Multivariate analysis of male mating success on morphometric traits and chromosome dosage in the grasshopper *Sinipta dalmani*

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Sinipta dalmani is a South American grasshopper that is chromosomally polymorphic for a pericentric inversion in the M4 pair. The inversion affects morphology, showing a negative average effect on body size. In the present work, male mating success was analysed in competition cages and possible correlations examined between this fitness component, body size and inversion polymorphism. The outcome of the study revealed that mated males were larger and had lower inversion dosage than unmated ones. The analysis of standardized selection differentials (S') demonstrated that directional selection operated on chromosome dosage and 3rd tibia, 3rd femur, thorax and tegmina lengths. The analysis of karyotype frequencies showed that mated males had a higher frequency of the standard homozygote karyotype. The analysis of selection gradient (β') and the transformed logistic regression (α) showed an important effect of selection on 3rd tibia length. Identical conclusions were obtained when linear and logistic multiple regression were performed on the first three components of PCA analyses. Our results indicate that differences in some body size-related traits are determined by the karyotype, and that of these length of 3rd tibia provides the greatest contribution to variation in fitness. The selective effects detected on chromosome dosage and the other morphological traits may be considered as indirect ones caused by correlated effects.

Keywords: grasshopper, mating success, morphometric traits, pericentric inversion, *Sinipta dalmani*.

Introduction

The detection, demonstration and description of selection in nature constitute the central aim of many evolutionary studies (Endler, 1986; Brodie *et al.*, 1995).

There are several direct and indirect methods to evaluate selection in nature. Perhaps the most efficient method to detect natural selection is the direct analysis of fitness components, which evaluate the differences in a certain trait between two or more adjacent life-history stages (Endler, 1986). In spite of the relative abundance of such studies in *Drosophila* (Robertson, 1957; Butlin *et al.*, 1982; Ruiz *et al.*, 1986; Santos *et al.*, 1988, 1992; Hasson *et al.*, 1992, 1993; Norry *et al.*, 1995), there are few in species of the order Orthoptera (López León *et al.*, 1992; Colombo, 1993; Martín-Alganza *et al.*, 1997). Because selection acts primarily at the phenotypic level, several studies in *Drosophila* have dealt with the

relationship between certain components of fitness and morphological traits. In particular, size-related traits are often related to adult fitness components such as mating success, fecundity and longevity (Robertson, 1957; Santos *et al.*, 1988, 1992; Hasson *et al.*, 1993; Norry *et al.*, 1995). Several studies have also shown that chromosomal factors may be associated with morphological variation in natural populations, and in some evidence of natural selection on chromosome polymorphism has been documented (Butlin *et al.*, 1982; Ruiz *et al.*, 1986; Hasson *et al.*, 1992).

Lande & Arnold (1983) proposed a method for analysing selection on multivariate phenotypes through multiple regression of relative fitness on measured traits. The introduction of this approach represented a significant advance in the understanding of microevolutionary processes. Lande and Arnold's method suggests that the coefficients of multiple regression of relative fitness on these traits should be used to estimate direct and indirect effects of directional selection. However, some evolutionary biologists have recognized a number of difficulties in using this method (Mitchell-Olds & Shaw,

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1987; Rausher, 1992; Janzen & Stern, 1998). Janzen & Stern (1998) suggested an alternative statistical method employing a logistic regression for estimating selection coefficients on multiple phenotypes when fitness is dichotomous. This method does not rely on assumptions about normality of the predictor variables or the errors and it allows the selection effect to vary nonlinearly. The combination of logistic regression and a principal component analysis (PCA), to avoid some of the multicollinearity problems, is a good approach for evaluating natural selection in species where genetic analyses are limited by long life cycles or there are difficulties in rearing large populations in laboratory conditions.

The present work investigated the effect of chromosomal and phenotypic variation on male mating success in the grasshopper *Sinipta dalmani*. This South American species is chromosomally polymorphic in some areas of its distribution (Remis, 1989, 1990). There is a considerable variation in adult male size in natural populations of *S. dalmani* in the El Palmar National Park and part of this variation was shown to be caused by the polymorphic M_4 inversion which has a negative average effect on size-related traits (Remis, 1997). In the present paper, male mating success of wild-caught *S. dalmani* grasshoppers in competition cages was analysed in relation to chromosome constitution and five size-related traits.

Materials and methods

Experimental design

Sinipta dalmani, like most Acridids, provides an interesting opportunity for selection analysis, because generations are annual and synchronized. Thus, individuals belonging to the same sample are approximately the same age.

In this species it is very difficult to observe adequate numbers of mating pairs in the field. Thus mating behaviour of wild individuals was analysed in mating cages.

A total of 158 individuals of *S. dalmani* collected in November 1995, 1996 and 1997 at Arroyo Los Loros, National Park El Palmar (Entre Ríos, Argentina), was used to evaluate male mating success. Immediately after sampling, females were separated and kept in different cages until the experiment was carried out.

The experimental procedure was performed in a chamber under controlled conditions of temperature, humidity and light. Crosses were conducted by placing one female and four males into individual mating cages. The first copulating male was removed and scored as 1 (mated) and the remaining males were scored as 0 (unmated).

Morphological analysis

All males (mated and unmated) were measured for five morphological traits: total body, 3rd femur, 3rd tibia, tegmina, and thorax lengths. The measurements were made using a stereoscopic microscope with an ocular micrometer (1 mm: 48 ocular units).

Chromosome analysis

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

Statistical analysis

To analyse the effect of chromosome constitution on male mating success, inversion dosage per individual was scored as 0 (standard homozygotes), 1 (heterozygotes) or 2 (inversion homozygotes).

Chromosomal dosage and morphological data of each mating cage were transformed to standardized deviations from the mean value for each trait within a cage to avoid intercage variation in subsequent statistical analyses. Standardization has the additional advantage of transforming chromosome dosage into a continuous variable.

For each trait, differences between mated and unmated males were tested by one-way ANOVA using mating success as the main effect. Chromosome variation associated with mating success was also tested using a 'sign test'.

Directional sexual selection was tested for pooled data across cages, to estimate the following.

1 The standardized directional selection differential (S'), which represents the difference between the standardized mean values of mated males and the whole population for each trait (mean values after and before selection). Statistical significance was evaluated through Spearman's correlations between fitness and each trait, assuming that the covariance between relative fitness and a quantitative trait is equivalent mathematically to the shift in the mean within a generation (following Arnold & Wade, 1984).

2 The standardized selection gradient (β'). The statistical significance of β' was estimated through regression ANOVA. Statistical analyses mentioned above were carried out with the STATISTICA program (Statistica Statsoft Inc. 1996).

3 Logistic multiple regression. The logistic regression model is more appropriate than linear regression when fitness is scored in discrete classes (Janzen & Stern, 1998). Statistical significance of logistic regression coefficients (α) was estimated by the STATISTIX program (Statistix Analytical Software, 1998). Transformed logistic regression coefficients (β_{avggrad}) were calculated according to Janzen & Stern (1998). This transformation, as these authors recognized, allows the estimation of linear rates of change.

To avoid errors in the results attributable to bias caused by multicollinearity among traits, we estimated the regression of fitness on the first three axes from a principal components analysis (PCA) of morphological and/or chromosomal variables.

Results

Sexual phenotypic selection in males

Male mating success was measured in 32 competition cages. During the courtship, the female chose a suitable male and rejected the others. Aggressive interactions among males were not observed in any mating cage. Under laboratory conditions copulation lasted about 20 min.

In all samples (1995, 1996 and 1997) mated males were larger than unmated ones for all studied traits. Table 1 summarizes mean values and standard errors for total body length and 3rd femur, 3rd tibia, tegmina, and thorax length for pooled data across years. The one-way ANOVA, employing standardized data from all mating cages, revealed that there were significant or highly significant differences between mated and unmated males for 3rd femur, 3rd tibia, tegmina and thorax lengths ($F_{1,157} = 5.39$, $P = 0.02$; $F_{1,157} = 10.83$, $P = 0.001$; $F_{1,157} = 7.04$, $P = 0.009$ and $F_{1,151} = 7.66$, $P = 0.0063$, respectively).

Standardized selection differentials (S') are shown in Table 2. The S' mean values, as well as the individual

Table 1 Mean values ($\bar{x}$) and standard errors (SE) (in mm) of mated, unmated and all males for five morphometric traits from pooled data. N , number of analysed individuals

	Mated ($N = 40$)		Unmated ($N = 118$)		All ($N = 158$)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Total	29.10	0.19	28.62	0.13	28.74	0.11
Femur	15.79	0.11	15.40	0.07	15.50	0.06
Tibia	12.41	0.11	12.03	0.06	12.12	0.05
Tegmina	19.54	0.15	18.96	0.11	19.11	0.09
Thorax	5.17	0.05	5.07	0.03	5.10	0.02

values of each mating cage (data not shown), tended to be positive in all samples for all analysed traits. Selection differentials were significant for 3rd femur, 3rd tibia, tegmina and thorax lengths for pooled data (Table 2).

Table 3 summarizes the results of linear and logistic regressions for males. There is a consistency in the signs of β' , α and β'_{avggrad} , although some differences in the statistical significance of regression coefficients were observed between these approaches. The β' analysis, based on morphology, suggested that sexual selection acts positively on 3rd tibia, tegmina and thorax lengths under the experimental conditions. The analysis of logistic regression indicated significant effects of 3rd tibia length.

Both uni- and multivariate analyses suggest that 3rd tibia length is related to sexual selection.

Chromosomal sexual selection in males

The frequency of the M4 inversion did not differ among the three samples collected during consecutive years in the Los Loros (LL) population ($\chi^2_2 = 4.31$, $P = 0.12$). In the same way, the frequency of the inversion remained similar when the comparison included data from other samples collected since 1986 (data from Remis, 1991; Remis *et al.*, 1998) ($\chi^2_6 = 8.42$, $P = 0.21$).

Table 2 Standardized directional selection differentials (S') for morphometric traits and inversion dosage of *Sinipta dalmani* males estimated for the mating cages of 1995, 1996 and 1997 (a, b and c, respectively) and for pooled data (d) showing their statistical significance levels from Spearman's partial correlation

	Total	Femur	Tibia	Tegmina	Thorax	Inversion dosage
(a) 1995 S'	0.19	0.12	0.64	-0.06	0.12	-0.15
(b) 1996 S'	0.49	0.42	0.57	0.49	0.44	-0.37
(c) 1997 S'	0.01	0.25	0.21	0.36	0.64	-0.59
(d) Pooled data						
S'	0.3	0.3	0.51	0.31	0.36	-0.26
r	0.12	0.17	0.38	0.24	0.21	-0.21
P	0.14	0.03	$< 10^{-4}$	0.003	0.01	0.04

Table 3 Directional selection gradient (β') and logistic regression coefficient (α) and transformed logistic regression coefficient (β_{avggrad}) obtained from multiple regression analysis of the relative fitness on morphometric traits in *Sinipta dalmani* males

	Linear regression analysis			Logistic regression analysis			
	β	SE	P	α	SE	P	β_{avggrad}
Total	-0.27	0.23	0.26	-0.42	0.37	0.30	-0.06
Femur	-0.03	0.24	0.91	-0.47	0.42	0.26	-0.07
Tibia	0.27	0.10	0.007	1.46	0.37	0.0001	0.22
Tegmina	0.47	0.18	0.01	0.52	0.28	0.06	0.08
Thorax	0.37	0.18	0.04	0.39	0.28	0.17	0.06

Because inversion frequencies and trends did not vary between years, statistical analyses were performed using pooled data of experiments carried out in years 1995, 1996 and 1997. The dosage of the inversion in relation to the copulatory success of males could be analysed in 25 competition cages. Mated males were less likely to carry the inversion (Table 2). The comparison of standardized inversion dosage showed that there are significant differences between mated and unmated males ($F_{1,49} = 4.92$, $P = 0.029$). Similar results were found when a sign test was performed ($Z = 2.25$, $P = 0.024$). The standardized selection differentials (S') were calculated again for each mating cage. The mean values of S' , like most of the individual S' values per cage (data not shown), tended to be negative (Table 2). Chromosomal selection differential was significant for pooled data ($r = -0.21$, $P = 0.04$). Thus, these results suggest selection acting to decrease the frequency of the inverted chromosome.

Karyotype frequencies in each sample were also scored (Table 4). The frequencies of standard homozygotes and inversion carriers in mated males were similar in the three sampled years ($\chi^2_2 = 4.7$, $P = 0.10$). The same holds for the three samples of unmated males ($\chi^2_2 = 4.9$, $P = 0.09$).

Contingency tests, using the data from all mating cages, revealed that the standard homozygote and the rest of the karyotype frequencies differed between mated and unmated males ($\chi^2_1 = 5.16$, $P = 0.02$). This result may be explained by sexual selection favouring the karyotype in which the standard chromosome is present in double dosage.

Simultaneous analysis of chromosomal and morphological selection

In order to evaluate simultaneously chromosomal and phenotypic selection, the data from all mating cages in which both chromosomal and phenotypic data could be scored were considered. In this analysis we examined

Table 4 Frequencies of inverted chromosomes (q) and karyotypes (standard homozygotes (A/A), heterozygotes (A/SM) and inverted homozygotes (SM/SM)) in mated and unmated *Sinipta dalmani* males from 1995, 1996 and 1997 samples (a, b and c, respectively)

	q	A/A	A/SM	SM/SM	N
(a) 1995 Sample					
Mated	0	1	0	0	7
Unmated	0.28	0.55	0.35	0.1	20
Total	0.2	0.68	0.26	0.07	27
(b) 1996 Sample					
Mated	0.27	0.53	0.4	0.07	15
Unmated	0.35	0.42	0.47	0.12	43
Total	0.33	0.45	0.45	0.1	58
(c) 1997 Sample					
Mated	0.33	0.67	0	0.33	3
Unmated	0.45	0.11	0.89	0	9
Total	0.42	0.25	0.67	0.08	12

only the morphological traits that showed significant standardized selection differentials in the previous analysis.

The multivariate analysis of variance (MANOVA) revealed again that the observed differences between mated and unmated males in morphological and chromosomal traits (jointly analysed) were statistically significant (Wilks' Lambda $_{5,86} = 0.79$, $P = 0.0008$). The main effects of mating success were statistically significant for 3rd tibia ($F_{1,90} = 12.02$, $P = 0.0008$), tegmina ($F_{1,90} = 3.86$, $P = 0.02$), and thorax ($F_{1,90} = 4.28$, $P = 0.01$) lengths, and inverted chromosome dosage ($F_{1,90} = 3.52$, $P = 0.029$).

The analysis of standardized selection gradients showed significant effects of 3rd tibia and thorax lengths and marginally significant effects of 3rd femur length (Table 5). The logistic regression analysis was consistent with the linear regression approach, showing significant differences for 3rd tibia length, whereas the differences

Table 5 Directional selection gradient (β') and logistic regression coefficient (α) and transformed logistic regression coefficient (β_{avggrad}) obtained from multiple regression analysis of the relative fitness on morphometric and chromosomal data of *Sinipia dalmani* males

	Linear regression analysis			Logistic regression analysis			
	β	SE	P	α	SE	P	β_{avggrad}
Inversion	-0.29	0.20	0.14	-0.53	0.36	0.14	-0.08
Femur	-0.51	0.28	0.07	-0.95	0.49	0.05	-0.14
Tibia	0.68	0.26	0.01	1.18	0.49	0.01	0.18
Tegmina	0.21	0.21	0.33	0.28	0.34	0.40	0.04
Thorax	0.47	0.23	0.04	0.80	0.42	0.05	0.12

Table 6 Factor loadings on the three first principal components (PC) for the total sample of pooled males showing the highest loading of the trait on each component (with asterisks)

	PC1	PC2	PC3
Femur	0.86*	0.22	-0.07
Tibia	0.84*	-0.02	0.04
Tegmina	0.59	-0.39	0.65
Thorax	0.69	0.42	-0.32
Inversion	-0.28	0.82*	0.49

for thorax and 3rd femur lengths were marginally significant. Neither analysis showed evidence of chromosome selection.

To eliminate bias arising from collinearity among traits, a principal component analysis was performed. The three components accounted for about 83% of total variance and the trait loadings on these components are shown in Table 6. Both linear and logistic regression analyses employing these axes revealed that only the first component, which is defined by 3rd femur and 3rd tibia lengths, shows selective effects (Table 7).

Discussion

There are several examples of sexual selection analysis involving differences in mating success in males and/or

females. Most of them demonstrated an association between this fitness component and morphology (Wilkinson, 1987; Taylor & Kekic, 1988; Gilburn & Day, 1992; Santos *et al.*, 1992; Norry *et al.*, 1995). In *Drosophila*, virility or male mating success showed a positive correlation with size-related traits (Wilkinson, 1987; Taylor & Kekic, 1988; Santos *et al.*, 1992; Norry *et al.*, 1995). Females of seaweed flies (*Coelopa frigida*) express a mating preference for large males in several populations of northern Europe (Gilburn & Day, 1992). In agreement with these results, our data on mating success in *Sinipia dalmani* show that larger males have mating advantage over smaller ones. The analysis of the standardized selection differential (S') was consistent between different years and demonstrated that directional selection could be acting on size-related traits.

Remis (1997) previously demonstrated a significant reduction of body size in homozygotes for the M4 inversion in natural populations of *S. dalmani*. In the current work, we observed significant differences in inversion frequency between mated and unmated males. The analysis of S' , using standardized chromosome dosage, suggested that directional selection should decrease the dosage of inverted chromosomes because of the advantage of standard homozygotes in mating success.

We employed the standardized selection gradient (β'), in an exploratory manner, to evaluate possible direct and indirect effects of selection. This analysis suggested

Table 7 Linear and logistic multiple regression analysis employing principal components analysis (PC1, PC2 and PC3) in place of the original morphological and chromosome variables

	Linear regression analysis			Logistic regression analysis			
	β	SE	P	α	SE	P	β_{avggrad}
PC1	0.15	0.04	0.001	0.85	0.28	0.002	0.14
PC2	-0.64	0.04	0.15	-0.38	0.27	0.16	-0.06
PC3	-0.02	0.04	0.69	-0.13	0.26	0.62	-0.02

that directional sexual selection may be acting on 3rd tibia, tegmina and thorax lengths whereas the effects on 3rd femur length may be considered indirect because of correlated influences. When chromosome data were also included in the β' analysis, an important result was drawn. Chromosome selection may be considered as an indirect effect related to residual effects of correlated traits.

Logistic regression analyses as well as linear regression showed an important effect of selection on tibia length. Identical conclusions were obtained when linear and logistic multiple regression were estimated on the first three principal components of morphometric variables.

Our results may be interpreted as indicating that differences in some size-related traits are determined by the karyotype and that the length of the 3rd tibia has the greatest contribution to variation in fitness. Remis (1997) observed that 3rd tibia length was the only trait in which inversion heterozygotes were intermediate between homozygotes, with standard homozygotes showing the highest phenotypic values. Phenotypic selection seems to favour individuals with larger 3rd tibia and accordingly standard homozygotes seem to be the fittest karyotype.

Sexual selection may act through competition between males (intrasexual selection) or female choice (intersexual selection) (see Hartl & Clark, 1997). In *S. dalmani*, there is no evidence of aggressive interactions among males in the experimental condition of our test. The superiority associated with morphological differences may be related to signals affecting female preference or higher activity of males. These conclusions, however, are valid for experimental conditions that may be different from nature. The interactions among fitness components in the field may be much more complex and an interesting question is why the M4 inversion seems to be temporally stable (Remis, 1991 and the present paper). Studies examining other fitness components may be helpful in reaching a better understanding for the evolution of chromosome polymorphism and morphological intraspecific variation in nature.

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