GENETIC ANALYSIS OF A POPULATION OF TRIBOLIUM

I. CORN OIL SENSITIVITY AND SELECTION RESPONSE*

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1. INTRODUCTION

In most quantitative genetic studies the hypothesis of polygenic inheritance and random environmental effects is plausible. However, in one of several populations of *Tribolium castaneum* developed by Yamada and Bell (1963) to study the effectiveness of selection for growth in different environments this hypothesis was not satisfactory. The selection response in this particular population suggested a peculiar genotype by environment interaction. Although the situation was confirmed by biometrical analyses, these techniques did not suffice to answer questions concerning the underlying genetic mechanism on which selection had acted. It was our objective to identify the genetic basis of this peculiar response to selection and consequently more precisely define genotypes and genotypic differences. A preliminary report of this work was given by Costantino *et al.* (1966).

2. MATERIALS AND METHODS

The genetic material for this study was a selected population (GS_2) of the flour beetle *Tribolium castaneum* (Yamada and Bell, 1963), and its base population, Purdue Foundation (+). The mating and selection procedure was as follows: During the first 16 generations of selection the population consisted of 40 single pair matings. Each pair was placed in " standard " medium (95 per cent. wheat flour, 5 per cent. yeast) for a 48-hour egg collection followed by four consecutive 24-hour egg collection intervals made alternately in the " good " and " poor " media of table 1. (For further details regarding these diets see Hardin, Rogler and Bell, 1967.) The criterion for selection was small 13-day larvæ as determined by weighing two random groups of five progeny, one from each of the two intervals the mated pair was on the " good " medium. The eight families that produced the smallest 13-day larvæ were selected. Five males and five females from each selected family were taken from those sibs grown on the " standard " medium (to avoid confounding possible genetic gain with environmental effects) and were mated at random with the exception of full-sib matings. Except for a reduction in selection intensity to 10/30 families, the above procedures were continued for another 14 generations or a total of 30 generations of selection. Parents of generation 16, and 26 through 30 (stored at 18·5° C. and 60 per cent. relative humidity) were available for the present investigation.

An unselected control population consisting of 20 single pair matings (originating from the same base as GS_2) was maintained for the first 16 generations of the selection experiment. Offspring were cultured similarly to the selected population each

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generation on the " good " and " poor " media and were weighed as 13-day larvæ to monitor environmental time trends.

The culturing environment for all the studies to be discussed consisted of a climate controlled chamber maintained at 33° C. and 70 per cent. relative humidity. Egg collection was achieved by placing mated adult beetles in media as specified for a particular experiment and then removing the adults after a defined interval by passing the contents of the mating creamer through a coarse screen.

TABLE 1

Diets used to investigate the peculiar growth of the GS_2 *population observed in fig. 1*

Component	Diet (per cent. weight)					
	Good	Poor	1	2	3	4
Corn meal	58	85	80	75	80	78
Soybean oil meal	17	12	12	12	17	12
Vitamin premix*	10	3	3	3	3	10
Dried brewer's yeast	10	0	0	10	0	0
Corn oil	5	0	5	0	0	0

* The vitamin premix consisted of pyridoxine, 0·5 g.; thiamine, 1 g.; folic acid, 0·1 g.; biotin, 0·01 g.; inositol, 10 g.; riboflavin, 0·5 g.; niacin, 2 g.; calcium pantothenate, 1 g.; vitamin B_{12}, 0·7 g.; choline, 20 g.; ascorbic acid, 0·8 g.; para amino benzoic acid, 1·5 g.; corn meal, 961·89 g.

3. RESULTS

The direct response of the GS_2 population in the environment of selection (" good "), its correlated response in the " poor " medium, along with the performance of the respective environmental control populations are shown in fig. 1. It should be noted that for the first eleven generations of selection the average larval weight of GS_2 was larger in the " good " medium (hence the name " good "); however, beyond generation 11 the population's mean 13-day larval weight was larger in the " poor " medium. Further comments will be made later regarding this response to selection.

(i) *Growth inhibitor*

Qualitatively, the " good " and " poor " diets differ in the presence or absence of brewer's yeast and corn oil. In our search for the one or more dietary ingredients responsible for the peculiar growth of the GS_2 population observed in fig. 1, the experimental diets described in table 1 were developed. Growth on these diets was measured as 13-day larval weight and are given in table 2 for offspring from parents of generations 16 and 26 of the GS_2 population. Note that growth on diets " poor ", 3 and 4 was reasonably similar in all three replications and that these three diets were qualitatively alike in being devoid of yeast and corn oil. The composition of diet 2 was similar to the three noted above except for the addition of dried brewer's yeast, yet growth on diet 2 as seen in table 2 was more than doubled. This indicates

that the yeast in the " good " diet was not a growth inhibitor of the GS_2 population; in fact, yeast acted as a growth stimulator.

Experimental diet 1 was qualitatively like " poor ", 3 and 4 except it contained 5 per cent. corn oil. Growth on this diet as seen in table 2 was reduced three- to four-fold and incriminated corn oil as the inhibitor of growth in the GS_2 population. The superior performance of

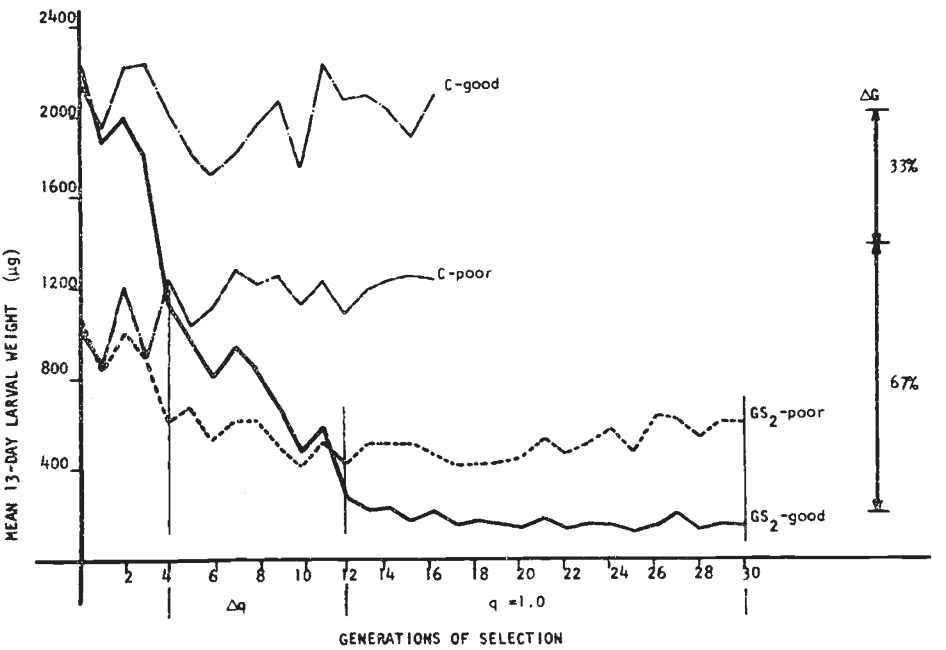

FIG. 1.—Generation means of the selected (GS_2) and control (C) populations on the " good " and " poor " diets.

this population on the " good " diet in comparison to diet 1 was probably due to the presence of dried brewer's yeast in the " good " diet.

It is important to note in table 2 that generations 16 and 26 reacted alike to the various diets. Thus the property of being sensitive to corn oil was present in the selected population as early as generation 16.

While the above results established that corn oil was a growth inhibitor for GS_2, a more definitive study of its quantitative effect on this population as well as on the unselected base population seemed desirable. The six experimental diets described in table 3 were formulated such that all the ingredients were uniform except corn oil, which was replaced by increments of one part glucose monohydrate. Growth on these diets is summarised in table 4 for both populations in two replications. It is readily apparent in both replications that the base population showed little change in growth with different concentrations of corn oil while growth of the selected (GS_2) population varied inversely

TABLE 2

Mean larval weight of generations 16 and 26 of the selected population on the diets defined in table 1

Diet	Generation 16	Generation 26	
		Replication 1	Replication 2
Good	360± 37*	323±21	378±21
Poor	680± 55	854±49	958±51
1	214± 32	199±19	221±15
2	1495±128	1340±59	1662±77
3	728± 72	725±30	1023±46
4	593± 33	747±23	1007±46

* Mean 13-day larval weight (μg.) ± standard error of mean; 25 observations per mean for generations 16 and 50 observations per mean for each replication of generation 26.

TABLE 3

Diets designed to study the quantitative response of the selected and base populations to concentration of corn oil

Component	(Diet (per cent. weight)					
	0	1	2	3	4	5
Corn meal	58	58	58	58	58	58
Soybean oil meal	17	17	17	17	17	17
Vitamin premix	10	10	10	10	10	10
Dried brewer's yeast	10	10	10	10	10	10
Corn oil	0	1	2	3	4	5
Glucose monohydrate	5	4	3	2	1	0

TABLE 4

Response of the selected and base populations to several concentrations of corn oil in the diet

Diet	Replication 1		Replication 2	
	Base	Selected	Base	Selected
0	2402±50*	1358±59	2253±68	1174±70
1	2190±76	783±50	2522±27	664±45
2	2483±71	568±32	2359±33	470±24
3	2359±27	371±22	2314±41	372±36
4	2204±66	292±20	2372±39	295±40
5	2247±49	243±17	2171±72	223±30

* Mean 13-day larval weight (μg.) ± standard error of mean; 50 observations per mean.

to corn oil concentration. In fig. 2 these data are plotted as means of the natural logarithm of larval weight ($\hat{\mu}$) on concentration of corn oil in the diet (X). The linear regressions are given for each population and replication. The regression coefficients for the base population were quite small in both replications while those for GS_2 were negative and statistically significant.

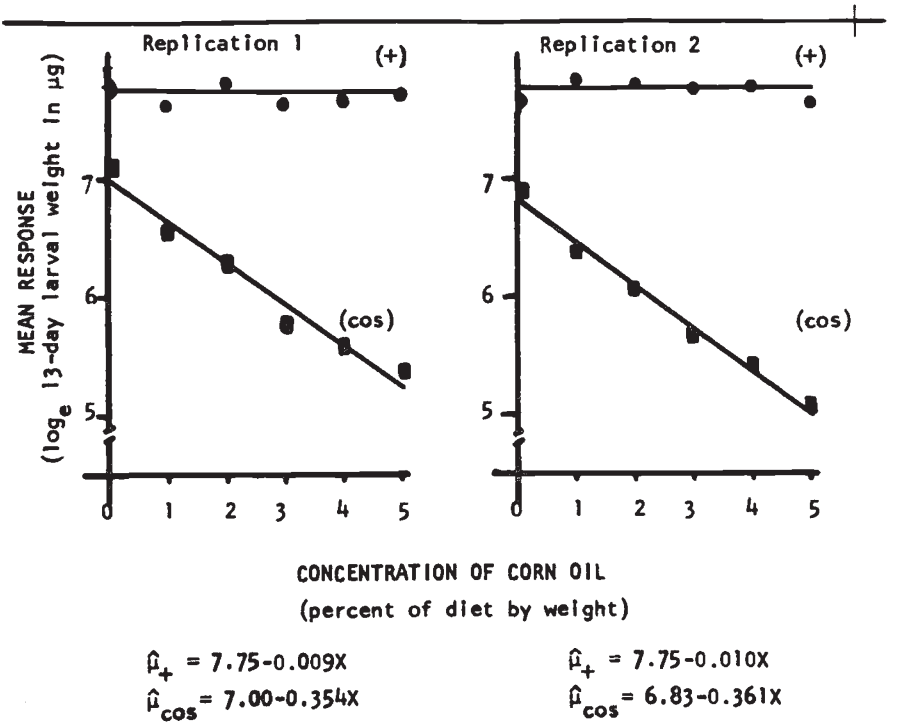

FIG. 2.—Mean response ($\hat{\mu}$) of the base (+) and selected (cos) populations to corn oil concentration (X) in the diet.

This study indicates that the inhibition or suppression of larval growth in the selected population is quantitative in nature, *i.e.* growth is a function of the concentration of corn oil in the diet. Furthermore, the base population's mean larval weight is essentially unchanged by level of corn oil in the diet. (This is reflected in that the hypothesis $\beta = 0$ was accepted at the 0·05 level of probability in both replications.)

(ii) *Inheritance of corn oil sensitivity*

In order to study the inheritance of the sensitivity to corn oil as manifested by the GS_2 population (and not by its base population), single pair matings were made between these populations and continued into the F_2 and test-crosses. All offspring were cultured on the " good " diet and were individually weighed as 13-day larvæ. The results of the inheritance studies are summarised in table 5. Histograms of the

parental, F_1, and test-cross data are presented in fig. 3. The data strongly support the hypothesis that an autosomal recessive gene is responsible for the corn oil sensitivity. The symbol " cos " for *c*orn *o*il *s*ensitive is suggested. The degree of dominance appears to be essentially complete; of course, this does not imply that physiological differences between the heterozygote and homozygous dominant genotypes do not exist. It was noted that in both the F_2 and test-cross data the total segregation differed markedly from the expected 3:1

TABLE 5

Inheritance of corn oil sensitivity

Mating type ♂ ♀	No. of matings	Normal (wt. ⩾ 1000 μg.)		Sensitive (wt. < 1000 μg.)	
		No. observed	$\bar{x}\pm$s.e.	No. observed	$\bar{x}\pm$s.e.
Parental data:					
1. +/+ × +/+	(20)	100	2343±41	0	...
2. cos/cos × cos/cos	(20)	0	...	100	297±14
F_1 data:					
3. +/+ × cos/cos	(20)	100	2226±35	0	...
4. cos/cos × +/+	(20)	100	2267±36	0	...
F_2 data:					
5. $F_1 \times F_1$ (from mating 3)	(24)	778	2275±26	172	478±33
6. $F_1 \times F_1$ (from mating 4)	(25)	899	2214±15	224	378±24
Test-cross data:					
7. +/cos × cos/cos	(32)	407	2150±14	303	213±10
8. cos/cos × +/cos	(30)	572	2139±14	425	300±8

and 1:1 ratios ($P<0{\cdot}01$). A heterogeneity analysis based on the observed segregation, as outlined by Mather (1957), indicated that both sets of data were consistent in showing a deficiency of homozygous corn oil sensitive beetles. This suggested that the viability of cos/cos individuals is poor, resulting in some deaths prior to 13 days of age.

To study the viability of the normal (+/+) and corn oil sensitive beetles (cos/cos), random samples of 100 eggs of each type were placed on the experimental diets listed in table 3 and viable larvæ were counted at 13 days of age. The results, shown in table 6, reveal that the viability of the cos/cos beetles was as good as normal or wild type in the absence of corn oil (diet 0). However, as the amount of corn oil in the diet increased, the number of viable cos/cos larvæ decreased while the normal genotype was unaffected. Since the culturing diet for the inheritance studies had corn oil at the level of diet 5 of table 6, the deficiency in the cos/cos class would be expected from the reduced viability of this genotype.

4. DISCUSSION

Two basic properties were identified for a population of Tribolium which had reflected a peculiar genotype by environment interaction.

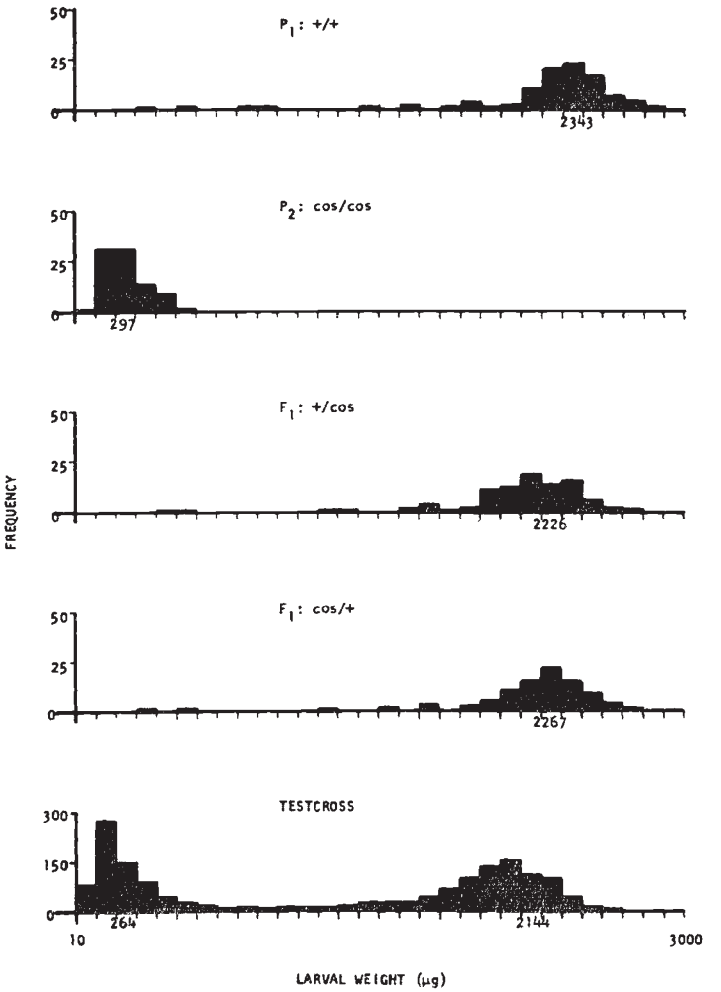

FIG. 3.—Distribution of progeny from the parental, F_1, and test-cross matings.

TABLE 6

Viability of normal (+/+) and sensitive (cos/cos) genotypes versus concentration of corn oil in the diet

Diet	Per cent. viability at 13 days	
	+/+	cos/cos
0	86	82
1	85	82
2	81	72
3	88	66
4	89	66
5	85	51

The first property is that corn oil inhibits larval growth and that the degree of inhibition is a function of the concentration of corn oil in the diet. Furthermore, the viability of the corn oil sensitive homozygote is also a function of the concentration of corn oil in the diet. The results of gas-liquid chromatography analyses of the base and selected populations grown on diets with and without corn oil showed an excessive accumulation of linoleic acid in the tissues of beetles of the selected population fed the diet with supplemental corn oil (Costantino, Bell and Rogler, 1966). Since corn oil contains approximately 58 per cent. linoleic acid, it appears that the inability of the population to metabolise this fatty acid is the primary cause of the observed growth inhibition. Analysis of the population's response to saturated and unsaturated free fatty acids at various concentrations constitutes further research which is currently underway. Inheritance studies suggest the second basic property, namely, that the genetic basis of the sensitivity to corn oil is a single autosomal recessive gene, *cos*.

As a means of integrating these two properties, the data from the two replications of table 4 were combined and are presented in fig. 4 (as natural logarithm of larval weight). When there is no corn oil in the environment (diet 0) the two populations appear to form a single continuous distribution. However, as the concentration of corn oil increases the selected (cos/cos) and base (+/+) populations diverge. Thus a genetic system with no major effect in an environment devoid of corn oil assumes major significance in differentiating these two populations as this environmental factor increases.

Let us now look more critically at the direct and correlated response of the GS_2 population (fig. 1). Note that while selection was based solely on performance in " good ", positive responses (in terms of selection) were observed during the first four generations in both the " good " and " poor " environments. The observed correlated response in " poor " suggests a relatively large positive genetic correlation between larval weight in the two environments. (Hardin and Bell, 1967 estimated this genetic correlation to be $0{\cdot}60 \pm 0{\cdot}21$ for this same base population.) From generation 4 to 30 the correlated response in " poor " was essentially zero even though response continued in the " good " environment until about generation 12. Apparently, the genetic correlation was zero during this period. It is obvious now that the genetic basis of the selection response from generation 4 to 12 was a system interacting with a factor unique to the " good " medium, viz, corn oil.

Another source of information on the selection response is the variance among families (table 7). Although these are estimates and subject to sampling variation, a definite trend is apparent. The variance increased during the initial generations, perhaps as the frequency of the *cos* gene increased, and then fluctuated through generation 11. However, at generation 12 a marked decrease in the variance occurred beyond which it was essentially zero and the population was

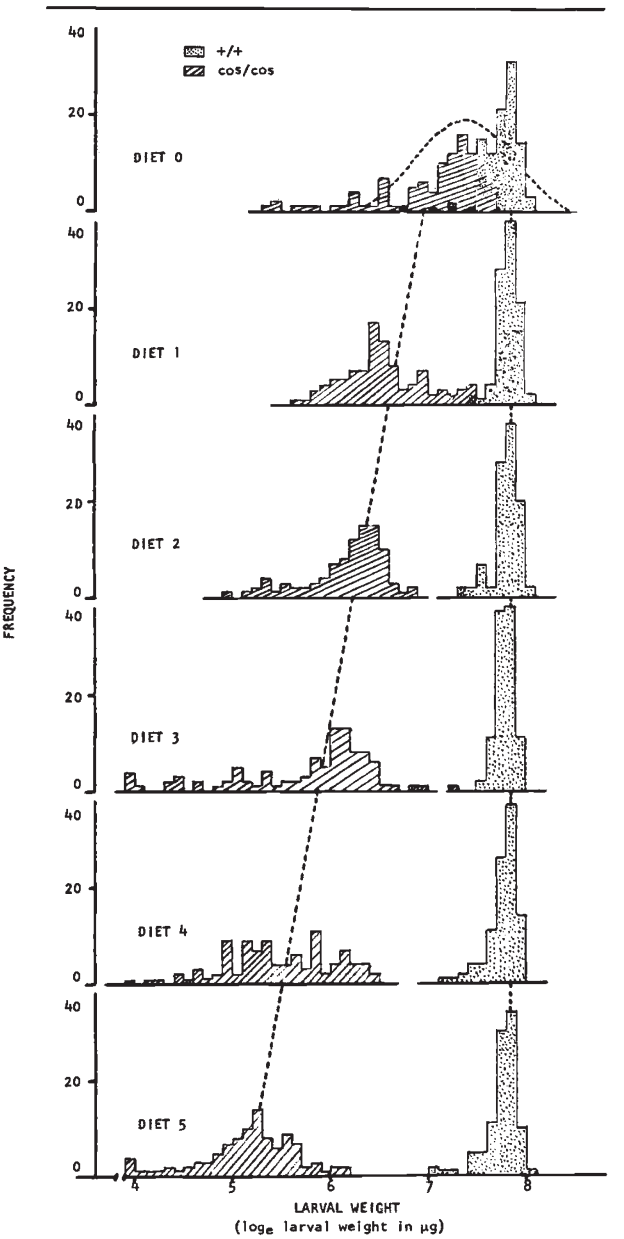

FIG. 4.—Histograms showing the divergence of the selected (cos/cos) and base (+/+) populations as the level of corn oil in the diet increased.

thought to be homozygous for the corn oil sensitive gene. The selection response expressed as a deviation from the control is also listed in table 7 and shows a correspondence between large family variance and subsequent selection progress. The close relationship between selection response and genetic variation is also evident if the latter is expressed relative to the population mean, last column of table 7.

If our hypothesis of selection response is realistic, then offspring of parents of generation 30 (cos/cos) grown on a diet the same as the "good" but devoid of corn oil should have a mean larval weight similar to the generation when the frequency of the *cos* gene was relatively low and not yet the genetic basis of the selection response.

TABLE 7

Variance among family means ($\hat{\sigma}_F^2$), *selection response* ($\Delta\hat{G}$), *and the coefficient of variation* ($\hat{\sigma}_F/\bar{x}$)

Generation	Selected population		
	$\hat{\sigma}_F^2$	$\Delta\hat{G}^*$	$\hat{\sigma}_F/\bar{x}$
0	341	+ 8	0·083
1	540	− 7	0·125
2	840	− 22	0·146
3	1056	− 43	0·180
4	835	− 85	0·248
5	851	− 85	0·297
6	331	− 91	0·223
7	992	− 85	0·326
8	426	−109	0·240
9	580	−136	0·345
10	425	−128	0·428
11	717	−163	0·448
12	170	−179	0·460
13	13	−186	0·163
14	18	−178	0·179
15	2	−172	0·082
16	16	−187	0·190

* Expressed as deviation from the control.

Under our hypothesis, this is generation 4. The results of our experiments indicated that progeny of generation 30 had a mean larval weight of 1340 μg. which is comparable to the mean larval weight of the population at generation 4 of 1160 μg. It is also possible to say that approximately 67 per cent. of the total response can be attributed to the corn oil sensitive system.

5. SUMMARY

1. In a population of *Tribolium castaneum* which had developed a peculiar growth response during 30 generations of selection for small 13-day larval weight, corn oil inhibits larval growth and the degree of inhibition is a function of the concentration of corn oil in the diet.

2. The genetic basis of this inhibition is an autosomal recessive gene symbolised *cos* (*c*orn *o*il *s*ensitive).

3. An explanation of the population's response to selection is proposed.

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