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A Further Assessment of the Role of Founder Effects in the Outcome of *Tribolium* Competition Experiments*,†

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Abstract. A novel experimental design was employed to further investigate the importance of founder effects in determining the outcome of competition between the flour beetles *Tribolium castaneum* and *T. confusum*. Each of 10 replicate cultures in seven competition series was started with 10 pairs of adults of each species that were, in turn, the progeny of 1, 2, 4, 8, 16, 32, or 64 pairs of adults, respectively, from the foundation population. Thus the founder effect occurred one generation prior to the initiation of competition. An analysis of variability among replicate cultures for the first eight monthly censuses showed a significant increase in variation with smaller numbers of original pairs, confirming the hypothesis previously proposed to explain the indeterminate outcome of replicate competition cultures initiated with small numbers of founders. In addition, there was a significant reduction in the rate of elimination of *T. confusum* with smaller numbers of original pairs. It is suggested that *T. castaneum* is more sensitive to inbreeding, and that the differential response of the two populations to inbreeding reflects a difference in their homeostatic properties.

Introduction. A long-term series of laboratory experiments by Park and his associates on interspecific competition between the flour beetles *Tribolium castaneum* and *T. confusum* has provided a wealth of information on the process of competition in a uniform environment. A consistently indeterminate pattern of competitive outcome under a regimen of 29°C, 70% relative humidity, where *T. castaneum* was the sole survivor in approximately 84% of replicate cultures over several experiments,[1] has been interpreted as a stochastic process.[2] Later, on the basis of extensive experiments with highly inbred lines, Lerner and Dempster[3] postulated that the indeterminacy observed by Park could be accounted for on the basis of founder effects. Park's cultures were initiated with small numbers of adults (two pairs of each species), and thus, under this hypothesis, would not be expected to receive a representative sample of the gene pools of the two strains utilized. When replicate cultures were started with 10 pairs of adults, the outcome was completely determinate.[4]

In a later study, Dawson and Lerner,[5] using several strains of each species, compared the outcomes of paired experiments initiated with 2 and 10 pairs of adults but treated identically in all other respects. A greater amount of indeterminacy was observed in the two-pair series, and, further, a measure of variation in the proportion of one species between replicate cultures within any experiment showed a significantly greater variance for the two-pair series, thus supporting the hypothesis of Lerner and Dempster. However, it can be argued that differences in initial density in these paired experiments may be in part responsible for the differences in both the pattern and the outcome of competition. Hence, a further test of the hypothesis was made using an experimental design under which all cultures were initiated with identical numbers of beetles.

Experimental Design. The beetles used in these experiments were from new, highly heterogeneous synthetic populations produced in late 1967 and early 1968 from systematic crosses among six strains of each species obtained from a variety of sources. The Berkeley strain of *T. castaneum* is marked with *sooty*, a dark body color mutant, which was also incorporated into the new synthetic population.

Several months after the new stocks had been prepared, large numbers of pupae were collected, sexed, and stored separately. Ten replicate matings were set up simultaneously for each species using 1, 2, 4, 8, 16, 32, or 64 pairs of beetles taken at random from the storage bottles. Two of the mating series were set up each week following a randomly determined schedule. From each of the 10 replicate matings within each mating series, 10 male and 10 female pupae were isolated and later used to establish one competition culture. Thus, for example, each of 10 replicate cultures for the one-pair series was initiated with 10 pairs of adults of each species that were, in turn, the progeny of one pair of adults. Under this procedure, the bottleneck in population size occurred one generation prior to the start of the competition tests; all 70 competition cultures were started with identical numbers of founders.

Two separate control competition series were also established in the usual manner using 10 pairs of adults obtained from random collections of large numbers of pupae from the synthetic strains. All nine series were coded and treated identically, and the technicians who performed most of the censuses had no knowledge of what series they were working with. Adults were discarded at the time of census (transfer), which was conducted at 4-week intervals. Cultures were maintained in 6-dram shell vials containing 7 g of a mixture of whole wheat flour supplemented with 5% brewer's yeast and kept in a constant temperature room at 29°C (±1°), and 65–75% relative humidity. All of the cultures were retained for a minimum of eight transfers, in order to provide data necessary for transformation of percentages (discussed later), and thereafter were discarded when the outcome of competition had been determined.

Toward the end of the eighth month my laboratory was moved. Furthermore, an unknown "disease" (possibly one of the sporozoan parasites frequently found in *Tribolium* cultures) was discovered in some vials at the seventh or eighth month. Some of the cultures seemingly recovered from the "disease" and returned to a "normal" condition. It is quite possible, however, that the time period required for elimination of the losing species was modified in diseased cultures; this should be kept in mind when the results are examined. Subsequent to the ninth transfer, all affected cultures were discarded. For these reasons, detailed analyses were only performed for the first eight transfers.

Results and Discussion. The data analyzed in this study were the means and variances of the percentage of *T. castaneum* in the 10 replicate cultures of each series. In order to account for differences in means between series, coefficients of variation rather than variances were used to assess variability between replicate cultures. Because the procedure of discarding adults leads to fluctuations in the frequency of each species as well as in absolute numbers,[6] the means and coefficients were converted to shifting two-point averages prior to analysis.

Percentage data were converted to angles by an arcsin transformation and were also corrected for spurious variance arising from values of 0 or 100%.[7] The transformation did not significantly alter the analysis of variability (Table 1), but the results are included for completeness.

TABLE 1. *Regression coefficients from regression of three dependent variables on logarithm of the number of pairs of founder adults for each culture.*

Dependent variable	Transfer (shifting average)					
	2 + 3	3 + 4	4 + 5	5 + 6	6 + 7	7 + 8
Mean	13.1*	14.4*	12.4†	9.0†	9.4*	8.5*
CV (original data)	−5.2‡	−10.4§	−9.5‡	−6.4‡	−8.7§	−10.7‡
CV (transformed data)	−2.9	−6.6§	−5.6‡	−4.3‡	−6.5‡	−8.3‡

The dependent variables are mean percentage of *T. castaneum* in 10 replicate cultures, and coefficients of variation (CV) of the percentage of *T. castaneum* from both original data and data subjected to angular transformation as described in the text.
* Significantly different from zero by t test, $p < 0.05$.
† Significantly different from zero by t test, $p < 0.01$.
‡ Significantly less than zero by t test, $p < 0.05$.
§ Significantly less than zero by t test, $p < 0.01$.

The primary hypothesis of interest in this investigation was as follows: There should be greater variability between replicate cultures when small numbers of founders are used. This hypothesis was tested by regression analysis in which the coefficient of variation and the *logarithm* of the number of original founder pairs were used as the dependent and independent variables respectively. The use of logarithms was designed to make the analysis extremely conservative; significant differences obtained in this manner would be greatly magnified if the original numbers were utilized as the independent variable. The null hypothesis was tested against the alternative that the regression coefficient is less than zero, hence a one-sided t test was used. As the study proceeded, it became evident that the mean percentage of *T. castaneum* was also of interest, and a similar regression analysis was performed using means. Here, however, there was no *a priori* alternative hypothesis, so a two-sided t test had to be used.

Prior to this analysis, a preliminary calculation of regression of coefficients of variation on transfer was conducted separately for each series in order to determine if there were systematic changes in the coefficients through time. For the period analyzed (transfers 2–8), none of the experimental series changed significantly (three of the regression coefficients were negative and four were positive), although in both control series the coefficients of variation decreased significantly. This result would be expected eventually in all series since there would be no variability after one species is completely eliminated.

Figures 1 and 2 and Table 1 contain the data relevant to the original intent of this investigation. Analysis of the coefficients of variation demonstrates that there is greater between-culture variation in the series initiated with the progeny of small numbers of founders. Regression of coefficients of variation on logarithm of the number of founder pairs was significantly less than zero for six out of six comparisons using the original data and for five out of six using transformed data. Figure 2 shows a composite regression line obtained using the average coefficient of variation over the first eight transfers; this regression coefficient was also significantly less than zero. These data support the hypothesis of Lerner and Dempster, since there is significantly more interculture variation in those series where each culture presumably had a lower probability of receiving representative samples of the gene pools of the two synthetic populations.

An unexpected and potentially more interesting result was obtained when the

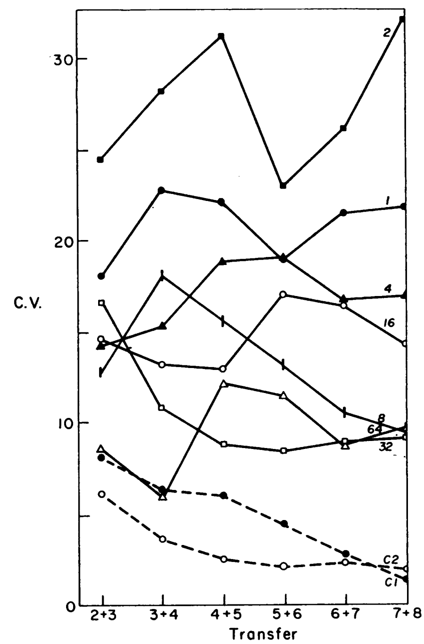

FIG. 1.—Coefficients of variation (CV) for percentage *T. castaneum* in 10 replicate cultures from competition series initiated with the progeny of different numbers of pairs of adults. *C1* and *C2* are controls. Data are plotted as shifting two-point averages (see text).

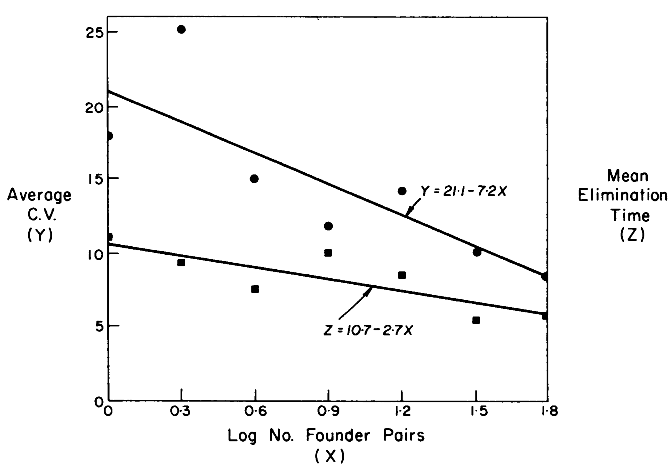

FIG. 2.—Regression of average coefficient of variation (CV) from transfers 2–8 and mean number of transfers for elimination on logarithm of the number of pairs of founder adults for each culture.

mean percentage of *T. castaneum* in the various series was analyzed (Table 1 and Fig. 3). There is a significantly slower elimination of *T. confusum* in the series where there were fewer original founders per culture; regression coefficients are significantly different from zero in all six of the comparisons made.

These data are consistent with some previous speculations[8] based on an analysis of the effects of inbreeding and artificial selection on the two species. In the

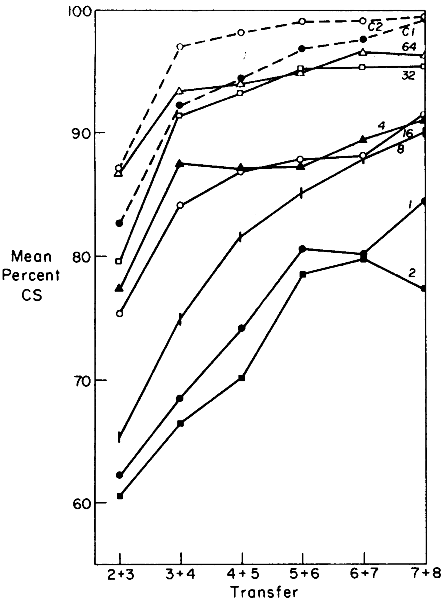

FIG. 3.—Same as Fig. 1 for mean percentage of *T. castaneum*.

previous study, inbreeding depression was greater in *T. castaneum* for both productivity and developmental rate. Further, the results of selection for developmental rate indicated that *T. confusum* was less affected by the pressure of artificial selection as measured by correlated responses in other fitness traits.[8] It was suggested that *T. confusum* populations possess higher levels of both genetic and developmental homeostasis. This was attributed to a greater reliance of this species on superior heterozygous genes or gene combinations accumulated as a result of natural selection for adaptation to a more variable, more extreme, and more unpredictable environment.

The bottlenecks in population size, similar to founder effects,[9] utilized in this study are essentially a form of inbreeding. For example, in the competition series initiated with the progeny of one pair of adults, all of the founders in any one culture are full sibs. If the above speculations are valid, *T. castaneum* should be more affected by inbreeding and should perform poorly in cultures initiated with the progeny of fewer founders. *T. confusum*, less affected by inbreeding, should do relatively better in these cultures. The data on changes in mean percentage of *T. castaneum* (Fig. 3) indicate that this is the case.

Table 2 shows the outcome of competition. Elimination of one species has occurred in all nondiseased cultures except one from the one-pair series. In

TABLE 2. *Outcome of competition between T. castaneum (CS) and T. confusum (CF) in experimental and control series initiated with the progeny of different numbers of pairs of founder adults.*

No. pairs founders	"Normal" Cultures CS won	CF won	"Diseased" Cultures CS won	Outcome uncertain*	Mean no. of transfers for elimination
1	7	0	1	1	>11.0 ± 1.1†
2	6	1	1	2	9.4 ± 0.9
4	7	0	1	2	7.6 ± 1.2
8	8	0	0	2	10.0 ± 0.5
16	6	0	2	2	8.5 ± 0.5
32	7	0	1	2	5.5 ± 0.7
64	10	0	0	0	5.8 ± 0.8
Control 1	8	0	2	0	7.1 ± 0.8
Control 2	10	0	0	0	5.9 ± 0.8

Each series was started with 10 replicates, but some cultures were discarded prior to elimination of one species because of an unidentified "disease" (see text).
* Both species present when "diseased" cultures were discarded.
† One culture remaining at 16th transfer (see text).

that replicate, *T. confusum* performed very well for the first 14 transfers, reaching a maximum of 68% at the ninth transfer. Subsequently a reversal occurred and by the 16th transfer the percentage was reduced to 28. The outcome was decided in all remaining cultures by the 14th transfer. The single culture in which *T. confusum* was the winner in competition occurred in the two-pair series.

Previously[6] I have used the mean number of transfers for elimination as a measure of competitive ability. This criterion is less satisfactory in the present study because of the "disease" problem and the fact that the experiment was moved while still in progress. Nevertheless, a regression analysis of mean number of transfers for elimination on the logarithm of the number of pairs of founders (Fig. 2) showed a significant increase in elimination time with small numbers of founders (one-tailed $t = 3.38$, $p < 0.01$). There were no significant differences among the experimental series with respect to coefficient of variation in the number of transfers required for elimination.

The results of the present experiment prompted a reanalysis of the data reported by Dawson and Lerner.[5] In that study, several strains of both species were utilized in 10 paired competitions started with 2 and 10 pairs of adults of each species. Table 3 presents a summary of the means and coefficients of variation for the number of transfers required for elimination of the losing species.

TABLE 3. *Means and coefficients of variation for the number of transfers required for elimination in paired experiments initiated with 2 and 10 pairs of adults.*

Strains*		10 Pairs		2 Pairs	
CS	CF	$\bar{X}$	CV	$\bar{X}$	CV
Stock	Stock	5.3	32.3	4.5	28.2
Fast	Stock	2.6	32.4	3.7	22.3
Fast	Fast	3.5	20.2	5.5	24.6
Fast	11a	3.1	18.3	3.5	27.8
Fast	11	2.1	15.1	3.6	64.4
Slow	Stock	9.5	38.5	10.0	59.6
Slow	Fast	6.1	22.5	6.1	30.4
Slow	11a	7.3	23.3	9.7	39.5
Slow	11	3.4	20.6	4.5	36.7
Stock	Fast	3.1	23.8	3.7	25.6

Each experiment had 10 replicates.
* For a description of the various *T. castaneum* (CS) and *T. confusum* (CF) strains see Dawson and Lerner.[5]

The nonparametric Wilcoxon signed-ranks test[10] was used to test the null hypothesis of no difference between the 2- and 10-pair series against the alternative that both means and coefficients of variation should be greater in the two-pair series. For means this was true in eight out of nine comparisons (ties are ignored in this test) and for coefficients of variation it was true eight out of ten times. The probability of obtaining such results by chance under the hypothesis tested is less than 0.05 for both cases. Thus the earlier results are concordant with the observations made in the present experiments.

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* This paper is dedicated to Theodosius Dobzhansky on the occasion of his 70th birthday.

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