Chromosome, genetic and morphometric variation of the Aquatic Grasshopper *Cornops aquaticum* (Bruner) (Acrididae: Leptysminae) in the Middle and Lower Paraná River, Argentina.

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INTRODUCTION

The water-hyacinth grasshopper *Cornops aquaticum* is a neotropical grasshopper whose distribution extends from the south of México (23ºN) up to Uruguay and North east of Argentina (35ºS). *C. aquaticum* has 2n=23 chromosomes in males and 24 in females, and in the lower course of the Paraná River, its karyotype includes three centric fusions (1/6, 2/5 and 3/4). Fusion frequencies increase southwards, showing a geographical cline.
This grasshopper lives in close relationship to species from the genera *Eichornia* and *Pontedaria*, on which it feeds and lays eggs. The water-hyacinth *Eichornia crassipes* is considered "the world's worst water weed": introduced elsewhere, this plant has become invasive specially as a result of the lack of natural enemies. In attempts to control this weed has led to the proposal of several control methods, having limited success so far. Ever since 1974, when Perkins found that *C.aquaticum* was one of the most harmful insects associated to water-hyacinths, its release is being proposed as a form of biological control.
Two of the most important features to consider when analyzing strategies related to insects are genetic diversity and population structure. Like other introduced species, *C. aquaticum* will have to face similar constraints as those who face isolated populations and also, new selective pressures.
In this study, four chromosomally differentiated populations were analyzed assessing morphometric variation and genetic diversity through microsatellite markers. This information will be helpful when trying to understand its adaptive strategies. This will allow the proposal of suitable release methods of this specie in the biological control programs.

AIMS

- To study the effect of centric fusions over morphometric traits in the polymorphic populations, in order to infer the influence of chromosome rearrangements on exophenotypic traits.
- To study the molecular variability in chromosomally differentiated populations, what would contribute to the interpretation of its adaptive strategies

RESULTS

- Choromosome polymorphisms for three centric fusions were found in three of the four analyzed populations (*Figure 1*).
- There are significant differences in the frequency of centric fusions in the studied populations ($p<0,05$ for all fusions). Fusion frequency increases when latitude grows.

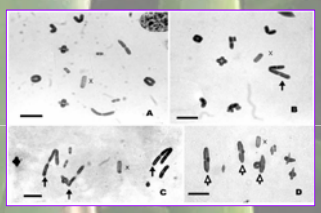

Figure 2. Metaphase I plates of *Cornops aquaticum* males with different karyotypes. A. Standard homozygote without fusions, B. heterozyfote for the 1/6 centric fusion, C. heterozygote for all three centric fusions, D. homozygote for all centric fusions. Full arrows: trivalents. Open arrows: Robertsonian bivalents. Bar= 10 µm. (Colombo, 2008)

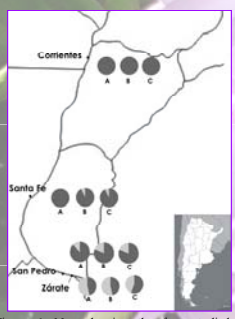

Figure 1. Map showing the four studied populations. Graphs show the frequency of fused chromosomes (dark gray) and non-fused chromosomes (light gray). A. 2/5 fusion, B. 1/6 fusion, and C. 3/ 4 fusion.

CYTOLOGICAL AND MORPHOMETRIC STUDIES.

- *C. aquaticum* has 2n=23 acrocentric chromosomes in males and 24 in females. (Mesa,1956)
- Both populations of Zárate and San Pedro are polymorphic for Robertsonian rearrangements between chromosome pairs 1 an 6 (fusion 1/6), 2 and 5 (2/5) and 3 and 4 (3/4) (Colombo 2008)(*Figure 2*).
- In both populations, fused individuals showed bigger body size than those with standard karyotype.
- Positive and significant correlations between fusion 1/6 and femur length (r= 0.26; p= 0.04), tibia length (r= 0.28; p=0.03) and tegmina length (r= 0.26; p= 0.04) were found. (*Figure 3a*)
- Fusion 3/ 4 showed a positive and significant relation with tegmina length (r= 0.28; p= 0.028)(*Figure 3b*).

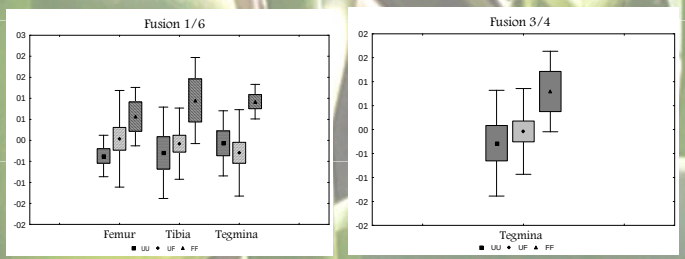

Figure 3. mean values (primary symbol), standard error (boxes) and standard deviations (bars) for morphometric traits related to karyotype on standard homozygote (UU), heterozygote (UF) and homozygote for the fusion (UF). (a) Fusion 1/6 (b) Fusion 3/4

MATERIALS AND METHODS

a) Biological material and cytological studies
In this study, individuals from Zárate (34.07° S, 59.02° O), San Pedro (33.59° S, 59.41° O), Santa Fe (31.35°S, 60.69°W) and Corrientes (27.3°S, 58.49°W) were sampled and cytologically analyzed. Testes were dissected and fixed in ethanol-acetic acid (3:1) for cytological studies. Cytological analyses were performed by squashing some follicles in propionic haematoxylin. To determine which chromosomes were involved in the Robertsonian translocation and consequently, the karyotype, trivalents and bivalents were carefully analyzed in at least 20 cells in metaphase.

b) Morphometric analysis
Individuals from Zárate and San Pedro were used for these analysis. Six morphological characters were measured (total length (TL), femur length (FL), tibia length (TiL), thorax length (TxL) and tegmina length (TegL)) on an stereoscopic microscope and an ocular micrometer (1 mm : 48 ocular units).
Statistical analysis. Statistical analysis of both populations were evaluated together. Data was transformed to standardized deviations from the mean value for each trait to avoid interpopulation variation. In order to analyze the effect of karyotype on the morphometric traits, centric fusion dosage was consider as "0" (standard unfused UU), "1" (heterozygote UF) and "2" (fused homozygote FF). Relations between the dosage of the centric fusion and morphometric data were analyzed through Kendall correlation. Data was analyzed using the software STATISTICA (StatSoft-Ink, Tulsa, OK, USA).

c) Molecular analysis
For these analysis, individuals from Zárate, Santa Fe and Corrientes were used. Molecular variation among chromosomally differentiated populations was analyzed using microsatellite markers.
I) DNA extraction: DNA was extracted from tissue (usually legs) using a modified phenol-chloroform protocol designed to extract DNA from frozen insect samples according to Sesarini & Remis (2008).
II) Microsatellite amplification: microsatellite loci were amplified by polymerase chain reaction (PCR) using specific primers and protocol designed by Brede and Beebee (2005). Amplification products were analyzed on an ABI Prism 3130 xl Genetic Analyzer (Applied Biosystems, Inc.) from Servicio de Secuenciación y Genotipificado from Facultad de Ciencias Exactas y Naturales, UBA, against the GeneScan TM 500LIZ (Applied Biosystems Inc.) internal standard. Data analysis was done with GeneMapper v4.4 (Applied Biosystems Inc.)
Data analysis:
Genetic diversity estimation: intraspecific genetic diversity was analyzed through mean number of alleles (A), observed (H_O) and expected (H_E) heterozygosis according to Nei (1978) using the software GENEPOP 4.0 (Raymond and Rousset 1995) and Arlequin v3.11 (Schneider *et al.* 2000). Allelic richness was calculated using FSTAT 2.9.3 (Goudet 2001).
Population structure analysis: genetic differentiation between and among populations was studied through a Molecular Analysis of Variance (AMOVA) using the program Arlequin v3.11 (Excoffier *et al.* 2005).

MOLECULAR ANALYSIS

- The analysis of microsatellite *loci* showed that the studied populations display high levels of genetic diversity evaluated through the mean number of alleles, expected heterozygosis and allelic richness (*Table 1*). The levels of genetic diversity are comparable among different populations.

Population	N	A (± SD)	AR (± SD)	Fis	He (± SD)
Corrientes	11	12.500 ± 3.416	10,22 ± 0,9	0,22	0.94793 ± 0.02049
Santa Fe	7	6.250 ± 2.630	8,35 ± 2,247	0,4	0.89043 ± 0.02425
Zárate	14	12.500 ± 3.697	9,00 ± 1,141	0,22	0.92473 ± 0.02884

Table 1. Preliminary genetic diversity indexes for the studied populations: mean number of alleles (A), allelic richness (AR), coefficients Fis and expected heterozygosis (He). N: number of individuals analyzed, SD: standard deviations.

- The AMOVA showed that most of the observed variation was within the populations, while just a little, the 2,51% of the variation was due to variation between populations (p=0,1974) (*table 2, figure 4*)

	DF	SC	MS	% of Variation	F	*P*
Between Populations	2	1,465	0,01234	2.51%	F_{ST}=0.025	0.1974
Within Populations	61	29,192	0,47855	97.49%		
Total	63	30.656	0.49089	100%		

Table 2. Analysis of molecular variation (AMOVA) between the tree populations of *C. aquaticum* using microsatellite *loci*.

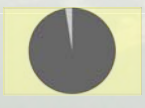

Figure 4. Percentage of genetic variation within and between populations evaluated through microsatellite *loci*. Dark gray: within populations, Light gray: between populations.

CONCLUSSIONS

- Fusion 1/6 and 3/ 4 are related with an increase body size in *C. aquaticum* males.
- Our results would support the hypothesis that fusions could modify the position of chromosomes in the nucleus, altering gene expression. Another possibility is that fused chromosomes bear genes that increase body size in fusion carriers. These genes would not be transferred to unfused homologues given that chiasma position in heterozygotes is shifted towards distal position (Colombo, 2007).
- The effect of centric fusions on phenotype could be related to the maintenance of chromosome polymorphisms.
- The populations of *C. aquaticum* from Middle and Lower Parana River studied show high levels of genetic diversity and gene flow between them, comparable to those found by Brede *et al* (2007) in populations from Middle and Upper Parana River.
- The lack of genetic differentiation between populations in the course of Parana River suggests a high migratory ability of this tucura species along the river´s stream.
- The disagreement between chromosome and molecular differentiation between populations reflects different evolutionary histories for both markers and points out the importance of adaptive effects of chromosome polymorphisms and coadapted gene complexes.
- The water-hyacinth grasshopper *Cornops aquaticum* shows levels of genetic diversity that may be explained by the interaction between genetic drive and migration, while the chromosomal variation would be related to the adaptation to different environments.