Phenotypic pattern over centric fusion clinal variation in the water-hyacinth grasshopper, *Cornops aquaticum* (Orthoptera: Acrididae)

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Abstract. The water-hyacinth grasshopper, *Cornops aquaticum*, occurs in freshwater environments in the New World between latitudes 23°N and 35°S. At the southernmost margin of this distribution the populations are polymorphic for three centric fusions (Robertsonian translocations). The frequencies of these chromosome rearrangements increase southwards and the recombination in structural homozygotes and heterozygotes diminishes both along the middle and lower courses of the Paraná River. In the present paper we report a similar cline along the southward flowing Uruguay River. In addition, we report the morphological effects of two of these centric fusion polymorphisms, namely the fusions between chromosomes 2 and 5 of the standard complement (fusion 2/5) and chromosomes 3 and 4 (fusion 3/4) and extend this study to the Uruguay River. There is a strong inverse correlation of fusion frequency with temperature, which indicates that these polymorphisms may be related to increased tolerance of colder climates in this originally tropical species, or some other correlated variable. This study is a further example of chromosomal clines correlated with latitude and is one of a few examples of chromosome polymorphisms associated with phenotypic effects. Finally, it indicates ways of using this species for controlling pests.

INTRODUCTION

The family Acrididae of the insect order Orthoptera are well studied because of their relatively big chromosomes and the clarity of their meiotic figures. Their karyotype is rather conservative, with 23 chromosomes in males and 24 chromosomes in females due to an X0/XX sex determination system. Most of their chromosomes are acrocentric or telocentric. However, quite a few of their species are chromosomally polymorphic, on occasion remarkably so (Hewitt, 1979; John, 1983). The study of chromosome polymorphisms in acridid grasshoppers has revealed that most of them are pericentric inversions (White, 1951; Colombo & Confalonieri, 1996; Remis, 1989) and Robertsonian translocations (centric fusions) (Colombo, 2013), along with supernumerary segments and B chromosome polymorphisms (Hewitt, 1979; Camacho et al., 2000; Rosetti & Remis, 2017). Polymorphic centric fusions in acridid grasshoppers occur in just a few species, such as *Hesperotettix viridis* (McClung, 1917), *Oedaleonotus enigma* (Hewitt & Schroeter, 1967); *Leptysma argentina* (Bidau & Hasson, 1984; Colombo, 1989), *Dichroplus pratensis* (Bidau

& Mirol, 1988; Bidau & Martí, 2005); *Sinipta dalmani* (Remis, 1990); (Remis, 2008), *Cornops aquaticum* (Mesa, 1956; Colombo, 2008), *Dichroplus fuscus* (Taffarel et al., 2015) and *Scotussa cliens* (Martí et al., 2017), all of them in the New World and six out of eight from South America (Colombo, 2013).

The water-hyacinth grasshopper, *Cornops aquaticum,* occurs in the New World between latitudes 23°N, in Mexico, and 35°S in Central Argentina and Uruguay following the distribution of its host plants, i.e. different species of water-hyacinths (family Pontederiaceae) (Adis et al., 2007; Capello et al., 2010). The recent worldwide expansion of the water-hyacinth *Eichhornia crassipes* as a serious freshwater weed in tropical and subtropical, and even temperate, regions of the world (Center et al., 2002) has led to the use of *C. aquaticum* as a biological control agent (Oberholzer & Hill, 2001; Coetzee et al., 2011; Bownes et al., 2013). Its basic karyotype consists of 22 acrocentric autosomes, with an X0/XX sex determination system (França Rocha et al., 2004). The cytological study of natural populations have revealed the presence of three centric fusion poly-

morphisms (= Robertsonian translocations) (Mesa, 1956; Mesa et al., 1982). Recent work at the southern margin of this species' distribution, between latitudes 27°S and 34°S along the Paraná River in Argentina, revealed the existence of a N-S cline in fused chromosome frequencies, namely fusions 2/5, 1/6 and 3/4 (Colombo, 2008). These fusions severely reduce recombination in fusion homozygotes and heterozygotes (Colombo, 2007).

Populations of orthopteran species, particularly those with a wide geographic distribution, usually show significant variations in body size. Some of the body size-related studies explain the variation in terms of Bergmann's rule, which predicts an inverse correlation between temperature and body size in nature. This rule was first established for endotherms; later studies report that Bergmann's or converse Bergmann's rule may also apply to ectotherms, but in some of them, especially insects, life history-related processes, such as generation span, are frequently more important. In fact, in Orthoptera most of the species so far studied follow the converse Bergmann's rule, whereby body size decreases with latitude or altitude, but some report they follow Bergmann's rule, with larger individuals at high latitudes and altitudes (for review see Blackburn et al., 1999; Blanckenhorn & Demont, 2004; Whitman, 2008).

Colombo & Remis (2015), using a continent-wide morphometric dataset published by Adis et al. (2008) and Romero et al. (2014), which includes several South-American populations of *C. aquaticum* distributed between lat. 10°N and 35°S, suggest that this species' body size-related variables are correlated positively with latitude. However, although the latitudinal area studied was very large (45 degrees of latitude), there are considerable gaps in the locations of the samples analysed and the study is based only on tegmen length, the only variable measured in the same way by both us and Adis et al. (2008).

Moreover, a preliminary morphometric study of two polymorphic populations indicates an association between fusion 1/6 and body size (Romero et al., 2014). As one of the centric fusions is correlated with body size-related variables (Romero et al., 2014), we suggest that the increasing cline for fusion frequency in the southern populations could be the indirect result of selection for increased body size (Colombo & Remis, 2015). However, we did not reject the operation of other factors, such as a correlation with as yet undisclosed environmental variables or, given the geographic marginal situation of the polymorphic populations, stochastic factors.

In the present contribution we extend the cytological and morphometric studies to populations sited along another large tributary of the River Plate, namely the Uruguay River, thus adding new highly polymorphic populations to the cytogenetic analysis and allowing a new and wider joint morphometric approach that allows the testing of these and other hypotheses.

MATERIALS AND METHODS

Biological material and cytogenetic analyses

We collected 240 individuals of *Cornops aquaticum* from 6 Argentine populations at Monte Caseros (37 individuals), Salto Grande (12 individuals), Colón (45 individuals), Concepción del Uruguay (66 individuals), Gualeguaychú (42 individuals) and Villa Paranacito (38 individuals) along the Uruguay River. The chromosome studies were on males; testes were dissected and fixed in ethanol-acetic acid (3 : 1). Cytological analysis was performed by squashing some follicles in propionic haematoxylin. Karyotypes were determined using male metaphase I plates. In order to identify the chromosome pairs involved in centric fusions in each individual, trivalents or sub metacentric bivalents with terminal chiasmata were carefully measured. When there are interstitial or proximal chiasmata the pairs of chromosomes involved in the rearrangement cannot be identified, but otherwise they are easily and accurately identifiable. However, due to poor fixation, the fusion number for the Salto Grande individuals could be determined but fusions could not be identified. Only males were analysed because male sample size was large enough for drawing reliable statistical conclusions, and because as yet unpublished research has shown that, within these limitations, the frequency of fusions per individual per population does not vary between sexes. Currently, with a larger sampling effort, we are carrying out a morphometric and cytological studies that include females, which will be a subject for a future publication (P.C. Colombo & M.I. Remis, unpubl. data).

The environmental variables for each site were obtained using the programme NewLocClim. The correlations between the fusion frequencies and average number of centric fusions per individual per population (fpi) with climatic data were obtained using nonparametric Kendall correlations.

Morphometric analyses

All specimens were measured for five morphometric traits: length of fastigium/third coxa, P/CL), length of third femur (FL), length of third tibia (TiL), length of thorax (TxL) and length of tegmen (TegL). Morphometric traits were measured on the left side of each insect using an ocular micrometre (1 mm = 48 ocular units).

Morphometric variation among populations was tested using nonparametric multivariate analyses of variance (np-MANOVA) and Euclidean distances, and Kruskal-Wallis (KW) tests considering "population" as the independent factor and each morphometric trait as a dependent variable. Both analyses were done using PAST 2.16 software (PAST, 2012).

The relationships between chromosome karyotype and body size-related traits were obtained by analysing datasets from the polymorphic populations (Colón, Concepción del Uruguay, Gualeguaychú and Villa Paranacito) sampled in 2014 and 2015. Data were transformed to standardized deviations from the mean value for each trait in each population to reduce between-population variation. To analyse the effect of chromosome constitution on the morphometric traits, fusion dosage per individual per centric fusion was coded as "0" (unfused homozygote), "1" (heterozygote), or "2" (fusion homozygote). Variations in body size-related traits due to the fusion dosage were analysed using nonparametric Kendall correlations in the package STATISTICA (StatSoft-Inc, Tulsa, OK, USA, 1999).
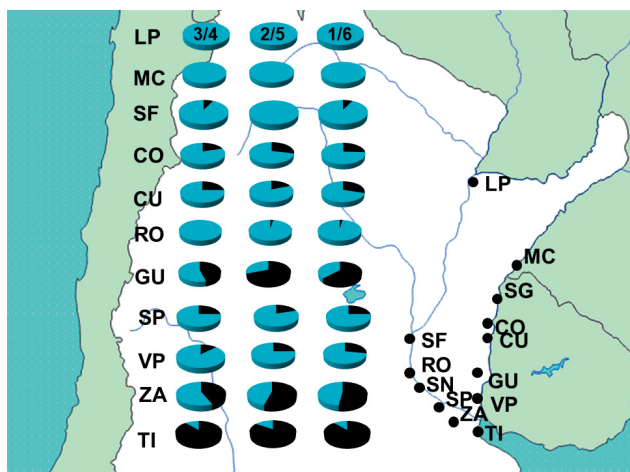
Fig. 1. Map showing the geographic distribution of the populations of *Cornops aquaticum* analysed. LP – Laguna Pampín*; MC – Monte Caseros; SG – Salto Grande; SF – Santa Fe*; RO – Rosario*; CO – Colón; CU – Concepción del Uruguay; SN – San Nicolás; GU – Gualeguaychú; VP – Villa Paranacito; SP – San Pedro*; ZA – Zárate*; TI – Tigre*. The pie diagrams show the frequency of fusion 2/5 (left circle), 1/6 (middle circle) and 3/4 (right circle) with the exception of SN and SG (see text). Those marked with an asterisk are reported in Colombo (2008).

RESULTS

Uruguay River Basin

Simultaneous patterns of karyotype and morphometric variation

We studied six populations of *C. aquaticum* in the Uruguay River basin (Fig. 1, Table 1). Five out of six of the populations analysed were polymorphic for three centric fusions previously detected along the Paraná River. In Table 1 and Fig. 1 the frequencies of centric fusions (1/6, 2/5 and 3/4) and fpi (fusion number per individual, so as to include Salto Grande, where fusion number could be determined but fusions could not be individualized due to poor fixation) are listed and depicted, as well as climatic and geographic variables. Along the Uruguay River there is a clear latitudinal cline in fusion frequencies. The Kendall correlations indicate that fpi (average number of fu-

Table 2. Mean morphometric values (with standard error between parentheses) for populations collected at the sample sites included in this study.

	P/CL	FL	TiL	TxL	TegL
Monte Caseros	10.8 (0.038)	14.6 (0.061)	11.1 (0.038)	4.81 (0.009)	21.74 (0.087)
Salto Grande	11.4 (0.028)	14.84 (0.066)	11.48 (0.092)	4.62 (0.098)	21.5 (0.369)
Colón	11.3 (0.022)	15.4 (0.027)	11.8 (0.023)	4.93 (0.007)	22.5 (0.041)
Concepción	10.9 (0.020)	14.2 (0.015)	11.1 (0.014)	4.97 (0.005)	21.6 (0.030)
Gualeguaychú	10.6 (0.088)	14.1 (0.072)	11.7 (0.058)	4.85 (0.019)	20.7 (0.129)
Villa Paranacito	9.6 (0.045)	13.9 (0.052)	10.8 (0.044)	4.46 (0.015)	18.7 (0.073)
Laguna Pampín	10.51 (0.010)	14.5 (0.011)	11.8 (0.013)	5.02 (0.006)	22.8 (0.019)
Santa Fe	10.73 (0.017)	14 (0.026)	11.8 (0.026)	4.85 (0.014)	22.8 (0.053)
Rosario	9.85 (0.040)	14.1 (0.084)	11.23 (0.083)	4.68 (0.015)	20.2 (0.076)
San Nicolás	10.19 (0.041)	13.87 (0.052)	10.51 (0.044)	4.44 (0.031)	19.6 (0.093)
San Pedro*	10.25 (0.029)	14.4 (0.028)	11.8 (0.019)	4.8 (0.014)	21.7 (0.056)
Zárate*	10.92 (0.020)	14.2 (0.040)	12 (0.025)	4.63 (0.015)	24.3 (0.056)
Tigre	10.15 (0.125)	13.93 (0.151)	10.76 (0.107)	4.27 (0.042)	21.0 (0.277)

Populations marked with an asterisk are reported in Romero et al. (2014). P/CL – phastigium/length of third coxa; FL – length of 3rd femur; TiL – 3rd tibia length; TxL – length of thorax; TegL – length of tegmen. All variables are expressed in mm.

sions per individual) correlates positively with latitude ($r = 1.00$, $P = 0.00483$) (Fig. 1) and negatively with maximum temperature ($r_s = –0.97$, $P = 0.00648$), rainfall ($r = –0.83$, $P = 0.0196$) and minimum temperature ($r_s = –0.99$, $P = 0.0196$).

There is considerable variation in body size in the populations studied. The dataset of five body size related traits is given in Table 2. Non parametric MANOVA revealed significant differences among populations ($F = 3.15$; $P =$

Table 1. Populations of *Cornops aquaticum*, centric fusion frequency and environmental and geographic variables analysed. The populations already reported in Colombo (2008) are marked with an asterisk.

	Fusion 2/5	Fusion 1/6	Fusion 3/4	fpi	lat	long	avt	mint	maxt	rainfall
Laguna Pampín*	0	0	0	0	27.5	58.8	21.7	17.15	27.34	117.7
Monte Caseros	0	0	0	0	30.3	57.7	19.6	14.3	25.9	113.0
Salto Grande	n/d	n/d	n/d	0.6	31.4	58.0	18.6	13.15	24.91	107.6
Santa Fe*	0.03	0.08	0.05	0.125	31.6	60.7	18.5	13.69	25.67	92.5
Colón	0.29	0.26	0.2	1.54	32.2	58.1	17.9	12.18	23.77	101.5
Concepción	0.19	0.3	0.25	1.57	32.5	58.2	18.3	12.18	23.77	101.5
Rosario*	0.03	0.026	0	0.11	32.5	60.5	16.9	11.73	23.98	82.4
Gualeguaychú	0.69	0.62	0.46	3.3	33	58.5	17.7	11.14	23.43	94.4
San Nicolás	n/d	n/d	n/d	0.57	33.6	60.2	17.1	12.29	24.36	77.4
San Pedro*	0.2	0.25	0.25	1.37	33.7	59.7	16.9	11.83	23.63	97.6
Villa Paranacito	0.25	0.28	0.125	1.65	34.0	58.7	16.9	11.25	22.9	78.5
Zárate*	0.54	0.52	0.44	3.07	34.1	59.0	16.4	11.18	22.94	97.3
Tigre*	0.85	0.88	0.88	5.14	34.5	58.6	16.5	13.49	21.18	84.5

fpi – average number of centric fusions per individual per per population; lat – latitude S (in degrees); long – longitude W (in degrees); avt – average temperature (in °C); mint – average minimum temperature (in °C); maxt – average maximum temperature (in °C); rainfall – average monthly rainfall (in mm).
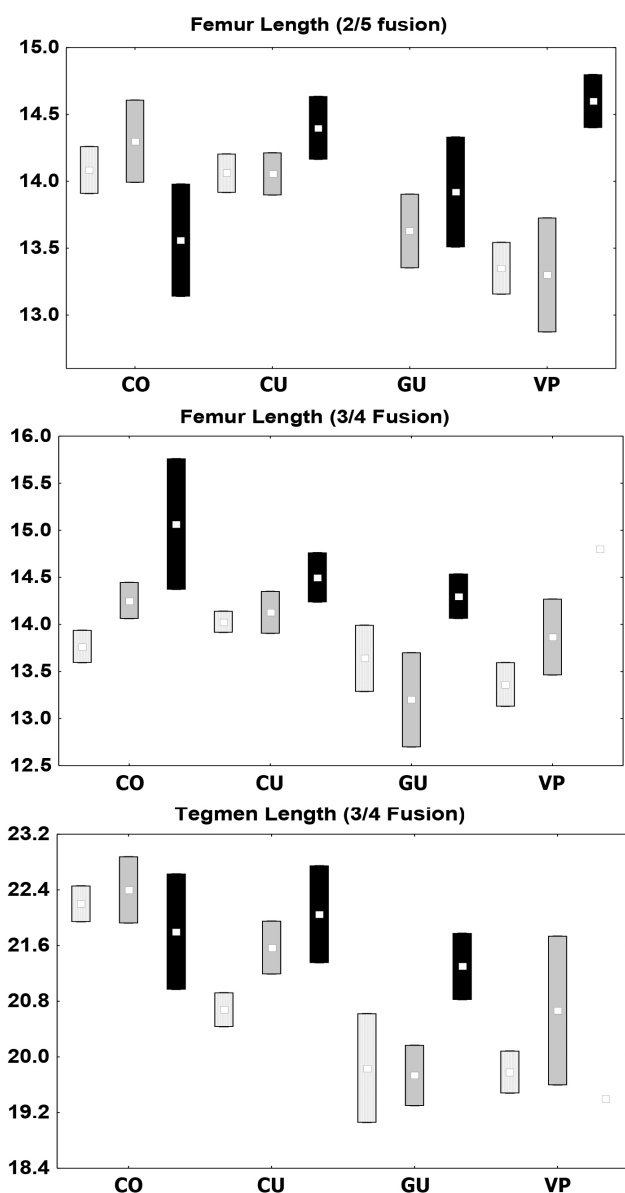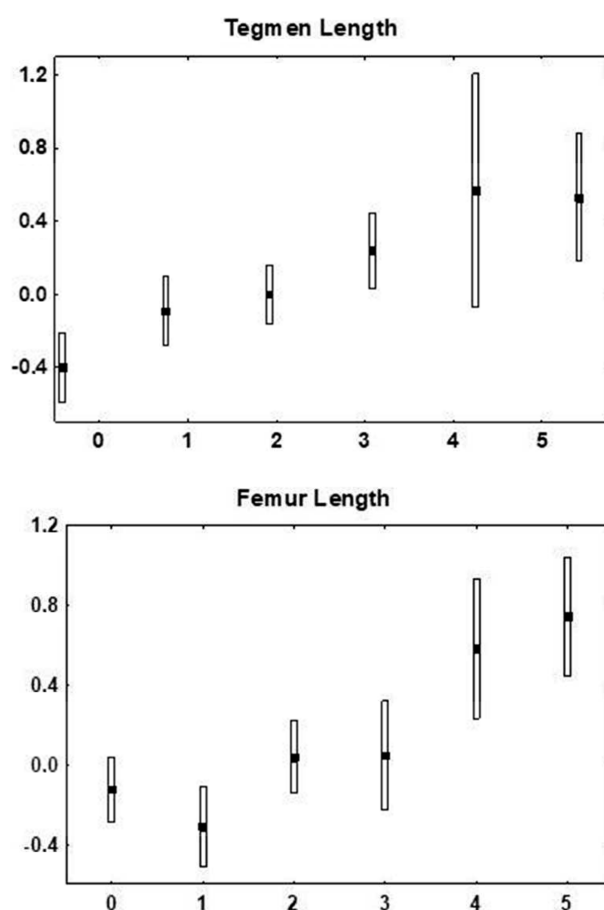
Fig. 2. Mean morphometric values (white spots) plus standard deviations (boxes) for unfused homozygotes (dotted boxes), heterozygotes (striped boxes) and fusion homozygotes (black boxes) for fusion 2/5 (upper graph) and fusion 3/4 (middle and bottom graphs). Ordinates in mm. Abbreviations as in Fig. 1.

Fig. 3. Mean morphometric variables (black spots) plus standard deviations (boxes) for length of tegmen (upper graph) and length of femur (bottom graph) for individuals from SP, ZA, SG, CU, CO, GU and VP (pooled data). Y axis: standardized values (see Materials and Methods). X axis: number of fusions per individual. Abbreviations as in Fig. 1.

0.00001). Individual Kruskal Wallis analyses showed highly significant differences for P/CL ($H = 20.01$; $P = 0.0002$), and length of tegmen ($H = 20.02$; $P = 0.0002$) ($H = 9.03$; $P = 0.02$). There is a tendency for body size to decrease with increasing latitude (Table 2). However, no significant correlations between any body size-related traits and geographic or climatic variables were detected after the Bonferroni correction.

Relationships between karyotype and morphometric variables

We performed for each rearrangement nonparametric Kendall correlations between fusion dosage and morphometric variables in the polymorphic populations. In these studies the individual values were standardized with respect to the mean and SD values to reduce the effect of between-population variation, which allowed the joint analysis of all four (or five, in the case of fpi, given that in Salto Grande fusions could be counted but not identified) polymorphic populations (Monte Caseros is monomorphic without fusions), which includes a respectable number of unfused homozygotes, heterozygotes and structural homozygotes for each centric fusion polymorphism.

In general, fusions 2/5 and 3/4 are associated with the larger sized individuals. Kruskal Wallis Anova analyses revealed highly significant differences among karyotypes for 2/5 centric fusion for TegL ($H_{2;\,106} = 10.06$; $P = 0.0065$) whereas the different karyotypes for 3/4 fusion exhibited highly significant differences for FL ($H_{2;\,106} = 11.20$; $P = 0.003$) and marginally significant differences for TegL ($H_{2;\,106} = 5.54$; $P = 0.05$) (Fig. 2). Accordingly, after Bonferroni corrections, fusion 2/5 dosage is highly correlated positively with TegL ($r = 0.2189$; $P = 0.0002$) and fusion 3/4 dosage is correlated positively with FL ($r = 0.1903$; $P = 0.0009$) and with TegL ($r = 0.1696$; $P = 0.0034$). Fusion 1/6 dosage was not found to be correlated with any morphometric variable.

As a consequence, fpi (including Salto Grande), a variable that fluctuates between 0 to 6, showed a significant positive correlation with FL ($r = 0.164244$; $P = 0.0122$),

and a highly significant one with TegL ($r = 0.2183$; $P = 0.0009$) when standardized individual data were considered (Fig. 3). However, fpi is not correlated with any of the mean morphometric variables per population.

Uruguay and Paraná River Basins

Correlations of karyotype with geographic and climatic variables

A joint correlation between fpi and geographic and climatic variables for all the 13 populations sampled by us in both river basins (Colombo, 2008; this paper), revealed a highly significantly positive Kendall correlation with latitude ($r = 0.64$, $P = 0.0023$) and a highly significant but negative one for maximum temperature ($r = –0.75$, $P = 0.000338$). Some body size-related variables are significantly (P/CL, $P = 0.031624$; TegL, $P = 0.018852$) or highly significantly (FL, $P = 0.000391$) correlated with rainfall.

Correlations of karyotype with morphometric variables

We performed nonparametric Kendall correlations between fusion dosage for each rearrangement and morphometric variables in the nine polymorphic populations so far sampled by us where fusions could be distinguished (excluding Laguna Pampín and Monte Caseros, given that they are monomorphic, and San Nicolás and Salto Grande, where fusions could not be identified). In this analysis the individual values were also standardized with respect to the mean and SD values to reduce between-population variation and allow the joint analysis of all populations.

The analysis considering both the results for the Paraná and Uruguay River basins corroborated the association between centric fusion dosage and body size. Fusion 2/5 dosage is highly significantly correlated with TegL ($r = 0.2163$, $P = 0.0002$) whereas fusion 3/4 dosage is correlated with FL ($r = 0.1903$; $P = 0.0009$) and with TegL ($r = 0.1587$; $P = 0.0059$). Once more, fusion 1/6 dosage was not found to be correlated with any morphometric variable. As a consequence, fpi (including the monomorphic populations as well as San Nicolás and Salto Grande) was significantly correlated with TxL ($r = 0.1389$; $P = 0.0156$), and highly significantly so with FL ($r = 0.1768$; $P = 0.0021$) and TegL ($r = 0.2202$; $P = 0.0001$). However, fpi is not correlated with mean morphometric variables in any of the populations.

DISCUSSION

Endler (1986) points out that clinal variation is often the first evidence, if indirect, of natural selection occurring in wild populations. More recently, Adrion et al. (2015) state that clines suggest the operation of natural selection, especially if they are stable. The repetition of these clines in similar environments would strengthen the case for a selective explanation. Classic cases are the latitudinal clines for inversion polymorphisms recorded in *Drosophila subobscura*, in which the European latitudinal clines match a comparable pattern in North America and another in South America, following the accidental colonization of the New World by recent invaders (Prevosti et al., 1988; Balanyà et al., 2009). A similar pattern in latitudinal clines in both

hemispheres exists for *D. melanogaster* (Lemeunier & Aulard, 1992). In the case of *C. aquaticum* the finding of two southward flowing large rivers with N-S parallel clines in what is a temperate area for this mostly tropical and subtropical species indicates that the pattern reported for centric fusions may be an adaptive one.

In the polymorphic centric fusions of *C. aquaticum*, the regions of the metacentric fused chromosomes that are close to the centromere are devoid of recombination, when compared with their unfused homologues (Colombo, 2007), a frequent feature in centric fusion polymorphisms both in grasshoppers (Colombo, 1987, 1990; Bidau, 1990; Martí & Bidau, 1995; Remis, 1990; Taffarel et al., 2015; see Colombo, 2013, for a review) and for mice (Bidau et al., 2001; Dumas & Britton-Davidian, 2002). These regions may contain an arrangement of genes sited on the metacentric chromosomes that are not present in the corresponding acrocentric, unfused ones. The genetic differentiation between rearranged and standard chromosomes due to reduced recombination in the structural heterozygotes is the usual adaptive explanation of chromosomal rearrangement clines (Rieseberg, 2001), although a more recent molecular genetic study of *Helianthus* claims that this restriction is only valid around breakpoints (Strasburg et al., 2009). Direct molecular genetic evidence for recombination reduction in structural heterozygotes, above all near inversion breakpoints, comes from comparisons of rates of genetic divergence between inverted and standard regions in *Drosophila* species (Faria & Navarro, 2010), or in centric fusion hybrids zones of house mouse (Franchini et al., 2010) and shrews (Marques-Bonet & Navarro, 2005). In addition, in populations of *Drosophila subobscura* that diverge repeatedly in their inversion frequencies in response to thermal selection regimes, genes with different expression levels are more commonly found within inversions (Laayouni et al., 2007).

In an earlier study it was found that centric fusions in *C. aquaticum* are associated with increase in body size-related variables in two polymorphic populations (Romero et al., 2014), a result confirmed here with the addition of the polymorphic populations along the Uruguay River. Reports linking the association of centric fusions and morphometric variables are not frequent in grasshoppers: another case is that of the South-American grasshopper *Leptysma argentina*, in which a centric fusion is consistently associated with increased body size (Colombo, 1989, 1997; Colombo et al., 2004). This polymorphism is subject to longevity selection (older individuals have higher frequencies of this fusion) (Colombo, 1993, 2000), possibly linked to the fact that old individuals are significantly larger (Norry & Colombo, 1999; Colombo, 2012). Another case of association between centric fusions and phenotypic effects is reported in *Mus musculus domesticus*, in which two chromosome races with different centric fusion karyotypes have significant differences in mandible shape (Franchini et al., 2016). However, in the latter example the between-populations phenotypic variation is present in alternative chromosome races due to fusion polytypism, even when the samples

were collected at sites where the two races coexist. In *Leptysma argentina* and *C. aquaticum*, however, the diverse morphological features occur in populations because they are caused by fusion polymorphisms.

In the present study fusions 2/5 and 3/4 are highly significantly associated with body size-related variables. This effect was detected by the within-population analysis (standardized data, to reduce between-population differences, thus allowing the pooling of all observations across populations in a single dataset). Earlier we reported a significant correlation between body size and latitude at a macro geographic level in South-America, between latitudes 10°N and 35°S (Colombo & Remis, 2015), based on a dataset published by Adis et al. (2008) and Romero et al. (2014). Although in the previous large-scale geographic study (−10 to 35 degrees in the southern hemisphere) we report a positive correlation between body size-related variables and latitude, in the samples analysed in the present paper (27°S to 34°S), body size (non-standardized data) seems to decrease southwards (thus indicating a converse Bergmann pattern), whereas correlations between body size and latitude, if negative, are not significant. In addition, at a micro geographic level, body size is highly significantly and positively correlated with rainfall, a pattern not found at a larger scale. Therefore, our previous hypothesis that fusion frequencies increase southwards due to the combined action of the phenotypic effect of the fusions and Bergmann's rule (Colombo & Remis, 2015) is not supported by present evidence.

What are the possible reasons for the latitudinal cline in three fusions? There are several alternatives: either the cline is not adaptive (i; ii), or is adaptive, but associated with environmental variables so far not taken into account (iii).

(i) A simple scenario to explain non adaptive clinal variation is that centric fusions originated in the southernmost populations, such as Tigre and Zarate, and then spread northwards. This hypothesis requires an upstream spread along the rivers, which is untenable because the development, feeding and oviposition of *C. aquaticum* take place on floating plants (specially water-hyacinths) (Franceschini et al., 2011) that are carried downstream by the river's current, favouring a North-South dispersal (Romero et al., 2017).

(ii) Hallatschek et al. (2007) and Excoffier & Ray (2009) advance a non-adaptive explanation of genetic clines. Their mathematical model indicates that during range expansions, low-frequency alleles may increase in incidence due to genetic drift at the advancing edge. Several features of this model are a close fit to *C. aquaticum*, such as a possible recent range expansion from the Amazon rainforest further north to southern low-variability marginal populations. These downstream populations differ genetically from the northern, more central ones, as shown by a recent microsatellite study of populations collected by us along the Paraná basin (Romero et al., 2017). In fact, in that study the downstream and chromosomally highly polymorphic populations (Zárate and Tigre) have deplet-

ed microsatellite variability at within-population level, as well as a significant differentiation between them and the populations located further north. Furthermore, the greater effort needed to collect individuals in the downstream populations indicate they are less abundant there and as a consequence genetic drift is likely to be greater there than in northern populations. This hypothesis fits and is attractive. In addition, our proposal that the parallel and southward flowing Paraná and Uruguay Rivers constitute two independent clines may be challenged: their basins, although not in contact, are close enough to allow migration from the tributaries of one river to the other. However, a serious problem with the genetic drift explanation is that it is difficult to imagine how stochastic forces may have led to the establishment not of one, but of three parallel clines in centric fusions. Studies on dispersal based on mitochondrial DNA sequences and past demographic history may offer complementary information on this contentious issue.

(iii) We found a highly significant negative correlation between fpi and maximum temperature at micro and macro geographic scales, which remains significant after applying Bonferroni's correction. A tentative adaptive explanation of this association is that centric fusions maintain a certain arrangement of genes that enable the individuals from the southernmost, downstream populations to tolerate what is an uncharacteristically colder climate for a tropical and subtropical species. In fact, the northern limit of this species distribution is latitude 23°N and we speculate that it has only extended its southern range to latitude 35°S, where a temperate climate and frequent frosts in winter are the norm, because their aquatic host plants (water-hyacinths) are transported southwards by the currents of both rivers. In fact, there are no natural populations of water-hyacinths or of *C. aquaticum* south of 35°S, where both rivers meet in the estuary of the River Plate and subsequently flow into the sea. Of course, a correlation does not mean causation, so this statistical relation may also be a side effect of temperature-related factors, such as population densities, forage, local pathogens and interspecific competition (Ciplak et al., 2008; Lehmann & Lehmann, 2008).

The association between centric fusions and the morphology of *Cornops aquaticum*, as well as their geographic distribution both at the micro geographic and continental scales, indicate that the confirmed and consistent within-populations phenotypic effects of centric fusions on morphology are not related to between-population differences in fusion frequency or temperature, as suggested earlier, although it may be relevant for adaptation to other environmental conditions such as rainfall. However, the strong negative association of fusion frequency with maximum temperature, as well as the consistent repetition of clines, is an indirect but suggestive indication that it is adaptive.

In the future we plan to carry out a phylogeographic study to test our hypothesis that *C. aquaticum* arrived in the River Plate area by migrating southwards from a centre of distribution, possibly in the Amazonian basin, and for this we will need to widen our sample area in order to test biogeographical hypotheses. A small-scale resam-

pling of the area already studied will allow us to carry out further cytological studies to analyse other possible effects of the centric fusions, such as the loss of rDNA and large heterochromatic regions or the formation of new C-positive regions and clusters of rDNA, processes that may be by-products of Robertsonian translocations (Wolff & Schwartz, 1992; Dolskiy et al., 2018).

C. aquaticum is being introduced into South Africa for controlling *Eichhornia crassipes* (Bownes, 2009). The released population was founded by individuals collected in Brazil, Venezuela, Trinidad and Mexico and was monitored in pre-release studies over 15 years (Coetzee et al., 2011; Bownes et al., 2013). It is suggested that the inclusion in the introduced populations of individuals adapted to the low temperatures experienced in the area around Buenos Aires, may increase the establishment success of the introduced populations (Coetzee et al., 2011) (as Capetown is at the same latitude as Buenos Aires). If this happens, we speculate that the same southward cline of centric fusions reported here will become established in the freshwater bodies in Southern Africa. This would be the ultimate experiment for confirming whether these polymorphisms are adaptive.

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