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Microsatellite DNA analysis of population structure in *Cornops aquaticum* (Orthoptera: Acrididae), over a cline for three Robertsonian translocations

María Luciana Romero[1] · Pablo César Colombo[1] ·
María Isabel Remis[1]

Abstract The grasshopper *Cornops aquaticum* occurs between Mexico (23°N) and Uruguay and Central Argentina (35°S). It was recently introduced as a pest control agent of the neotropical water-hyacinth *Eichhornia crassipes* in South Africa. The information about the amount and distribution of genetic variability of the native populations may optimise the results of biological control programmes. Here we analyse microsatellite variability at the south of *C. aquaticum*'s distribution, coinciding with a cline for three polymorphic Robertsonian translocations along the Paraná River in order to: (1) estimate the amount of intrapopulation variation and its correlation with geographic/climatic variables, (2) infer interpopulation genetic variation and assess connectivity between local populations and (3) compare chromosome, morphometric and molecular variation patterns to analyse the probable causes involved in the maintenance of intraspecific variation. Our sample of 170 individuals of *C. aquaticum* from seven Argentine populations between latitudes 27°S to 34°S showed 211 alleles across seven microsatellite loci. Genetic diversity was estimated through average number of alleles, allelic richness, expected heterozygosity and observed heterozygosity. The analysis of molecular variance showed significant genetic differentiation among populations. Pairwise comparisons of F_{ST}/R_{ST} and Bayesian population assignment method and the discriminant analysis of principal components revealed that the two southernmost populations are more differentiated. Genetic diversity is negatively correlated with Southern latitude and with Robertsonian translocation frequencies. Our

✉ María Isabel Remis
mariar@ege.fcen.uba.ar

1 Laboratory of Population Structure, Department of Ecology, Genetics and Evolution, Faculty of Exact and Natural Sciences, University of Buenos Aires, Ciudad Universitaria, 1428 Buenos Aires, Argentina

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results showed that the Paraná River's middle course populations are genetically undifferentiated and more genetically diverse than the highly chromosomally polymorphic downstream ones. The chromosomal polymorphisms are associated with increased body size in the direction in which larger size is adaptive. This may be relevant for *C. aquaticum*'s role as a pest control agent, since chromosome variability would enhance the ability of the species for a successful settlement in its new habitats, especially in temperate regions of the world.

Introduction

The knowledge of natural population variability may be useful in the biological control organisms. In fact, the information on the amount and distribution of variability in the native range of a biocontrol agent gives complementary information to maximise the efficiency of these programs (Oberholzer and Hill 2001; Wajnberg 2004). DNA markers are particularly useful tools to analyze population variability by assessing divergence in the allele frequencies at neutral marker loci. Neutral molecular markers provide an effective approach to follow population dynamics (McGuigan 2006).

The water-hyacinth grasshopper *Cornops aquaticum* Bruner is widely distributed along the Americas from Mexico (23°N) to Uruguay and Central Argentina (35°S), a wide region that includes the Atlantic and Pacific coasts of Central America and the Atlantic coast of South-America, up to 2184 m above sea level (Adis et al. 2007). Their host plants are aquatic Pontederiaceae (water-hyacinths), on which they exclusively feed (Adis et al. 2007) and on which their females endophytically lay their eggs (Capello et al. 2010). One of these host plants, the water-hyacinth *Eichhornia crassipes*, has become a world-wide pest of fresh water ecosystems, and its eradication became a priority in many tropical, subtropical and even temperate countries, extending as far north as Japan.

Cornops aquaticum is one of the most important herbivores in the native area of water-hyacinth (Franceschini et al. 2011), and hence it was contemplated as a possible biological control agent in non-native ecosystems (Center et al. 2002). It has recently been released in four initial sites of South Africa as a biocontrol agent. The released population was founded by individuals collected in Brazil, Venezuela, Trinidad and Mexico and was monitored through pre-release studies over 15 years. Today these sites are being examined to assess establishment, and subsequent pest control (Bownes et al. 2011; Coetzee et al. 2011).

Population genetic approaches provide valuable information about genetic diversity and population structure in biological control species (Lowe et al. 2004). The amount and distribution of genetic variability of the populations in their native range as well as the possible factors/processes that shaped this diversity will allow us to take initiatives that may optimise the results of biological control programmes.

In an earlier study, populations distributed in a South American scale, with the northernmost extreme being Trinidad and Tobago (10°N) and the southernmost Santa Fe (Argentina, on the Middle Paraná River, 30°S) had been analysed in order to characterise the genetic variability of *C. aquaticum*. Studies using microsatellites loci developed by Brede and Beebee (2005) had shown high levels of genetic diversity and low differentiation among local populations (Brede et al. 2008). Phenotypic studies had shown considerable

variation in body size and part of this variation depends on that of the host plant (Adis et al. 2008).

In the last few years we began to analyse intraspecific diversity of *C. aquaticum* in the downstream populations corresponding to the southern area of the Medium course and Lower course of the Paraná River. Previous cytogenetic analysis in *C. aquaticum* from this area covered by our present study revealed that this species is polymorphic for three Robertsonian translocations (Mesa 1956; Mesa et al. 1982). Our own cytogenetic population studies showed that there is a latitudinal cline for three Robertsonian translocations (=centric fusions), with a maximum of fusion frequency in the marginal populations of the Paraná Delta (lower course) (Colombo 2007, 2008). They reduce recombination by changing the number and position of chiasmata to ensure the proper orientation and segregation of the meiotic trivalents of heterozygotes, and by reducing the number of independent linkage groups (Colombo 2007, see review in Colombo 2013). Polymorphic translocations do not usually affect viability neither in carriers nor in between-populations hybrids, but it may reduce fertility in heterozygotes (Bidau 1991). However, in a previous study on polymorphic populations of *C. aquaticum*, the orientation and segregation of Robertsonian trivalents in fusion heterozygotes showed to be balanced and does not cause aneuploidy in metaphase II, the major cause of fertility reduction (Colombo 2009). Multivalents may form when there are polytypisms (i.e. different populations bearing different centric fusions) (Bidau 1991), but in *C. aquaticum* the rearrangements are the same across populations, although with different frequencies.

Moreover, simultaneous phenotypic and chromosome studies showed that these Robertsonian translocations are associated with an increase in body size-related variables, and are poised to have an influence on the adaptedness of the species to the environment (Romero et al. 2014); in fact, an increased body size is adaptive for low temperature tolerance (Bergmann effect) (Colombo and Remis 2015).

In the southernmost area of the species distribution, genetic diversity within populations may change as a result of chromosome polymorphisms affecting genetic recombination and/or due to historical factors. Moreover, the geographical features of the river basin and/or the environmental conditions may lead to a strong divergence of *C aquaticum* populations, unless gene flow counteracts the population divergence.

To improve our understanding of intraspecific variation in *C. aquatium* and to analyse the hypotheses mentioned before we extended the analysis of genetic variation to the southern extreme of *C. aquaticum*'s distribution, and we studied by means of a microsatellite loci survey the genetic diversity of seven Argentine populations between latitudes 27°S to 34°S, most of which are polymorphic for chromosome rearrangements in the medium and lower course of the Paraná River.

Our objectives were: (1) to estimate the amount of intrapopulation variation and determine the probable associations between genetic variation with geographic/climatic variables, (2) to infer interpopulation genetic variation and assess connectivity between local populations, and (3) to compare chromosome, morphometric and molecular variation patterns with the aim to infer the relative importance of adaptive and stochastic processes in the maintenance of intraspecific variation.

This analysis could render a better characterization of diversity throughout the native range and give us a glimpse of future developments in the non-native areas where this grasshopper is meant to be released as a pest control.
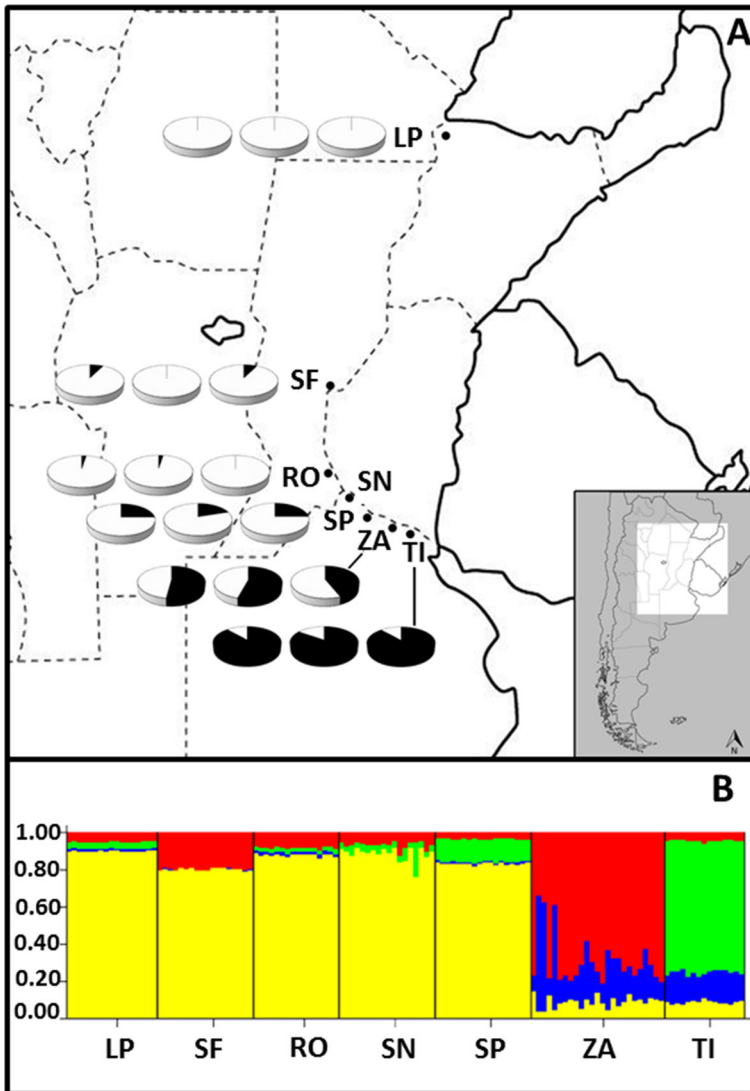
Fig. 1 **a** Geographical distribution of the studied *C. aquaticum* populations along the Paraná River basin in Northeastern Argentina. *filled areas in pie diagrams* represent the relative frequency of 2/5 (*left circle*), 1/6 (*center circle*) and 3/4 (*right circle*) centric fusions. **b** Results of genetic assignment of individuals analysis based on Bayesian method implemented in STRUCTURE assuming correlated frequencies and admixed origin of populations for K = 4. *LP* Laguna Pampín, *SF* Santa Fe, *RO* Rosario, *SN* San Nicolás, *SP* San Pedro, *ZA* Zárate, *TI* Tigre

Materials and methods

Biological material

A total of 170 individuals of *Cornops aquaticum* were collected on the medium and the lower course of the Paraná River. The sites of collection (Fig. 1a), along with their coordinates and the year of sampling are summarised in Table 1.

Geographical distribution of Robertsonian translocation

Robertsonian translocation frequencies previously estimated in the six natural Argentinean populations of *C. aquaticum* were considered to analyze geographic distribution of chromosome polymorphisms (Colombo 2007, 2008) (Fig. 1a). The same population samples analysed at chromosome level were used for molecular analysis. In some cases some additional capture efforts were needed to obtain high quality DNA.

DNA extraction and microsatellite genotyping

We assessed at molecular level seven populations, six of which were previously analysed at the chromosome level. Total genomic DNA was isolated according to Sesarini and Remis (2008). Extracted genomic DNA was used as template DNA to amplify seven microsatellite loci (Ca10b4, Ca14a5, Ca15b3, Ca3g10, Ca11b1, Ca23c1, and Ca10c5 in Brede and Beebee (2005).

These loci were amplified using fluorescently labelled polymerase chain reaction (PCR) primers following Brede and Beebee (2005). Alleles were analysed with an ABI Prism 3130 xl Genetic Analyzer (Applied Biosystems, Inc.) from the Biotechnology Institute of the National Institute of Agricultural Technology (INTA, Argentina) and genotypes were scored using GeneMarker v.2.4 (SoftGenetics LLC). We checked the presence of null alleles using MICRO-CHECKER 2.2.3 program (van Oosterhout et al. 2004).

Data analyses

Analyses of Hardy–Weinberg equilibrium and genetic diversity were performed on all dataset whereas further analyses were performed on the loci which did not show evidence of null alleles (five microsatellite loci dataset).

Table 1 Geographic location and years of sampling for the *Cornops aquaticum* populations described

Site	Longitude	Latitude	Year of sampling	N
Laguna Pampín	58°51′W	27°28′S	2005–2011	36
Santa Fe	60°43′W	31°65′S	2005–2011	20
Rosario	60°39′W	32°57′S	2005	22
San Nicolás	60°13′W	33°19′S	2011	22
San Pedro	59°41′W	33°39′S	2005–2011	23
Zárate	59°02′W	34°06′S	2005–2011	29
Tigre	58°35′W	34°25′S	2011	18

N sample size

Hardy–Weinberg equilibrium and genetic diversity

Linkage disequilibrium (LD) for each pair of loci using the exact Fisher's test, and departures from Hardy–Weinberg equilibrium in each locus for all populations using an exact test (Guo and Thompson 1992) were tested using Genepop v.4.0 (Rousset 2008). We also estimated the inbreeding coefficient (F_{IS}) (Wright 1951) using the estimator of Weir and Cockerham (1984) as implemented in the software FSTAT Version 2.9.3 (Goudet 2001). In all cases Bonferroni's sequential correction for multiple comparisons was applied (Rice 1989). Genetic variability was estimated by means of the average number of alleles (A), the observed (Ho) and expected (He) heterozygosity and the allelic richness (AR) as implemented in Genepop 4.0 (Rousset 2008), Arlequin 3.11 (Excoffier et al. 2005) and FSTAT 2.9.3 (Goudet 2001) softwares. The estimation of allelic richness was corrected based on minimum simple size (18 diploid individuals) according to El Mousadik and Petit (1996).

Geographical distribution of molecular and chromosome variability

With the aim of studying the spatial distribution of molecular and chromosome variability we analysed the relationship between the diversity indices (AR) and fusion frequencies with geographical variables (south latitude and west longitude) and/or climatic variables (mean, maximum and minimum temperature) through Kendall correlations implemented in Statistica software (Statistica Statsoft Inc 1999).

Genetic structure analysis

We analysed population structure through different approaches with the aim of knowing if the individuals are from a homogeneous population or from a population containing subgroups that are genetically distinct.

Analysis of molecular variance (AMOVA) using both F_{ST} (IAM) and R_{ST} (SMM) values in were conducted to investigate differentiation among populations with the software Arlequin 3.11 (Excoffier et al. 2005). Genetic differentiation between pairs of populations was analysed through pair-wise F_{ST}/R_{ST} comparisons. The statistical significance of each of the variance components of the AMOVA and the paired comparisons was determined by nonparametric procedures using 1000 random permutations. Bonferroni's sequential correction for multiple comparisons was applied.

Genetic structure was also investigated using Bayesian population assignment methods implemented in STRUCTURE 2.3.2 (Pritchard et al. 2000). For K population clusters, the software estimates the probability of the data Pr (K/X) and the probability of individual association to each cluster using a Markov Chain Monte Carlo (MCMC) method. The most likely structure was inferred under the admixture model with correlated allele frequencies, using 5 independent runs for each value of K (1–7) with a 4.10^6 repetition burn-in period and 10^6 Markov chain Monte Carlo randomisations. The identification of panmictic units was made with Structure Harvester (Earl and von Holdt 2012), which uses the Evanno's method (Evanno et al. 2005) to evaluate the change in the logarithmic probability a posteriori estimated by Structure as K changes.

Analysis of population structure was also tested through discriminant analysis of principal components (DAPC), without assumptions about Hardy–Weinberg or linkage equilibriums (Jombart et al. 2010).

Simultaneous analysis of chromosomal and molecular variation

In order to study the possible association between chromosome and molecular variability we used Kendall correlations between the estimated diversity indices (AR) and the frequency of each centric fusion previously described by Colombo (2008). The correlation analysis was performed with the *Statistica* package (Statistica Statsoft Inc. 1999).

We also compared matrices of genetic diversity and chromosome differences between populations. The chromosome (fusion centric) and genetic diversity (AR) dissimilarities were estimated as Euclidean distances. The relationships between two distance matrices were analysed using the Mantel test (1967) (ISOLDE program, GENEPOP package, Rousset 2008). For all tests, the significance was estimated with 10,000 permutations.

Results

Genetic diversity and Hardy–Weinberg equilibrium

A total of 211 alleles were observed across populations of *C. aquaticum* considering the seven successfully amplified *loci* (Table S1). We found no evidence of linkage disequilibrium among loci ($P > 0.05$ in all cases), indicating that we can assume independent segregation for all seven loci. The use of Micro-checker suggested the presence of null alleles at Ca15b3 and Ca11b1 loci in some population samples (Santa Fe, Rosario, San Nicolás, San Pedro and Zárate).

The inbreeding coefficient (F_{IS}) was estimated per locus in each studied population sample. Some population samples (Zárate, San Nicolás and San Pedro) showed excess of homozygotes for Ca15b3 and Ca11b1 loci (Table 2). Hardy–Weinberg (HW) adjustment per locus per population by means of the HW exact test and later correction for multiple

Table 2 *Cornops aquaticum*

	Ca14a5	Ca10c5	Ca3g10	Ca10b4	Ca23c1	Ca15b3	Ca11b1
Lag. Pampín	0.102 (0.1183)	0.081 (0.0118)	0.047 (0.1392)	0.119 (0.0213)	0.057 (0.1167)	0.107 (0.0825)	0.086 (0.0666)
Santa Fe	0.101 (0.1099)	0.108 (0.0108)	0.078 (0.1687)	0.035 (0.1626)	0.023 (0.4336)	0.238 (0.0046)	0.2 (**0.0003**)
Rosario	−0.064 (1.0000)	0.09 (0.2352)	0.082 (0.0514)	0.071 (0.0724)	0.002 (0.6511)	0.267 (0.0017)	0.19 (0.0055)
San Nicolás	0.104 (0.1003)	0.034 (0.1221)	0.016 (0.3904)	0.058 (0.1651)	0.057 (0.3073)	0.28 (**0.0009**)	**0.509** (**0.0000**)
San Pedro	0.008 (0.4271)	−0.114 (1.0000)	−0.044 (1.0000)	0.103 (0.0237)	0.012 (0.5909)	**0.465** (**0.0000**)	0.233 (0.0073)
Zárate	−0.042 (0.7896)	0.111 (0.0988)	0.01 (0.1403)	0.068 (0.1478)	0.036 (0.1373)	0.167 (**0.0000**)	**0.651** (**0.0000**)
Tigre	0.064 (0.1632)	−0.066 (0.5626)	−0.034 (1.0000)	0.142 (0.032)	0.017 (0.3262)	0.178 (0.0868)	0.107 (0.3902)

F_{IS} values and Guo and Thompson Exact Hardy–Weinberg test significance values (between parentheses) for each locus in every population. Significant values ($P < 0.05$) are marked in bold type. Sample size as in Table 1

comparisons showed that only Ca15b3 and Ca11b1 loci showed significant deviations from HW equilibrium in Santa Fe, San Nicolás, San Pedro and Zárate (Table 2). These results could be due to null alleles present in populations at these loci in which null alleles had been previously detected (Carlsson 2008). At all other loci, alleles were distributed according to Hardy–Weinberg expectations.

Genetic diversity was also estimated for each population by means of the average number of allele (A), allelic richness (AR), inbreeding coefficient F_{IS}, expected heterozygosity (He) and observed heterozygosity (Ho) (Table 3). Similar genetic diversity indices were obtained from both seven (all analysed loci) and with five loci (loci with no null alleles). When all seven loci were taken into account, all population samples showed heterozygote deficit, except Tigre (Table 3). When five loci dataset were considered, only two population samples exhibited heterozygote deficit, namely Laguna Pampín and Santa Fe.

Geographical distribution of molecular variability

With the purpose of analysing whether the genetic diversity varies toward the southern-most and geographically most marginal populations we estimated Kendall correlations between the allelic richness and latitude (S) and longitude (W). A negative highly significant association was found between AR with latitude (r = −0.905, P = 0.001). As a general pattern, genetic diversity diminishes southwards (coinciding with the Robertsonian translocation-bearing population samples).

Table 3 Genetic diversity indices estimated for the seven loci set (upper line) and for the five loci set (lower line) for all populations of *C. aquaticum*

Population	N	A (±SD)	AR	Ho (±SD)	He (±SD)	F_{IS} (P)
Laguna Pampín	36	20.000 (4.726)	9.355	0.873 (0.035)	0.952 (0.016)	0.085 (**0.001**)
		20.000 (5.320)	15.757	0.877 (0.039)	0.952 (0.018)	0.081 (**0.004**)
Santa Fe	20	16.857 (4.100)	8.889	0.830 (0.102)	0.938 (0.029)	0.118 (**0.001**)
		18.200 (4.025)	15.458	0.885 (0.047)	0.945 (0.022)	0.108 (**0.006**)
Rosario	22	17.000 (4.163)	9.071	0.863 (0.110)	0.945 (0.023)	0.09 (**0.001**)
		18.400 (3.362)	15.417	0.917 (0.057)	0.951 (0.012)	0.048 (0.089)
San Nicolás	22	17.000 (4.967)	8.794	0.799 (0.178)	0.934 (0.029)	0.15 (**0.001**)
		18.800 (4.438)	15.101	0.897 (0.039)	0.947 (0.022)	0.089 (0.016)
San Pedro	23	17.857 (4.337)	9.075	0.856 (0.181)	0.943 (0.025)	0.096 (**0.003**)
		19.200 (4.382)	15.401	0.948 (0.065)	0.943 (0.025)	−0.006 (563)
Zárate	29	17.714 (6.130)	8.596	0.801 (0.219)	0.931 (0.031)	0.144 (**0.001**)
		18.600 (6.542)	13.786	0.896 (0.007)	0.928 (0.036)	0.036 (0.067)
Tigre	18	13 (3.873)	8.371	0.876 (0.087)	0.928 (0.031)	0.059 (0.042)
		14.400 (3.578)	13.433	0.910 (0.073)	0.933 (0.036)	0.025 (0.201)

A average number of alleles, *AR* allelic richness, *Ho* observed heterozygosity, *He* expected heterozygosity, F_{IS} inbreeding coefficient, *SD* standard deviation

Significant *P* (*P* < 0.05) is shown in bold type

Analysis of population structure

The analysis of molecular variance (AMOVA) showed small but significant differentiation among population samples (0.76%; FST = 0.0077, $P < 10^{-4}$; 2.6% R_{ST} = 0.027, P = 0.008). The remaining variation is due to differences among individuals within groups.

Pairwise comparisons of F_{ST}/R_{ST} values revealed that genetic differentiation was driven largely by differentiation in the southernmost population samples (Tigre and Zárate) (Table 4).

Population structure was examined using the Bayesian clustering approach implemented in STRUCTURE. Results under the admixture ancestry model with correlated allele frequencies demonstrated that the posterior probability for each value of K was highest in K = 4 (−3902.64). The Evanno method showed that the maximum delta K was detected at K = 4. One cluster recognised Zárate population, the second cluster identified Tigre population, while the rest of the populations seem to be very similar and it is difficult to associate any specific cluster and population (Fig. 1b).

Population genetic structure was also evaluated by means of a discriminant analysis of principal components (DAPC). In a first analysis, the sampled areas were used as a priori groups for the discriminant analysis. Most of the genetic variation was captured by four discriminant functions (DFs) out of a total of six. The representation in two dimensions of the two first DFs shows that there is not a complete separation of the populations (Fig. 2). The collection sites "Laguna Pampín", "Santa Fe", "Rosario", "San Nicolás" and "San Pedro" are projected as overlapped one on the other. The collection sites "Zárate" and "Tigre", by contrast, show a lesser degree of overlapping between them and with respect to the other population samples, what would be indicating a certain degree of differentiation.

Analysis of the genetic and chromosomal variation

In the studied area centric fusion frequencies increase southwards (Colombo 2008). We verified a negative correlation between maximum and minimum temperatures and fusion frequency (r = −0.82, P = 0.01 for fusion 1/6; r = −0.92, P = 0.008 for fusion 2/5 and r = −0.78, P = 0.02 for fusion 3/4).

Table 4 F_{ST} (above the diagonal) and R_{ST} (below the diagonal) indices for all pairs of populations of *C. aquaticum* to estimate genetic differentiation

	Lag. Pampín	Santa Fe	Rosario	San Nicolás	San Pedro	Zárate	Tigre
Lag. Pampín	0	−0.008	−0.011	0.006	−0.001	0.009	**0.018**
Santa Fe	−0.02997	0	−0.002	0.005	−0.006	0.009	**0.016**
Rosario	0.00425	−0.00212	0	0.000	0.002	0.011	**0.016**
San Nicolás	−0.01412	0.00120	−0.01499	0	0.007	**0.015**	0.015
San Pedro	0.03150	0.00337	−0.01895	0.03457	0	**0.021**	0.007
Zárate	**0.04523**	**0.03600**	0.00952	**0.03459**	**0.04030**	0	**0.017**
Tigre	0.04751	0.03120	0.00515	0.01288	0.02317	0.01017	0

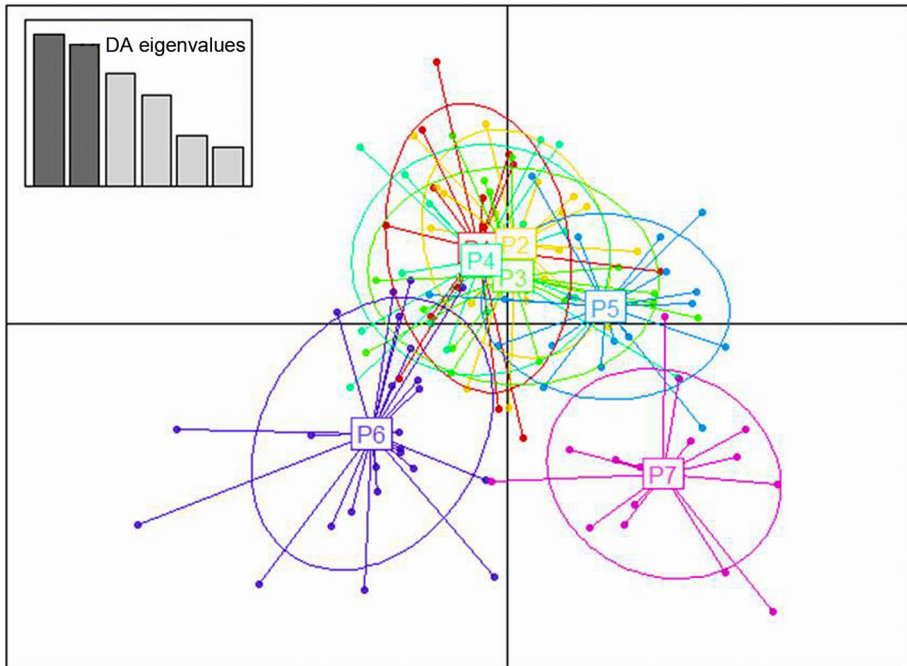Significant values of P ($P < 0.05$) are shown in bold type

Fig. 2 Scatterplots of discriminant analysis principal component (DAPC) showing the genetic differentiation among *Cornops aquaticum* sampling localities. In the figure the two dimensions represent the first two principal components. *P1* Lag. Pampín, *P2* Santa Fe, *P3* Rosario, *P4* San Nicolás, *P5* San Pedro, *P6* Zárate, *P7* Tigre. *Inset* represents the relative contribution of individual discriminant functions to overall variability

As a general feature genetic diversity decreases when the frequency of centric fusions increases. We detected negative significant correlations between AR and the frequency of all three fusions (r = −0.867, P = 0.015 for fusion 1/6; r = −0.966, P = 0.006 for fusion 2/5; r = −0.82, P = 0.02 for fusion 3/4) (Fig. 3a–c). The Mantel tests revealed that the pair differences in the incidence of fusion 1/6 correlated with pair differences in AR as a proxy matrix (P = 0.005) whereas only marginally significant differences were detected for the other fusion (P = 0.07). These results verified a pattern of decreasing diversity (evaluated through AR) with increasing frequency of the 1/6 Robertsonian translocation.

Discussion

Several approaches were used to analyse intraspecific variation in the semiaquatic grasshopper *C. aquaticum,* proposed as a biological control agent of the introduced *Eichhornia crassipes*. In an earlier work, Brede et al. (2008) analysed the genetic variability using microsatellite loci in 10 South-American populations of *C. aquaticum* located between latitudes 10°N and 30°S (among which two came from isolated environments) and another from a laboratory-kept population from South-Africa. That study does not include the area of highest chromosomal variability, between latitudes 30°S and 34°S with three polymorphic centric fusions whose frequency increases southwards (Colombo 2008).
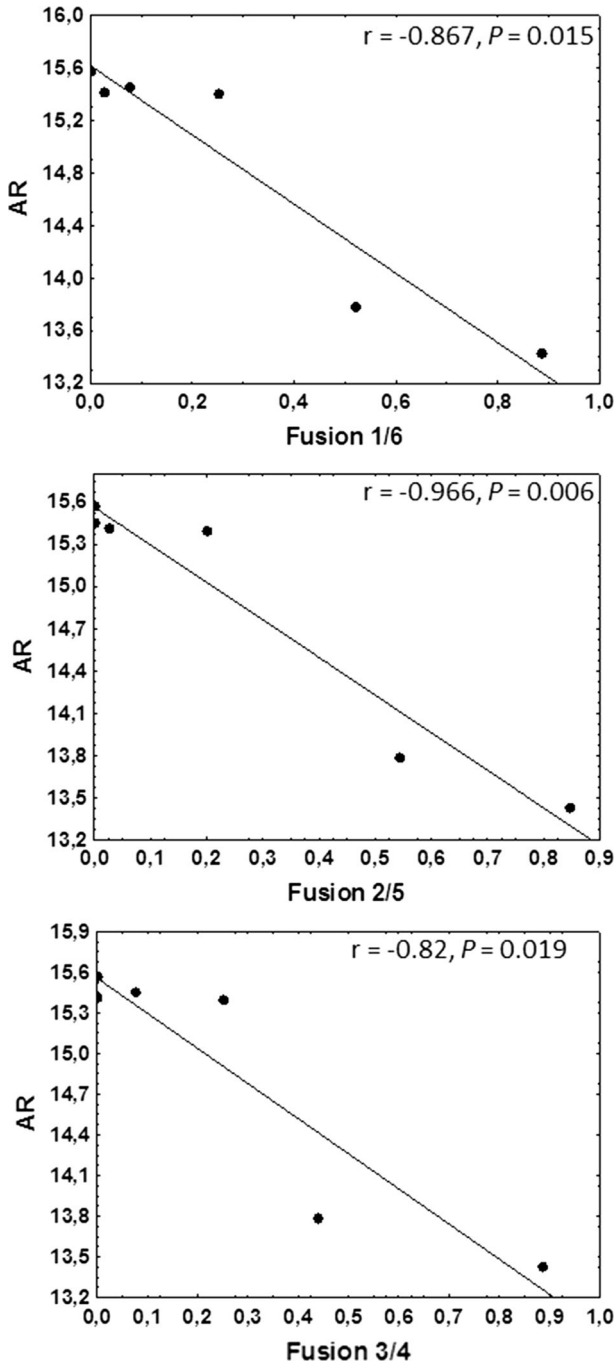
Fig. 3 Relationships (Kendall correlations) between the diversity indices estimated on five loci dataset and each centric fusion frequency. *AR* allelic richness

The analysis of genetic variability and population structure of our paper that corresponds to seven Argentine chromosome polymorphic populations from the Medium and Lower Paraná River was carried out with seven microsatellite loci described by Brede and Beebee ([2005]). Populations in the studied area showed considerable genetic variation as evaluated through He and A. As a general result, genetic diversity diminishes gradually southwards in the studied area.

Moreover, the AMOVA indicated that the highest proportion of the observed variability was detected between individuals within populations and to a lesser extent between populations, although the global analysis detected the presence of significant genetic structure. The pairwise comparisons between populations suggested some heterogeneity in the populations, revealing significant differences between Tigre and Santa Fe, and Rosario and Zárate. The Bayesian population clustering analysis using STRUCTURE recognised Zárate and Tigre populations as different genetic clusters, while the remaining populations do not show a clear difference. Accordingly the DAPC represents an alternative to the Bayesian approach, especially when the assumptions of assignment of individuals to panmictic units are not met. The DAPC carried out on the sampling sites also demonstrated that the downstream populations of Zárate and Tigre would show a higher degree of differentiation between them and with respect to the other sampling sites.

Our results evidenced that in the studied area *C. aquaticum* showed a low signal of population genetic structure, with genetic differentiation centred in the southernmost area of this species, in the Lower Paraná River. The populations sited in the middle course and in the northern border of the lower course are more stable and have higher population size (judged by capture efforts) and maintained high genetic homogeneity explained by shared ancestral polymorphisms or, more likely, by high gene flow (high connectivity). Water-hyacinths are floating plants that are dragged downstream by the river's current, which may help in the dispersal (Romero et al. [2011]). The southernmost and most marginal populations (Zárate and Tigre), with higher genetic differentiation, are located in the youngest region of the lower Delta Paraná that comprises deltaic islands and represents an intricate environmental mosaic, where the hydrological regime interacts with the geomorphic pattern (Kandus et al. [2006]).The water-level fluctuations of distinct magnitudes and duration in this complex region may cause different habitat connectivity. Zarate and Tigre populations, which experience both a more unstable environment as well as variations in population sizes, are subject to intense stochastic processes.

The decrease of genetic diversity southwards may be related with the fact that genetic variation in the newly colonized areas of a species generally represents a fraction of the original population's variability (Nei et al. [1975]; Simberloff [2009]). Populations analysed here and located below 32°S are included in the Paraná Delta and are more recent. The decrease in genetic diversity in downstream populations may be the result of more recent settlement. Thus in these populations the stochastic processes could explain both the detected interpopulation differentiation and the reduced genetic diversity in molecular neutral markers.

We also evaluated the relationship between the chromosomal and genetic variability of *C. aquaticum* in the southernmost area of the species' distribution. There is a significant and inverse relation between microsatellite variability and fusion frequency. In fact, southern marginal populations have a higher fusion frequency and a lower level of genetic variability.

In *C. aquaticum* the decrease in genetic variability as well as the greater genetic differentiation in the most marginal populations may also be associated with chromosome polymorphisms. The variability expressed in terms of neutral loci analysed in this paper

could be reflecting the restriction in genetic recombination imposed by the chromosomal rearrangements in the downstream populations. We already mentioned that centric fusions usually reduce recombination in the chromosomes involved in the rearrangement, both by the loss of linkage groups and due to chiasma frequency decrease, a frequent feature of polymorphic centric fusions (Colombo 2013; Taffarel et al. 2015) and inversions (Rieseberg 2001; Faria and Navarro 2010).

There is widespread literature about cytological evidence of fusions restricting recombination, both in grasshoppers (John and Hewitt 1970; see review in Colombo 2013; Taffarel et al. 2015) and elsewhere (Bidau et al. 2001; Dumas and Britton-Davidian 2002). Some recent molecular reports support the hypothesis of recombination decrease associated with chromosome rearrangement. Evidence for recombination reduction in structural heterozygotes, above all near inversion breakpoints, comes from comparisons of rates of genic divergence between inverted and standard regions (Faria and Navarro 2010). Recent studies in *Helianthus* only noticed the phenomenon of genetic differentiation close to the breakpoints (Strasburg et al. 2009). Among animals, in populations of *Drosophila subobscura* that showed clines in their inversion frequencies in response to selection due to temperature gradients, genes with different expression levels are more frequent within inversions (Laayouni et al. 2007). As for centric fusions, a restriction of gene flow was noticed near centromeres of fused chromosomes in a hybrid zone between chromosomal races of the house mouse *Mus musculus domesticus* (Franchini et al. 2010).

Chromosome clinal patterns may be shaped by adaptive and non-adaptive causes (Endler 1977; Werle 2005; Vasemagi 2006; Strand et al. 2012; Miño et al. 2011). Under the adaptive hypothesis it may be argued that the reduction in recombination may lead to the genetic diversification between rearranged and colinear regions, thus allowing the appearance of coadapted gene complexes (Rieseberg 2001). It has been pointed out that parallel clines are an indirect evidence of an adaptive pattern (Adrion et al. 2015), as in the case of latitudinal clines for chromosomal inversions in *Drosophila subobscura* in Europe, North America and South America (Prevosti et al. 1988; Ayala et al. 1989; Adrion et al. 2015). In fact, the cline found along the Paraná River is repeated further east, on the Uruguay River, which also flows southwards and whose current merges with that of the Paraná River to give rise to the River Plate estuary at the Buenos Aires latitude (34.5°S) (Colombo and Remis, in preparation).

Besides, it should be remembered that the centric fusions in *C. aquaticum* are accompanied by phenotypic effects (Romero et al. 2014). Among endotherms, the Bergmann effect (i.e., the correlation between body size-related variables and latitude) has traditionally been explained as growing cold climate tolerance due to an increased body mass/body surface ratio; in ectotherms there may be a positive Bergmann effect (due to cold climate tolerance) or a negative one (attributed to life history causes, such as a shortened life span due to a decreased season length) (Blackburn et al. 1999; Blanckenhorn and Demont 2004). In a continent-wide morphometric study (Adis et al. 2008), *C. aquaticum* was shown to have a positive Bergmann pattern (Colombo and Remis 2015), so the increased body size of fusion carriers would be consistent both with the Bergmann pattern and with the correlation between fusion frequency and latitude. This would be in line with the negative correlations between fusion frequencies and minimum and maximum temperatures reported in this paper, thus suggesting another adaptive cause for the chromosomal clines.

Conversely, an alternative and attractive explanation of chromosome clines is purely stochastic and was put forward by Hallatschek et al. (2007) and Excoffier and Ray (2008). These authors demonstrated that during range expansions (for example, expansion towards

higher latitudes following the end of the last glaciations), low-frequency alleles may increase in frequency due to genetic drift in the wave of advance—a phenomenon called "surfing". This explanation would rule out the traditional explanation of clines as due to selective causes. Several features of this model clearly accommodate the case of *C. aquaticum*, such as relatively recent range expansion from the Amazon basin further north and variability-depleted marginal populations with some extent of genetic differentiation with respect to northern, more central ones. Furthermore, these marginal populations show lower density when compared with northern ones, as judged by increased capture effort, thus easing the action of genetic drift. This phenomenon could also explain the intrapopulational decrease of genetic diversity and the between-population differentiation of Zárate and Tigre.

Cornops aquaticum is being released in populations of South Africa (Bownes 2009; Bownes et al. 2011; Coetzee et al. 2011) which are sited at the same latitude than the temperate area here studied in Argentina, and their settlement success is being checked. The source of these individuals is a largely inbred laboratory-kept population from South Africa, started from individuals originally captured in Central America (Oberholzer and Hill 2001). It has been pointed out that the inclusion of individuals from divergent habitats in new collections could improve the chances of success of the biological control agents, and increase the probability of survival in an alien environment (Taylor et al. 2011; Wajnberg 2004). In particular, it had been suggested that the inclusion in the introduced populations of individuals adapted to lower temperatures, as those experimented in the area around Buenos Aires, may increase the establishment success of the introduced populations (Coetzee et al. 2011). In fact, our results may be relevant in the context of *C. aquaticum*'s role as a pest control agent, since chromosome variability would enhance the ability of the species for a successful settlement to their new habitats, especially in temperate regions of the world.

Our results completed the analysis of genetic diversity and population structure in the grasshopper *C. aquaticum* across a native environment, the Paraná River. Populations from the middle course and in the northern border of the lower course appear to be genetically undifferentiated and exhibited more genetic diversity with respect to the southernmost ones, which display higher chromosome variability. The chromosomal polymorphisms are associated with a well-defined morphometric pattern, and this pattern occurred in the direction in which latitudinal phenotypic variation was adaptive. We suggest that the genetic pattern may be explained by both historic and stochastic forces, given that the two downstream populations were more recently invaded and live in a marginal and unstable habitat; the chromosomal pattern may be due to natural selection, perhaps on body size-related features, but a stochastic cause, such as "genetic surfing", cannot be discarded at this point of our research. Further studies based on mitochondrial DNA sequences about dispersal and past demographic history can offer complementary information to improve our understanding of population structure in this species.

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