Proc. Nat. Acad. Sci. USA
Vol. 73, No. 4, pp. 1368–1372, April 1976
Zoology

An experimental analysis of competitive indeterminacy in *Tribolium*

(genetic founder effect/interspecies competition/stochastic competition theory)

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Communicated by Alfred E. Emerson, January 29, 1976

ABSTRACT **This report reexamines experimentally the problem of competitive indeterminacy in mixed-species populations of the flour beetles, *Tribolium confusum* and *T. castaneum*. Indeterminacy takes the form of alternative competitive outcomes: in some replicate cultures one species exterminates the other with a probability, say *p*, whereas in others, the opposing species wins with a complementary probability, 1 − *p*. The conventional explanation for this is the genetic founder effect hypothesis—an explanation based on genetic stochasticity. The experiment reported here partitioned indeterminacy into founder effect and nonfounder effect components. The results implicate demographic stochasticity, not classical genetic founder effect, as a factor influencing the identity of the winning species.**

This report presents findings from an experiment designed to resolve the biological mechanisms underlying competitive indeterminacy in mixed-species populations of the flour beetles, *Tribolium castaneum* and *T. confusum*. A detailed account will be published later.

Both flour beetle species are pests of stored grain products, particularly flours, and have become model organisms for laboratory investigations of interspecies competition. Thomas Park (1) first reported indeterminacy in this competitive system in 1948, and it has been a recurring phenomenon ever since in studies in his own laboratory (2–6) and in the experiments of I. M. Lerner, P. S. Dawson, and their associates (7–11). There are several published reviews of this work (12–15).

Competitive indeterminacy has been observed and characterized in terms of the following kind of experiments. Initial populations of *T. confusum* (hereafter called *b*) and *T. castaneum* (*c*) are introduced together in a controlled climate and supplied with standard flour medium which serves as nutrient source and physical habitat. At regular intervals censuses are taken and the flour is renewed. Invariably in these experiments, either *b* exterminates *c* or the opposite occurs; i.e., permanent coexistence has never been achieved. With a few minor exceptions, the winning populations, as well as single-species control cultures, persist indefinitely in the absence of the competing species. Depending upon climate, nutritive conditions, initial population size, and the genetic strains of *b* and *c*, competitive indeterminacy may occur. This takes the form of alternative eliminations: in some replicate cultures *b* is the winning species, and, in others, *c* wins. In terms of the variables subject to experimental control, the replicates are identical, but, for each one, there is a probability *p* associated with *b* winning and a complementary probability, 1 − *p*, that the reverse will occur.

The conventional explanation for competitive indeterminacy is the genetic founder effect hypothesis proposed by Lerner and Dempster (8). Their interpretation is that the identity of the winning species can be attributed mainly to the genetic characteristics of the founding populations. If *b* wins, this implies that, initially, a genetically superior collection of *b* beetles was matched with a relatively inferior collection of *c* beetles. If *c* wins, the reverse is presumably true. Because the founding populations consist of a haphazard collection of genotypes from a larger stock culture, the heterogeneity between replicates is attributed to random sampling error among the genotypes represented in the initial cultures. Replicated small samples should be more heterogeneous than large ones, so this hypothesis predicts the highest levels of competitive indeterminacy for the smallest founding populations. This hypothesis need not be all inclusive. If the two founding populations are fairly evenly matched in terms of their competitive strengths, then other factors that arise during the course of the competitive encounter may be invoked to account for the identity of the winner. It thus becomes a matter of degree, but, among the potential contributing causes, genetic founder effect is judged to be of predominant importance.

Five lines of experimental evidence have been established in support of the genetic founder effect hypothesis: (*a*) Inbred strains of *b* and *c*, which, because of their increased homozygosity, would be expected to provide more homogeneous founding populations, have indeed given wholly determinate outcomes in a number of experiments (5, 7, 8). (*b*) Larger founding populations of *b* and *c* (10 pairs each), which would be expected to provide less genotypic sampling error, give less indeterminacy than smaller founding populations (2 pairs each) (7, 8). (*c*) Less inbred *b* cultures that displayed indeterminacy in competition with *c* responded to selection for shorter developmental time. Highly inbred *b* beetles, which produced wholly determinate outcomes with the same *c* stocks, did not respond to such selection (9). (*d*) Variation in the percentage of each species early in the course of competition was greater in cultures founded with two pairs of *b* and *c* than in ones founded with 10 pairs. This was taken as indication of genetic variability of the sort required for the genetic founder effect to be operative (10). (*e*) Dawson (11) set up founder cultures to produce 10 pairs each of *b* and *c* which were placed into competition in the next generation. The smaller the precompetition founder cultures, the greater the tendency toward indeterminacy. Superficially, statements (*b*) and (*e*) would seem to constitute strongest evidence for the genetic founder effect hypothesis. However, we describe below how statement (*b*) could be reinterpreted in light of stochastic competition theory; the experiment alluded to in (*e*), to its detriment, gave a low overall incidence of alternative outcomes. The variation in time to elimination of *b* was taken as an index of indeterminacy, but this may be inappropriate.

An alternative to the genetic founder effect hypothesis is found in stochastic competition theory, developed partly in connection with the *Tribolium* studies but never seriously

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defended as an explanation of competitive indeterminacy. A model devised by P. H. Leslie (16) is used here. It is a discrete time formulation based on:

$$\begin{cases} E[N_{t+1}|N_t,M_t] = \lambda_1 N_t/(1 + \alpha_1 N_t + \beta_1 M_t) \\ E[M_{t+1}|N_t,M_t] = \lambda_2 M_t/(1 + \alpha_2 M_t + \beta_2 N_t). \end{cases} \quad [1]$$

Here, N and M are the adult numbers of b and c respectively, and the expressions on the left are conditional expectations at time $t + 1$ given the population sizes at t. $\lambda(>1)$ and $\alpha,\beta(>0)$ are parameters explained by Leslie (16). Additional parameters d_1 and d_2, representing constant death rates, are required to obtain expressions for the variances of N and M. Both species are assumed to conform explicitly to a simple birth and death process over the interval, t to $t + 1$, with parameters calculated from the equations and d_1 and d_2. Using Monte Carlo simulation with normal approximations for N and M, artificial census realizations may be generated for any pair of initial populations (17). The unstable stationary state, with b exterminating c with a probability, say p, and c winning with probability $1 - p$, is obtained when

$$\beta_1\beta_2/\alpha_1\alpha_2 > K_2\beta_1/\alpha_1 K_1 > 1, \quad [2]$$

where $K_i = (\lambda_i - 1)/\alpha_i$. The initial N and M determine the numerical value for p, and alternative outcomes are attributed to random variation with time in N and M. Thus competitive indeterminacy occurs as a result of what May (18) calls demographic stochasticity.

Eqs. 1 can be shown to be similar to the continuous time Volterra-Lotka two-species competition equations (5, 16). Similar unstable stationary states owing to demographic stochasticity can also be obtained using more ecologically realistic, stochastic competition models (19–22).

With a few exceptions the model used here has been successful in the analysis of *Tribolium* competition experiments in the following respects: (*a*) it has correctly identified those competitive systems that should exhibit an unstable stationary state, (*b*) it has given good approximate estimates of p, and (*c*) it has given good approximations for extinction times for the losing species (5, 6). Fig. 1C and D illustrate still another application of the model: projecting zones of competitive determinacy and indeterminacy based upon the initial populations of b and c (23).

Fig. 1 contrasts the predictions based on the genetic founder effect hypothesis (A and B) with those generated using Leslie's model (C and D). Indeterminacy is to be expected in the shaded areas of the graphs; the probability of alternative outcomes is negligible in the unshaded areas.

In Fig. 1A, indeterminacy, as described earlier, results from a genetic founder effect associated with low initial numbers of b and/or c. In Fig. 1B, however, it is postulated that the founder effect influences the properties of the competing c beetles, but not the b beetles; hence, indeterminacy occurs only when the initial numbers of c are low. This assumption gains plausibility from the published reports that c may be more responsive to inbreeding than b (11, 24).

Stochastic competition theory predicts a different configuration for the zone of indeterminacy: a fan-shaped area bordered on the b-axis by a determinate zone with b the winner, and on the c-axis by a second determinate zone with c the winner (Fig. 1C and D). In Fig. 1C, c is the superior competitor, so the zone of indeterminacy is closer to the b-axis; i.e., b requires an initial numerical advantage to overcome c's competitive superiority. In Fig. 1D the two species are more equally matched, so the zone of indeterminacy embraces the diagonal.

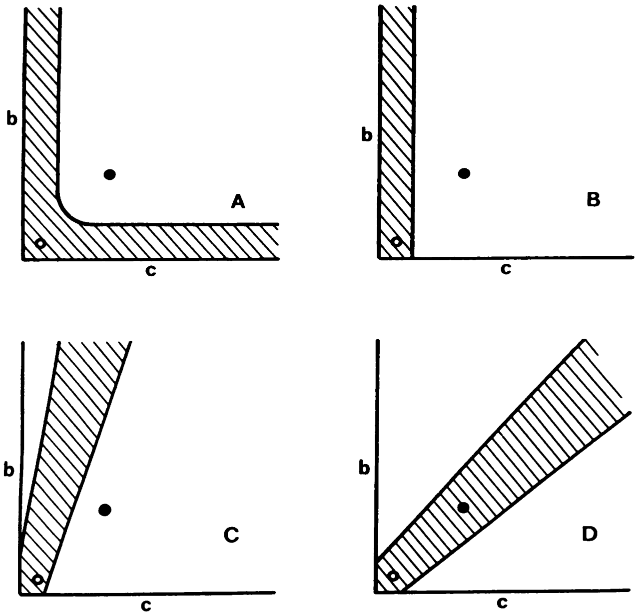

FIG. 1. Contrasting zones of competitive indeterminacy (shaded areas) predicted by the genetic founder effect hypothesis (A and B) and by Leslie's stochastic competition model (C and D). The b- and c-axes represent the initial numbers of adult competitors of each species. See *text* for further explanation. After Mertz (14).

The open circles in Fig. 1 represent low and equal founding numbers of b and c, i.e., two pairs each as in many of Park's experiments. The closed circles represent larger, but still equal, founding populations, i.e., 10 pairs each as in many of Lerner's experiments. In Fig. 1A–C indeterminacy is associated with low initial numbers but disappears as these numbers are increased. Thus the association of indeterminacy with small founder populations does not vindicate the genetic founder effect hypothesis; rather, if the two species are dissimilar in competitive prowess, the same result would be predicted by either model. A more searching test for the alternative models would be a characterization of the zone of indeterminacy. This test is embodied into the experimental design described in the next section.

In this study, realizations generated from Leslie's model were used to draft Fig. 1C and D, and, in connection with the experimental design, to choose the climate, the initial numbers of b and c, and overall replication. We believe that this constitutes an unusually appropriate example of the prospective use of a stochastic population model in the planning of ecological experimentation.

MATERIALS AND METHODS

The experiment was designed with the intent of partitioning competitive indeterminacy into the following components: founder effects (i.e., genetic and, possibly, ecological attributes of the founder beetles), starting densities, and statistical interactions between the founder and density effects. As discussed earlier, c has been reported to be more responsive than b to inbreeding, so only c was subjected to the varying founder treatments listed in the columns of Table 1. Borrowing from the experimental design of Dawson (11), the founder treatments were removed one generation into the prior history of the competing c beetles. Specifically, one generation before being placed into competition, virgin adults were mated in cultures consisting of 1, 2, 4, or 125

Table 1. Design of the experiment (20 replicate cultures for each of the 12 treatments)

Initial population	Number of pairs of founder adults in prior history culture			
	1	2	4	>125 (*P*)
40*b*:10*c* (A)	A1	A2	A4	AP
10*b*:10*c* (B)	B1	B2	B4	BP
10*b*:40*c* (C)	C1	C2	C4	CP

pairs. Progeny from the 125-pair prior history cultures, designated *P* for panmixia, provided the initial *c* beetles for several competition cultures. Otherwise one prior history culture produced all of the initial *c* competitors for only a single population. Thus, the parental beetles in the competition cultures were not inbred, but their F_1 offspring had theoretical inbreeding coefficients of ½ for 1 pair of founders, ¼ for 2 pairs, ⅛ for 4 pairs, and 0 for *P*. The *b* beetles for starting the experiment were husbanded like the *c* beetles in the *P*-treatment.

The rows in Table 1 describe the initial numbers of competing *b* and *c* adults. These were chosen in order to span the theoretical zones of indeterminacy shown in Fig. 1C and D.

The experiment was organized into five completely randomized blocks, each with four replicates for every one of the twelve treatments shown in Table 1. A given block was set up during the first four days of a 6-day interval, and new blocks were started every six days for 24 days. They were also separated spatially, each in its own rack on one of three shelves in the same incubator. Starting orders and positions within blocks were randomized.

The formal possibility of a statistical block effect proved to be of no concern here, since blocks had no confirmable effect on the identity of the winning species. All that remain to be considered are the row, column, and interaction elements for the cells in Table 1. In the event that rows, but not columns, should reveal statistical differences in competitive outcome, this may be construed *prima faciae* as evidence for the unstable state predicted by stochastic competition theory. Contrariwise, a column effect would indicate that a founder effect is present. If both factors are operative, as seems most likely, the relative importance of each may be assessed. The remaining statistical possibility—interaction between rows and columns—is to be expected if both the unstable stationary state and founder effect influence competitive outcome and modify each other's effect.

Park (3), starting with two pairs of the "Chicago" *b* and *c* strains, the same ones used here, reported *c* as the winner in 86% of his replicates in a climate of 29°, 70% relative humidity; at 29°, 30% relative humidity *c*'s winning frequency was reduced to 13%. An intermediate humidity would seem to be called for in order to maximize competitive indeterminacy. However, we had reason to believe that *c* is now more tolerant of dry climates than it used to be, so we chose a climate of 29°, 25% relative humidity.

The populations were husbanded in 8 dram shell vials containing 8 g of the standard flour medium described by Park (1). Censuses and flour renewal occurred at 30-day intervals. Only the adult beetles were diagnosed as to species and counted, but all life stages were returned to the vials.

Fully sclerotized virgin adults, less than 18 days post-eclosion, were used to initiate the competition cultures. Most were 12–18 days post-eclosion, but younger adults had to be used to obtain a full complement of beetles for some of the founder treatments. These age differences and unavoidable density differences in the founder cultures are the reason why ecological effects cannot be discounted as part of the founder treatments.

None of the cultures exhibited symptoms of parasitic disease during the course of the census histories reported here. Single-species control cultures, which were also included in the experiment, are of no interpretative value for the present results and will be discussed in the later report.

RESULTS

To date the 780-day censuses are complete, and the identity of the winning species is known or can be projected accurately for 238 of the 240 competing populations. The results are shown in Table 2. The following conclusions are quite obvious without resorting to statistical analysis: (*a*) Of the 238 decided contests, *c* won in 126 (53%) and *b* in 112 (47%). Thus the experiment achieved a close match between the two competing species. (*b*) Initial population sizes had the greatest effect on competitive outcome. For an initial population of 40*b*:10*c*, *b* won 75 of 80 contests (94%). For the reverse ratio, 10*b*:40*c*, *b*'s winning frequency dropped to 7 of 78 (9%). With even initial populations, 10*b*:10*c*, *b* won in 30 of 80 (38%). (*c*) The initial population effect described in (*b*) above is evident in every column of the table. Regardless of the founder treatment, the populations appear to exhibit an unstable stationary state of the type predicted by stochastic competition theory. (*d*) There is a detectable column effect in the table, but it is much smaller than the row effect. Moving from left to right across the four columns, *b* won respectively in 69%, 47%, 39%, and 33% of the cultures. (*e*) Although the column effect must definitely be the outcome of the founder treatments, it seems just as definitely not to have been caused by the classical genetic founder effect of the type hypothesized by Lerner, Dawson, and their coworkers (7–10). The evidence for this assertion is that competitive indeterminacy is not generally associated with low founder numbers in the prior history cultures. (*f*) There is an interaction between rows and columns in Table 2 such that indeterminacy is associated with large prior history populations of *c* when *b* starts with superior numbers and with small prior history populations of *c* when *c* is given the initial advantage. In the middle row of the table the degree of indeterminacy seems not to be associated with the founder treatments. (*g*) In every row of Table 2 the competitive strength of *c* seems to increase with the size of its prior history population.

Statement (*g*) above raises the specter of a founder effect of a different type than has been hypothesized earlier for competing *Tribolium* populations. It was our intent that the major component of the founder treatments should be genetic, and perhaps this was true. The competitive weakness of the *c* beetles from smaller founder populations could conceivably be related to inbreeding depression. We are, however, skeptical of this interpretation. The overall level of inbreeding depression suggested by all rows of the table would seem to require an implausibly high genetic load. Speculation aside, our results call for further experimental analysis of the genetic and ecological components involved with the founder treatments.

We conclude that the competitive indeterminacy exhibited between *b* and *c* in this experiment is compatible with

Table 2. The winner (or projected winner) in each of the 240 populations after 780 days of census history*

Initial population	Number of pairs in the prior history population of *c*				
	1 pr.	2 prs.	4 prs.	>125 prs. (*P*)	Totals
40*b*:10*c*	20*b*;0*c*	20*b*;0*c*	19*b*;1*c*	16*b*;4*c*	75*b*;5*c*
10*b*:10*c*	16*b*;4*c*	6*b*;14*c*	4*b*;16*c*	4*b*;16*c*	30*b*;50*c*
10*b*:40*c*	5*b*;14*c* (1?)	2*b*;18*c*	0*b*;19*c* (1?)	0*b*;20*c*	7*b*;71*c* (2?)
Totals	41*b*;18*c* (1?)	28*b*;32*c*	23*b*;36*c* (1?)	20*b*;40*c*	112*b*;126*c* (2?)

* Undecided contests are marked with a question mark.

levels of demographic stochasticity predicted by stochastic competition theory. Classical genetic founder effect seems to have played an undetectably small part in determining the experimental outcomes. However, in confirmation of earlier reports (5, 7), the genetic and/or ecological properties of the founder beetles can, and do, affect the identity of the winning species.

PERSPECTIVE

The thrust of this paper has been to examine empirically the phenomenon of "indeterminacy"—a phenomenon sometimes realized when two species of flour beetles (*Tribolium*) engage in competition. As stressed earlier, this is not a new problem. It has occupied the attention of a number of laboratory workers and theoreticians, and, peripherally, the interests of field ecologists and quite a few textbook authors. By indeterminacy, as discussed earlier, we simply mean that it may be a matter of probability which species survives the competitive process as the other drifts to extinction. The opposite case, "determinacy," occurs when, under a given set of conditions, the outcome is not probabilistic, but, rather, one species is the invariable winner. We do not concern ourselves with determinacy in this report, other than to note that it is realized under certain conditions in *Tribolium* experiments.

The demonstration of indeterminacy in populations of *Tribolium* was reported in earlier, adequately replicated, experiments by Park (*vide supra*). In retrospect, it is perhaps informative that Park initially was not motivated by questions of stochastic events but, rather, with the demonstration that certain reasonably realistic ecological factors could affect the survival of one species over another when both were competing. It is also amusing to record, again in retrospect, that Park's growing appreciation of indeterminacy was nurtured by the data themselves; by his association with the statisticians P. H. Leslie, J. Neyman, and M. S. Bartlett, who were interested, each in his own way, in the *Tribolium* experiments; and, finally, that Park had an increasing awareness of the fact that ecological systems are indeed stochastic—that a population's survival, even in the absence of environmental stochasticity, is related intimately to a component which has recently, and meaningfully, been described as "gambling for existence" (25).

As various examples of indeterminacy continued to be published, the question of causation quite properly came more and more to the forefront. The probabilists could erect *models* that predicted alternative survival of competing species, and did so. The experimentalists continued to demonstrate the existence of the phenomenon using a variety of controlled conditions. The whole matter came under challenge when geneticists (especially Lerner and associates) asserted that the essential causation lay in the hereditary composition of those beetles that were used to *initiate* the mixed-species cultures—a most reasonable *a priori* assertion. To their credit, these investigators went beyond the domain of speculation and set up experiments to test their hypothesis. Their findings afforded evidence that the eventual competitive outcomes were indeed related in some way to the genetics of the founding beetles. In consequence, the "founder effect" became an accepted, and lauded, part of competition theory. This concept maximizes genetic stochasticity and minimizes ecological stochasticity as causes of indeterminacy.

The founder effect principle really motivated the experiment that is reported here. Actually, we were not persuaded that the causation of indeterminacy had been revealed either by the experiments in Park's laboratory or by those of Lerner and his associates. This led us to design an experiment which would reexamine the question—this time in accord with accepted stochastic theory. The data reported in the rows of Table 2 clearly show that species survival in the face of competition is much more affected by population densities achieved during competition than by founder effects. We reassert: regardless of the founder treatment, the populations appear to exhibit an unstable stationary state of the type predicted by stochastic competition theory. This is the essential conclusion of our work. We do discern some impact of the founder treatments (columns of Table 2) but, by the very nature of the experimental results, this is clearly different from the founder effect hypothesized by Lerner and his associates.

In sum, we believe on the basis of *empirical* evidence, that we have helped to restore the concept of demographic stochasticity to its rightful status as an integral part of population biology.

We are grateful to Ms. Joan Bieler and Mr. Ralph Cornell for assistance in the laboratory. This research was supported by National Science Foundation Grants GB-24818 and BMS75-18178 (D.B.M. principal investigator).

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