherms along latitudinal clines represents adaptation to local environmental conditions, especially adaptation to variation in season length and, hence, to the favorable conditions for growth and reproduction (Blanckenhorn and Demont 2004, Angilletta et al. 2004). Furthermore, geographic variation patterns often differ between females and males, e.g., differences in sexual dimorphism along latitudinal clines (Stillwell et al. 2007).

In many species, the selective processes molded differences in body size between sexes (sexual size dimorphism [SSD]). The degree and direction of SSD might vary among species and among populations of the same species, contributing to intraspecific variation (Blanckenhorn et al. 2007, Hochkirch and Gröning 2008, Stillwell et al. 2010). The relationship between the extent of SSD and body size was formalized in Rensch's rule (Rensch 1960, Fairbairn 1997). SSD generally increases with body size in taxa where males are larger than females, whereas when females are larger than males, SSD decreases with body size (Rensch 1960, Fairbairn 1997). When log male size is regressed on log female size, and the slope (b) is 1, a geometric similarity or isometry is verified. $b > 1$ establishes an allometric relationship consistent with Rensch's in which male size varies faster among populations than female

size, whereas $b < 1$ defines an allometric relationship in which female size varies faster among populations than male size, indicating the reversion of Rensch's rule (Fairbairn 1997). Although Rensch's rule was originally proposed to describe allometry for SSD among closely related species, this rule has been applied to describe allometric patterns for SSD among populations of the same species (Fairbairn and Preziosi 1994, Nosil 2001, Fairbairn 2005, Tamate and Maekawa 2006, Bidau and Marti 2007, Lengkeek et al. 2008).

A widely accepted hypothesis explaining the existence of intersexual variation is the "differential equilibrium hypothesis" (Blanckenhorn 2000). This hypothesis proposes that SSD is the result of selection favoring larger body size (female fecundity selection and male sexual selection) and selection against large body size (viability selection; Blanckenhorn 2000, Blanckenhorn et al. 2007, Stillwell et al. 2007, Hochkirch and Gröning 2008).

Fecundity is an important life history trait invoked to explain SSD. Fecundity studies should be helpful in understanding population dynamics, which is one of the bases from which changes in population size are determined through the incidence of environmental factors. Furthermore, in many insect species, female body size and fecundity are positively correlated (Fitt 1990, Calvo and Molina 2005, Akman and Whitman 2008). The analysis of relationships between body size and fecundity across space provide valuable information to examine phenotypic intraspecific variation as

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well as to investigate the magnitude and direction of SSD in nature. While the occurrence and importance of SSD among animals is widely recognized, it is important to understand how environmental variables and evolutionary processes affect phenotype. New studies of intraspecific variation in body size and its relationships with female reproductive potential in grasshopper species may be useful to understand phenotype evolution in this insect group.

Dichroplus elongatus Giglio-Tos is an abundant South American grasshopper with a wide distribution in Argentina. It has the ability to dramatically increase in population size, making it an agricultural pest for forage grasses of great economic importance (Lieberman 1944, Ronderos 1985).

Morphometric analysis in *D. elongatus* has indicated that body size-related traits in males showed a “saw tooth” pattern of variation being small at low and high latitudes and large at intermediate latitudes in most of the analyzed populations. These results may be explained by local adaptations to the length of the favorable season along a latitudinal gradient (Rosetti and Remis 2013). Genotyping of *D. elongatus* based on minisatellite markers verified a genetic population structure explained by interactions between effective migration and genetic drift (Rosetti and Remis 2012). The comparison between the differentiations of body size traits with molecular neutral markers supports the relative importance of selection shaping male phenotype variation (Rosetti and Remis 2013). The analysis of variation in body size and fecundity in females of this grasshopper species can improve our knowledge of intraspecific phenotypic variation in different environments.

In the present article, we examine several female body size-related traits in natural populations of the grasshopper *D. elongatus* from central-eastern Argentina previously analyzed at male phenotypic level. Our particular goals were 1) to analyze the body size variation pattern and the relationship between reproductive potential and morphometric traits in females, 2) to analyze the relationship between morphometric traits in both sexes to provide a first assessment of the SSD pattern, and 3) to compare body size related traits and SSD against a set of neutral dataset to evaluate the relative importance of selective processes on phenotypic variation in *D. elongatus*.

Materials and Methods

Biological Material. One hundred and twenty-two adult females of *D. elongatus* were collected during summer from eight natural populations in Argentina distributed along a latitudinal range (~850 km). One sample per locality was obtained in February. The collection localities and climatic parameters are listed in Table 1. Food and Agriculture Organization World Climate Database applying the New LocClim software (Grieser et al. 2006) was used to estimate the climate data for each location.

Body Size and Reproductive Potential-Related Trait Measurements. All individuals were measured for five morphometric traits: total body, third femur,

third tibia, tegmen, and thorax lengths. Total body size was measured from the phastigium to the articulation between the third coxa and third femur. All measurements were performed on the right side of the insect, with a stereoscopic microscope and an ocular micrometer (1 mm = 48 ocular units). Potential fecundity was estimated through the number of ovarioles per ovary and the egg clutch size from wild females (Akman and Whitman 2008, Taylor and Whitman 2010). The number of follicles in the right ovary in 37 noninseminated females, and the egg clutch size from 31 wild females were counted. All measurements were performed using a stereoscopic microscope.

Statistical Analysis. *Body Size Variation.* To estimate morphometric differences among populations, the mean values of the five morphometric traits were compared, through a multivariate analysis of variance (MANOVA). We tested for statistical significance among populations through conventional one-way analyses of variance (ANOVAs), considering the population as the independent factor and body size-related traits as the dependent variables. We analyzed population variation on a reduced set of independent variables to avoid the problem of pseudoreplication, principal components analyses (PCA) of body size-related variables were also performed. We tested for variation in the first principal components (PCs) by means of one-way ANOVAs, considering the population as the independent factor and PCs as dependent ones. The relationships between female body size and climatic variables were analyzed using Spearman partial correlations. Bonferroni's test for multiple comparisons was applied.

Reproductive Potential in Females. The variation of reproductive potential among populations was assessed through one-way ANOVAs. The reproductive potential was also evaluated by means of nonparametric Spearman correlation index.

Sexual Dimorphism Analysis. To estimate morphometric differences between sexes and among populations, the mean values of the five morphometric traits were compared through a MANOVA considering the population and sexes as the independent factors and body size-related traits as the dependent variables. PCA of body size-related variables were also used to examine morphometric differences between males and females.

Deviations from isometry were analyzed through a reduced major axis regression (RMA) using log (male size) on log (female size) as variables (Fairbairn 1997). Estimated slopes (b) were used to describe the scaling of SSD with body size. We tested the null hypotheses that $b = 1$ (no allometry for SSD) with Clarke's *t* statistic with adjusted degrees of freedom (Clarke 1980).

We calculated the ratio defined as the arithmetic mean of each morphometric trait in the larger sex (females) divided by the smaller sex (males) to visualize the amount of SSD (Smith 1999). To evaluate the relationship of SSD indexes with climatic variables, nonparametric Spearman correlations were carried out.

All statistical analyses aforementioned were carried out with the software STATISTICA (Statistica Statsoft

Table 1. Geographic and climatic variables of eight natural populations of *D. elongatus* collected in Argentina

Population	Symbol	Latitude	Longitude	Altitude (m)	Mean Temp. °C	Max temp °C	Min. temp °C	Precipitations (mm per month)
Yapeyú	YAP	29° 28' S	56° 49S' W	40	20.60	26.72	14.60	127.75
Monte Caseros	MCA	30° 17' S	57° 38' W	60	19.75	25.91	14.31	113
Mocoretá	MOC	30° 38' S	57° 58' W	60	19.74	25.90	14.31	112
Concordia	CON	31° 24' S	58° 02' W	20	19.03	24.91	13.15	93.76
Río Cuarto	RCA	33° 08' S	64° 20' W	300	16.33	22.98	10.97	69.92
Río los Sauces	RLS	31° 40' S	63° 55' W	360	16.56	23.17	9.86	67.58
Santa Catalina	SCA	34° 09' S	63° 22' W	120	16.25	23.83	9.85	70.56
Colón	COB	33° 52' S	61° 05' W	40	16.40	22.84	10.34	80.97

Inc. 1996) and lmodel2 package for the R version 2.15.1 software (Legendre and Legendre 2012).

Analysis of Phenotype and SSD Variation in Relation to Molecular Neutral Data. The evaluation of statistical estimators of population differentiation based on diverse datasets has been used to examine the role of genetic drift and selection (Gillespie and Oxford 1998, Storz 2002, Latta 2004, Roff and Mousseau 2005, Lehtonen et al. 2009, Rosetti and Remis 2013). Neutral loci should show similar patterns of genetic divergence among populations by interactions between migration and genetic drift. It is expected that the phenotypic differences responsible for adaptive population differentiation can be identified by a different degree of population difference than expected under neutral scenario (Latta 2004). The analysis of a representative number of neutral molecular markers gave a suitable estimation of average genetic differentiation under neutral expectations against which to contrast the phenotypic differentiation. Thus, estimates of neutral genetic molecular variation provide a null hypothesis or neutral expectations to the alternative hypothesis of adaptive divergence (Pertoldi et al. 2012).

Correlation between one set of data and the neutral marker data set indicates that interaction between genetic drift and migration may explain the pattern of variation in the first set of data. Alternatively, if there are significant differences between estimates of population differentiation from the two data sets, genetic drift could not be responsible for differences in the second set of data.

To infer the relative importance of genetic drift and natural selection on body size, fecundity, and SSD variation, we compared estimation of morphometric, reproductive potential, and SSD differentiation with variation in neutral genetic markers according to Roff and Mousseau (2005).

Phenotypic, fecundity, and SSD variation between populations was estimated through the differences in mean values. We compared phenotypic reproductive potential and SSD differences between populations with Nei's genetic distances previously estimated from the same populations analyzed here using 155 loci DAMD (Direct Amplification of Minisatellite region DNA; Rosetti and Remis 2012).

Comparisons between Nei's genetic distances and differences in phenotype, reproductive potential, and SSD were carried out through standard Mantel test

Table 2. Mean length values (in mm) and SE in brackets for five body size-related traits in females (A) and males (B) of *D. elongatus*

Population	Total	Thorax	Femur	Tibia	Tegmen	N
A) Females						
YAP	10.962 (0.056)	4.727 (0.043)	13.942 (0.213)	11.747 (0.194)	20.417 (0.289)	13
MCA	11.005 (0.054)	4.853 (0.043)	15.306 (0.186)	12.218 (0.169)	21.005 (0.253)	17
MOC	8.725 (0.054)	4.645 (0.037)	13.051 (0.186)	10.870 (0.170)	19.877 (0.252)	17
CON	8.792 (0.058)	4.778 (0.040)	13.611 (0.198)	11.083 (0.181)	20.472 (0.269)	15
RLS	8.958 (0.059)	4.821 (0.041)	14.018 (0.205)	10.536 (0.187)	20.223 (0.278)	14
RCA	9.233 (0.065)	5.019 (0.033)	14.018 (0.205)	11.401 (0.149)	22.386 (0.222)	22
COB	9.270 (0.065)	4.965 (0.045)	14.323 (0.221)	11.024 (0.202)	21.319 (0.301)	12
SCA	9.236 (0.065)	4.826 (0.045)	13.906 (0.221)	11.388 (0.202)	20.885 (0.301)	12
B) Males ^a						
YAP	8.148 (0.031)	3.935 (0.023)	11.004 (0.095)	8.560 (0.085)	17.055 (0.138)	44
MCA	8.182 (0.035)	3.996 (0.027)	11.629 (0.109)	9.008 (0.098)	17.708 (0.160)	33
MOC	8.098 (0.036)	3.958 (0.029)	10.457 (0.090)	8.058 (0.087)	16.176 (0.156)	31
CON	8.103 (0.024)	3.988 (0.027)	10.484 (0.049)	8.311 (0.051)	16.495 (0.111)	29
RLS	8.361 (0.045)	4.014 (0.048)	10.889 (0.190)	8.500 (0.106)	17.139 (0.238)	15
RCA	8.451 (0.037)	4.130 (0.025)	11.241 (0.076)	8.669 (0.135)	17.509 (0.172)	23
COB	9.052 (0.054)	4.505 (0.033)	11.865 (0.076)	8.937 (0.059)	17.963 (0.134)	40
SCA	8.526 (0.037)	4.215 (0.035)	11.410 (0.125)	8.894 (0.104)	18.061 (0.114)	13

N = sample size.

^a Data from Rosetti and Remis (2013).

using the PASSAGE v.2 software (Rosenberg and Anderson 2011). For all tests, the significance was estimated with 10,000 permutations.

Results

Body Size Variation in Females. To assess the phenotypic variation in females of *D. elongatus*, five morphometric traits were analyzed in 122 individuals from Argentinean populations (Table 2). MANOVA demonstrated significant differences in body size

Table 3. Factor loadings, eigenvalues, and cumulative % of variance on the three PCs for females of *D. elongatus* showing the highest loading of each trait in bold type

Trait	PC1	PC2	PC3
Total	-0.478	0.818	0.303
Thorax	-0.812	-0.464	0.271
Femur	-0.932	-0.029	0.003
Tibia	-0.825	0.385	-0.376
Tegmen	-0.873	-0.348	-0.064
Eigenvalues	3.200	1.155	0.311
Cumulative % of variance	64.007	87.118	93.344

among populations (Wilks' lambda_{35,465} = 0.007; $P < 10^{-4}$). The individual (ANOVAs) revealed highly significant differences in all analyzed body size traits (total: $F_{7,114} = 258.7$; thorax: $F_{7,114} = 14.4$; femur: $F_{7,114} = 17.9$; tibia: $F_{7,114} = 8.8$; tegmen: $F_{7,114} = 10.7$; $P < 10^{-4}$ in all cases; Table 2).

To analyze population variation on a reduced set of independent variables, PCA of morphometric variables were also performed (Table 3). The first PC (PC₁) accounted for 64.01% of total variance, the highly positive correlations found for all body size-related traits allow us to consider this component as an adequate estimator of overall body size. The second PC (PC₂) explained 23.11% of total variation and may be a good indicator of body shape (Table 3). Because the two first PCs explained ~84% of the total variation, they were used for further analyses. The individual ANOVAs using PCs instead of original data, revealed highly significant differences among populations for PC₁ and PC₂ (PC₁: $F_{7,114} = 14.1$; PC₂: $F_{7,114} = 137.5$; $P < 10^{-4}$ in all cases). There were no clear tendencies to describe body size variation in relation to climatic and/or geographic variables ($P > 0.05$ in all cases).

Reproductive Potential in Females. To evaluate the variation of reproductive potential in females, the number of ovarioles per ovary and eggs per clutch was studied in 68 individuals (37 and 31 females, respectively; Fig. 1). ANOVA revealed significant differences in the number of eggs per clutch among populations ($F_{7,45} = 2.73$; $P = 0.018$). The number of ovarioles per ovary did not differ among populations. Nevertheless, the mean number of ovarioles per ovary was positively correlated with latitude ($r = -0.88$; $P = 0.0003$), mean temperature ($r = 3.12$; $P = 0.014$), minimum temperature ($r = 5.11$; $P = 0.0009$), and precipitation ($r = 3.49$; $P = 0.008$), suggesting that variation in this reproductive potential trait occurs more gradually.

Spearman's partial correlation showed significant association between body size and number of eggs per clutch. In general, bigger females produce a larger number of eggs per clutch (Fig. 2 A–E). Femur and tibia lengths also exhibited positive and significant correlations with ovariole number, whereas tegmen length is marginally correlated with the number of ovarioles (Fig. 2 F–H).

Likewise, the number of eggs per clutch and ovarioles per ovary increases at lower PC₁ values, thus indicating that females with larger body size generate a higher number of ovarioles and eggs. Therefore, larger

female body size increases reproductive potential (Fig. 3A and C). The number of eggs per clutch increases slightly at lower PC₂ values, suggesting that the female shape might also be related with this reproductive trait (Fig. 3B), whereas no relationship has been detected between the number of ovarioles and PC₂ (Fig. 3D).

Sexual Dimorphism. Male phenotypic variation was previously examined in natural population of *D. elongatus* (Rosetti and Remis 2013). We considered male morphometric data set from the eight Argentinean populations studied earlier to analyze SSD (Tables 2 and 4).

MANOVA demonstrated that females were considerably larger than males (Wilks' lambda_{35,1386} = 0.095; $P < 0.0001$) and again, there were highly significant differences among populations (Wilks' lambda_{5,329} = 0.037; $P < 0.0001$). Significant interactions were detected between main factors (sex × population; Wilks' lambda_{35,1386} = 0.060; $P < 0.0001$), suggesting some differences in SSD among populations. Individual ANOVAs showed significant differences for the five morphometric traits between sexes and among populations ($P < 0.0001$ in all cases; Table 4). Similarly, MANOVA based in the first three PCs, which explained ~98% of the total variance, demonstrated highly significant differences among populations, between sexes and for interaction between main factors (Wilks' lambda_{21,951} = 0.060, $P < 0.0001$; Wilks' lambda_{3,331} = 0.011, $P < 0.0001$; Wilks' lambda_{21,951} = 0.087, $P < 0.0001$).

To visualize directly morphometric differences between sexes, we have estimated SSD as the ratio between the arithmetic mean of each measured character of females, and the corresponding mean of males, in the eight populations. Additionally, we considered the data from two populations analyzed previously (Raco and Horco Molle) (Rosetti et al. 2007; Table 5). SSD varied substantially with latitude ($r_{\text{THORAX}} = -0.733$, $P = 0.015$, $r_{\text{TIBIA}} = -0.757$, $P = 0.011$), grasshoppers decreased in the degree of dimorphism with increasing latitude. We detected positive correlations between tibia SSD and mean temperature ($r = 0.656$; $P = 0.039$), minimum temperature ($r = 0.766$; $P = 0.009$), maximum temperature ($r = 0.765$; $P = 0.009$), and precipitations ($r = 0.790$; $P = 0.006$).

To test allometry for SSD among populations, we estimated regression of log(male size) on log(female size). Whereas the variation in mean total length among populations is not correlated between males and females ($P > 0.05$), the between-sex allometric slopes for femur, tibia, and tegmen lengths do not differ significantly from 1.0. This demonstrates that these traits scale isometrically (Table 6). In contrast, the RMA slope for thorax is marginally different from 1.0 ($b < 1$), suggesting SSD allometry for this trait. Female thorax length increases relatively faster compared with male thorax length (Table 6).

Analysis of Reproductive Potential and SSD Differences in Relation to Molecular Neutral Variation. Fecundity evaluated through mean differences of reproductive potential-related traits did not

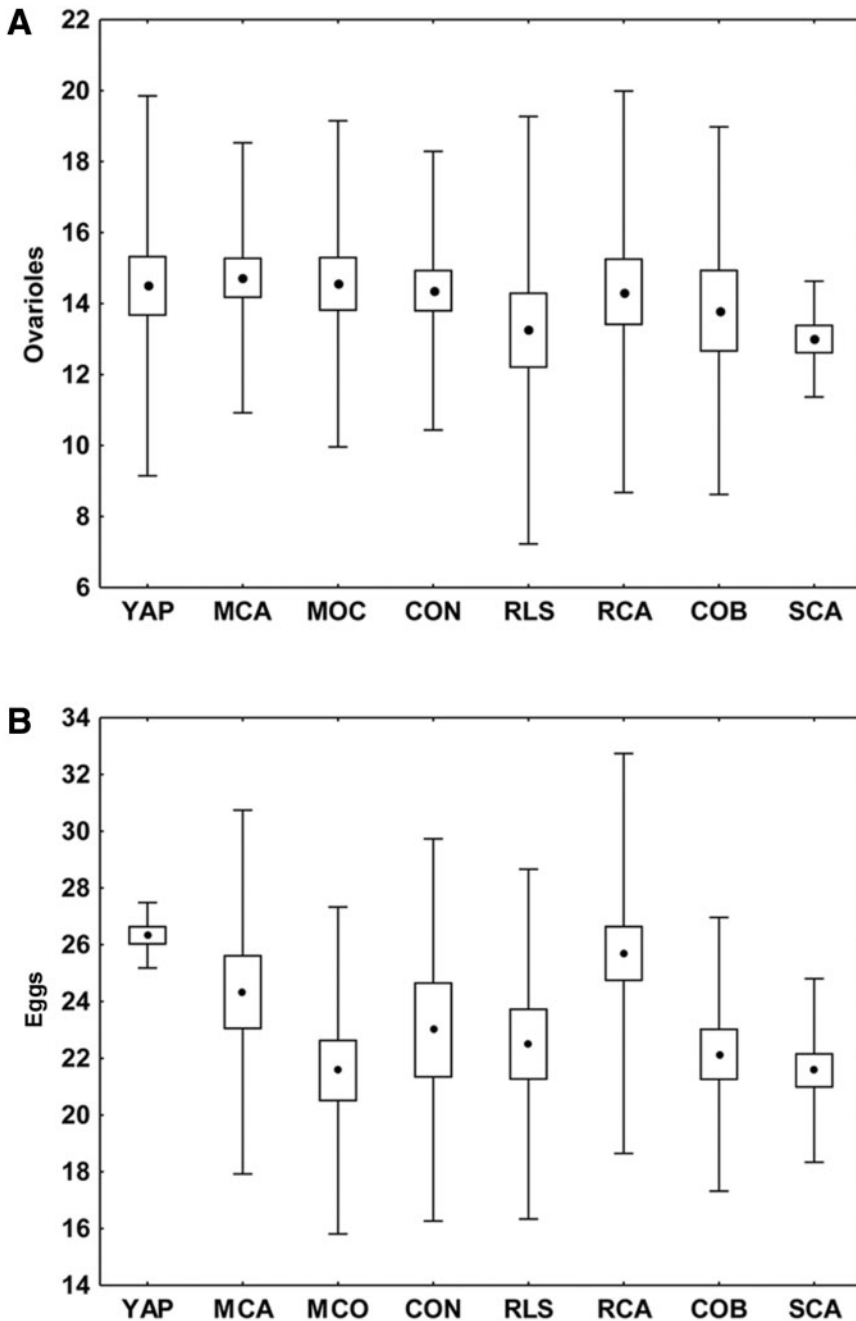


Fig. 1. Mean numbers of ovarioles per ovary (A) and eggs per clutch (B) (primaré symbols), SEs (boxes) and SDs (bars) in eight populations of *D. elongatus*.

correlate with genetic distances (ovariole: $r=0.33$, $P=0.16$; eggs: $r=-0.25$, $P=0.27$), which indicates that reproductive potential variation in females cannot be molded only by genetic drift.

A significant relationship between differences in SSD means for total length and genetic distances was evident ($r=-0.49$; $P=0.004$), suggesting that genetic drift has a strong influence over the variation of this

trait in particular. However, no significant correlation was detected between molecular data and the differences in SSD mean values of the other morphometric traits (thorax: $r=0.32$, $P=0.29$; femur: $r=-0.19$, $P=0.36$; tibia: $r=-0.19$, $P=0.36$; tegmen: $r=0.02$, $P=0.95$), which shows the importance of natural selection in shaping SSD variation in the studied populations.

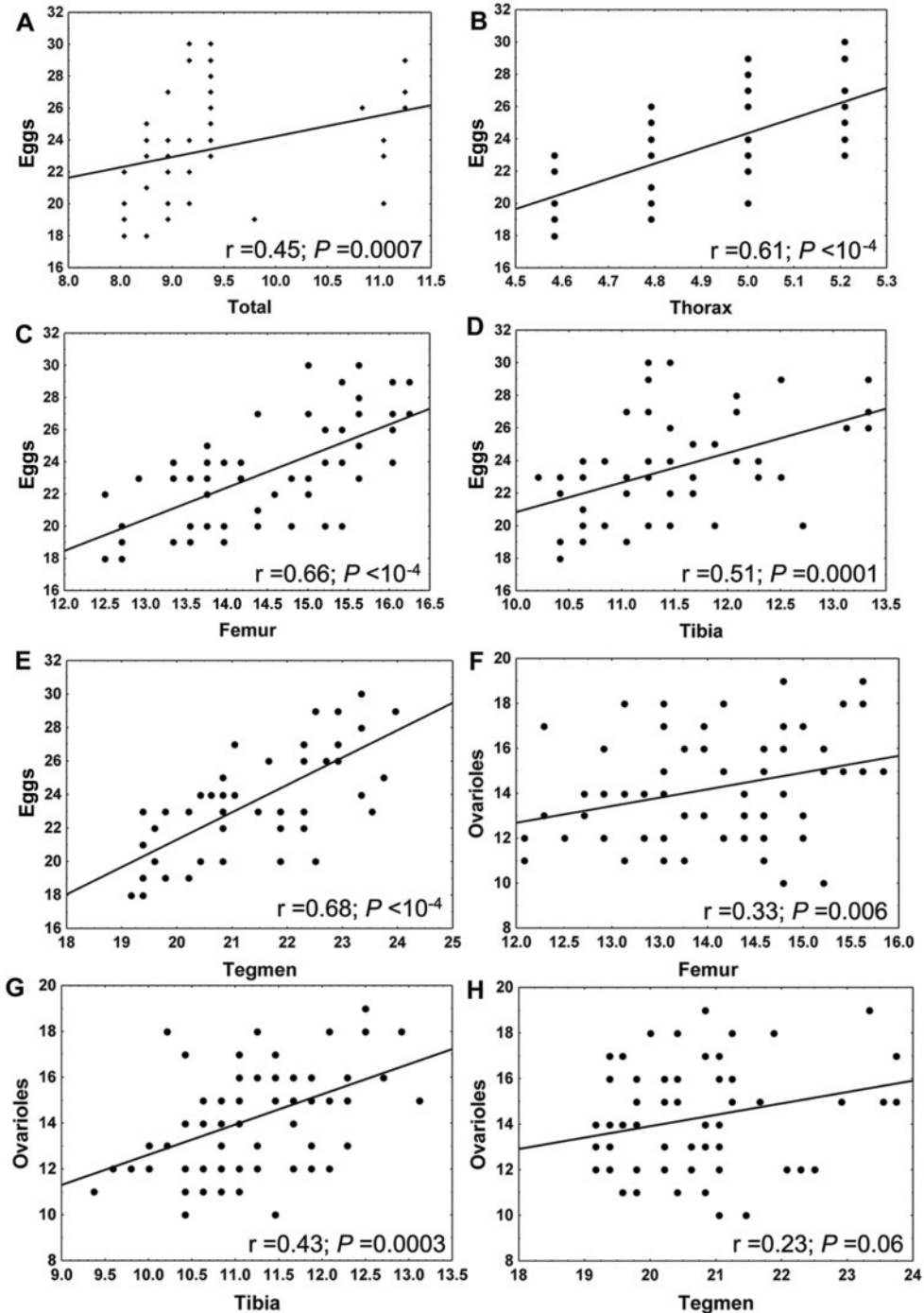


Fig. 2. Linear relationship between mean number of eggs per clutch (A–E) and mean number of ovarioles per ovary (F–H) and morphometric traits of *D. elongatus* females. Only significant correlations are shown.

Discussion

Previous studies showed a saw-tooth clinal pattern in male body size according to south latitude. The observed phenotypic variation is likely due to local adaptation to season length along the latitudinal

gradient and further studies are required to advance our understanding of the relationship between the number of generations per year and the detected body size variation (Rosetti and Remis 2013). Blanckenhorn and Demont (2004) have suggested that clinal patterns

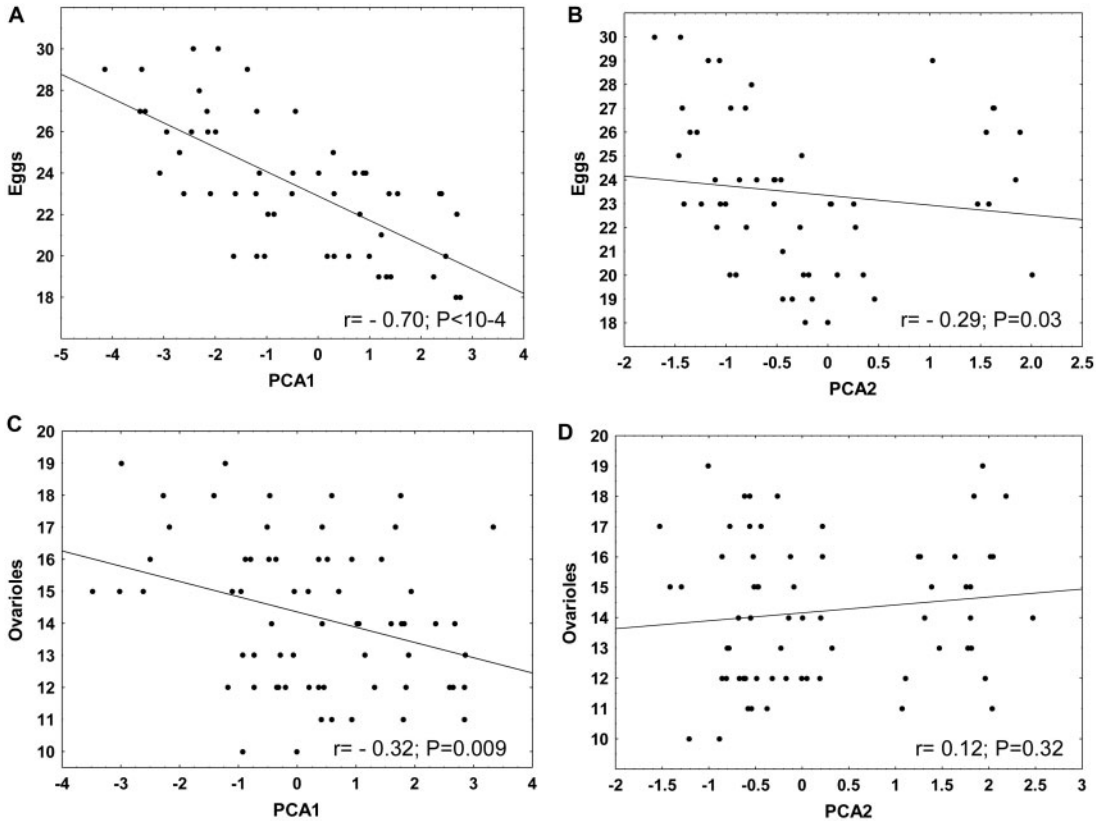


Fig. 3. Correlations between mean number of eggs per clutch (A and B) and mean number of ovarioles per ovary (C and D), and the two PCs that best explain the variance.

Table 4. *F* statistic values and their significance levels (*P*) from individual two-way ANOVAs analyzing morphometric differences among sampled populations

Source of Variation	Total	Thorax	Femur	Tibia	Tegmen
Among populations	<i>F</i> 210.5 <i>P</i> 0.001	35.3 <10 ⁻⁶	37.5 <10 ⁻⁶	18.3 <10 ⁻⁶	21.1 <10 ⁻⁶
Between sexes	<i>F</i> 2,375.7 <i>P</i> 0.001	1,670.5 <10 ⁻⁶	1,708.5 <10 ⁻⁶	1,613.9 <10 ⁻⁶	1,096.1 <10 ⁻⁶
Population × Sexes	<i>F</i> 266.6 <i>P</i> 0.001	9.4 <10 ⁻⁶	7.8 <10 ⁻⁶	5.5 6 × 10 ⁻⁵	4.7 5 × 10 ⁻⁴

Sex and population was considered as independent factors.

Table 5. SSD indexes calculated as mean size of the larger sex/mean size of the smallest sex, for five morphometric traits evaluated in eight natural populations of *D. elongatus*

Population	Total	Thorax	Femur	Tibia	Tegmen
RCO	1.249	1.389	1.219	1.326	1.125
HCM	1.298	1.428	1.341	1.381	1.127
YAP	1.345	1.214	1.316	1.356	1.186
MCA	1.345	1.202	1.267	1.369	1.197
MOC	1.077	1.173	1.248	1.349	1.229
CON	1.085	1.198	1.298	1.334	1.241
RLS	1.071	1.201	1.287	1.239	1.179
RCA	1.093	1.231	1.354	1.315	1.278
COB	1.024	1.102	1.207	1.233	1.187
SCA	1.083	1.145	1.306	1.280	1.156

Data of Raco (RCO) and Horco Molle (HCM) populations from Rosetti et al. (2007).

Table 6. RMA analysis of log (mean male size) on log (mean female size) from 10 populations of *D. elongatus*

Trait	b	<i>t</i>	df	<i>P</i>
Thorax	0.408	-2.012	9.902	0.07*
Femur	0.769	-1.051	8.579	0.32
Tibia	0.740	-1.025	9.094	0.33
Tegmen	0.838	-0.642	8.139	0.53

Clarke's *t* statistic tests the null hypotheses that the RMA slope (b) = 1, which signifies isometry.

*Marginally significant differences.

might be part of a continuum of adaptive responses to ambient temperature or length of growing season. Females of *D. elongatus* exhibited large body size variation among populations but phenotypic variation did not follow saw-tooth pattern, possibly because of the limited number of populations analyzed at high latitudes.

We also found a strong correlation between body size and reproductive potential evaluated through both the number of ovarioles per ovary and the number of eggs per clutch. Several examples showing positive relationships between body size and reproductive traits have been well documented in Orthoptera (Lewis 1984, Cueva del Castillo et al. 1999). Positive correlations between body size and reproductive potential are

expected to be stronger in insects that acquire resources as nymphs for subsequent reproduction than in insects that acquire resources for reproduction as adults, where environmental constraints are likely to be more important in determining fecundity (Honěk 1993, Tammaru et al. 1996). In the pyrgomorphid *Sphenarium purpurascens* Bruner, that there is a positive association between body size and the number of eggs per pod (Cueva del Castillo et al. 1999). Lewis (1984) has demonstrated that larger females of *Melanoplus differentialis* (Thomas) produce more eggs per pod, more pods per female, and also larger eggs. These examples, including *D. elongatus*, suggest an important resource acquisition during nymphal instars for following reproduction.

Moreover, in *D. elongatus*, the number of ovarioles per ovary increases in populations with higher temperature. Our results for *D. elongatus* agree with those detected by Bellinger and Pienkowski (1985), who found that in grasshopper species, the number of ovarioles per ovary is higher in populations from warmer locations. Depending on the type of life cycle, populations subject to favorable conditions for growth (in general, given in warmer locations) are likely to have an increased ovariole number and thus more eggs could be produced in the same amount of time as a smaller pod in a cooler environment.

Our results have also demonstrated that, overall, females are substantially larger than males, consistent with other studies showing female-biased size dimorphism in this genus (Bidau and Marti 2008). As a general factor, female-biased SSD is frequent among Orthoptera (Blanckenhorn et al. 2007). An exhaustive interspecific analysis revealed that SSD is rather uniform in Ensifera, with females being on average 9% larger than males. In contrast, SSD is usually much stronger in Caelifera (37%) and also more variable (Hochkirch and Gröning 2008).

In a widely accepted adaptive scenario, the differential equilibrium hypothesis supports that SSD is molded by selection favoring larger body size (female fecundity selection and male sexual selection) and selection rejecting large body size (viability selection; Fairbairn 1997, Blanckenhorn 2000, Blanckenhorn et al. 2007, Hochkirch and Gröning 2008, Stillwell et al. 2010).

In insects, fecundity selection is one of the major sources of selection favoring larger size in females. As detected in *D. elongatus*, females may increase their fitness, reaching higher adult body size, which allows them to produce more eggs.

Organism growth and development might also be considered shaping intraspecific body size variation (Roff 1992). Orthoptera females are usually associated not only with higher number of nymphal instars, leading to an earlier emergence of adult males (protandry; Esperk et al. 2007), but also with more variation in instar number (Bellinger and Pienkowski 1989), which leads to higher body size variation in females. In *D. elongatus*, the number of nymphal instars is five in males and five or six in females (Turk and Barrera 1979). Further studies may be necessary to analyze the relative

importance of instar number on both between sexes and among females from population with different seasonal stations molding phenotypic variation. Yet, the faster emergence of adult males of *D. elongatus* might confer adaptive advantages, allowing them to ensure high quality of territory and fertilize virgin females (Teder and Tammaru 2005, Berner and Blanckenhorn 2006, Blanckenhorn et al. 2007, Hochkirch and Gröning 2008).

Moreover, the magnitude and direction of SSD can vary among populations within species (Teder and Tammaru 2005). Latitudinal or altitudinal variation in body size can differ between sexes, creating geographic patterns in SSD. SSD differences at intraspecific level have been well documented and this variation was attributed to both adaptive and phenotypic plasticity hypotheses (Fairbairn 2005, Teder and Tammaru 2005, Stillwell et al. 2007).

In *D. elongatus*, we also identified clinal latitudinal variation in SSD for tibia and thorax lengths. In tibia length, no allometry for SSD was detected, indicating that males and females vary in a similar fashion across the studied area; however, thorax length seems to scale allometrically. Although marginally significant, the slope (b) of the relationship $\log(\text{male size})$ versus $\log(\text{female size})$ for thorax length was <1 , suggesting that female size varies more among populations than male size. In other words, our results indicate that SSD for thorax length increases with increasing body size following the converse to Rensch's rule (Blanckenhorn 2000).

A general approach to infer the role of selection in maintaining clinal variation is to compare relative levels of between-population divergence in quantitative traits and neutral markers (Storz 2002, Latta 2004, Roff and Mousseau 2005). To gain insights into the evolutionary processes driving clinal variation, we analyzed the changes in trait means in relation to genetic differentiation detected at the molecular level. We used the genetic distance matrix from DAMD data as a predictor matrix for neutral variation patterns previously obtained (Rosetti and Remis 2012). We found no association between Nei's genetic distances and differences in female reproductive potential-related traits, suggesting the adaptive significance of the variation patterns. In accordance with the foregoing results, no significant relationships were detected between Nei's genetic distances and SSD for thorax and tibia lengths, which provide indirect evidence about the relative importance of selection molding sexual dimorphism in nature. Our results have also shown that dimorphism for thorax length is greatest in warmer environmental conditions. Though environmental variables may not be the causal factor for phenotypic variation, they may be indicator variables of other agents (selective), leading to different effects for male versus female body size.

In the present preliminary analysis of female phenotype variation, we have detected significant differences in body size among populations of *D. elongatus* from central-east Argentina. Our results would indicate some evidences about adaptive variation in phenotype and suggest the relative importance of female fecundity selection molding SSD in this species.

Further, phenotype analysis of this grasshopper species from throughout the range of the species distribution would improve our knowledge about phenotype variation in nature.

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References Cited

- Akman, O., and D. Whitman.** 2008. Analysis of body size and fecundity in a grasshopper. *J. Orth. Res.* 17: 249–257.
- Angilletta, M. J., P. H. Niewiarowski, A. E. Dunham, A. Leache, and W. P. Porter.** 2004. Bergmann's clines in ectotherms: illustrating a life-historical perspective with sceloporine lizards. *Am. Nat.* 164: E168–E183.
- Bellinger, R. G., and R. L. Pienkowski.** 1985. Interspecific variation in ovariole number in Melanopline grasshoppers (Orthoptera: Acrididae). *Ann. Entomol. Soc. of Am.* 78: 127–130.
- Bellinger, R. G., and R. L. Pienkowski.** 1989. Polymorphic development in relation to the life history of *Melanoplus femurrubrum* (Orthoptera: Acrididae). *Ann. Entomol. Soc. Am.* 82: 166–171.
- Berner, D., and W. U. Blanckenhorn.** 2006. Grasshopper ontogeny in relation to time constraints: adaptive divergence and stasis. *J. Anim. Ecol.* 75: 130–139.
- Bidau, C. J., and D. A. Marti.** 2007. Clinal variation of body size in *Dichroplus pratensis* (Orthoptera: Acrididae): Inversion of Bergmann's and Rensch's rules. *Ann. Entomol. Soc. Am.* 10: 850–860.
- Bidau, C. J., and D. A. Marti.** 2008. Contrasting patterns of sexual size dimorphism in the grasshoppers *Dichroplus vittatus* and *D. pratensis* (Acrididae, Melanoplineae). *J. Orthop. Res.* 17: 201–211.
- Blanckenhorn, W. U.** 2000. The evolution of body size: what keeps organisms small? *Q. Rev. Biol.* 75: 385–407.
- Blanckenhorn, W. U., and M. Demont.** 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? *Integr. Comp. Biol.* 44: 413–424.
- Blanckenhorn, W. U., R. Meier, and T. Teder.** 2007. Rensch's rule in insects: patterns among and within species, pp. 60–70. *In* D. J. Fairbairn, W. U. Blanckenhorn, and T. Székely (eds.), *Sex, size and gender roles. Evolutionary studies of sexual size dimorphism*. Oxford University Press, New York, NY.
- Calvo, D., and J. M. Molina.** 2005. Fecundity–body size relationship and other reproductive aspects of *Streblothe panda* (Lepidoptera: Lasiocampidae). *Ann. Entomol. Soc. Am.* 98: 191–196.
- Clarke, M.R.B.** 1980. The reduced major axis of a bivariate sample. *Biometrika*, 67: 441–446.
- Cueva del Castillo, R. J. Z., Nuñez-Farfan and Cano-Santana.** 1999. The role of body size in mating success of *Sphenarium purpurascens* in Central Mexico. *Ecol. Entomol.*, 24: 146–155.
- Espærk, T. T. S., Tammaru T., Nylin and Teder.** 2007. Achieving high sexual size dimorphism in insects: Females add instars. *Ecol. Entomol.* 32: 243–256.
- Fairbairn, D. J., and R. F. Preziosi.** 1994. Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. *Am. Nat.* 144: 101–18.
- Fairbairn, D. J.** 1997. Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annu. Rev. Ecol. Syst.* 28: 659–687.
- Fairbairn, D. J.** 2005. Allometry for sexual size dimorphism: Testing two hypotheses for Rensch's rule in the water strider *Aquarius remigis*. *Am. Nat.* 166: S69–S84.
- Fitt, G. P.** 1990. Variation in ovariole number and egg size of species of *Dacus* (Diptera, Tephritidae) and their relation to host specialization. *Ecol. Entomol.* 15: 255–264.
- Gillespie, R. G., and G. S. Oxford.** 1998. Selection on the colour polymorphism in Hawaiian Happy-Face spiders: Evidence from genetic structure and temporal fluctuations. *Evolution* 52: 775–783.
- Grieser, J., R. Gommers, and M. Bernardi.** 2006. New LocClim – the Local Climate Estimator of FAO. Geophysical Research Abstracts 8, 08305. (http://www.fao.org/nr/climpag/pub/en3_051002_en.asp)
- Hochkirch, A., and J. Gröning.** 2008. Sexual size dimorphism in Orthoptera (*sens. str.*)—a review. *J. Orthopt. Res.* 17: 189–196.
- Honěk, A.** 1993. Intraspecific variation in body size and fecundity in insects: A general relationship. *Oikos* 66: 483–492.
- Latta, R. G.** 2004. Gene flow, adaptive population divergence and comparative population structure across loci. *N. Phytologist*. 161: 51–58.
- Legendre, P., and L.F.J. Legendre.** 2012. Numerical ecology, 3rd English edition. Elsevier Science BV, Amsterdam.
- Lengkeek, W. K., I. M. Didderen, E. M., Côté, R. C., van der Zee, J. Snoek, and D. Reynolds.** 2008. Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (Blenniidae). *Can. J. Zool.* 86: 1173–1178.
- Lehtonen, P. K., T. Laaksonen, A. V. Artemyev, E. Belskii, C. Both, and S. Bures.** 2009. Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). *Mol. Ecol.* 18: 4463–4476.
- Lewis, A. C.** 1984. Plant quality and grasshopper feeding: Effects of sunflower condition on preference and performance in *Melanoplus differentialis*. *Ecology* 65: 836–843.
- Lieberman, J.** 1944. Los acridoideos de Chile. *Rev. Chil. Hist.* 48: 3–158.
- Nosil, P.** 2001. Tarsal asymmetry, trait size, and extreme phenotypes in a sexually size-dimorphic water boatman *Callicorixa vulnerata*. *Can. J. Zool.* 79: 1114–1119.
- Pertoldi, C., H.B.H. Jørgensen, E. Randi, L. F. Jensen, A. Kjærsgaard, V. Loeschcke, and S. Faurby.** 2012. Implementation of mixture analysis on quantitative traits in studies of neutral versus selective divergence. *Evol. Ecol. Res.* 14: 881–895.
- Rensch, B.** 1960. Evolution above the species level. Columbia University Press, New York, NY.
- Roff, D. A.** 1992. The evolution of life histories. Chapman and Hall, New York, NY.
- Roff, D., and A. T. Mousseau.** 2005. The evolution of the phenotypic covariance matrix: Evidence for selection and drift in *Melanoplus*. *J. Evol. Biol.* 18: 1104–1114.
- Ronderos, R. A.** 1985. Consideraciones sobre la biogeografía de los Melanoplineae en Sudamérica (Orthoptera: Acrididae), pp. 53–54. *In* Proceedings of the 3rd Triennial Meeting of the Pan American Acridological Society, 5–10 July 1981, Pan American Acridological Society, Detroit, MI.
- Rosenberg, M. S., and C. D. Anderson.** 2011. PASSaGE: Pattern analysis, spatial statistics and geographic exegesis. Version 2. *Methods Ecol. Evol.* 2: 229–232.
- Rosetti, N.J.C. M. I., Vilarde Remis.** 2007. Effects of B chromosome and supernumerary segments on morphometric traits and adult fitness components in the grasshopper, *Dichroplus elongatus* (Acrididae). *J. Evol. Biol.* 20: 249–259.
- Rosetti, N., and M. I. Remis.** 2012. Spatial genetic structure and mitochondrial DNA phylogeography of Argentinean population of the grasshopper *Dichroplus elongatus*. *PLoS ONE* 7: e40807. doi:10.1371/journal.pone.0040807.

- Rosetti, N., and M. I. Remis. 2013.** Latitudinal cline in the grasshopper *Dichroplus elongatus*: Coevolution of the A genome and B chromosomes? *J. Evol. Biol.* 26: 719–732.
- Smith, R. J. 1999.** Statistics of sexual size dimorphism. *J. Hum. Evol.* 36: 423–459.
- Statistica Statsoft Inc. 1996.** Statistica 5 for Windows (Computer Program Manual). Statistica, Tulsa, OK.
- Stillwell, R. C., G. E. Morse, and C. W. Fox. 2007.** Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *Am. Nat.* 170: 358–369.
- Stillwell, R. C., W. U. Blanckenhorn, T. Teder, G. Davidowitz, and C. W. Fox. 2010.** Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: From physiology to evolution. *Annu. Rev. Entomol.* 55: 227–245.
- Storz, J. F. 2002.** Contrasting patterns of evolution in quantitative traits and neutral DNA markers: Analysis of clinal variation. *Mol. Ecol.* 11: 2583–2551.
- Tamate, T., and K. Maekawa. 2006.** Latitudinal variation in sexual size dimorphism of sea run masu salmon, *Oncorhynchus masou*. *Evolution* 60: 196–201.
- Tammaru, T., K. Ruomaki, and K. Saikonen. 1996.** Components of male fitness in relation to body size in *Epirrita autumnata* (Lepidoptera, Geometridae). *Ecol. Entomol.* 21: 185–192.
- Taylor, B. J., and D. W. Whitman. 2010.** A test of three hypotheses for ovariole number determination in the grasshopper *Romalea microptera*. *Physiol. Entomol.* 35: 214–221.
- Teder, T., and T. Tammaru. 2005.** Sexual size dimorphism within species increases with body size in insects. *Oikos* 108: 321–334.
- Turk, S. Z., and M. Barrera. 1979.** Acrididos del NOA-III. Estudio bio-ecológico sobre siete especies del género *Dichroplus* Stal (Orthoptera, Acrididae). *Acta Zool. Lilloana* 24: 786–805.

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