Elytra colour polymorphism and randomness of matings in *Chauliognathus fallax* Germar 1824 from southern Brazil (Coleoptera, Cantharidae)

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Abstract. Beetles of the species *Chauliognathus fallax* Germar 1824 are polymorphic for elytron colouration with six morphs distinguished on the basis of black pigmentation on a yellow background. We investigated samples of *C. fallax* taken in eight consecutive weeks aiming to determine the frequency of the morphs which were grouped, for statistical analysis, into three classes according to elytra pigmentation as well as the possible occurrence of assortative mating. Our results showed a variation in the frequency of the classes throughout the season, both in males and females, with the maximum frequency of each class at the fourth and fifth week. The three classes (A, B, C) showed the same pattern of variation, and class B was always the more frequent. To test randomness of matings two approaches were taken: in the first, we compared the frequency of each class in copulating and non-copulating insects. In the second, the frequency of each class in the whole sample was taken as the probability of occurrence of the respective class; then, using the criterion of the probability of independent events we calculated the expected proportion of copulating insects for each pair of events. Both methods gave non-significant differences, suggesting that the matings were random.

Keywords. Assortative mating; *Chauliognathus;* Coleoptera; colour polymorphism.

INTRODUCTION

The maintenance of genetic polymorphism is considered one of the central problems in population genetics. However, the emphasis in the last decades on molecular polymorphisms has displaced studies on colour polymorphism (Hoffmann *et al.* 1995). On the other hand, animal colours have been strongly discussed under the heading of evolutionary ecology, exemplifying mainly the communication between prey and predator as well as that between the sexes (Endler 1978, 1993 a, b).

Beetles of the genus *Chauliognathus* Hentz, 1930 (Coleoptera, Cantharidae) show several features that make them highly suitable for studies both in population genetics and evolutionary ecology. Some species are polymorphic for colour pattern of elytra and/or pronotum, such as *C. flavipes* Fabricius, 1781 from the State of Rio Grande do Sul, Brazil, where the extensive geographical variation contrasts with the temporal stability of the polymorphism (Machado & Araújo

1995). Also for the North American species *C. basalis* Lacordaire, 1837, it has been reported a high degree of polymorphism for pronotum colouration in populations from Colorado, USA (Bernstein & Bernstein 1996).

The phenomenon of assortative mating has already been described in species of that genus. McCauley & Wade (1978) and McLain (1982), showed the occurrence of preferential mating related to size in the species *C. pennsylvanicus* De Geer, 1774, which is however a monomorphic species for colour. Bernstein & Bernstein (1998) also ascertained size-related assortative mating in *C. basalis* and *C. deceptus* Fender, 1964. Southern Brazilian populations of the polymorphic species *C. flavipes* have been studied by Diehl-Fleig & Araújo (1991) and by Machado & Araújo (1999), using elytra colour pattern as the variable involved in the preference with contradictory results. The first authors found positive assortative mating, while this was not found by Machado & Araújo, which explain the possible causes for the different results.

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Table I. Number and percentage for each morph and sex of *Chauliognathus fallax* at every week of sampling.   A - males; B - females.

Week/morphs	1	%	2	%	3	%	4	%	5	%	6	%	Total
A													
07/XI/1996	7	5	2	40	17	2	3	2	3	1	0	0	32
14/XI/1996	15	11	1	20	124	11	22	14	30	10	2	50	194
21/XI/1996	8	5	0	0	186	17	15	9	45	15	0	0	254
28/XI/1996	35	25	1	20	240	22	37	23	77	26	0	0	390
05/XII/1996	33	23	1	20	177	16	38	24	54	18	2	50	305
12/XII/1996	12	8	0	0	138	13	17	11	45	15	0	0	212
19/XII/1996	18	13	0	0	126	12	18	11	34	11	0	0	196
26/XII/1996	14	10	0	0	78	7	10	6	13	4	0	0	115
Total	142		5		1086		160		301		4		1698
B													
07/XI/1996	0	0	2	18	30	3	10	2	2	25	0	0	44
14/XI/1996	9	15	4	36	142	13	65	16	1	12	0	0	221
21/XI/1996	5	8	0	0	164	15	53	13	0	0	0	0	222
28/XI/1996	8	14	2	18	284	26	99	25	1	12	0	0	394
05/XII/1996	20	34	0	0	210	19	86	22	0	0	0	0	316
12/XII/1996	3	5	0	0	115	10	36	9	1	12	0	0	155
19/XII/1996	8	14	2	18	85	8	26	6	0	0	0	0	121
26/XII/1996	6	10	1	9	67	6	28	7	3	39	0	0	105
Total	59		11		1097		403		8		0		1578

Here we studied the frequency of different colour morphs in *Chauliognathus fallax* Germar, 1824 throughout the season and analysed whether there is random or assortative mating in order to find out one of the possible causes in the maintenance of the elytra polymorphism.

MATERIAL AND METHODS

Study sites. Beetles were collected at Fazenda Santa Isabel, municipality of Guaíba, RS, Brazil. Two natural fields (0.5 ha each) were examined for the presence of the beetles between October 17th, 1996 and January 2nd, 1997; they appeared only on November 7th, being absent from December 26th on, which represents the first and the last of the eight weeks referred latter. The fields were about 100 m apart from each other and were separated by a small wood. The vegetation consisted predominantly of grasses and *Eryngium* **sp.** (Tourn.) L. (Apiaceae). Beetles on flowers were sampled one day per week (between 10h and 12h); the insects were captured by hand, scored for the type of morph and then stored in glasses to be later released (pairs in copulation were stored in individual glasses). Although always feeding on pollen they changed hosts, first using *Myrceugenia campestris* (DC.) Legr. et Kaus. (Myrtaceae) and *Struthantus polyrhizus* Mart. (Loranthaceae) in the first week and then *Eryngium* **sp.** until the last week. For statistical analyses, we used contingency or heterogeneity chi-square (Sokal & Rohlf 1995). A sample comprising all the phenotypes found were deposited in the Cantharidae collection of the Laboratório de Genética from UNISINOS.

Colour polymorphism and matings. Colour patterns of *C. fallax* were classified basically by the presence of black spots or stripes on the elytra; six morphs were recognized and numbered 1 through 6 (Table I; Fig. 1). For the analysis we joined morphs according to the increased degree of melanization of elytra. In accordance with this criterion, morphs 1 and 2 form class A; class B consisted only of morph 3, and morphs 4 and 5 formed class C. Morph 6 (the more pigmented) appeared in only four individuals, and so was excluded from the analysis. To analyse the randomness of matings, the morphs of both partners of copulating pairs were recorded and compared with non-copulating individuals. Moreover, the overall frequency of each class (A, B, C) was used as the probability of finding
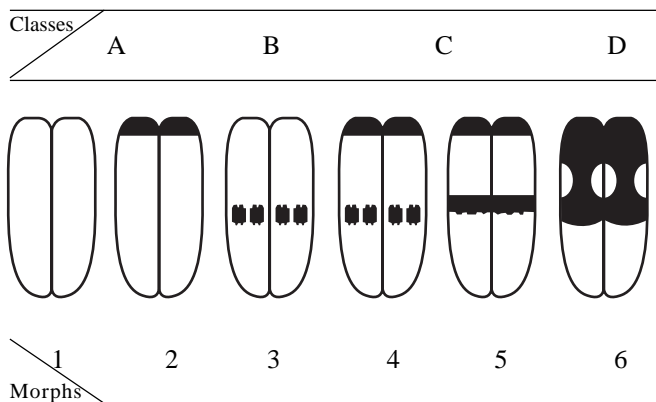

Fig. 1. Elytra of the six morphs found in *Chauliognathus fallax* grouped into four classes. Class D was excluded of the analysis due to the small number of individuals (only 4).

Table II. Test of independence between the number of *C. fallax* for each phenotypic class and week (morphs 1,2, class A; morph 3, class B; morphs 4, 5, class C; morph 6 discarded due to small number of individuals – see Table I). Heterogeneity χ^2 between the sexes not significant.

Males	A	B	C	χ^2	d.f.	P
07/XI/1996	9	17	6	32.428	14	<0.01
14/XI/1996	16	124	52			
21/XI/1996	8	186	60			
28/XI/1996	36	240	114			
05/XII/1996	34	177	92			
12/XII/1996	12	138	62			
19/XII/1996	18	126	52			
26/XII/1996	14	78	23			
Total	147	1086	461			

Females	A	B	C	χ^2	d.f.	P
07/XI/1996	2	30	12	23.46	14	N. S.
14/XI/1996	13	142	66			
21/XI/1996	5	164	53			
28/XI/1996	10	284	100			
05/XII/1996	20	210	86			
12/XII/1996	3	115	37			
19/XII/1996	10	85	26			
26/XII/1996	7	67	31			
Total	70	1097	411			
			Total χ^2	55.89	28	<0.01
			Pooled χ^2	42.86	14	<0.01
			Heterog. χ^2	13.03	14	N. S.

such class to estimate the expected frequency of each mating.

RESULTS

Colour polymorphism. The data were analysed in two complementary ways: first taking into account each sample (week) separately to estimate the frequency of the classes for each sex (per week); this is showed in Table II, for absolute numbers. These numbers were used as entries in a contingency table (8 x 3) to test for an association between week and class. Males showed highly significant differences in the frequency of classes A, B, and C per week ($\chi^2 = 32.43$; 14 d.f.; P < 0.01). Females, on the other hand, are close to statistical significance ($\chi^2 = 23.46$; 14 d.f.; 0.05<P<0.10). Both sexes, however, showed the same pattern, as suggested by the non-significant heterogeneity chi-square ($\chi^2 = 13.03$; 14 d.f.; P > 0.50). Due to this reason they are pooled in Fig. 2; the range of variation for each class was, respectively for class A, B, and C, 62-74%, 24-29 % and 3-14 %.

For descriptive purposes, we also considered the number of beetles in each class and week in relation to the total number caught in the respective class; these frequencies can be considered an estimate of the recruitment rate per class (Table III, Fig. 3). There is a peak of recruitment at the fourth/fifth week of sampling.

Randomness of matings. To test for randomness of matings

two approaches were taken: in the first we tested for an association between the frequency of the respective class in copulating and non-copulating beetles (Table IV); no association was detected. A second test consisted in comparing the expected frequency of each pair in copulation with the observed one (see Materials and Methods for details); again, a non-significant result was obtained (Table V). In order to eliminate the effects of a biased sex ratio for each class of morphs, chi-square statistics were applied each week to test for sex ratio proportions. Only in the sixth and seventh week of sampling there was a significant departure from the expected 1 male :1 female ($\chi^2 = 8.85$; 1 d.f.; $P < 0,01$).

DISCUSSION

Soldier beetles had been used to study the process and consequences of assortative mating. The variables employed in these studies are the spot length (*Chauliognathus pennsylvanicus* see MASON 1972), spot width, dry weight and antennal scape diameter (*C. pennsylvanicus* see MCLAIN 1982, 1985), polymorphism of elytra colour in *C. fallax* and *C. flavipes* (DIEHL-FLEIG & ARAÚJO 1991; MACHADO & ARAÚJO 1995, 1999), and elytra length (*C. basalis* and *C. deceptus*) (BERNSTEIN & BERNSTEIN 1998). Whichever the variables used to evaluate the occurrence of assortative mating, the findings of positive assortative mating reported in the above papers are inconsistent. It seems that the detection of non-random

Table III. Number of individuals for each phenotypic class (A, B, C) every week. M= males; F=females. Percentages were estimated for each week considering males plus females (T), in relation to the total number of beetles during the eight weeks (here defined as recruitment).

Week/morphs	A				B				C			
	M	F	T	%	M	F	T	%	M	F	T	%
1	9	2	11	5	17	30	47	2	6	12	18	2
2	16	13	29	13	124	142	266	12	52	66	120	14
3	8	5	13	6	186	164	350	16	60	53	113	13
4	36	10	46	21	240	284	524	24	114	100	214	24
5	34	20	54	25	177	210	387	18	92	86	180	21
6	12	3	15	7	138	115	253	12	62	37	99	11
7	18	10	28	13	126	85	211	10	52	26	78	9
8	14	7	21	10	78	67	145	7	23	31	54	6
Total	147	70	217		1086	1097	2183		461	411	876	

Table IV. Test of the association between the number of males (a) and females (b) for each phenotypic class and copulating / non-copulating individuals of *C. fallax*.

	Class	A	B	C	χ^2	d.f.	P
(a)	Copulating	49	419	167			
	Non-copulating	57	446	217	2.635	2	N. S.
(b)	Copulating	29	451	155		2	N. S.
	Non-copulating	22	464	187	3.040		

Table V. Test of randomness of matings by comparing observed and expected number of copulating *C. fallax* according to the phenotypic class.

	A x A	A x B	A x C	B x B	B x C	C x C	χ^2	d.f.	P
Observed	4.0	57.0	13.0	303.0	209.0	49.0	7.787	5	N. S.
Expected	2.3	5.1	20.6	286.1	229.7	46.3			

mating in some populations but not in others, is related to other variables, as, for instance, the density of individuals or the presence/absence of a sympatric species.

This report deals with a single population, which could be a disadvantage as compared to other studies; however the fact that the population was followed during eight consecutive weeks is relevant (this procedure was already used in our previous papers, MACHADO & ARAÚJO 1998, 1999). In those species studied so far in the State of Rio Grande do Sul,

Southern Brazil, recruitment rates of the phenotypic classes are different between the sexes, implying that at a given time the frequency of a copulating pair (as far as colour is concerned) could be over/underestimated. So, the occurrence of nonrandom mating could be only an artifact. We think even that this could explain some of the inconsistencies reported in the literature. It is relevant to this matter the following remark by BERNSTEIN & BERNSTEIN (1998): "Temporal covariation may cause positive assortative mating if the sizes of males and
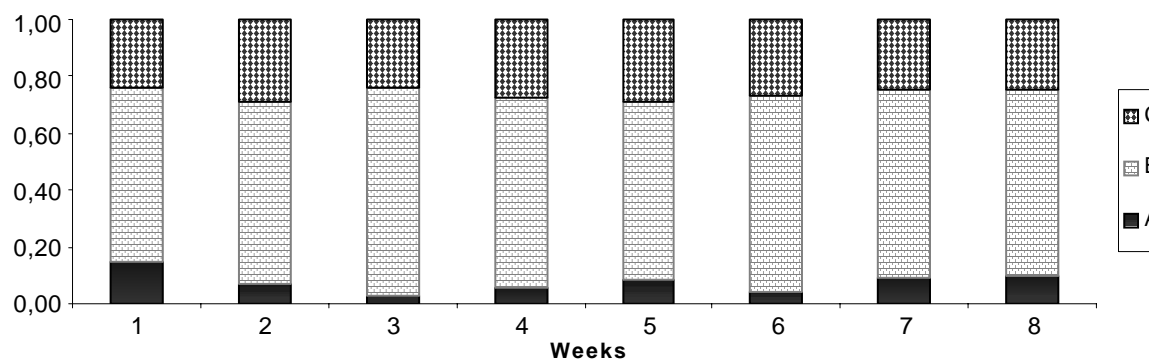

Fig. 2. Relative frequencies of individuals (%) of each colour class of *Chauliognathus fallax*, per sample.
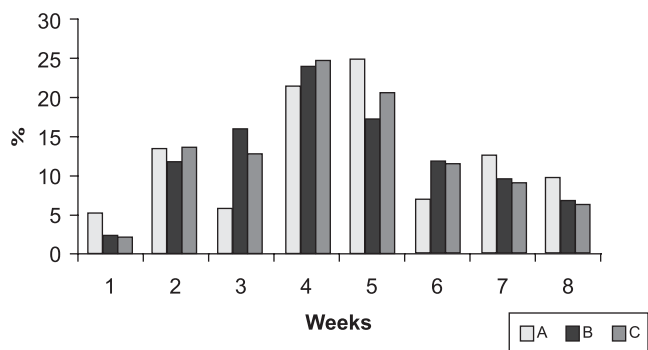
Fig. 3. Variation in the recruitment (%, see Table III) of each colour class of *Chauliognathus fallax* during the period of study.

females vary in a similar way over time, and pairs remain coupled for periods of time that are relatively long compared to emergence time (or if samples taken at different times are pooled). Thus we reasoned that if the beetles that breed earlier are larger or smaller than the beetles that breed later, then a sample taken midway through the season may include pairs that have been mated for a relatively long time (the early breeders, of a similar size) as well as pairs that have just begun to mate (the late breeders, of a similar size but larger or smaller than the beetles that began mating earlier). This particular sample would show positive assortative mating whereas a sample taken earlier or later in the season may not" (p. 63). They discussed for size what we are discussing for colour pattern.

In the present paper we approached the question of randomness of matings in two ways. Both analyses showed no evidence of preferential mating (Tables IV and V). Moreover, as what might be seen as a third way to analyse the data (not showed here), each week was independently analysed; applying the same procedure as before, again no significant results were obtained. It seems then that *C. fallax* mates at random as far as colour of the morphs is concerned, as does *C. flavipes* (MACHADO & ARAÚJO 1999).

The results reported here, as well as others from the literature, seem to indicate the necessity to study different aspects of the biology of *Chauliognathus* **spp**. For instance, how does the male courtship behaviour differ from species to species? Does female behaviour change in response to feeding conditions? (RAUSHER & FOWLER 1979, for instance, found a positive association for the latter question). Could female sexual maturity determine the acceptance or rejection of a male? If so, the acceptance of a given male would be independent of his colour morph.

The reason for the maintenance of the polymorphism for elytra colour (if caused by assortative mating, natural selection or another agent) still remains to be explained satisfactorily. It is remarkable that several species of the genus

Chauliognathus show polymorphism for elytra and/or pronotum colour pattern. In the State of Rio Grande do Sul, for example, 5 out of 9 species already identified are polymorphic for elytra colour (Machado, unpublished). A survey of the literature showed that *C. pennsylvanicus*, *C. basalis*, and *C. deceptus* are also polymorphic for colour pattern (McLAIN 1982, 1985; BERNSTEIN & BERNSTEIN 1996, 1998). Could be there a common denominator for all these findings? Much remains to be done in order to provide an adequate answer to this question.

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