Genet Sel Evol (1993) 25, 31-40

Original article

Larval competition and genetic diversity in *Tribolium castaneum*

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(Received 9 March 1992; accepted 24 November 1992)

Summary – The study of the relationship between genetic homogenity and intensity of competition in groups of organisms may help to explain the widespread existence of sexual reproduction and it can also be used to design efficient crop mixtures. To study this, we compared the survival of sib groups and random groups of larvae of the beetle *Tribolium castaneum* maintained at high population density: every group was formed by introducing 150 eggs in 1 g of culture medium. The larvae in every group were counted weekly. The random groups survived longer, as they had more larvae in the last weeks. This advantage was related to a higher early mortality, which reduced competition in the long run in these groups. Therefore, in the early stages of development, our results did not confirm the hypothesis that genetically heterogeneous groups reduce competition through diversification in the use of environmental resources. In addition, a clear increase in between-group variability for survival was found in the sib groups, implying the presence of genetic variance for competitive ability at constant initial densities in this species.

genetic homogeneity / elbow-room model / genetic variance for competition / *Tribolium castaneum*

Résumé – Compétition larvaire et diversité génétique chez *Tribolium castaneum*. *L'étude de la relation entre l'homogénéité génétique et l'intensité de la compétition au sein de groupes d'organismes peut servir à expliquer la prédominance de la reproduction sexuée dans la nature, et elle pourrait aussi être utilisée pour mettre au point des mélanges de cultures. Dans ce but, on a comparé la viabilité en haute densité de populations de larves de* Tribolium castaneum *maintenues en groupes de frères ou bien en groupes aléatoires. Chaque groupe était établi en introduisant 150 œufs sur 1 g de milieu de culture. Les larves de chaque groupe étaient comptées chaque semaine. Les groupes aléatoires ont survécu plus de temps, mais cet avantage était associé à une plus haute mortalité initiale, ce qui a réduit*

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la compétition à long terme dans ces groupes. En conséquence, dans les premiers stades de développement, on n'a pas confirmé l'hypothèse selon laquelle des groupes génétiquement hétérogènes pourraient subir une moindre compétition par une utilisation plus diversifiée de l'environnement. On a observé que la variabilité pour la viabilité était plus grande entre les groupes de frères, ce qui indique l'existence d'une variance génétique pour l'aptitude à survivre à la compétition dans cette espèce.

homogénéité génétique / modèle *elbow-room* / variance génétique pour la compétition / *Tribolium castaneum*

INTRODUCTION

The study of the relationship between genetic homogeneity and intensity of competition within a group of individuals has both theoretical and practical interest in modern biology (Bell, 1985). Firstly, the subject is related to the evolution and maintenance of sexual reproduction. It has been claimed that sexually reproducing parents could be at an advantage over asexually reproducing ones because their offspring are genetically more variable (Williams and Mitton, 1973; Maynard Smith, 1976). If this increase in genetic variation results in lower competition between sibs and smaller offspring mortality, sexual parents could have a greater fitness. This is the "elbow-room" model of sib competition (Young, 1981), based on the assumption that individuals with less similar genotypes have less similar ecological requirements. Some empirical tests of the model have been made using different plant and animal species, and the results were very diverse, the relationship between genetic homogeneity and the intensity of competition being positive in some cases (Pérez-Tomé and Toro, 1982; Ellstrand and Antonovics, 1985; Kelley *et al*, 1988; Martín *et al*, 1988) and negative in others (Jasienski, 1988; Jasienski *et al*, 1988). In addition, no relationship was found in some experiments (Fowler and Partridge, 1986; Willson *et al*, 1987; Kelley, 1989; Tonsor, 1989). Secondly, the topic has a bearing on the agronomic advantage of mixed crops over monocultures (Valentine, 1982; Spitters, 1983). Mixed crops may outyield monocultures because different strains or varieties complement each other in their canopies, root systems, or nutritional requirements (Trenbath, 1974). Mixtures may also have greater resistance to diseases (Wolfe and Barret, 1980). Thus, a better understanding of the nature of the relationship between genetic homogeneity and competition would make the result of crop mixture more predictable and, therefore, its use will be more efficient (Bell, 1985).

We carried out an experiment on sib competition using the beetle *Tribolium castaneum* to analyse in detail possible differences in the competition process between similar and non-similar genotypes. *Tribolium castaneum* is a useful organism to model these situations, because its population size is regulated to a great extent by competition. Moreover, there is an ample bibliography on competition in this species (Park *et al*, 1964; King and Dawson, 1972; Mertz, 1972).

We used a very high population density to ensure strong competition. By doing so, we expected to increase the probability of detecting differences in competition between our experimental groups. This strategy has the additional advantage of

simplifying the analysis of the competition process. Competition is complex in *Tribolium*, because it is dependent on many mechanisms involving different life stages, such as eggs, larvae, pupae or adults. However, pupation is inhibited at high population densities (Botella and Mensua, 1986), such that no pupae or adults develop. Thus, only mechanisms involving competition among eggs and larvae need to be taken into account when interpreting the experimental results.

MATERIALS AND METHODS

Beetles were randomly sampled 7 d after their adult emergence from the Consejo laboratory population. All individuals were maintained at 30°C and 60% relative humidity. Culture medium consisted of 95% whole wheat flour and 5% dried brewer's yeast.

Two random samples of parents were used. Each sample consisted of 25 males, each male being mated to 20 virgin females during 8 days. To increase egg harvest, each female was subsequently transferred to a separate 3 × 3.5 cm glass vial with 2 g culture medium which was sifted 24 h later to recover the eggs. These eggs were used to set up competition vials (3 × 3.5 cm glass vials with a plastic cap and 1 g of culture medium). As we expected to have high population densities in the vials, we made a hole of $\approx$ 6 mm in diameter in the caps, and covered it with a fine wire mesh to improve ventilation and to prevent an excessive accumulation of humidity.

There were 2 experimental treatments. In the first, a random sample of 150 eggs fertilized by a single male parent were put together in a competition vial. Thus, genetically homogeneous groups were obtained, individuals sharing the same vial being related at least as half-sibs. In the second, eggs sired by all males were pooled, random samples of 150 being taken from the pool and introduced into competition vials. These gave rise to genetically heteregeneous groups.

Setting up competition vials took 4 d. From the first sample of parents, 25 homogeneous groups were obtained on the lst and 25 heterogeneous groups on the third. Likewise, 25 heterogeneous groups and 25 homogeneous groups were obtained from the second sample of parents on the second and fourth d, respectively. Thus, the same parents provided the eggs for the homogeneous and heterogeneous groups. In what follows, the set of competition vials corresponding to the first sample of parents will be called repetition A, and that corresponding to the second sample of parents will be called repetition B.

Homogeneous competition vials started from < 150 eggs were discarded. Thus, only data from 34 homogeneous and 49 heterogenous competition vials were analysed.

The numbers of larvae, pupae and adults per competition vial were counted 2 wk after the vials were established. Larvae, pupae and culture medium were returned to the vial, and the adults were removed. These counts were repeated weekly, as long as living animals were found in the vials. The culture medium was not changed.

To analyse the survival of the individuals in the homogeneous and heterogeneous groups, we carried out a log rank test for the comparison of the survival in 2 samples, as described in Cox and Oakes (1984), chapter 7. This method considers that the survival function takes a log-linear form. The test involves the calculation of the

first and second derivatives of the log likelihood survival function, which, for the null hypothesis of no difference in survival between the 2 groups, are:

$$U = \sum_j \left[d_{1j} - (d_j r_{1j}/r_j) \right]$$

and

$$I = \sum_j \left\{ d_j r_{0j} r_{1j} (r_j - d_j) / \left[r_j^2 (r_j - 1) \right] \right\}$$

Where $d_j = d_{0j} + d_{1j}$, d_{0j} and d_{1j} being the number of individuals dying from time j to time $j+1$ in the homogeneous and heterogeneous groups, respectively, and $r_j = r_{0j} + r_{1j}$, r_{0j} and r_{1j} being the number of individuals alive at time j in the homogeneous and heterogeneous groups. The statistic $W_U = U^2/I$ has, under the null hypothesis, approximately a χ^2 distribution with 1 degree of freedom. This test can also be obtained formally by setting up a separate 2×2 contingency table for every time j, with rows corresponding to the kind of group and columns to survival, and carrying out the combined test for association according to the method of Mantel and Haenszel (Cox and Oakes, 1984).

The second week counts for the homogeneous groups of repetition B were missing. For this reason, we eliminated that week from the analyses. Also for this reason, in the calculation of the correlation between the initial and the final densities in the vials, we estimated the initial density as the mean number of larvae in wk 1 and 3. Final density was estimated as the mean number of larvae found in counts 9 and 10. Later counts were not considered because larval numbers were too low and many vials were empty.

RESULTS

As intended, the competition intensity attained in the vials was very high. Only 16 of the 5 100 eggs used in the homogeneous groups became adults, while 6 adults emerged from 7 350 eggs in the heterogeneous groups. As the number of pupae found was also very low, only larvae number was analyzed. In table I, it can be seen that the number of larvae surviving in the last weeks was greater in the heterogeneous groups in both repetitions. A log rank test to compare the larval survivals in the 2 kinds of groups failed to find significant differences in repetition A, but found them in repetition B. When the data of both repetitions were pooled, the log rank test detected a significant advantage in survival for the heterogeneous groups (table II). Thus, the heterogeneous groups survived longer than the homogeneous groups. However, in the first weeks, the homogeneous groups tended to have a higher survival than the heterogeneous groups, but tended also to have a lower survival in the last weeks (fig 1). A χ^2 test of contingency for the number of individuals alive and dead in each kind of group found these differences in survival as significant in some weeks (table I). However, the χ^2 tests in the same repetition were not independent, as they corresponded to the same vials. In fact, those vials having higher initial numbers of larvae in the first weeks tended to be among those with fewer larvae in the last weeks. In repetition A, the correlation between initial

Table I. Number of individuals alive and dead in each week and repetition in the homogeneous and heterogeneous groups.

Week	*Repetition A*					*Repetition B*				
	Homogeneous Eggs= 3 450		*Heterogenous Eggs = 3 750*		χ^2	*Homogeneous Eggs = 1 650*		*Heterogeneous Eggs = 3 600*		χ^2
	Alive	*Dead*	*Alive*	*Dead*		*Alive*	*Dead*	*Alive*	*Dead*	
1	2 940	510	3 226	524	1.0	1 471	179	2 718	882	130.8**
2	2 607	333	2 755	471	14.6**	1 212	259	–	–	–
3	1 869	738	1 874	881	8.6**	850	362	1 895	823	60.2**
4	1 269	600	1 338	536	5.4*	584	266	1 464	431	22.6**
5	819	450	907	431	3.1	382	202	1 034	430	5.3*
6	424	395	517	390	4.7*	225	157	666	368	3.6
7	197	227	253	264	0.6	111	114	387	279	5.3*
8	72	125	87	166	0.2	33	78	185	202	11.4**
9	17	55	21	66	0.0	10	23	81	104	2.1
10	3	14	4	17	0.0	2	8	26	55	0.6
11	0	3	0	4	–	0	2	9	17	–
12	0	0	0	0	–	0	0	1	8	–
13	0	0	0	0	–	0	0	0	1	–

Eggs is the total number of eggs used in each repetition and kind of group. Under the heading χ^2 are the values of χ^2 2 × 2 contingency tests of the number of individuals alive and dead in each kind of group and week. * $P < 0.050$; ** $P < 0.005$. The test for the third week of Repetition B used the sum of the dead individuals in the second and third week in the homogeneous groups (*ie* 621), as this was the only datum available for the heterogeneous groups.

and final larval densities was −0.49 ($n = 23$, $P < 0.05$) in the homogeneous groups and −0.33 ($n = 25$, NS) in the heterogeneous groups. In repetition B, these correlations were −0.50 ($n = 11$, NS) and −0.12 ($n = 24$, NS). When data were pooled across repetitions, correlations of −0.48 ($n = 34$, $P < 0.005$) and −0.41 ($n = 49$, $P < 0.005$) were obtained for the homogeneous and heterogeneous groups, respectively.

Table II. Log rank test for the comparison of the survival in the homogeneous and heterogeneous groups.

	U	I	W_U	*Probability*
Repetition A	4.8	1 220.5	0.019	0.8904
Repetition B	−82.6	735.5	9.284	0.0023
Both repetitions	−132.2	1 936.1	9.030	0.0027

Analyses were carried out for each repetition and for the pool for both repetitions. The probability given is that of a random variable with χ^2 distribution with one degree of freedom exceeds the observed value of W_U. See the *Materials and Methods* section for a description of the method.

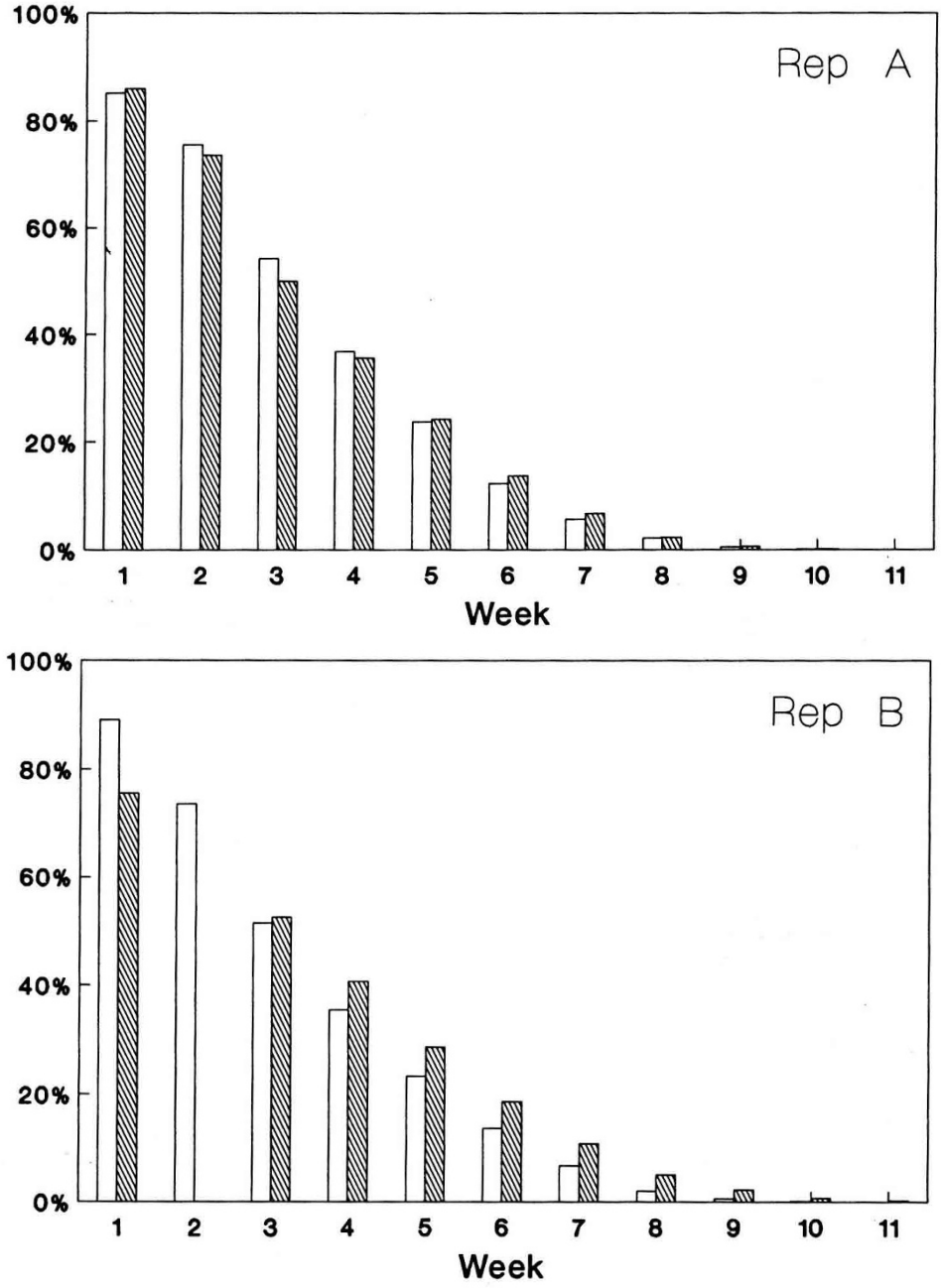

Fig 1. Number of larvae as a percentage of the number of eggs introduced in the vials in each repetition. White bars: homogeneous groups; shaded bars: heterogeneous groups.

We calculated the between-vial variance for the number of larvae in each week. As these variances showed some dependence on the mean larvae number, we calculated also the coefficient of variation for this variable (fig 2). It can be seen that, with the exception of the last week of repetition B, in which the number of larvae was already very low, there was always a greater variability between vials in the homogeneous groups.

DISCUSSION

The longer survival found in the heterogeneous groups could seem to be in agreement with the prediction of the elbow-room model of sib competition that less similar genotypes could partition the environmental resources with greater efficiency. However, the situation was more complex, as the homogeneous groups tended to maintain higher population densities in the first weeks. The reason for this initial advantage of the homogeneous groups is not clear. It could be explained by the observation by Fogle and Englert (1976) that larvae of 2 strains of *Tribolium castaneum* prefer to eat eggs of the opposite strain. Jasienski *et al* (1988) found a reduction in developmental time of homogeneous groups of *Tribolium confusum.* This was attributed to a reduction in the behavioural antagonism between related individuals that could have evolved by kin selection. However, they did not find the same effect in *Tribolium castaneum.* Further experimental work should be done to ascertain the nature of the mechanisms responsible for this apparent sib cooperation in *Tribolium castaneum.*

Part of the longer survival of the heterogeneous groups could be related to their lower number of larvae in the first weeks. Homogeneous groups had greater initial densities, but, as medium conditioning is faster at high densities (Park, 1934) and flour was not replaced in our experiment, these groups lived in a worse medium and had lower viability in the long run. This interpretation is supported by the fact that the same outcome was observed within treatments. In both treatments, there was a negative correlation between initial and final vial population densities. We found similar results in a previous experiment carried out at a lower density (García and Toro, 1992). In it, we found and initial advantage in larval production for the homogeneous groups. Nevertheless, this did not result in higher adult production, because these variables were negatively correlated.

Rather than a consistent advantage in larvae numbers for the heterogeneous groups in all weeks, which could be interpreted as the result of resource partitioning between different genotypes, we have found that the differences between treatments for this character changed with time, and that these changes were related to a negative correlation between initial and final larvae numbers. This is a compensating mechanism that can mask real between-treatment differences in competition experiments, especially if only final or average outcomes are evaluated. Therefore, the entire development of the competition process should be followed to be able to detect differences between treatments. These negative correlations can be generated by simple mechanisms, such as the depletion of a given environmental resource.

Our results indicate that there was genetic variability for competition intensity between larvae, as the variation between homogeneous groups was greater than

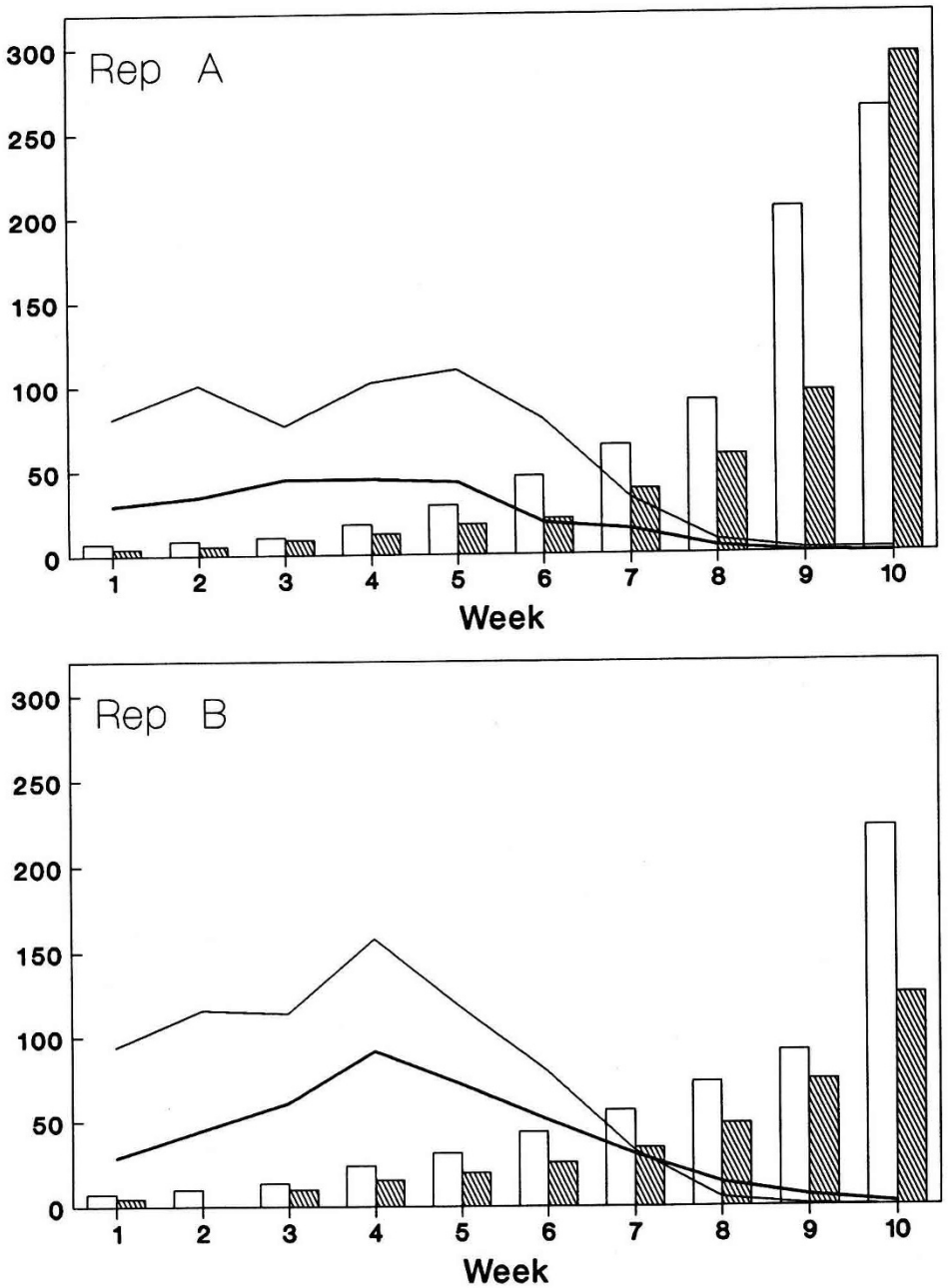

Fig 2. Between-vial variance (lines) and coefficient of variation (bars) for the number of larvae in each week. Simple lines and white bars: homogeneous groups; bold lines and shaded bars: heterogeneous groups.

between heterogeneous groups. This could not be due to genetic differences in parent productivity, because the number of eggs in every vial was the same. The greater variability observed in the homogeneous groups must be related to greater variability for other characters, such as egg hatchability at high densities, tolerance to conditioned medium, aggressive behaviour, etc. The detection of between-group genetic differences for the intensity of competition is consistent with our previous work (García and Toro, 1990), in which we obtained a positive response to group selection for productivity in *Tribolium castaneum* under competition conditions. It is likely that the increase in productivity found in group selected lines of that experiment was related to a reduction in competition intensity.

Our experiment indicates that between-group genetic variance for production may be available for selection even in situations of strong competition. Furthermore, it also shows how this variance can be detected. Efficient selection techniques should be designed to use this variance in the improvement of the productivity of populations in situations of competition.

ACKNOWLEDGMENT

We thank C López-Fanjul for helpful comments on the manuscript.

REFERENCES

Bell G (1985) Two theories of sex and variation. *Experientia* 41, 1235-1245

Botella LM, Mensua JL (1986) Larval arrest in development of *Tribolium castaneum* (Coleoptera, Tenebrionidae). *Environ Entomol* 15, 1264-1267

Cox DR, Oakes D (1984) *The Analysis of Survival Data.* Chapman and Hall, London

Ellstrand NC, Antonovics J (1985) Experimental studies of the evolutionary significance of sexual reproduction. II. A test of the density-dependent selection hypothesis. *Evolution* 39, 657-666

Fogle TA, Englert DC (1976) Differential cannibalism among larvae of *Tribolium castaneum* as influenced by the antennapedia mutation. *Can J Genet Cytol* 18, 179-187

Fowler K, Partridge L (1986) Variation in male fertility explain an apparent effect of genotypic diversity on success in larval competition in *Drosophila melanogaster*. *Heredity* 57, 31-36

García C, Toro MA (1990) Individual and group selection for productivity in *Tribolium castaneum*. *Theor Appl Genet* 79, 256-260

García C, Toro MA (1992) Sib competition in *Tribolium*: a test of the elbow-room model. *Heredity* 68, 529-536

Jasienski M (1988) Kinship ecology of competition: size hierarchies in kin and nonkin laboratory cohorts of tadpoles. *Oecologia* 77, 407-413

Jasienski M, Korzeniak U, Lomnicki A (1988) Ecology of kin and nonkin larval interactions in *Tribolium* beetles. *Behav Ecol Sociobiol* 22, 277-284

Kelley SE, Antonovics J, Schmistt J (1988) A test of the short-term advantage of sexual reproduction. *Nature* 331, 714-716

Kelley SE (1989) Experimental studies of the evolutionary significance of sexual reproduction. VI. Greenhouse test of sib- competition hypothesis. *Evolution* 43, 1066-1074

King CE, Dawson PS (1972) Population biology and the *Tribolium* model. *Evol Biol* 5, 133-227

Martín MJ, Pérez-Tomé JM, Toro MA (1988) Competition and genotypic variability in *Drosophila melanogaster. Heredity* 60, 119-123

Maynard Smith J (1976) A short-term advantage for sex and recombination through sib-competition. *J Theor Biol* 63, 245-258

Mertz DB (1972) The *Tribolium* model and the mathematics of population growth. *Annu Rev Ecol Syst* 3, 51-78

Park T (1934) Studies in population physiology. III. The effect of conditioned flour upon the productivity and population decline of *Tribolium confusum. J. Exp Zool* 68, 167-182

Park T, Leslie RH, Mertz DB (1964) Genetic strains and competition in populations of *Tribolium. Physiol Zool* 37, 97-162

Pérez-Tomé JM, Toro MA (1982) Competition of similar and non-similar genotypes. *Nature* 229, 153-154

Spitters CJT (1983) An alternative approach to the analysis of mixed cropping experiments. 1. Estimation of competition effects. *Neth J Agric Sci* 31, 1-11

Tonsor SJ (1989) Relatedness and intraspecific competition in *Plantago lanceolata. Am Nat* 134, 897-906

Trenbath BR (1974) Biomass productivity of mixtures. *Adv Agron* 26, 177-210

Valentine J (1982) Variation in monoculture and in mixture for grain yield and other characters in spring barley. *Ann Appl Biol* 101, 127-141

Williams GC, Mitton JB (1973) Why reproduce sexually? *J Theor Biol* 39, 545-554

Willson MF, Thomas PA, Hoppes WG, Katusic-Malmborg PL, Goldman DA, Bothwell JL (1987) Sibling competition in plants: an experimental study. *Am Nat* 129, 304-311

Wolfe MS, Barret JA (1980) Can we lead the pathogen astray? *Plant Dis* 64, 148-155

Young JPW (1981) Sib competition can favor sex in two ways. *J Theor Biol* 88, 755-756