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## RESEARCH

# Morphometric Differentiation in *Cornops aquaticum* (Orthoptera: Acrididae): Associations With Sex, Chromosome, and Geographic Conditions

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**ABSTRACT.** The water-hyacinth grasshopper *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae) is native to South America and inhabits lowlands from southern Mexico to central Argentina and Uruguay. This grasshopper feeds and lays eggs on species from the genera *Eichhornia* and *Pontederia*. Particularly, *Eichhornia crassipes* is considered “the world’s worst water weed,” and the release of *C. aquaticum* was proposed as a form of biological control. Morphometric variation on the chromosomally differentiated populations from the middle and lower Paraná River and its possible association with geographic, sex, and chromosomal conditions was analyzed. Significant phenotype variation in *C. aquaticum* population was detected. *C. aquaticum* presents body-size sexual dimorphism, females being bigger than males. Female-biased sexual size dimorphism for all five analyzed traits was detected. The assessment of variation in sexual size dimorphism for tegmen length showed that this trait scaled allometrically, indicating that males and females did not vary in a similar fashion. The detected allometry was consistent with Rensch’s rule demonstrating greater evolutionary divergence in male size than in female size and suggests that males are more sensitive to environmental condition. The analysis of morphometric variation in the context of chromosome constitution showed that the presence of fusion 1/6 was related to body-size variation. Fusion carriers displayed bigger body size than standard homozygotes. Besides, a positive relationship between tegmen length and the number of fused chromosomes was detected, showing a chromosome dose effect. Because the highest frequency of fusions has been found in the lower Paraná River, a marginal environment for this species, the results found would support the hypothesis that some supergenes located in the fusions may be favored in the southern populations, thus contributing to the establishment and maintenance of the polymorphism.

**Key Words:** morphometric variation, semiaquatic grasshopper, sex dimorphism, chromosome rearrangement

The water-hyacinth grasshopper *Cornops aquaticum* (Bruner) (Orthoptera: Acrididae) is native to South America and inhabits lowlands from southern Mexico (23° N) to central Argentina and Uruguay (35° S; Adis et al. 2007). This grasshopper lives in close relationship to species from the genera *Eichhornia* and *Pontederia*, on which it feeds and lays eggs (Adis and Junk 2003, Adis et al. 2004). Particularly, the water hyacinth *Eichhornia crassipes* is considered “the world’s worst water weed”: being introduced elsewhere as an ornamental, this plant has become invasive especially as a result of the lack of natural enemies, obstructing canals, clogging ditches, overgrowing lakes and rivers, and preventing people from free access to water (Center et al. 2002, Aguilar et al. 2003). Attempts to control this weed have led to several control method proposals, expensive and with limited success (Oberholzer and Hill 2001, Aguilar et al. 2003, Albright et al. 2004). Because Perkins (1974) found that *C. aquaticum* was one of the most harmful insects associated with water hyacinths, its release was proposed as a form of biological control (Ferreira and Vasconcellos-Neto 2001, Oberholzer and Hill 2001, Aguilar et al. 2003). Although *C. aquaticum* can feed on a variety of aquatic plants, its life cycle is closely associated with the genus *Eichhornia* because other plant species are unsuitable for their endophytic oviposition (Capello et al. 2010).

Understanding the success of this semiaquatic species as a biological control requires detailed information about diversity and spatial structure of populations.

Recently, chromosome studies have revealed that in the middle and lower courses of the Paraná River, the southernmost part of its distribution, this grasshopper species exhibits karyotype variation. The chromosome number of *C. aquaticum* is modified by the occurrence of

three centric fusions, also called Robertsonian (Rb) translocations (Mesa 1956; Mesa et al. 1982; Colombo 2007, 2008, 2009). As many other chromosomal rearrangements, centric fusions are thought to cause reduction in fertility in structural heterozygotes due to meiotic irregularities (Hewitt 1979). As a matter of fact, proximal chiasma frequency in the trivalents of structural heterozygotes is correlated with malorientation of the trivalent, thus leading to aneuploid gametes (Colombo 2009). However, Colombo (2007, 2009) found that in these populations, chiasma redistribution occurs in both fusion homozygotes and heterozygotes, reducing proximal chiasma frequency and overall recombination due to the loss of independent segregation. Although areas in the middle and lower Paraná River are considered as ecologically marginal environments for this species, Rb translocations increase southward, showing a geographical cline (Colombo 2008).

Body size-related traits have been the center of many evolutionary studies describing intraspecific variability because they are easily observable characters on which natural selection may act. Many studies in insects have shown that body-size characters are usually associated with several adult fitness components in natural populations (Santos et al. 1988, 1992; Hasson et al. 1993; Norry et al. 1995; Remis et al. 2000; Colombo et al. 2001; Rosetti et al. 2007). Effects of natural selection shaping morphometric variation could differ over males and females, thus creating sexual size dimorphism (SSD) patterns (Slatkin 1984, Fairbairn 1997). Sexual dimorphism was defined as “any consistent difference between males and females beyond the basic functional portions of the sex organs” (Wilson 1975). There are several examples on which females display larger body sizes than males (Ridley 1983, Fairbairn 1997). Larger females would have higher fecundity, measured

through ovariole number or laid egg number and egg size (Roff 1992, Blanckenhorn 2000, Akman and Whitman 2008).

Body size-related traits exhibit continuous variation as a consequence of polygenic segregation and environmental influence (Falconer and Mackay 1996). There is evidence of several chromosomal rearrangements, including Rb translocations, shaping the number and position of chiasmata and therefore affecting potentially genetic recombination (Bidau 1990, 1993; Bidau and Martí 2005; Colombo 2007, 2008). As a consequence, chromosomal rearrangement polymorphisms have been also associated with morphometric traits, introducing further variation on which natural selection may act (Butlin et al. 1982, Colombo 1989, Hasson et al. 1992, Remis 1997).

The aim of our study was to analyze morphometric variation on the chromosomally differentiated populations of *C. aquaticum* belonging to the middle and lower Paraná River and to evaluate its possible association with geographic, sex, and chromosomal conditions.

Materials and Methods

**Biological Material and Morphometric Measurements.** Adult males and females of *C. aquaticum* (Orthoptera: Leptysminae) were collected during March–April from six natural Argentine populations: “Laguna Pampin” located in the Corrientes Province; “Santa Fe” and “Rosario” belonging to the Santa Fe Province; and “San Pedro,” “Tigre,” and “Zarate” localized in the Buenos Aires Province. The first set of data includes 108 males sampled in five localities during 2005 (Table 1). The second dataset consists of 78 individuals (41 females and 37 males) from five localities sampled during 2011 (Table 1). Grasshoppers from the Laguna Pampin population were collected on *Eichhornia azurea*, whereas individuals from all other populations were collected on *E. crassipes*.

All specimens were measured for six morphometric traits: total length (length from the phastigium to the articulation between the third coxa and third femur), third femur length, third tibia length, thorax length, thorax height, and tegmen length. Morphometric traits were measured on the right side of the insect, and by the same observer (P.C.C.), with an ocular micrometer (1 mm = 48 ocular units).

Data Analysis.

**Morphometric Variation Among Populations.** We tested the statistical significance of differences in morphometric traits among populations and between sexes using nonparametric multivariate analyses of variance (np-MANOVA). np-MANOVA evaluates differences between two or more groups based on any distance measure and does not require the assumption of multivariate normality (Anderson 2001). This technique was applied using the Euclidean distance.

We also used nonparametric Kruskal–Wallis (KW) tests considering “population” as the independent factor and each morphometric trait as a dependent variable to identify phenotype among-population differences within 2005 male, 2011 male, and 2011 female samples. In the same way, KW tests were applied to analyze temporal phenotype differences within each population considering “year” as the independent factor and each morphometric trait as a dependent variable, based on samples of males collected in 2005 and 2011.

We performed a nonparametric multidimensional scaling (np-MDS) to get a graphical representation of individuals based on morphometric

Euclidean dissimilarities. np-MDS is a multivariate ordering technique that represents the relationships among objects in a reduced number of dimensions, taking into consideration their similarities and dissimilarities. The “stress” value was used as a measure of the model’s fitting (Kruskal 1964): the higher the value of the stress, the worse the fitting of the model. According to Kruskal (1964), stress values of 0.1 are considered acceptable, while the optimal stress value is 0.

These nonparametric analyses were applied because variables including thorax measurements did not fit the normal distribution even after using different transformations.

**Sexual Size Dimorphism.** To study SSD, we considered tegmen length as a measure of body size because it allows us to extend our dataset by incorporating the data of natural South American populations of *C. aquaticum* previously collected on *E. crassipes* and measured by Adis et al. (2008). This trait was measured in both studies according to the same criteria (sensu Carbonell 2002). To graphically analyze sexual dimorphism, we estimated SSD as the ratio of the male mean value to the female mean value (Smith 1999).

As suggested by Fairbairn (1997), allometry for SSD was estimated from the log–log plot of male size against female size. We used reduced major axis (RMA) regression to estimate the slope (*b*) of the relationship between log10-transformed measurements for males and females (Fairbairn 1997). We tested the hypothesis that *b* = 1 (no allometry for SSD) with the Clarke’s *T* statistic with adjusted degrees of freedom (Clarke 1980).

**Morphometric Variation Associated With Karyotype.** The standard karyotype of *C. aquaticum* is composed of 22 acrocentric autosomes and an X0–XX sex determination system (Mesa 1956, Mesa et al. 1982, De França Rocha et al. 2004). Natural Argentine populations of this species are polymorphic for three centric fusions (Fig. 1). Previous cytogenetic studies showed that the Laguna Pampin population is monomorphic without fusions and the Santa Fe and Rosario populations have very low fusion frequencies, whereas in Tigre the opposite situation is found: almost all individuals are homozygous for the three centric fusions. Only the San Pedro and Zarate populations are polymorphic, the first with low fusion frequencies (Colombo 2008).

To assess the relationships between chromosome rearrangements and phenotypes, we scored body- size-related traits of individuals from polymorphic populations where different karyotype classes had been previously characterized at the chromosome level (Colombo 2008).

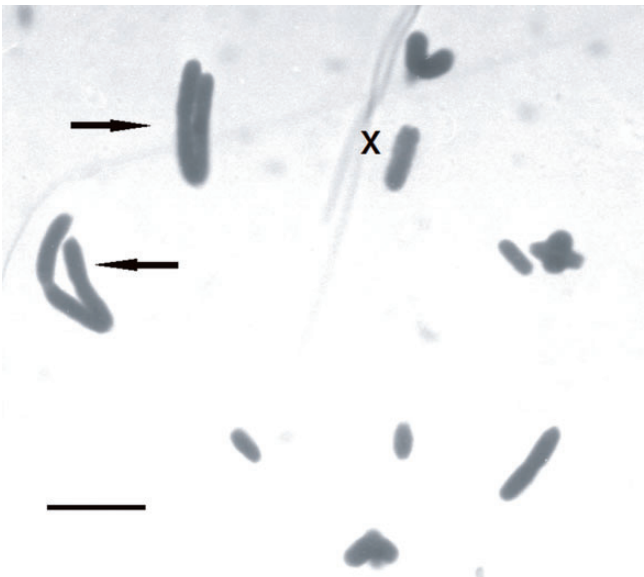


Fig. 1. Metaphase I plate of male *C. aquaticum* heterozygote for two fusions (full arrow). Scale bar = 10  $\mu$ m.

Table 1. Geographic data of natural populations of *C. aquaticum* collected in Argentina

Populations	Longitude (°)	Latitude (°)	Sample year(s)
Laguna Pampin	58° 51' W	27° 28' S	2005–2011
Santa Fe	60° 43' W	31° 65' S	2005–2011
Rosario	60° 39' W	32° 57' S	2005
San Pedro	59° 41' W	33° 39' S	2005–2011
Zarate	59° 02' W	34° 06' S	2005–2011
Tigre	58° 35' W	34° 25' S	2011

Statistical analyses were performed using datasets from Zárate and San Pedro populations sampled during 2005. Data were transformed to standardized deviations from the mean value for each trait in each population to avoid between-population variation. To analyze the effect of chromosome constitution on the morphometric traits, fusion dosage per individual was coded as “0” (unfused homozygote), “1” (heterozygote), or “2” (fusion homozygote). Variations in body size-related traits due to the fusion dosage were analyzed by nonparametric KW tests, considering “fusion dosage per individual” as the independent factor and each morphometric trait as the dependent variable, and by means of Kendall correlations.

np-MANOVA was performed to see overall differences among karyotypes for the group of morphometric traits. np-MDS was also performed to describe multivariate similarities among individuals with the same karyotype.

KW tests and correlation analyses were performed using STATISTICA (Statistica Statsoft Inc. 1996). RMA regression was performed with lmodel2 package for R version 2.15.1 software (Legendre 2012). np-MANOVA and np-MDS analyses were performed using PAST 2.16 software (PAST, 2012) and Vegan package for R version 2-16-32 software (Vegan, 2013).

## Results

**Morphometric Variation Among Populations.** Considerable body-size variation was observed among natural populations of the grasshopper *C. aquaticum* (Table 2). MDS ordination of the Euclidean distance matrix based on the 2011 dataset has shown a clear spatial distribution between sexes, whereas weak differentiation among populations has been revealed within male and female groups (stress value = 0.038; Fig. 2). The np-MANOVA based on the first two coordinates of individuals in MDS clearly demonstrated highly significant differences between sexes ( $F = 175.89$ ,  $P = 0.0005$ ) and marginally significant differences among populations ( $F = 1.95$ ,  $P = 0.07$ ). No interaction (population  $\times$  sex) was found, with females exhibiting higher mean values for each trait measured ( $F = 1.99$ ,  $P = 0.094$ ). As males and females had shown differences in the mean value of each morphometric trait, both sexes were analyzed independently in further studies.

np-MANOVA using the complete male dataset (2005 and 2011) revealed highly significant differences among populations ( $F = 5.47$ ,  $P = 0.0005$ ) and between years ( $F = 9.22$ ,  $P = 0.0009$ ). Furthermore, significant interaction (population  $\times$  year) was detected ( $F = 2.86$ ,  $P = 0.0124$ ).

Individual KW tests allowed gaining further insight about spatial phenotypic differences in males. We detected significant heterogeneity

among populations for thorax length in 2011 males and for total, femur, tibia, and tegmen lengths in 2005 males (Table 3). To analyze temporal phenotypic differences in males, only populations collected in both years were considered. Greater temporal heterogeneity was detected in the Santa Fe and Laguna Pampin populations, with four of the six analyzed traits exhibiting significant or highly significant differences. Minor temporal heterogeneity was detected (for thorax length) in the Zárate population, whereas the San Pedro population was temporally homogeneous (Table 3).

np-MANOVA applied to the female dataset (2011) showed significant differences among populations ( $F = 497$ ,  $P = 0.0001$ ), whereas KW tests detected significant differences for thorax length and thorax height (Table 3).

**Sexual Dimorphism.** Phenotypic variation was previously examined in nine natural South American populations of *C. aquaticum* (Brede et al. 2008). To evaluate SSD, we considered the tegmen length, i.e., the only trait analyzed simultaneously by Brede et al. (2008) and in this study.

To visualize directly differences, we have estimated SSD as the ratio of the arithmetic mean of each measured character of females to the corresponding mean of males, in the different populations (Table 4). The SSD values for tegmen length were higher than 1, confirming female-biased size dimorphism in all analyzed populations. Variations in SSD were not correlated with variations in latitude or longitude ( $P > 0.05$  in both cases).

To test allometry for SSD among populations, regression between log (male size) and log (female size) was set up. The RMA slope for tegmen length was higher than 1.0 ( $b = 1.528$ ,  $T = 3.57$ ,  $P = 0.004$ ), suggesting SSD allometry for this trait (Fig. 3). Similar results were obtained when the Laguna Pampin population of *C. aquaticum* collected in another host plant (*E. azureae*) was excluded ( $b = 1.533$ ,  $T = 3.43$ ,  $P = 0.005$ ). Our results indicated that tegmen size increases relatively faster in males than in females.

**Relationships Between Morphometric Traits and Karyotype (Chromosome Constitution).** Populations of *C. aquaticum* from Argentina are polymorphic for three centric fusions. To analyze the relationships between karyotype and phenotype, we considered population samples with a representative number of individuals within each karyotype class (Zárate 2005 and San Pedro 2005). Males with 1/6 fusion are bigger than standard males (Table 5; Fig. 4). When jointly analyzing standardized data of both populations, we detected significant relationship among 1/6 karyotypes for tegmen length (KW test,  $H = 7.280$ ,  $P = 0.026$ ).

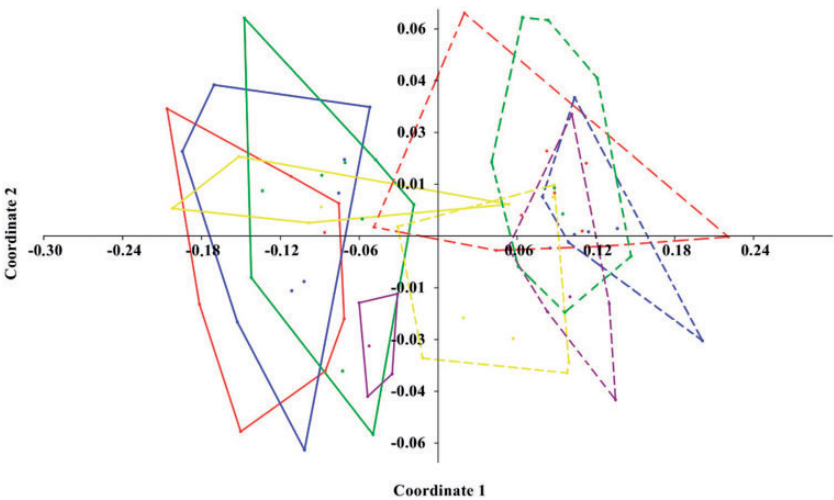
Variation in body-size traits related to the fusion dosage was also evaluated by correlating chromosome constitution with morphometric

**Table 2. Mean values (in cm) and standard errors (in brackets) for morphometric traits in males and females from the populations sampled**

	TL	FL	TiL	TxL	TxH	TegL	N
<b>Males 2005</b>							
Laguna Pampin	1.052 (0.007)	1.399 (0.013)	1.127 (0.011)	0.474 (0.004)	0.482 (0.005)	2.221 (0.017)	43
Santa Fe	1.073 (0.010)	1.417 (0.018)	1.145 (0.015)	0.478 (0.006)	0.492 (0.007)	2.234 (0.024)	21
Rosario	0.989 (0.013)	1.409 (0.022)	1.123 (0.019)	0.471 (0.008)	0.474 (0.008)	2.027 (0.030)	14
San Pedro	1.003 (0.018)	1.417 (0.031)	1.149 (0.026)	0.480 (0.011)	0.486 (0.012)	2.146 (0.042)	7
Zárate	1.076 (0.010)	1.443 (0.017)	1.164 (0.015)	0.465 (0.006)	0.488 (0.006)	2.297 (0.023)	23
<b>Males 2011</b>							
Laguna Pampin	0.985 (0.034)	1.340 (0.032)	1.043 (0.033)	0.446 (0.008)	0.418 (0.015)	2.113 (0.051)	8
Santa Fe	1.004 (0.032)	1.333 (0.031)	1.062 (0.031)	0.480 (0.008)	0.469 (0.014)	2.107 (0.048)	9
Rosario	1.052 (0.030)	1.426 (0.029)	1.074 (0.029)	0.489 (0.007)	0.466 (0.013)	2.148 (0.045)	10
San Pedro	1.048 (0.043)	1.388 (0.041)	1.076 (0.041)	0.488 (0.010)	0.462 (0.019)	2.112 (0.064)	5
Zárate	1.044 (0.043)	1.420 (0.041)	1.080 (0.041)	0.500 (0.010)	0.448 (0.019)	2.320 (0.064)	5
<b>Females 2011</b>							
Laguna Pampin	1.266 (0.030)	1.622 (0.027)	1.274 (0.017)	0.567 (0.010)	0.555 (0.019)	2.494 (0.042)	10
Santa Fe	1.257 (0.036)	1.700 (0.032)	1.314 (0.021)	0.603 (0.012)	0.596 (0.022)	2.577 (0.050)	7
Rosario	1.260 (0.032)	1.629 (0.029)	1.307 (0.018)	0.603 (0.010)	0.579 (0.020)	2.489 (0.044)	9
San Pedro	1.209 (0.036)	1.674 (0.032)	1.251 (0.021)	0.600 (0.012)	0.561 (0.022)	2.560 (0.050)	7
Zárate	1.125 (0.034)	1.610 (0.030)	1.260 (0.019)	0.568 (0.011)	0.504 (0.021)	2.460 (0.047)	8

TL, total length; FL, femur length; TiL, tibia length; TxL, thorax length; TxH, thorax height; TegL, tegmen length; N, number of individuals sampled.





**Fig. 2.** MDS analysis applied to the Euclidean distance matrix describing similarities and dissimilarities among five populations of *C. aquaticum* males (full line) and females (dashed line), based on six morphometric traits. Convex polygons encompass the individuals from the Laguna Pampin (red), Santa Fe (blue), San Pedro (green), Zarate (violet), and Tigre (yellow) populations.

**Table 3.** *H* and *P* values from KW tests analyzing morphometric differences among sampled populations of 2005 males, 2011 males and 2011 females, and between 2005 and 2011 male sampled populations collected simultaneously in both years

	TL	FL	TiL	TxL	TxH	TegL
Among populations						
Males 2005						
<i>H</i>	31.232	13.680	13.028	4.961	4.047	36.927
<i>P</i>	<b>&lt;10<sup>-4</sup></b>	<b>0.008</b>	<b>0.011</b>	0.291	0.400	<b>&lt;10<sup>-4</sup></b>
Males 2011						
<i>H</i>	4.465	9.416	2.772	17.852	7.876	7.913
<i>P</i>	0.347	0.052	0.597	<b>0.001</b>	0.096	0.095
Females 2011						
<i>H</i>	8.783	6.787	6.771	13.59	12.212	5.976
<i>P</i>	0.067	0.148	0.149	<b>0.009</b>	<b>0.016</b>	0.201
Between years						
Laguna Pampin						
<i>H</i>	5.732	3.924	11.020	5.033	14.436	3.753
<i>P</i>	<b>0.017</b>	0.048	<b>0.001</b>	<b>0.025</b>	<b>0.000</b>	0.053
Santa Fe						
<i>H</i>	2.380	6.459	5.724	0.025	3.920	5.666
<i>P</i>	0.123	<b>0.011</b>	<b>0.017</b>	0.874	<b>0.048</b>	<b>0.017</b>
San Pedro						
<i>H</i>	1.388	0.088	2.800	0.780	2.300	0.021
<i>P</i>	0.239	0.767	0.094	0.377	0.129	0.885
Zarate						
<i>H</i>	1.847	1.621	3.086	5.103	2.046	0.030
<i>P</i>	0.174	0.203	0.079	<b>0.024</b>	0.153	0.863

TL, total length; FL, femur length; TiL, tibia length; TxL, thorax length; TxH, thorax height; TegL, tegmen length.  
Significant values are in bold.

trait mean values. We found a positive nonparametric Kendall correlation between 1/6 fusion dosage and femur length ( $r=0.259$ ,  $P=0.044$ ), tibia length ( $r=0.275$ ,  $P=0.033$ ), and tegmen length ( $r=0.257$ ,  $P=0.045$ ; Fig. 4).

Two separated groups were highlighted by np-MSD analysis (stress value = 0.088): the first group clustered standard homozygotes and the second gathered fusion homozygotes. A bigger group containing heterozygotes occupied a larger area, including the two previous groups, revealing higher heterogeneity in heterozygotes when compared with the other karyotypes (Fig. 5). For fusions 2/5 and 3/4, the different dosages of fusion did not differ from one another with regard to the multivariate components extracted.

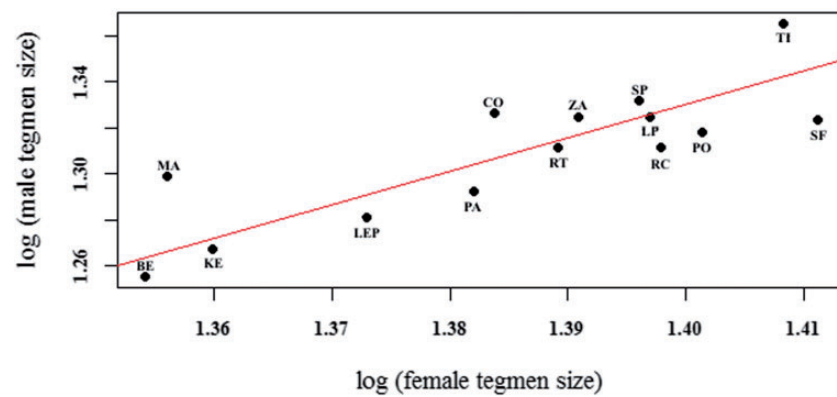
**Table 4.** SSD for tegmen length in 14 populations of *C. aquaticum*

Country	Population	SSD
Argentina	Laguna Pampin (LP)	1.181
	Santa Fe (SF)	1.223
	San Pedro (SP)	1.159
	Zarate (ZA)	1.060
	Tigre (TI)	1.212
	Rio Torrentoso (RT) <sup>a</sup>	1.195
	Las Cataratas (LC) <sup>a</sup>	1.220
	Laguna El Puesto (LEP) <sup>a</sup>	1.236
	Corrientes (CO) <sup>a</sup>	1.142
	Belém (BE) <sup>a</sup>	1.256
Brazil	Manaus (MA) <sup>a</sup>	1.141
	Pantanal (PA) <sup>a</sup>	1.230
	Kernahan (KE) <sup>a</sup>	1.238
Trinidad	Piriapolis (PI) <sup>a</sup>	1.212
Uruguay		

<sup>a</sup>SSD values were calculated with data from Adis et al. (2008).

**Discussion**

The water-hyacinth grasshopper *C. aquaticum* (Orthoptera: Acrididae) is a Neotropical grasshopper with a distribution extending from the south of Mexico up to Uruguay and the northeast of Argentina (Adis et al. 2007). South American populations have shown variability for voltinism. Northern populations are bivoltine, whereas southern populations are univoltine (Brede et al. 2007). In addition to studying the ecological consequences of the introduction of a new species as a biological control agent, due, for example, to its feeding and oviposition preferences (Ferreira and Vasconcellos-Neto 2001, Oberholzer and Hill 2001, Aguilar et al. 2003, Capello et al. 2010), examining natural variability of populations is useful for knowing which ones may be more successful when introduced in new environments, and thus improve weed control management programs. Like other introduced species, *C. aquaticum* will have to face not only similar constraints as those exhibiting isolated populations but also new selective pressures. Body-size variation among populations of arthropod species is a common phenomenon, and such variation has often been related to adaptation to different environmental conditions (Blanckenhorn and Demont 2004). Body size-related traits exhibit continuous variation as a consequence of polygenic segregation and environmental influence (Hedrick 1983, Falconer and Mackay 1996). Moreover, many examples have demonstrated, in natural populations, a close relationship between



**Fig. 3.** RMA regression (red line) of log male tegmen size on log female tegmen size of *C. aquaticum* populations. Population abbreviations are available in Table 4.

**Table 5.** Mean values (in cm) and standard errors (in brackets) for morphometric traits in males of *C. aquaticum* sampled in San Pedro and Zárate and their karyotype composition

	TL	FL	TiL	TxL	TxH	TegL
<b>San Pedro</b>						
Fusion 2/5						
UU (N = 7)	1.010 (0.071)	1.423 (0.065)	1.146 (0.046)	0.472 (0.032)	0.495 (0.029)	2.184 (0.155)
UF (N = 2)	1.053 (0.001)	1.42 (0.001)	1.180 (0.028)	0.502 (0.012)	0.502 (0.012)	2.140 (0.084)
FF (N = 0)	—	—	—	—	—	—
Fusion 1/6						
UU (N = 5)	0.983 (0.046)	1.392 (0.030)	1.132 (0.038)	0.460 (0.030)	0.496 (0.025)	2.125 (0.095)
UF (N = 3)	1.083 (0.053)	1.473 (0.050)	1.193 (0.011)	0.501 (0.008)	0.512 (0.005)	2.250 (0.240)
FF (N = 1)	1.010 (0.000)	—	—	0.500 (0.000)	0.450 (0.000)	2.200 (0.000)
Fusion 3/4						
UU (N = 5)	1.042 (0.077)	1.444 (0.053)	1.176 (0.032)	0.482 (0.026)	0.505 (0.013)	2.200 (0.156)
UF (N = 4)	0.991 (0.055)	1.386 (0.042)	1.120 (0.040)	0.474 (0.039)	0.486 (0.037)	2.133 (0.115)
FF (N = 0)	—	—	—	—	—	—
<b>Zárate</b>						
Fusion 2/5						
UU (N = 5)	1.066 (0.079)	1.460 (0.082)	1.160 (0.071)	0.474 (0.049)	0.476 (0.041)	2.256 (0.172)
UF (N = 11)	1.075 (0.042)	1.420 (0.107)	1.162 (0.059)	0.461 (0.033)	0.487 (0.033)	2.304 (0.119)
FF (N = 7)	1.073 (0.022)	1.457 (0.064)	1.163 (0.051)	0.465 (0.027)	0.485 (0.030)	2.314 (0.106)
Fusion 1/6						
UU (N = 3)	1.075 (0.042)	1.433 (0.012)	1.167 (0.083)	0.473 (0.009)	0.482 (0.048)	2.333 (0.103)
UF (N = 15)	1.066 (0.048)	1.427 (0.102)	1.147 (0.045)	0.458 (0.027)	0.479 (0.031)	2.245 (0.114)
FF (N = 5)	1.098 (0.013)	1.490 (0.062)	1.215 (0.057)	0.481 (0.030)	0.502 (0.031)	2.428 (0.030)
Fusion 3/4						
UU (N = 5)	1.037 (0.067)	1.436 (0.085)	1.116 (0.026)	0.451 (0.043)	0.468 (0.032)	2.212 (0.122)
UF (N = 14)	1.087 (0.028)	1.446 (0.102)	1.179 (0.061)	0.468 (0.03)	0.489 (0.036)	2.299 (0.114)
FF (N = 4)	1.066 (0.023)	1.413 (0.042)	1.160 (0.020)	0.476 (0.024)	0.489 (0.020)	2.395 (0.104)

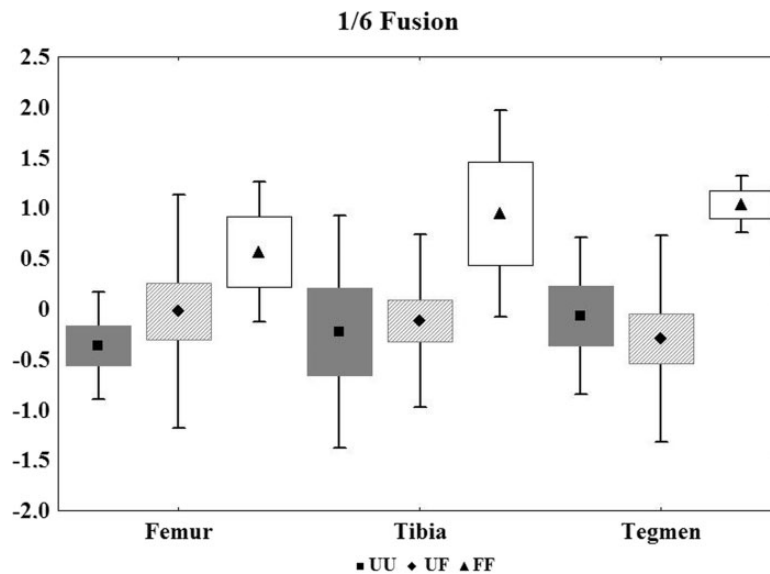
UU, unfused homozygotes; UF, heterozygotes; FF, fused homozygotes; TL, total length; FL, femur length; TiL, tibia length; TxL, thorax length; TxH, thorax height; TegL, tegmen length; N, number of individuals sampled.

variation in morphometric traits and fitness, a target of natural selection both in males and in females (Santos et al. 1988, Hasson et al. 1993, Norry et al. 1995, Akman and Whitman 2008). Knowing population body size-related patterns is relevant because it may provide information on the intensity of environmental constraints and the species adaptive responses (Stearns 1992, Berner and Blanckenhorn 2006, Huizenga et al. 2008), issues that may potentially influence species establishment success.

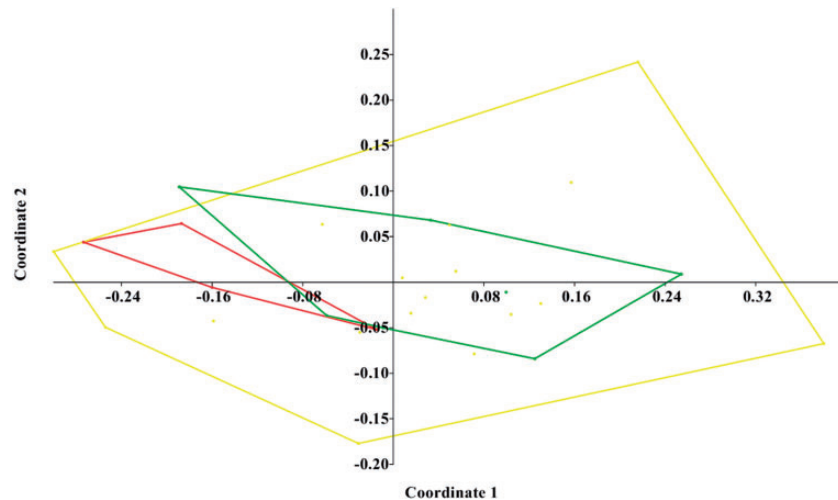
In this study, we have detected significant phenotype variation in the *C. aquaticum* population. np-MANOVA and KW tests have shown significant heterogeneity in most of the analyzed morphometric traits. Similar results were obtained by Adis et al. (2008) when analyzing 14 populations of this grasshopper species across South America. We have identified phenotypic variation across a more restricted geographic area (from Laguna Pampín to Tigre, Argentina) demonstrating the high phenotypic plasticity of this species in nature. np-MANOVA and np-MDS have pointed out that most of the phenotypic variation observed in the

studied area might be explained mainly by differences between sexes and, to a lesser extent, by among-population heterogeneity.

SSD is a widespread phenomenon in different groups of animals, including Orthoptera, whose females are larger than males (Colombo et al. 2004; Bidau and Martí 2005, 2008; Rosetti et al. 2007; Hochkirch and Gröning 2008; Remis 2008; Huizenga et al. 2008). A widely accepted hypothesis explaining the existence of intersexual variation is the “differential equilibrium hypothesis,” which proposes that SSD would be the result of selective forces favoring larger body size (i.e., fecundity selection in females and sexual selection in males) and selective forces favoring smaller body size in both sexes (i.e., viability selection; Blanckenhorn 2000, Blanckenhorn et al. 2007, Stillwell et al. 2007). Organism growth and development might also be considered as factors affecting intraspecific body-size variation (Roff 1992). Males of Orthoptera are frequently associated with a lower number of nymphal instars than females, leading to earlier emergence (protandry; Esperk et al. 2007). The faster emergence of adult males might have adaptive



**Fig. 4.** Mean value (primary symbol), standard error (box), and standard deviations (bar) of standardized morphometric traits related to karyotype for standard homozygotes (UU), heterozygotes (UF), and fused homozygotes (FF) for fusion 1/6.



**Fig. 5.** MDS analysis applied to the Euclidean distance matrix describing similarities and dissimilarities among karyotypes for 1/6 centric fusion, based on six morphometric traits. Convex polygons encompass the fused homozygotes (FF) in red, heterozygotes (UF) in yellow, and unfused homozygotes (UU) in green.

significance ensuring higher quality of territory and higher probability to fertilize virgin females.

As seen for many insect species, *C. aquaticum* showed body-size sexual dimorphism, with females being bigger than males. We detected female-biased SSD for the five analyzed traits. Although population studies related to sexual selection, fecundity, and viability are rare, some interesting results are available about the development time in this species. The number of juvenile instars in *C. aquaticum* can vary from 5 to 7, and this variation might be related to photoperiod and temperature variation across the species distribution range (Adis et al. 2004). Laboratory studies have demonstrated that females from Argentina and Uruguay have five to six juvenile instars whereas males exhibit only five instars, suggesting the relative importance of development time in shaping SSD.

The SSD in Orthoptera may vary among populations following different patterns according to the relative importance of selective forces acting on phenotype evolution. SSD of the grasshoppers *Dichroplus vittatus* and *Dichroplus elongatus* increases with increasing body size following the converse Rensch's rule (Bidau and Marti 2008). On the

contrary, the bushcricket *Poecilimon thessalicus* and the grasshopper *Dichroplus pratensis* are examples illustrating Rensch's rule where SSD decreases when body size increases (Bidau and Marti 2008, Lehman and Lehman 2008).

Variation in SSD for tegmen length of *C. aquaticum* was assessed from a representative number of populations considering previous studies and this study. Tegmen length scaled allometrically, indicating that males and females do not vary in a similar fashion across the studied area. The between-sex allometric slope (*b*) for tegmen length is significantly higher than 1.0, indicating a greater change in male size than in female size. The detected allometry was consistent with Rensch's rule demonstrating greater evolutionary divergence in male size than in female size. This result suggests that males are more sensitive to environmental condition than females.

Several studies have demonstrated that arthropod populations can differ phenotypically. Such variation has been related to local adaptation, phenotypic plasticity, or both (Blanckenhorn and Demont, 2004, Stillwell et al. 2010). In this article, the multivariate and univariate analyses based on samples of males collected in both years have revealed

spatial and temporal phenotypic variation in *C. aquaticum*. Between-sample phenotypic differences within a short time period (this study) suggest a significant role of phenotypic plasticity to climatic variation, voltinism, and population density in shaping temporal phenotypic variation. The spatial phenotypic variation may also illustrate the local adaptation of populations to season length, along the latitudinal gradient even if we do not have strong arguments for rejecting an alternative scenario based again on plasticity mainly to explain phenotypic differences among populations. Moreover, we have detected significant population-year interaction in males. This effect may be also due to phenotype plasticity. Previous results analyzing nine populations of *C. aquaticum* from a wide range of South American areas (from Monte Dourada in northern Brazil to Santa Fe in Argentina) have demonstrated genetic homogeneity among populations (evaluated through microsatellite loci) and high heterogeneity at the phenotypic level (Brede et al. 2007). It was suggested that phenotypic plasticity with respect to the variable number of instars responding to different environmental conditions may explain the detected body-size variation.

Additionally, the *C. aquaticum* populations analyzed in this study exhibited chromosome variation in the lower course of the Paraná River: their karyotype includes three centric fusions (Mesa et al. 1982; Colombo 2007, 2008, 2009). The maintenance of these rearrangements in polymorphic state is associated with changes in the number and position of chiasmata, potentially affecting genetic recombination (White 1973; Bidau 1993; Colombo 2007, 2008). Furthermore, fusion frequencies in studied populations increase southward, showing a geographical cline (Colombo 2008). We found that, in polymorphic populations, the presence of fusion 1/6 was related to body size. Fusion carriers displayed bigger body size than standard homozygotes. KW tests demonstrated a chromosome component in the effect of karyomorph on tegmen length. Accordingly, we detected a positive relationship between tegmen length and the number of fused chromosomes with a chromosome dose effect.

Several studies on Orthoptera species have found that chromosomal rearrangements are associated with morphometric traits (Colombo 1989; Remis 1997, 2008; Colombo et al. 2001; Bidau and Martí 2005). Some examples have demonstrated similar effects as those detected in this article, i.e., heterozygotes for centric fusion or pericentric inversions in an intermediate position between both homozygote types for particular morphometric traits used as indices of body size (Colombo 1989, Remis 1997).

Colombo (2008) studied the behavior and geographical distribution of the Rb polymorphisms described for *C. aquaticum*. He found that chiasma redistribution occurs and affects both homozygotes and heterozygotes, reducing the amount of recombinations in the population with high frequency of metacentric chromosomes (Colombo 2008). Besides, the distribution pattern of the Rb translocations found in *C. aquaticum* seems to follow the opposite pattern observed for inversion polymorphisms in *Drosophila*, where higher levels of recombination are expected to occur in marginal areas (see Powell 1997 for a review). The lower Paraná River is a marginal environment for this species, and the highest frequency of fusions has been found in this river. *C. aquaticum* shows the lowest level of recombination in most marginal populations (Colombo 2008). Colombo (2008) has suggested that some supergenes may be favored in the southern populations, thus favoring the establishment of the polymorphism. In our case, we found a direct relationship between chromosome constitution and morphometric traits. Fusion carriers were larger than standard homozygotes, and a chromosome dose effect was found. Additionally, the MDS graph showed that the presence of 1/6 centric fusion is not only related to change in the mean morphometric values but also to phenotype variation, generating a greater number of phenotypes for heterozygotes. *C. aquaticum* maintains chromosome variability in their southernmost distribution, and this variation affects body size-related traits, leading to an increase in population phenotypic variation. Because *C. aquaticum* has been proposed in South Africa as a biological pest control (Oberholzer and Hill 2001),

it is tempting to consider that the levels of recombination and phenotype variation in the introduced samples may depend on the presence of centric fusions in the colonist populations.

This study increases our knowledge about the quantification and distribution of morphometric diversity in populations from the southernmost distribution area of *C. aquaticum*. We have confirmed the heterogeneous patterns observed in previous studies, and we have demonstrated chromosome constitution as a source of variability that needs to be taken into account. The adaptive significance of these effects remains an attractive question for further studies. Using molecular markers will certainly help to elucidate the sources of phenotype heterogeneity found in native populations of *C. aquaticum* in the middle and lower courses of the Paraná River.

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