

Chromosomal polymorphism, morphological traits and male mating success in *Leptysma argentina* (Orthoptera)

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Leptysma argentina (Acrididae: Orthoptera) is polymorphic for a centric fusion between pairs 3 and 6 of its basic chromosomal complement. With the aim of carrying out an analysis of selection components in this species, male mating success, in relation to karyotype and three morphometric traits, was assessed by using experimental contests with four males and one female per mating cage. Karyotype was assessed in terms of fused chromosome 3/6 dosage and coded as '0', '1' and '2' for unfused homozygotes, heterozygotes and fusion homozygotes, respectively. The individual ANOVA showed significant differences between 'successful' and 'unsuccessful' males in all four measured characters. Fusion carriers showed a higher relative fitness. Since the phenotypic correlations among traits were significant, the selective effects were assessed by means of a multivariate analysis, which demonstrated that selection acted significantly on femur length only, selection on karyotype being indirect. Given that the fusion frequency has been constant since 1985, the presence of selective trade-offs cannot be ruled out.

Keywords: centric fusion, *Leptysma argentina*, morphometric variables, Orthoptera, sexual selection.

Introduction

According to Darwin (1871) a distinction must be made between natural selection and sexual selection: the former drives a population towards adaptation, whereas the latter is not necessarily adaptive. Sexual selection is defined in terms of mating success, and natural selection is a consequence of other fitness components (see review in Santos *et al.*, 1988). Despite this distinction, however, these processes can be viewed as essentially similar, given that both can be assessed in terms of different selection components that act during the life of individuals (Endler, 1986). In this aspect, species with synchronized, discrete generations — like grasshoppers — offer an advantage over those with overlapping generations, because fitness components are easier to measure in the wild (Colombo, 1993b) and in the laboratory (Remis *et al.*, 2000).

Leptysma argentina Bruner (Acrididae: Orthoptera) is a South American grasshopper possessing several chromosomal polymorphisms whose cytological,

morphometric and selective properties have been well characterized (Colombo, 1989; 1993a, b; 1997; Norry & Colombo, 1999). It carries, among others, a polymorphism for a centric fusion between the acrocentric/telocentric pairs 3 and 6 (fusion 3/6). This centric fusion causes effects on morphometric characters (Colombo, 1989, 1997) which are favourably selected during the adult life of male individuals (Colombo, 1993b). The target of selection in this case has been identified as thorax height (TH), with other morphometric and karyotypic characteristics undergoing selection due to other correlations with this trait (Norry & Colombo, 1999).

Male sexual selection may be exerted at the level of male mating success or as selection acting through sperm competition (Martín-Alganza *et al.*, 1997). The present paper aims to detect possible effects of chromosome and morphometric variables on male mating success in the grasshopper *Leptysma argentina* by using univariate and multivariate approaches. The results are discussed in the light of the previous knowledge of natural selection acting on other fitness components in this species.

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Materials and methods

One-hundred and forty-five adult individuals of *Leptysma argentina* (Acrididae: Orthoptera) (115 males and 30 females) were captured in October and November 1997 in National Park 'El Palmar' (Entre Ríos, Argentina).

Experimental design

Crosses were made by placing a female with four males in individual mating cages inside a chamber under controlled conditions of temperature, humidity and light. The first copulating male was removed and scored as '1' (successful) whereas the other males were scored as '0' (unsuccessful).

Morphological analysis

All males (successful and unsuccessful) were measured for three morphological traits: third (3°) femur length and prothorax length and height. These three morphological traits were chosen on account of the effects that fusion 3/6 exerts on them (Colombo, 1989, 1997) and due to the fact that in the grasshopper species *Sinipta dalmani* femur length was shown to affect male mating success (Remis *et al.*, 2000).

Chromosome analysis

Males of each mating cage were dissected and their testes fixed in 3:1 ethanol: acetic acid and subsequently stored at 4°C. Cytological preparations were made by squashing some follicles in acetic haematoxylin. A minimum of 10 cells at metaphase I per individual were examined in order to determine their karyotype.

Statistical analysis

Fusion dosage per individual was coded as '0' (unfused homozygote), '1' (heterozygote) or '2' (fusion homozygote).

Chromosomal dosage and morphological data of each mating cage were transformed to standardized deviation from the mean value for each trait within a

cage to prevent inter-cage variation in all subsequent statistical analyses.

For each morphological character, differences between successful and unsuccessful males were assessed by means of a one-way analysis of variance (ANOVA) taking mating success as the main effect. Standardized and absolute fused chromosome dosage were compared between both subsamples by using ANOVA and chi-squared contingency tests. Fitness coefficients were estimated by the expression $w_{ij} = a_{ij}/b_{ij}$, where a_{ij} and b_{ij} denote the frequencies of each karyotype in the samples of mated males and total males, respectively (karyotype frequencies after and before sexual selection). The approximate variance of w_{ij} was estimated according to Clegg *et al.* (1978). We estimated the intensity of selection using the logistic multiple regression coefficient (α) and transformed logistic multiple selection coefficient (β_{avggrad}) proposed by Janzen & Stern (1998) to assess selection on multiple phenotypes for dichotomous fitness (see Remis *et al.*, 2000). This method constitutes an alternative to the widely applied Lande & Arnold (1983) approach, since the former does not rely on assumptions about normality of the predictor variable or the errors and it allows the selection effect to vary nonlinearly.

Results

Mating success and morphometric traits

Male mating success was measured in 30 mating cages. Females are larger than males and, during the courtship, no aggressive interactions among males were observed in any cage.

One of the main goals in the present paper was to determine whether the influence of the centric fusion on body-size-related traits confers any adaptive effect on male mating success. At first, we compared the mean values of thorax length (TL), thorax height (TH) and femur length of the third (3°) pair of legs (FL) for successful and unsuccessful males (Table 1). Consistent differences in size between successful and unsuccessful males were observed. In 26 and 21 of the 30 mating cages (data not shown), successful males were larger than non-mating ones for FL and TL, respectively. Sign

Table 1 Mean values and standard errors (in mm) in successful and unsuccessful males of all three morphological characters studied here in *Leptysma argentina* and their comparison using a one-way ANOVA

	<i>N</i>	Thorax length	Thorax height	Femur length
Successful	30	4.1666 ± 0.019	2.989 ± 0.016	7.795 ± 0.067
Unsuccessful	84	4.052 ± 0.027	2.894 ± 0.025	7.390 ± 0.054
Total	114	4.082	2.919	7.496
<i>F</i>		11.08	8.92	26.73
<i>P</i>		0.001	0.003	0.000001

tests show significant differences for both size-related traits ($P < 0.001$, $P = 0.037$). Accordingly, parametric comparisons between the average of body size-related traits for both male samples through one-way ANOVA employing the data of all mating cages, showed that larger grasshoppers had a mating advantage over smaller ones in all three morphometric traits (TL, TH and FL) (Table 1). However, before reaching a conclusion, we investigated directional sexual selection acting on multiple phenotypes through a multivariate approach using the data of all body-size-related traits. The intensity of sexual selection through logistic regression of relative fitness on the measured variables showed directional selective effects on FL ($\alpha = 1.393$, $P < 0.001$).

Male mating success and karyotype

Since phenotype directional selection favours larger males and the centric fusion affects morphology we also investigated the relationship between mating success and karyotype. We studied the dosage of the 3/6 fusion in relation to copulatory success of males in the same 30 mating cages (Table 2a). The comparison of fusion dosage using absolute frequencies between successful and unsuccessful males showed that there are highly significant differences in karyotype between successful and unsuccessful males ($\chi^2_1 = 9.86$, $P = 0.002$). Furthermore, when these male samples were compared by using the standardized data by means of one-way ANOVA the same result was found ($F_{1,83} = 7.53$, $P = 0.007$). Moreover, we detected a chromosome frequency change due to mating success, since the comparison between successful males and the whole population yielded significant differences ($\chi^2_1 = 5.61$, $P = 0.017$). This result suggests that this rearrangement confers higher mating success. Table 2a also gives karyotype frequencies of successful and unsuccessful males. In both samples, the χ^2 goodness of fit test did not indicate significant deviations from the expected values according to the Hardy–Weinberg law ($P > 0.050$ in both cases). However, a contingency test revealed that there are significant differences between karyotype frequencies of successful and unsuccessful males ($\chi^2_2 = 9.12$, $P = 0.010$), the former having a higher frequency of fused homozygotes. Fitness coefficients for each karyo-

Table 2b Estimated values of fitness in males of *Leptysmia argentina* according to their karyotype

	UU	FU	FF
Fitness	0.28 ± 0.03	0.58 ± 0.01	1.00 ± 0.09

Table 3 Logistic regression coefficient (α) and transformed logistic regression coefficient (β_{avggrad}) obtained from the multiple regression analysis of relative fitness on morphological and chromosomal characters of *Leptysmia argentina* simultaneously

	α	ES	P	β_{avggrad}
TL	0.0209	1.630	0.989	0.004
TH	1.5538	1.528	0.309	0.300
FL	1.2529	0.435	0.004	0.241
Fusion dosage	1.2792	1.373	0.352	0.246

type also revealed that different karyotypes had different fitnesses; the fittest karyotype seems to be fusion homozygote (Table 2b). These results are consistent with our hypothesis of directional selection favouring fusion 3/6.

Taking into account the karyotype and phenotype directional sexual selection, we should also consider morphometric and chromosome variables together through a multivariate approach to detect the direct effect of selection. The transformed logistic regression (α) again showed directional effects of FL and did not reveal any evidence of direct chromosome sexual selection (Table 3).

Discussion

Body size variation has been the subject of many evolutionary studies, since it affects numerous biological traits (e.g. duration of development) and may be a target of natural selection. Many studies of selection in connection with body-size-related traits in *Drosophila* (Wilkinson, 1987; Santos *et al.*, 1988; Taylor & Kekik, 1988; Gilburn *et al.*, 1992; Norry *et al.*, 1995) and other Diptera (Butlin *et al.*, 1982) have been consistent in

Table 2a Karyotypic frequencies of unfused homozygotes (UU), heterozygotes (FU) and fusion homozygotes (FF) in successful, unsuccessful and all males of *Leptysmia argentina* used in this study

	UU	FU	FF	F	N
Successful	0.233 (7)	0.467 (14)	0.300 (9)	0.533	30
Unsuccessful	0.494 (42)	0.400 (34)	0.106 (9)	0.306	85
Total	0.426 (49)	0.417 (48)	0.157 (18)	0.365	115

showing an advantage in mating success for larger individuals. Morphometric traits such as wing width and length, thorax width and length and tibia and femur lengths have been used as an index of body size (e.g. Ruiz *et al.*, 1986; Norry *et al.*, 1995; Colombo, 1997; Norry & Colombo, 1999; Remis *et al.*, 2000). In some cases (Colombo, 1997; Norry & Colombo, 1999; Remis *et al.*, 2000) chromosome polymorphisms have been associated with morphometric variation. Thus, since selection operates on phenotypic characters and some of them are modified by karyotype, this interaction is central to the understanding of the parallel evolution of chromosome and phenotype variation. Within Orthoptera, the scarce information about this issue is related to the few examples of chromosome polymorphism affecting morphometric traits (White & Andrew, 1960; Colombo, 1989, 1997; Remis, 1997).

Leptysma argentina constitutes an interesting case because it has a polymorphism for a centric fusion whose presence is strongly and systematically correlated with increased body size (Colombo, 1989, 1997). In the present work, which was focused on sexual selection on the chromosome and phenotypic traits, we detected significant differences between successful and unsuccessful males in three morphometric traits. Moreover, significant differences in fusion dosage before and after directional sexual selection were also found, the fusion homozygotes being the most favoured karyotype.

Although several body-size-related traits might be related with mating success, some of them could be the result of selection on other correlated traits. Recently, Janzen & Stern (1998) proposed a statistical method for analysing selection on multivariate phenotypes through logistic regression, which allows us to distinguish the target of selection for dichotomous fitness.

In the present work, the target of male mating selection in *Leptysma argentina* has been identified as the third femur length, with sexual selection on karyotype occurring as a correlated response. Analogously, in the grasshopper *Sinipta dalmani*, the analysis of sexual selection in populations polymorphic for a pericentric inversion showed that the differences in some body-size-related traits were determined by the karyotype; among these traits, third tibia length provided the greatest contribution to variation in fitness (Remis *et al.*, 2000). These results may indicate that the third pair of legs plays an important role in courtship among grasshoppers.

In a previous contribution, it has also been shown that fusion 3/6 carriers have enhanced viability, since they show an increased longevity when compared with unfused homozygotes in the wild (Colombo, 1993b). Yet another study has identified prothorax height as the target of viability selection, which is correlated to overall

size and hence to fusion 3/6 (Norry & Colombo, 1999). Thus, albeit indirectly, viability selection is directed towards an increase in the frequency of this chromosomal rearrangement.

The 3/6 fusion seems to be favoured by two different and independent components of selection. However, as the 3/6 fusion has been constant in this population since studies began in 1985, it must be subject to trade-offs in other components of natural or sexual selection which have not yet been identified. We plan to identify the nature of these trade-offs in the future.

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