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Consequences of inbreeding for the cowpea seed beetle, *Callosobruchus maculatus* (F.)(Coleoptera: Bruchidae)

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Inbreeding is said to reduce vigour and fitness. It may also determine how a population responds to selection. Local populations of *Callosobruchus maculatus,* the cowpea seed beetle, are established annually from small numbers of founders and the species has been distributed to many parts of the world where isolated populations may have been founded by very small numbers of individuals. After more than 20 generations of inbreeding, inbred lines have been shown to diverge from a common ancestral stock in similar directions with respect of some variables such as developmental speed, but haphazardly in respect of other parameters such as male weight. The respective roles of drift and of selection as effective evolutionary forces in inbred lines are discussed in the light of these results. It is argued that some intraspecific differences in *C. maculatus* may be explained as a product of periodic inbreeding, but that the process does not impair the ability to adapt to local conditions so contributing to the status of the species as a pest of international importance.

ADDITIONAL KEY WORDS:—Inbreeding – *Callosobruchus maculatus* – Coleoptera – Bruchidae – stored products pests – life history evolution – phenotypic variation – fitness components – selection.

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INTRODUCTION

The population biology of stored products insects is unusual since it concerns unusual biological cycles. The habitat of such pests is typically made of discontinuous units, the stores, each of which is unique. With the development of agriculture, the geographical distribution of these habitats has dramatically increased. The spatial and temporal distributions of the resource units impose a requirement for migration and colonization, which are followed by exponential increase in population numbers within the new unit. Human attempts to store and maintain pest-free resources accentuate cycles composed of bottlenecks with reduced numbers of founders and extremely dense populations which quickly develop from them. These cycles have imposed constraints on such species, the effects of which should be discernible in today's' populations. It is our hypothesis that colonization of a novel store by a very small number of founders will produce inbreeding. Successive phases of inbreeding in the past would have purged the genome of most deleterious genes, gradually producing populations with the ability to undergo subsequent inbreeding more easily.

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), the cowpea seed beetle, almost certainly evolved in that part of west Africa presently known as the Sahel. This area is currently occupied by Mali, Niger, Burkina Faso and part of northern Nigeria. From there the species has spread, probably through the transport of legume seeds, to many other parts of the tropical and sub-tropical world (Southgate, 1978). It is likely that new populations have frequently been established from very small numbers of progenitors, since the quantity of infested seeds which survived the journey would be small, and care would be taken to minimise the number of insects initially associated with the cowpeas. As in the case of the closely related *C. chinensis*, because this species is "one of the major pests of stored products *(it)* is likely to be a colonising species for heterogeneously distributed storages or host plants, (*and*) they may often encounter bottlenecks" (Tanaka, 1990). In extreme cases a single mated female may have been the basis of such populations. Thus, *C. maculatus* provides an appropriate model for the laboratory study of inbreeding and its effects on fitness since, as a species, it is likely to have been subjected to and survived similar pressures under natural conditions, and also there is solid basis of bionomic and life-history data already available.

Inbreeding will reduce genetic variance in an isolated population ('line' *sensu* Falconer (1989)) and lead to increased homozygosity among individuals. It can also be expected to produce combinations of characters conferring very different fitnesses and reveal associations between variables which affect the life history of the insect. However "the harmful effects of inbreeding on reproductive rate and general vigour are well known..." (Falconer, 1989). Inbreeding depression may thereby prevent the establishment of new

populations from small numbers of founders. The comparative study of inbred lines derived from a common ancestral population may provide information about the effects of inbreeding and isolation. This study concentrates on phenotypic rather than genotypic consequences because, as Falconer (1989) has explained, measurement of genetic heritability in such lines is difficult and has limited value.

It has to be recognized that in laboratory conditions the establishment and maintenance of inbred lines imposes selection pressures, which may be absent or unimportant in the parental population. However, individual progenitors of new populations, under field conditions, will also be exposed to a set of selective forces different from those experienced by established populations. Selection can counteract inbreeding depression, and "it is possible to get highly inbred lines that are at least as good as their original population in respect of the character selected" (Falconer, 1989). Whilst the selection pressures in a laboratory may differ from those in the field, the principles are applicable in the real world. One might hypothesise that in several inbred lines maintained under the same conditions and derived from a common base population, characters which contribute to enhanced fitness might change in a common direction, whilst those that do not so contribute may change at random.

Different experimental methods (Moller *et al.*, 1989a,b) have shown that many variables affecting the fitness of individual cowpea seed beetles are closely correlated (Moller *et al.*, 1989a,b). In this study, a number of phenotypic variables which could be expected to contribute to fitness have been studied in a number of lines inbred for more than 20 generations, using single pairs of siblings as ancestors of each generation (therefore with an inbreeding coefficient approaching 1), and others recently established from the same parental population. By comparing groups with each other, it was possible to look for cases where variables have changed in a common direction, or have moved haphazardly from the parental condition.

The objectives of this study are to measure the effects of inbreeding on selected life-history characteristics, and question its effects on fitness. We will also look for correlations between characteristics which have not become apparent using other methods, thereby providing an independent assessment of their importance in life-history evolution.

MATERIAL AND METHODS

The life cycle of Callosobruchus maculatus

Female beetles attach their eggs individually to the testa of seeds. The larvae hatch in around 5 days and chew through the seed coat beneath the egg into the seed where they complete their development. Adult eclosion occurs within the seed and usually, at temperatures of about 27°C, beetles emerge some 25–30 days after oviposition. They mate within a short time, and in the presence of a suitable host females normally begin ovipositing within 1 hour. The adult beetles do not require food or water. Oviposition is completed in about 8 days, and adults die about 10-12 days after emergence (Credland, 1987).

Origin and maintenance of culture

The population of *C. maculatus* employed in these studies originated from Campinas, Brazil, in 1975. It has been maintained on cowpea, using a Californian cultivar usually known as black-eye beans, since this date. The population is cultured using a standard procedure (Credland, 1987) which maintains a large number of insects in each generation. New generations are established with approximately 400 to 600 adults transferred onto about 800 ml of fresh cowpeas. The adults are derived from 2 previous subcultures; one in the early stages of adult production, and the other about 7 days older. Subculturing is conducted at an irregular time interval. Thus, there is no constant, direct, selection pressure on the developmental time. This basic population will be hereafter referred to as the stock.

The culture and all the experiments took place in a temperature and humidity controlled room, at 27 ± 1°C and 70 ± 10% r.h. The photoperiod is 14h L:10h D.

Establishment of inbred lines

In September 1990, five inbred lines were isolated from the stock population. Individual virgin females were isolated with a single male in a tube containing approximately 60 cowpea seeds. As each subsequent generation emerged, virgin adults were isolated from each tube and a female was provided with a number of full-sib males in a petri dish. As soon as she was seen to mate, the pair was removed and provided with 50 to 60 seeds on which the female could lay eggs. The generations derived from one of the five ancestral pairs is termed a line. Only one couple was kept for each line at each generation. One of the lines became extinct after 13 generations of inbreeding, due to the failure of the female to lay eggs (and a second one after the conclusion of the experiments reported below, after 28 generations).

Experimental protocol

Previous work on *C. maculatus* has shown the great importance of comparative studies being undertaken under rigidly standardized conditions (Mitchell, 1990). For example, in order to minimize the influence of larval competition (Credland *et al.*, 1986), the insects used in this study were all reared at a density of 1 larva per seed. Similarly, individual females were provided with the same number of standardised seeds since host availability is known to affect realised fecundity (Credland, 1986).

Individuals from the stock population

150 seeds were placed in an uncovered 7 cm diameter dish which was then lowered into a culture jar containing a large adult population and left for 45 min. Preliminary studies showed that more than 600 beetles entered the dish containing these seeds. All but one egg on each seed were destroyed before they could hatch. The seeds were then isolated in 'repli-dishes' (square, 100×100 mm plastic petri dishes subdivided into 25 individual compartments).

This protocol was repeated twice, on 2 consecutive days. Virgin adults which emerged from these seeds on the same day were pooled. They were allowed to mate freely. Couples were isolated during mating, and constituted the G1 adult generation for the stock; the individuals in the culture jar represented the G0 generation. 101 couples were isolated during 5 days of emergences. The number of couples kept each day was approximately proportional to the total number of emergences on that day.

Lines derived by controlled mating of individuals from the stock (CM)

Virgin adults were isolated on emergence from seeds taken from the stock culture. No control had been exercized over the number of larvae in these seeds. These adults, representing the G0 generation, were allowed to mate *ad libitum*. Five mating couples were each isolated on 50 to 60 seeds. Three days later, all but one egg on each seed were destroyed. The same pairs were then offered a further 50 new seeds, on which additional eggs at a density of one egg per seed were collected 3 days later. All the seeds were kept in 'repli-dishes'. The emerging adults represented the G1 generation of the 'controlled mating stock'. Pairs of G1 individuals were constituted as follows: virgin female offspring of each G0 couple were mated individually with their siblings or male progeny of the four other couples, three replicates were prepared for each combination. For each of the five G0 couples, 15 of their female progeny were therefore used in the G1 generation.

The inbred lines

Virgin adults were isolated at emergence from each of the four lines. Five couples (G0) from each inbred line were isolated and offered 50 to 60 seeds. After 3 days, the seeds were removed and all but one egg on each were destroyed. Fifty new seeds were provided and treated in the same way. The seeds were isolated in 'repli-dishes'. The emerging adults represented the G1 generation of the inbred lines and were inbred to their siblings as usual. An average of 16 (from 8 to 18) pairs were established using G1 adults for each of the five G0 couples from each line; there were between 71 and 85 pairs representing each line in the G1 generation.

Treatment

All G1 pairs were offered 40 seeds which had previously been standardized by sieving and visual inspection to minimize variability in size and seed coat appearance (Credland, 1987). The seeds were placed in 7.5×2.5 cm glass tubes, which had previously been lined with emery paper to deter animals from ovipositing anywhere except on the seeds provided. The necks of the tubes were stopped with porous foam bungs as in previous experiments (Credland, 1987).

Variables measured

The G1 generation

Every individual of the G1 was weighed within 24 h of its emergence. The stock and CM adults emerged in large numbers and were grouped to allow mating to occur. Pairs were removed *in copula*. As it was necessary to

regulate the pairs of inbred individuals which mated, each female was provided with only a single male. Individuals did not always mate in the first hour after emergence. In such cases the sexes were separated until the following day, when they were reintroduced to potential mates. This procedure was repeated as necessary until 5 days had elapsed after their emergence. At least 94% of the females and 79% of the males mated within 3 days of emergence and individuals which had failed to mate after 5 days were discarded. Delays in mating of up to 5 days from emergence had no effect on any of the variables measured.

The realized fecundity (Mitchell, 1990) was measured by recording the total number of eggs laid by each female. The number of hatched eggs was determined at least 15 days after mating, but before any progeny had emerged. This interval ensured that every egg was at least 6 days old and thus would have hatched if it was viable. No discrimination could be made between infertile and inviable eggs, and they were not segregated from those in which mortality occurred before the first instar larva penetrated the testa of the seed to which the egg was attached. The term 'egg mortality' is used to include all those eggs which failed to produce a larva which successfully entered the seed.

The number of eggs on each of the 40 seeds provided to each female was recorded. These values were used to calculate the U-index (Messina & Mitchell, 1989) which expresses the uniformity of egg distribution among the seeds. A simple transformation ([1+U]/2) followed by an arcsine-square root transformation was used to normalize the values of U for further analysis and comparison between treatments. Transformed values of U are identified as Ut.

The G2 generation

The number and sex of adults emerging from the 40 seeds was recorded daily for the entire period of emergence. The development time for each G2 beetle represents the amount of time which elapsed from the mating of the G1 adults until the emergence of the G2 adult; it therefore includes the duration of oviposition, embryonic, larval and pupal development, and the period spent within the seed by the adult between its eclosion and its emergence through the testa. Developmental speed was calculated as: 1/[(development time in days)-23], 23 days being the minimum developmental time for this population under the specified conditions. Some adults did not emerge after their eclosion, and died inside the seeds. The number of dead adults within each seed (adult mortality) at the conclusion of the experiment was recorded.

'Larval mortality' is the difference between the number of hatched eggs and the total of the number of adults which emerged summed with the number of dead adults in the seeds. The 'total mortality' is the difference between the total number of eggs and the total number of adults which emerged.

Statistical analysis

When not otherwise stated, minimum significance in all statistical tests was set at the usual probability of 95%. Homogeneity of variances was assessed

with Bartlett-Box *F*-tests for each variable. For the one normally distributed variable with homogeneous variances, a one-way analysis of variance (ANOVA) was conducted, followed by a Student-Newman-Keuls (SNK) *a posteriori* multiple range test. In the case of data which were not normally distributed, appropriate transformations were employed to normalize the distribution but, where this procedure was inappropriate or impossible, or when variances were heterogeneous, a Kruskal-Wallis non-parametric ANOVA on ranks was used. It was followed by a non-parametric multiple comparison test (Zar, 1984).

Spearman's rank correlation coefficients were used throughout. The complete set of variables was subjected to a Canonical Multiple Discriminant Analysis, in order to test the variables' ability to characterize the individual groups and to measure their relative importance in doing so.

RESULTS

Except where specified, only pairs which had more than 20 offspring were used in the statistical analysis. On this basis, 28 couples were excluded, all from inbred lines (8, 16, 1 and 3 respectively, for the lines A, B, C and D). Females which lay a very small number of eggs represent a discrete subset of the population, and not simply one tail of the normal distribution (Mitchell, Credland, Toquenaga & Fujii, in prep.).

Descriptive statistics

Emergence weights for each sex, the total number of eggs laid by each female and their distribution among the host seeds were determined for each G1 group of the ancestral stock, controlled mating and inbred lines. For each individual female of the G1 generation the mean and variance of the developmental speed of her offspring were calculated, and the total number of emergences, sex ratio and percentage mortalities in total and at each stage of development are given for the G2 generation. All these data are presented in Table 1.

Multiple discriminant analysis

The aim of this analysis was to discover the extent to which the nine lines and stock population identified in Table 1 can be separated, using combinations of the variables. The variance of developmental speed and the total mortality were excluded from the analysis since both are data which are already represented, leaving the 10 remaining parameters shown in Table 1.

The nine possible discriminant functions which explain the percentages of variance in the grouping variable (West, 1991) have been computed. Table 2 gives the percentages of variance, in the grouping variable, explained by each of the first five functions. It can be seen that the first four functions explain 99.1% of the variance which is why graphical representation (Figs 1 and 2) is restricted to them.

The contribution of each function to the classification or segregation of

TABLE 1. Mean values (±SE) of the 12 variables measured in each line and population examined. Weights of females and males at emergence; Fecundity = total number of eggs laid; Ut = U index of uniformity, normally transformed (see text for details); D.S. = developmental speed; Emerg. = total number of adult offspring which emerge; %Egg, %Larval, %Adult, %Total Mortalities = percentages of embryonic, larval, adults within the seeds, and cumulative (between oviposition and adult emergence from the seeds) mortality among the offspring

Lines	n	Female's Weight (mg)	Male's Weight (mg)	Fecundity	Ut	D.S. mean	D.S. variance	Emerg.	Sex-Ratio	%Egg Mortality	%Larval Mortality	%Adult Mortality	%Total Mortality
							Inbred lines						
A	77	6.02	4.16	113.34	0.79	9.78	8.22	90.04	0.48	9.06	11.89	0.52	20.28
		±0.060	±0.034	±1.666	±0.015	±0.077	±0.167	±1.471	±0.006	±0.556	±0.641	±0.077	±0.909
B	69	5.91	4.57	95.99	0.89	7.21	2.70	58.39	0.46	25.37	15.56	2.75	39.50
		±0.070	±0.057	±1.609	±0.013	±0.053	±0.075	±1.964	±0.009	±1.533	±0.717	±0.223	±1.599
C	74	5.25	3.20	90.61	0.84	11.15	9.88	73.46	0.49	11.67	7.06	0.68	18.58
		±0.072	±0.031	±1.308	±0.014	±0.071	±0.310	±1.178	±0.007	±0.573	±0.926	±0.095	±0.990
D	67	5.87	4.85	104.55	0.98	11.35	10.08	66.09	0.50	20.89	21.26	0.15	37.43
		±0.078	±0.068	±2.116	±0.009	±0.137	±0.368	±2.708	±0.009	±1.646	±1.492	±0.043	±1.958
							Stock						
	101	6.73	4.15	136.80	0.89	20.85	114.47	118.50	0.52	3.21	9.94	0.35	13.16
		±0.067	±0.060	±1.268	±0.010	±0.267	±6.197	±1.196	±0.005	±0.466	±0.518	±0.059	±0.680
							Stock CM						
CM1	15	6.51	4.33	138.13	0.81	20.57	107.93	114.87	0.50	2.11	14.60	0.43	16.77
		±0.113	±0.102	±2.870	±0.019	±0.541	±14.458	±4.429	±0.012	±0.409	±2.755	±0.166	±2.844
CM2	15	6.72	4.03	132.60	0.92	22.88	132.39	107.47	0.50	6.16	12.50	0.74	18.95
		±0.131	±0.099	±3.034	±0.021	±0.804	±15.779	±3.566	±0.013	±2.622	±1.461	±0.221	±1.924
CM3	16	7.06	4.33	134.56	0.94	22.46	147.01	112.50	0.53	4.22	12.35	0.06	16.14
		±0.180	±0.126	±2.978	±0.018	±0.910	±26.397	±3.462	±0.009	±2.010	±1.750	±0.055	±2.419
CM4	15	7.10	4.38	133.20	0.92	21.25	113.26	100.33	0.50	0.14	16.24	0.10	24.31
		±0.185	±0.156	±3.184	±0.020	±0.660	±12.817	±6.853	±0.013	±4.547	±3.130	±0.066	±4.984
CM5	15	6.94	4.14	146.67	0.89	18.85	76.47	123.07	0.52	1.99	14.43	0.13	16.21
		±0.195	±0.109	±3.293	±0.022	±0.556	±9.688	±5.259	±0.014	±0.373	±2.827	±0.070	±2.884

TABLE 2. Eigenvalues and percentages of variance explained by the first five functions of the discriminant analysis

	Eigenvalues	Percentage	Cumulative percentage
Function 1	14.46	82.0%	82.0%
Function 2	1.71	9.7%	91.7%
Function 3	0.72	4.1%	95.8%
Function 4	0.58	3.3%	99.1%
Function 5	0.08	0.5%	99.6%
Function 6	0.04	0.3%	99.8%

individual values was tested by calculation of Wilks' lambda and a conventional chi-squared test. As is shown in Table 3, the chi-squared values are no longer significant after the sixth function has been added. Thus, the functions 7, 8 and 9 are redundant.

In Figures 1 and 2 each individual pair of the G1 is represented by a letter, identifying its origin. In Figure 1 the first function, which is represented on the abscissa, separates two clouds: the inbred lines constitute one cloud, on the left, and the other cloud comprises the remaining individuals. There is no overlap between the two clouds. Within the cloud incorporating the inbred lines, the couples from line B (letter b) are separated from the others (letters d, a and c). The second function represented on the ordinate provides additional separation of the inbred lines. Line D (d) is separated from lines A (a) and C (c) and has moved in the opposite direction from the ancestral population. There is no obvious separation among the individuals of different origins which make up this other cloud.

Figure 2 provides less information to enable lines to be separated than that displayed in Figure 1. Individuals from the ancestral stock (e) are centrally located and not separated by function 3 from either of the inbred lines A or C. However, most individuals of the inbred line B have higher values and are to the right of E whilst most individuals of line D are separated with lower values than all the remainder. Lines derived from the stock by controlled mating (f to j) have not diverged to any great extent.

TABLE 3. The contribution of the first six functions to the separation of the populations studied. The 6th and further functions do not make a significant contribution to further separation

After function:	Wilks' lambda	χ^2	Degrees of Freedom	Significance
0	0.007	2218.01	90	0.0000
1	0.11	977.64	72	0.0000
2	0.31	525.11	56	0.0000
3	0.54	278.40	42	0.0000
4	0.85	70.96	30	0.0000
5	0.93	33.04	20	0.03
6	0.97	12.12	12	0.44

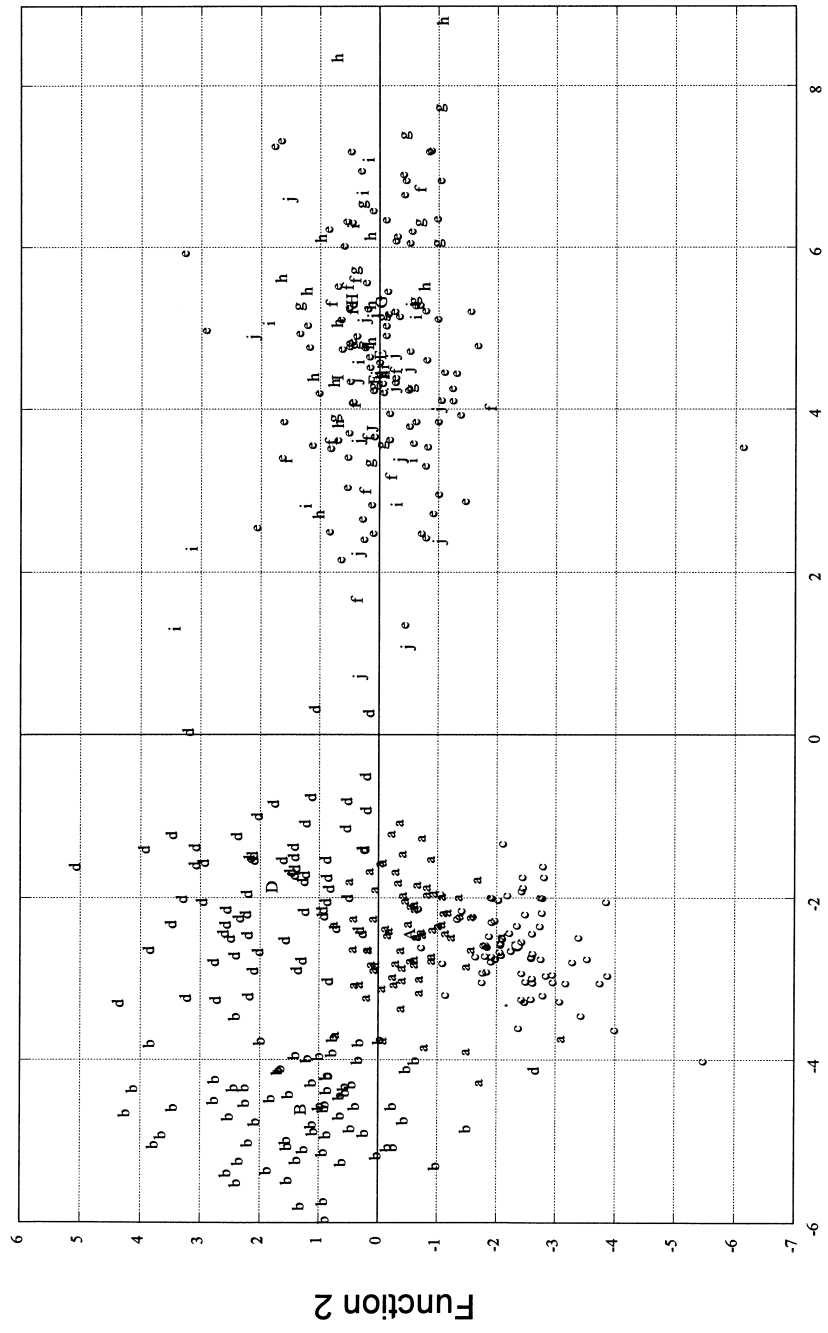

Figure 1. Dispositions of functions 1 and 2 obtained from a canonical discriminant analysis using 10 parameters measured in 10 populations, comprising 4 inbred lines (a, b, c, d), 5 lines produced by controlled mating (f to j) and the ancestral stock population of all the lines (e). Centroids are indicated by capital letters.

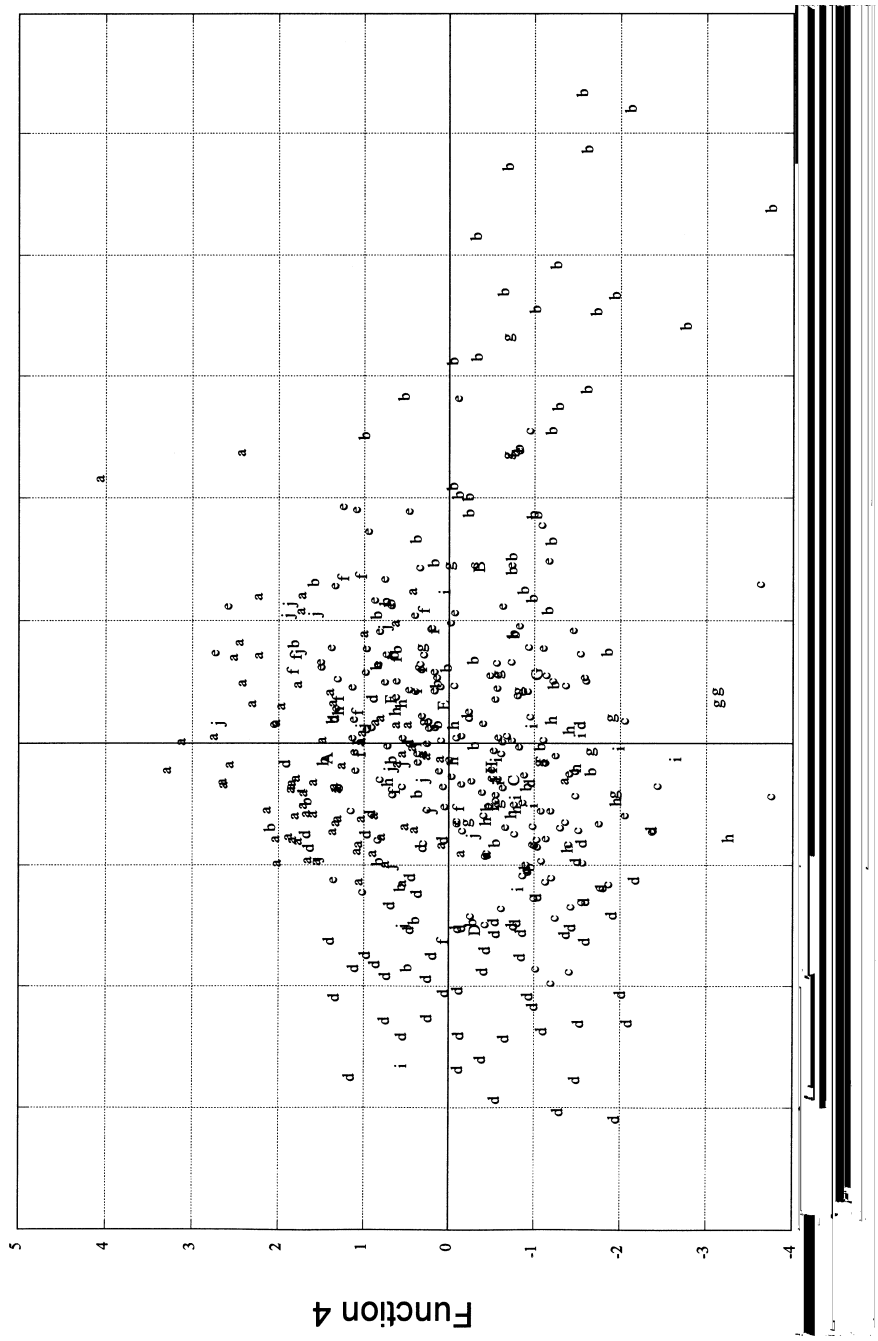

Figure 2. Dispositions of functions 3 and 4 obtained from a canonical discriminant analysis using 10 parameters measured in 10 populations, comprising 4 inbred lines (a, b, c, d), 5 lines produced by controlled mating (f to j) and the ancestral stock population of all the lines (e). Centroids are indicated by capital letters.

TABLE 4. The three variables having the highest correlations with the first six functions

Function 1	Function 2	Function 3	Function 4	Function 5	Function 6
D.S. mean (0.83)	Males' Weight (0.80)	% Adult Mortality (0.85)	Emerg. (0.64)	Females' Weight (−0.44)	Females' Weight (0.68)
Emerg. (0.36)	% Larval Mortality (0.38)	Ut (−0.22)	Fecundity (0.61)	Emerg. (0.30)	Ut (0.44)
Fecundity (0.34)	% Egg Mortality (0.34)	Females' Weight (0.22)	Ut (−0.44)	Ut (0.30)	Emerg. (0.33)

Function 4 provides minimal information but does suggest a separation between inbred line A and all the remainder.

Correlations between individual variables and the first six functions were calculated (Table 4). Developmental speed, the total number of adult progeny which emerged and the number of eggs are most closely correlated with function 1. The weight of newly emerged males and mortality during the different stages of the life cycle are highly correlated with the functions 2 and 3 whilst, for example, female weight and sex ratio are correlated with less important functions, or only loosely with those of primary importance.

As a final step, the individual pairs of the G1 generation and their offspring were tested for conformity with 10 predicted groups. These groups were based upon the calculated mean values of the discriminant functions and each pair was placed into the group to whose mean it was closest. The allocation is largely successful, and 71.6% of the pairs were placed in their correct groups. Actual and predicted groups, and their overlaps both in absolute values and percentages are given in Table 5.

Analysis of variance

Having shown that the lines could be separated by discriminant analysis, one-way ANOVAs followed by multiple range tests were performed, in order to determine which lines differed in respect of the variables studied.

The data for neither mean developmental speed nor the mean number of adults emerging were normally distributed and were therefore analysed by Kruskal-Wallis tests. In both cases there were very highly significant differences among the lines ($\chi^2_9 = 433.3$ and 331.5 respectively, $P < 0.001$). Developmental speed was greater in the stock population and lines recently derived from it than in the inbred lines (Table 1). A non-parametric multiple range test revealed significant differences between each inbred line and the stock population. Furthermore, the variance in developmental speed was much greater in these lines than those which have been inbred. Similarly, although there were considerable differences among the inbred lines, the number of adult progeny was lower in all of them than in the ancestral population.

The realized fecundity of females is a normally distributed parameter if all data are included, but variances were not homogeneous (Bartlett's $F = 12.0$, $P < 0.001$). A Kruskal-Wallis analysis showed that there were significant

TABLE 5. Classification of the populations according to the discriminant functions. Actual numbers and percentages attributed to each group are given. The predicted groups (1 to 10) were generated by the analysis and correspond, broadly speaking, with the 10 populations listed in the first column. When no value is given, it was equal to zero

Actual/Predicted	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Inbred line A	94.8%	1.3%	3.9%	.	.	.	.	.	.	.
	73	1	3							
Inbred line B	7.2%	84.1%	.	8.7%	.	.	.	.	.	.
	5	58		6						
Inbred line C	1.4%	.	98.6%	.	.	.	.	.	.	.
	1		73							
Inbred line D	7.5%	.	1.5%	91.0%	.	.	.	.	.	.
	5		1	61						
Stock	.	.	.	.	29.7%	11.9%	20.8%	13.9%	3%	20.8%
					30	12	21	14	3	21
Stock-CM	.	.	.	.	13.3%	66.7%	6.7%	.	.	13.3%
Family 1					2	10	1			2
Stock-CM	.	.	.	.	20%	6.7%	46.7%	13.3%	13.3%	.
Family 2					3	1	7	2	2	
Stock-CM	.	.	.	.	25%	6.3%	18.8%	37.5%	6.3%	6.3%
Family 3					4	1	3	6	1	1
Stock-CM	.	.	.	6.7%	6.7%	.	20%	40%	20%	6.7%
Family 4				1	1		3	6	3	1
Stock-CM	.	.	.	.	13.3%	.	.	6.7%	6.7%	73.3%
Family 5					2			1	1	11

differences among the lines tested ($\chi^2_9 = 327.35$, $P < 0.001$). Inbred lines C, B and D laid significantly fewer eggs than the stock population and lines recently derived from it.

Weights of males also showed very highly significant variation among the lines, with heterogeneous variances (Bartlett's $F = 8.8$, $P < 0.001$), ($\chi^2_9 = 274.04$, $P < 0.001$). Line C males were the lightest of all, whereas inbred line A did not differ from the stock population. Males of inbred lines B and D were heaviest of all and differed significantly from the parental population.

Mortality at each stage of the life cycle, including the death of adults within the seed prior to their emergence, differed among the lines examined (Table 1). For example, the egg mortality in the inbred lines was much greater than that among all the remainder. The total mortality within each line was expressed as a log transformed percentage of the number of eggs laid but variances were not homogeneous (Bartlett's $F = 5.4$, $P < 0.001$). There were again significant differences among the lines ($\chi^2_9 = 213.16$, $P < 0.001$); the lowest mortality was displayed in the ancestral population and the five lines established from it by controlled mating. Mortality in these lines did not differ significantly from inbred line C, but was lower than in line A. Inbred lines B and D suffered heavier mortality than all the remainder.

Variances were not homogeneous (Bartlett's $F = 3.7$, $P < 0.001$) among transformed values of U, the index of uniformity of eggs distribution, in each line. It varied among the lines examined ($\chi^2_9 = 121.86$, $P < 0.001$), but in an irregular pattern (Table 1). Although there was little variation within each line, especially in the ancestral stock population, the mean values differed considerably. Furthermore, there was no discernible pattern among

TABLE 6. Variances of the normally distributed variables in the 4 inbred lines and the original stock. The − and + signs indicate significant differences from the stock values. Every value was based on a sample size of at least 67

	Females' weight		Males' weight		Fecundity		Ut		Arcsine-$\sqrt{}$ (Sex-Ratio)		Log (% Total Mortality)	
A	0.2740	−	0.0881	−	606.68	+	0.0177	+	0.0028	NS	0.0288	−
B	0.3409	NS	0.2257	−	682.71	+	0.0112	NS	0.0054	+	0.0216	−
C	0.3784	NS	0.0704	−	132.99	NS	0.0139	NS	0.0037	NS	0.0308	−
D	0.4112	NS	0.3131	NS	337.53	+	0.0060	−	0.0052	+	0.0503	NS
Stock	0.4566		0.3587		162.28		0.0094		0.0029		0.0573	

the inbred lines; line D, uniquely, distributed its eggs more uniformly than the stock population and line A distributed its eggs more randomly than the parental stock.

Female weights at emergence were normally distributed. There were differences between the lines ($F_{9,454} = 43.0$, $P < 0.001$) and the SNK multiple range test revealed three homogeneous and distinct subgroups among the lines. Inbred line C was significantly lighter than lines D, B and A, which were not different from each other, but were all lighter than the remaining lines investigated (Table 1). Variances were homogeneous (Bartlett's $F = 1.4$, $P \geq 0.05$).

Arcsine square root transformations of the sex ratio among the adults of the G2 generation were normally distributed but variances were not homogeneous (Bartlett's $F = 2.7$, $P < 0.01$). A Kruskal-Wallis ANOVA revealed differences among the lines ($\chi^2_9 = 48.40$, $P < 0.001$) but this could be attributed to the inbred line B (Table 1) in which there was a shift towards excess male production. The remaining groups were homogeneous.

Comparisons of the variances

Variances of normally distributed variables in each line were calculated. They and the significance of the ratio of each value in the inbred lines to the corresponding value of the stock population are presented in Table 6. Variances determined for the CM families did not differ significantly from those of the original stock, perhaps due to the smaller sample sizes.

Correlations

If the data for the stock population and the inbred lines are treated separately, the effect of inbreeding becomes apparent. The upper right part of Table 7, representing correlations among variables for the stock population reveals that relatively few are significant, whereas the lower left portion, representing the corresponding information for the inbred lines reveals a larger number of significant correlations.

TABLE 7. Significance and sign of Spearman's rank correlations between variables for the individuals of the stock population (upper right) and the individuals of the 4 inbred lines (lower left)

	WF	WM	Dsm	Emer	Eggs	Ut	SR	% EM	% LM	% AM	% TM	Stock
WF		NS	NS	+ **	+ ***	NS	NS	NS	+ **	NS	+ **	WF
WM	+ ***		NS	NS	NS	NS	NS	NS	NS	NS	NS	WM
DSm	− ***	− ***		− ***	NS	NS	+ *	NS	+ ***	NS	+ ***	DSm
Emer	+ **	− **	+ **		+ ***	NS	NS	NS	− ***	NS	− ***	Emer
Eggs	+ ***	+ ***	NS	+ ***		NS	+ *	+ *	+ *	NS	+ **	Eggs
Ut	NS	+ **	+ **	− ***	NS		NS	NS	NS	NS	NS	Ut
SR	NS	NS	+ ***	NS	NS	NS		NS	NS	NS	NS	SR
%EM	NS	+ ***	− ***	− *	− ***	+ ***	NS		NS	NS	+ ***	%EM
%LM	+ ***	+ ***	− ***	− *	+ ***	+ **	NS	+ ***		NS	+ ***	%LM
%AM	NS	NS	− ***	− ***	− **	NS	− *	+ ***	NS		+ *	%AM
%TM	+ ***	+ ***	− ***	− ***	NS	+ ***	NS	+ ***	+ ***	+ ***		%TM
inbred lines	WF	WM	Dsm	Emer	Eggs	UT	SR	% EM	% LM	% AM	% TM	

***: $P < 0.001$ **: $P < 0.01$ *: $P < 0.05$ NS: $P \geqslant 0.05$.

Key to abbreviations: WF, females' weight; WM, males'; DSm, mean developmental speed; Emer, total number of adult offspring which emerge; Ut, transformed index of uniformity (see text for details); SR, sex-ratio; %EM, %LM, %AM, %TM, percentages of egg, larval, adults within the seeds, and cumulative (between oviposition and adult emergence from the seeds) mortality among the offspring.

DISCUSSION

Changes revealed by inbreeding

Twelve variables potentially affecting the fitness of *C. maculatus* have been measured in a laboratory stock population, a number of inbred lines and pairs derived from the stock by controlled mating. Many of the parameters measured have changed radically in the course of 22 generations of inbreeding (Table 1). The multiple discriminant analysis provided an unbiased quantitative description of the distinctions between each line and the population from which they were derived. The classification using discriminant functions (Table 5) provided strong evidence that the lines diverged rapidly and ultimately to a considerable extent (Fig. 1). In two of the five lines very recently established by controlled mating, approximately 70% of individuals were correctly classified. In the case of the inbred lines, at least 84% of individuals were allocated to their correct group.

Graphical representation (Figs 1, 2) demonstrated that all the inbred lines have diverged in a substantially similar direction from the base population.

Function 1, of which mean developmental speed is the most important component, provides the major part of this separation from the ancestral stock. As Moller *et al.* (1989a) have shown that developmental speed is correlated with both female adult weight and fecundity in an outbred population, it is not surprising that these latter two parameters provided little additional discrimination among lines (Table 4). The same table shows that male weight provided the next most important discriminating function (Fig. 1, function 2) and that the third function was substantially dependent on the pre-emergence mortality of adults; neither of these characters was considered by Moller *et al.* (1989a). Table 2 showed that 96% of the grouping could be explained primarily by these three functions and hence largely by the three variables associated with them. One might construct two hypotheses on the basis of this part of the study: (i) That reduction in developmental speed is a consequence of inbreeding depression. The character is probably determined polygenically and, furthermore, a decrease in the fitness of one or more pleiotropic genes may be reflected most readily in developmental speed. (ii) That developmental speed may be the driving force behind the phenotypic variation observed. The importance of developmental speed demonstrated in Table 4 might suggest that it determines the course of evolutionary change.

It was anticipated that genetic variance and heterozygosity would decrease with diminished population size and inbreeding although inbred individuals show greater sensitivity to environmental sources of variation (Falconer, 1989). However, there is an ambiguous association between genetic and environmental variance, so there are dangers in ascribing changes in phenotypic variance to a diminution in genetic variance. Table 6 indicates that changes in phenotypic variance have not always taken the same direction as a consequence of inbreeding. For example, in comparison with those of the stock population the variances of egg numbers and the U indices change in relative size among the inbred lines. As some inbred lines are more variable phenotypically than the non-inbred stock, there appears to have been an increase in the environmental variance of fecundity, sex-ratio and egg distribution in some cases. Considering the individual variables which have been measured, the total variance of each parameter within each inbred line is smaller than the variances among the lines, as would be predicted by Falconer (1989). Because environmental variance "interferes with the experimental study of the changes in variance,... we cannot put much reliance on the theoretical expectations concerning the genetic variance being manifest in the observed phenotypic variance" (Falconer, 1989). In the absence of an acceptable theoretical basis for so doing, no attempt has been made to mathematically partition the variance which has been observed. Studies which have attempted to partition variance surrounding some of the characters examined in this study, using conventional sib/half sib protocols, have been instructive (Moller *et al.*, 1989a; Messina, 1993) but practical constraints rendered this design inappropriate as part of this investigation involving entire families and numerous variables. Furthermore, Ofuya (1995) has shown that repeated mating by a male quickly leads to a reduction in fertile egg production by its mates. Thus the experimental design itself leads to variability among the results.

What is the cause of these changes?

The fundamental issue that remains is to consider whether the changes observed in the inbred lines can be attributed to inbreeding depression, to drift and/or to selection. Most characteristics of inbred lines appear to confer lower fitness, as is usually predicted (Falconer, 1989; Lopez Bueno *et al.*, 1993; Tanaka, 1993).

Fitness may be defined as "the *proportionate* contribution of individuals to future generations" (Begon *et al.*, 1990). Recent works on bruchids used measurement of fecundity and longevity (Tanaka, 1993), developmental rate, size and weight (Fox, 1993) as parameters of fitness. Fitness "... is a relative not an absolute term" (Begon *et al.*, 1990) and can be observed in competition experiments (Lopez Bueno *et al.*, 1993) but, by definition, fitness exists within a specific environment. Thus, care must be taken in making direct comparisons between the fitness of the inbred lines and the stock population from which they were derived.

Lower fecundity, higher total mortality, slower developmental speed and even a slightly skewed sex ratio confer lower fitness and may result from inbreeding. Furthermore, the frequency of individual females producing fewer than 20 adult progeny may be another manifestation of declining fitness. These traits moved in the same direction for all the inbred lines but deviations in other parameters occurred in both directions (Table 1).

Separate lines derived from a common base population are often found to differ in the changes that they show, as would be expected as a consequence of random drift of gene frequencies (Falconer, 1989). However, the inbred lines employed in this study were subjected to selection pressures by the regime under which they were maintained, whilst different pressures may be more important in the ancestral population. For example, in the ancestral population, all the ovipositing females competed for access to seeds on which to oviposit, and previous work (Bellows, 1982; Thanthianga & Mitchell, 1987; Toquenaga & Fujii, 1991) has shown that the first larva to penetrate a seed has a competitive advantage over others which may enter subsequently. Thus, in the ancestral population, there may be selection for rapid development, so that females have access to seeds on which to lay before they are similarly used by other females. The importance of developmental rate may, under such circumstances, be critical, and have evolved at the expense of larger somatic size and total fecundity. One might speculate that this explains the pre-eminent importance of developmental rate, which was revealed by the multiple discriminant analysis. However, in a newly established store containing many seeds but few beetles, high fecundity may be the attribute which most enhances fitness. Rapid development or the production of large numbers of eggs may not confer a selective advantage on females of the inbred lines, since hatching larvae would only compete with their siblings in the restricted number of seeds available; the siblings would, of course, exhibit minimal allelic diversity. The decline in the values of both parameters in the inbred lines could be considered as inbreeding depression, as hypothesised by Tanaka (1990) on *C. chinensis* for the diminished fecundity of inbred lines but it could also have resulted from release of selection pressures which were effective in the stock population.

In Figures 1 and 2, if the individual lines move in the same direction away from the base population, theory would dictate that (i) selection may be responsible, or (ii) if the position of the stock population represents a peak in the adaptive landscape representing the conditions in which they are cultured, then change may represent a consistent reduction in relative fitness (in the context of the stock population) among the inbred lines. The movement in a similar direction is probably due to a pleiotropic effect of alleles affecting developmental speed, similar to that proposed by Tanaka (1993) for *C. chinensis*.

If the groups had moved in different directions, then drift rather than selection may have been responsible. Function 1, which is primarily dependent upon the mean developmental speed, produced a uniform movement of the inbred lines, although those which had only recently been established showed no such consistency. This could indicate that selection consistently produced or permitted a reduction in developmental rate, compatible with the theory that females could 'afford' to develop more slowly, in the absence of competition, as postulated above. Alternatively, this could show that a slower developmental speed is indicative of lower fitness.

Functions 2, 3 and 4 do not all separate the inbred lines by moving them in the same direction, away from the base population. This could be explained as drift of characters which do not come under significant selective pressure, under the regime in which the inbred lines were maintained. Reference to Table 4, where the parameters making major contribution to these functions are listed, shows that two characters, male weight and percentage mortality of adults within the seeds, are of primary importance in the calculation of the functions. Neither has an obvious selective advantage or disadvantage in the inbred lines' culture regime where only a single pair of viable, fertile offspring are required to produce the next generation. Fecundity, which is the primary factor determining function 4, is a less important parameter under conditions lacking competition between females than in the base population, for the same reason as explained in the context of pre-emergence mortality.

In the stock population, relatively few correlations between the variables were discovered beyond those already known. However, correlations among the same variables measured in the inbred lines are very much more common. Many phenotypic characteristics are correlated, perhaps reflecting the decline in genetic variance and pleiotropic effects among the remaining alleles.

The importance of inbreeding in the biology of C. maculatus

Inbreeding of *C. maculatus*, to a coefficient of inbreeding of almost 1, does not invariably lead to extinction. Of five inbred lines one died out after 13 generations of inbreeding and another after 28; the remainder are still breeding after 58 generations. This is consistent with Tanaka's results (1993) in which he reported that in *C. chinensis* (L.) only three lines were lost out of 25 in the course of 12 generations. Within such inbred lines the capacity

for adaptation to novel environmental conditions apparently remains if the potential genetic options set (Sibly & Antonovics, 1992) is not too drastically diminished. It has been shown that the value of certain characteristics affecting fitness may decline, but other characteristics of the options set which influence life history evolution have moved in different directions with the release or change of selection pressure. If the change in the character sets of the inbred lines depicted in Figure 1 is adaptive, then inbreeding has not impeded a response to selection. However the changes observed in egg distribution or male weight which do not apparently affect fitness in the context of these experiments, exhibit the drift theoretically expected of inbred lines. Thus in *C. maculatus*, inbreeding does not appear to prevent adaptation to new environment or impose fitness costs which lead to extinction. Isolated lines could readily develop into new populations which diverge significantly from the parental or ancestral stock. There is therefore a good explanation in these observations for the phenotypic variation among geographical biotypes of *C. maculatus* which have been reported previously (Credland, 1990). The species probably lost the more deleterious elements from its genome through phases of bottlenecks followed by inbreeding. Tanaka (1990) has proposed that *C. chinensis*, which shares the same kind of life history, may have undergone a similar process.

The capacity of *C. maculatus* to survive phases of inbreeding successfully has probably been a critical element enabling it to become a cosmopolitan pest. Evidence for its natural occurrence was provided by Messina (1991), who observed heterosis in crosses between two populations. Another cowpea beetle, *Bruchidius atrolineatus* (Pic), is sympatric with *C. maculatus* in many parts of Africa, but has never become established outside that continent. Climatic conditions are undoubtedly important in restricting its movement (Monge *et al.*, 1989). However, Lenga (1991) and Tran (unpubl. obs.) have been unable to maintain inbred lines of *B. atrolineatus* for more than five generations. This inability to survive inbreeding may have prevented the distribution of the species, as small founder populations consistently failed to survive. *B. atrolineatus* is frequently the dominant pest for the first month or two of cowpea storage, but after only one or two generations, adults in reproductive diapause are produced. In the very same stores, populations of *C. maculatus* continue reproducing for seven or more generations during the storage period. Since the initial levels of infestation by both species are invariably small, being dependent on eggs laid on pods or seeds of the cowpea in the fields, one might speculate that the incidence of reproductive diapause provides a mechanism to circumvent the adverse effects of protracted inbreeding and represents an adaptation to life in patchily distributed food sources.

Sibly & Antonovics (1992) have suggested that inbreeding forces the "genetic options set" to move into a different dimension of the multi-dimensional "potential genetic options set". The results presented above indicate that inbreeding similarly may force phenotypic options to move into a different dimension, but also permits selection to remain an important force in evolution. Inbreeding thus creates new genotypic environments which are excellent bases for new evolution and adaptation to novel environments, such as changes in host, climate, type and rhythm of human activities, etc...

The ability to survive inbreeding would then not only allow populations of *C. maculatus* to go through bottlenecks in small stores, but could also increase the adaptive potential of these populations and, regrettably, their importance as pests.

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