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Heterozygosity in Inbred Lines of *Tribolium castaneum*

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Summary. Two-way selection for 21-day pupa weight was conducted in two highly inbred lines of *Tribolium castaneum*. The results, after 17 generations of selection, indicated that one of these lines (CSI-10) possessed a moderate amount of genetic variation for the trait selected (21-day pupa weight).

When the selected populations were allowed to mate at random for 13 generations, the mean pupa weight regressed to values close to the means in the populations prior to selection. Reciprocal crosses between the high and low select lines revealed that 80% of the variation was associated with the sex chromosomes.

The possibility that recurrent mutation was responsible for the genetic variation is discussed. It is concluded that natural selection favoring the heterozygous condition, rather than recurrent mutation, is responsible for the genetic variation. It is suggested that selection occurring between the sublines has reduced the rate at which the inbred line, CSI-10, is approaching complete homozygosity.

A fundamental concept of population genetics is that continuous inbreeding will systematically reduce the probability of an individual being heterozygous for a given gene. In essence, the theory of inbreeding predicts that a population will become subdivided into different lines and each line will eventually approach homozygosity at all loci. A number of cases have been reported where specific inbred lines have not reached the level of homozygosity predicted by theory (Carson, 1967).

In the experiments of Enfield *et al.* (1966) and Enfield *et al.* (1969), two inbred lines of *Tribolium castaneum* were crossed to produce a foundation population where the initial gene frequency would be .5 for all segregating loci. Two-way selection within each of the inbred lines revealed a moderate amount of genetic variation present in one of the lines (Enfield *et al.*, 1969).

In this paper these two-way selection experiments are reported. In addition, the nature of this genetic variation is investigated and a discussion of the most plausible explanation for its maintenance is presented.

Methods and Materials

Strains. Two highly inbred lines of *Tribolium castaneum* were used in this study. They were obtained from Dr. A. Sokoloff at the University of California, Berkeley, in 1963. These lines (CSI-10 and CSI-5) originated from a synthetic population carrying the body color mutant *sooty*, see Lerner and Ho (1961) for description.

Before the lines were obtained, they had passed through 35 generations of duplicate brother-sister matings. In each generation if the first of these was successful, the second was discarded; otherwise, the second was used to propagate the line. Brother-sister matings have been continued since arrival in this laboratory, with the only modification being that 15 single pair matings are made each generation. If certain of these matings are sterile, additional brother-sister pairs are chosen from the fertile matings, so that there are always 15 single pair matings to propagate the line. At the initiation of these experiments each of these lines had passed through 46 generations of brother-sister matings, resulting in a theoretical inbreeding coefficient of 0.99994.

Culturing Conditions. Standard culturing procedures for *Tribolium* were used in all phases of the experiments. The media consisted of 95% whole wheat flour and 5% brewer's yeast. All populations were placed in the same incubator, which maintained the temperature at 31° ± 0.5 °C. and the relative humidity at 65± 5%.

Two-Way Selection. Populations were established for the initiation of a two-way selection program as follows. The progeny of a single brother-sister mating in each of these strains were permitted to mate randomly and to expand into a large population. The expanded populations were then randomly sampled. These samples were randomly divided into two sub-samples, which are arbitrarily designated High and Low. The four resulting populations were considered closed populations. These populations, CSI-10 High, CSI-10 Low, CSI-5 High, and CSI-5 Low, will be referred to hereafter as 10 High, 10 Low, 5 High, and 5 Low, respectively. Within each closed population each selected male was randomly mated with 3 to 5 females. The progenies produced from these matings constituted generation 0 of the program in which males and females were selected in each generation to serve as parents for the next generation. The selection in the populations designated High was for heavier 21-day pupa weight, while selection in the Low populations was for lighter 21-day pupa weight. The actual number of males and females selected each generation was adjusted so that the population size each generation was between 40 and 50 full-sib families. Recurrent mass selection was practiced for 17 generations.

The matings were made in creamers (20 ml. bottles with cardboard pull-caps). After a seven-day mating period, females were transferred to individual creamers for a 5-day egg-laying period. This made it possible to record complete pedigree data on all individuals. Pupa weighing was performed on the 21st day, counting from the middle day of egg-laying. In each full-sib family, five males and

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five females (when the mating was fertile and left sufficient progeny) were randomly selected and weighed individually to the nearest 2 micrograms on a Cahn Electrogram balance. In addition to the progeny pupa weight, records were maintained for the sire's 21-day pupa weight and the dam's 21-day pupa weight.

It was evident after the early generations that selection was effective in changing the mean pupa weight in the lines derived from the CSI-10 inbred line. The repeatability of these results was examined by establishing an additional population from the CSI-10 line in the manner described above. The resulting lines were designated 10-N High and 10-N Low. The inbred strain had passed through an additional 13 generations of brother-sister matings by this time, so that the theoretical coefficient of inbreeding was 0.999997. Concurrent with the 12th generation of selection in the original lines, the above described procedures were initiated in the new population.

Reciprocal Crosses. Random selections were drawn from the 10-N High and 10-N Low populations. This sampling was done twice in generation 11 and once in generations 13, 15 and 17. These selected individuals were then used to make the four possible matings within a generation involving the 10-N High and Low lines. When making the matings of individuals drawn from generations 11, 13 and 15, the mating procedures followed were the same as described above in the two-way selection experiment. In generation 17, each of the four matings was made by placing ten individuals of each sex into a single creamer. Two replications of each type were made. Following maturation and a 5-day mating period, all adults were transferred to a half-pint bottle.

In each set of reciprocal crosses, the individual 21-day pupa weight was recorded. The progeny from these crosses were designated as HH, HL, LH and LL, where the first letter designates the line from which the sire originated (High or Low), and the second letter designates the line of the dam. The data for male and female progeny were analyzed separately. The number of pupa was limited in some of the crosses of generation 17. The two replicates were not significantly different and thus were combined to form a single set of data comparable in size to those collected in the four previous generations. These five sets of reciprocal crosses were then considered as replications, in time, in the same experiment.

The set of reciprocal cross populations from generation 17 of the 10-N line was maintained as a closed random breeding population for 12 generations. In addition, a fifth population was established where the foundation parents consisted of 5 males and 5 females from the High line and 5 males and 5 females from the Low line. This population was designated SY and maintained as a closed, random breeding population. The generations were kept discrete by discarding the adults every 33 days. The 21-day pupa weight was recorded in the first generation and the pupa were returned to the pint bottles. After the first generation, there was no method of determining the age of pupa, and no weighing was done. The pupa from the 12th generation were sexed. The males and females were placed in separate creamers and allowed to mature. Matings were set up by placing 1 adult male with 3 to 5 adult females in separate creamers. After a seven-day mating period, the adults were transferred to fresh media and the females were allowed to lay eggs for five days. The pupa were then weighed on the 21st day, counting from the middle day of egg-laying.

Experimental Results and Statistical Analysis

Scaling Effect. Underlying the statistical analysis of a quantitative character is the assumption that the character is measured on the "appropriate" scale or is transformed to the "appropriate" scale. Falconer (1960) and Wright (1968) have discussed several recognized criteria for deciding what is the appropriate scale. Chief among these are: (1) the distribution of phenotypic values should approximate a normal curve on this scale, and (2) the standard deviation should be independent of the mean on this scale.

Enfield (unpublished data) has found that pupa weight in the cross population obtained from the CSI-5 and CSI-10 lines has a normal distribution on the arithmetic scale of measurement.

Table 1. *The regression coefficients (b) and F statistics obtained in the regression of standard deviations on mean pupa weights*

	b	*F*
10 High males	.597	22.66**
10 High females	.525	49.00**
10 Low males	−.120	2.23 n. s.
10 Low females	−.050	<1
10-N High males	−.168	9.06**
10-N High females	−.080	<1
10-N Low males	−.054	2.85 n. s.
10-N Low females	−.024	<1
5 High males	.320	3.08 n. s.
5 High females	.160	2.47 n. s.
5 Low males	−.050	<1
5 Low females	.270	5.08*

* denotes significance at .05 level
** denotes significance at .01 level
n.s. nonsignificant at the .05 level

The possibility of a systematic relationship between the mean and variance was examined by regressing the standard deviation on the mean. The results are summarized in Table 1. A relationship between the mean and variance appears to be indicated for the 10 High males, 10 High females, 10-N High males and 5 Low females. However, the data in the 10 populations are inconsistent. In the 10 High lines, the standard deviation increases as the mean increases, while in the 10-N High males, the standard deviation decreases when the mean increases.

It will be reported later that while there was a linear response to selection in the 10 populations, selection was relatively ineffective in the 5 population (realized heritabilities of .04 and .02 for High and Low respectively). In the 10 populations, if a systematic relationship exists between the mean and variance, the coefficient of variability (i.e., the ratio of standard deviations to mean) should remain constant over the 17 generations of selection. In the 5 population, a change in the variance cannot be attributed to a scaling effect. The results of regressing the coefficients of variation on generations are shown in Table 2. In all cases, except the 10 Low females, there is a linear change in the coefficients of variation. Thus no systematic relationship between the mean and variance is indicated in the 10 populations.

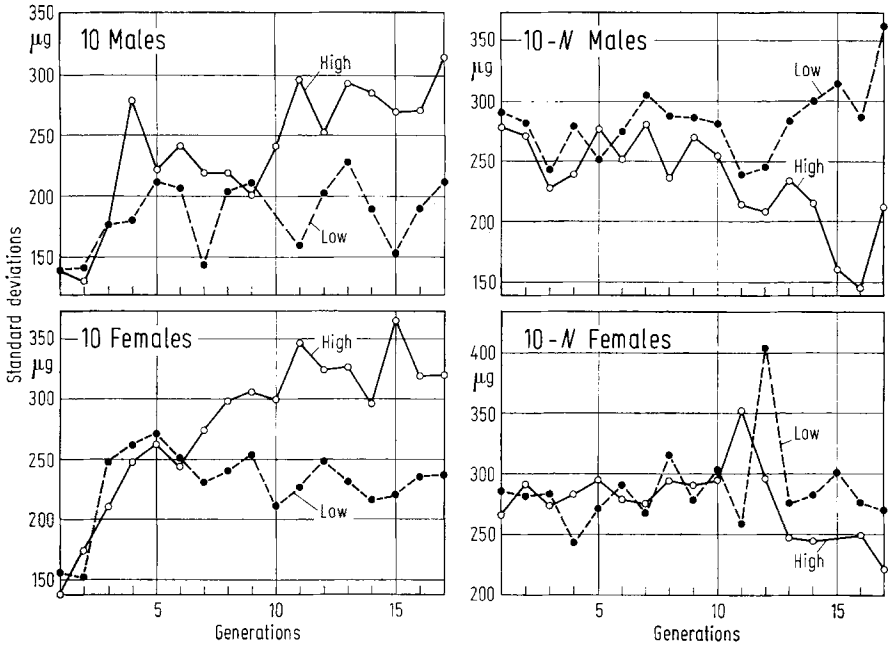

Fig. 1. Standard deviations for 21-day pupa weight in each sex in each generation for the 10 and 10-N populations

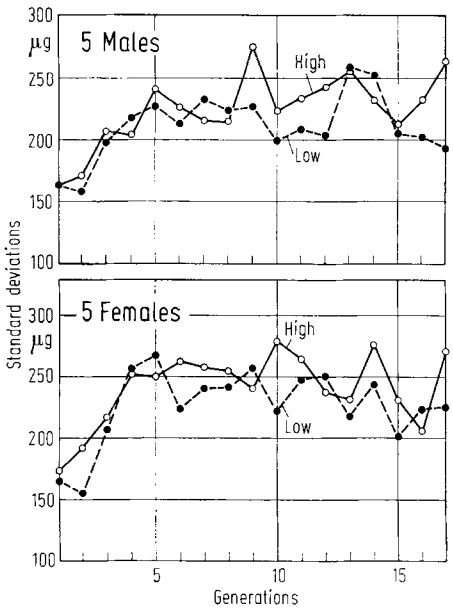

Fig. 2. Standard deviations for 21-day pupa weight in each sex in each generation for the 5 population

Table 2. *The regression coefficients (b) and F statistics obtained in regressing the coefficients of variation on generations of selection*

	b	*F*
10 High males	.30	19.00**
10 High females	.31	35.00**
10 Low males	.17	7.00*
10 Low females	.14	3.60 n. s.
10-N High males	−.28	21.00**
10-N High females	−.16	16.00**
10-N Low males	.37	15.00**
10-N Low females	.31	15.00**
5 High males	.08	3.00 n. s.
5 High females	.08	1.30 n. s.
5 Low males	.13	4.90*
5 Low females	.10	3.80 n. s.

* denotes significance at .05 level
** denotes significance at .01 level
n. s. nonsignificant at the .05 level

The graphs of the standard deviation (Figures 1 and 2) are useful in interpreting the changes in variance. The first point of interest is in the 10-N populations, where no substantial change in the standard deviations is seen until the more advanced generations. In the 10 population, a consistent increase in the standard deviation of both High and Low lines is observed in the first four generations. A similar pattern is observed in the 5 population, where no substantial change was observed in the means. This suggests that an environmental factor, rather than a change in the mean, is responsible for the increase in variance in these generations. After the fourth generation there is no consistent increase or decrease in the Low line of the 10 population. The standard deviation of the 10 High females continues to increase until about the 10th generation, after which it remains fairly constant. The standard deviation in the 10 High males remains reasonably constant between the 4th and 10th generations, at which time it increases and remains reasonably constant through generation 17. These patterns suggest that the population is becoming more susceptible to subtle changes in the environment.

Since by these two criteria there is no basis for transformation, the analyses were performed on the scale of measurement.

Two-Way Selection. The responses in the High-Low selection experiments are shown in Figure 3. (Note the 10-N lines were not contemporary with 10 and 5 lines.) The results of regressing the mean pupa weight on generations of selection are summarized in Table 3. The realized heritabilities, calculated by regressing the accumulated response on accumulated adjusted selection differentials (adjusted for diffe-

Table 3. *The regression coefficients (b), and standard error of b (S. E.) from the regression of average pupa weight on time for each of the sex lines*

Line	*b*	S.E.	r^2	*F*
10 High	14.87	1.21	.90	157.0**
10 Low	−18.35	2.23	.81	133.0**
10-N High	21.77	4.89	.83	88.0**
10-N Low	−43.26	4.06	.88	275.0**
5 High	6.80	1.75	.48	15.9**
5 Low	−5.30	1.52	.35	9.4**

** denotes significance at .01 level

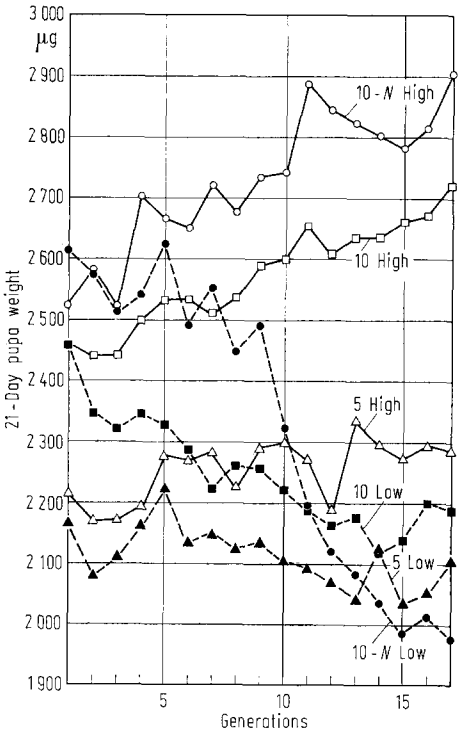

Fig. 3. Mean 21-day pupa weight in the 17 generations of high-low selection

rential rates of reproduction among selected parents), are shown in Table 4.

It is evident from these results that there was a significant change in the mean pupa weight in all lines. The coefficients of determination (Table 3) indicate that more than 80 percent of the variations in the generation means of the 10 and 10-N populations are associated with a linear change over time. This is true for less than 50 percent of the variation in the 5 population.

In all cases, the response in the low line exceeds the response in the high line. This asymmetry is not unexpected. The foundation populations of these lines originated from single pair matings which would restrict the initial gene frequency to 0.25, 0.50 or 0.75 at any segregating loci. Subsequent sampling would bias gene frequencies in the direction of 0.50 (Hill and Robertson, 1968). Falconer (1960) pointed out that asymmetrical response to two-way selection might be expected with intermediate gene frequencies and dominance in the direction of the high selection. Dominance is in the direction of higher pupa weight in *Tribolium* (Boylan and Wong, 1965, Enfield *et al.*, 1969).

Table 4. *Realized heritabilities computed by regressing accumulated response on accumulated adjusted selection differential*

	High	Low	Average
10	.04 ± .003	.16 ± .009	.10 ± .004
10-N	.09 ± .005	.11 ± .010	.10 ± .005
5	.04 ± .004	.02 ± .005	.03 ± .003

Despite this asymmetry, the realized heritabilities which are free from effects of differences in selection intensity do provide a basis for comparing the effectiveness of selection in the different populations. The responses in the 10 and 10-N populations are both much larger than the response in the 5 population. The average realized heritabilities were identical in the 10 and 10-N populations.

Estimates of Heritability. Heritability was estimated by the following methods: 1) sire-offspring regressions, 2) dam-offspring regressions, and 3) sib analysis, using the sire component of variance as an estimate of one-fourth of the additive genetic variance. The analysis of variance for a nested classification with unequal class size was used in estimating the sire, dam, and within full-sib family components of variance.

These estimates of heritability were first calculated on an intra-generation basis. When regression on time indicated no significant time trends, the intra-generation estimates were pooled over all generations. Comparisons among the three methods indicated no significant differences. The estimates were pooled to obtain the estimate of heritability for each line. The pooling in all cases was done by weighting the estimate by the inverse of its own variance, as discussed by Cochran and Carroll (1953). These pooled estimates of heritability are reported in Table 5.

Table 5. *Pooled estimates of heritability (h^2) using sire-offspring regression, dam-offspring regression, and sire component of variance*

Population	Males h^2	Females h^2	Pooled h^2
10 High	.08 ± .06	.08 ± .08	.08 ± .04
10 Low	.19 ± .11	.27 ± .09	.23 ± .06
Pooled	.11 ± .05	.15 ± .05	.13 ± .04
10-N High	.08 ± .07	.20 ± .07	.14 ± .04
10-N Low	.26 ± .09	.17 ± .09	.22 ± .06
Pooled	.15 ± .07	.19 ± .07	.17 ± .04
5 High	−.04 ± .05	−.01 ± .06	−.02 ± .04
5 Low	.06 ± .08	−.03 ± .07	.01 ± .05
Pooled	−.003 ± .04	−.02 ± .04	−.01 ± .03

Since heritability is a ratio of two variances, it is necessarily $\geqq 0$. The negative values reported in the 5 population serve as a reminder that these are merely estimates of the parameter. None of these are significantly different from zero, and may be interpreted as estimates of zero for the parameter. Their presence, however, does argue that the true value of the parameter does not differ greatly from zero. From these data, along with the fact that the 17 generations of selection resulted in very little change in the 21-day pupa weight, it is clear that there is very little genetic variation present for genes

affecting pupa weight in the 5 population. Since we have no evidence to the contrary, we conclude that the CSI-5 line has attained the level of homozygosity anticipated from the coefficient of inbreeding. In view of this, the remainder of this discussion will deal only with data pertinent to the 10 and 10-N populations.

In contrast to the CSI-5 inbred line, there appears to be considerable genetic variance present in the CSI-10 inbred line. There is very good agreement between the corresponding estimates of heritability in the 10 and 10-N populations. Pooling the estimates over sexes and lines results in values of 0.13 ± 0.04 and 0.17 ± 0.04 for the 10 and 10-N populations, respectively. These are both significantly different from zero ($P < .01$) while there is no significant difference between them.

It should be recalled that 13 generations of brother-sister matings in the CSI-10 line occurred between the establishment of the 10 and the 10-N populations. These data indicate that during this interval no further reduction in genetic variance occurred.

In both the 10 and 10-N populations, the realized heritabilities (Table 4) are somewhat smaller than the estimates of heritability in Table 5. However, there are no significant differences, and the realized heritabilities can be considered good estimates of heritability.

Table 6. *The mean pupa weight (in μg) and heterosis for the reciprocal cross between the 10-N High and 10-N Low lines*

	Males	Females
HH	2676 ± 23	2878 ± 30
HL	2304 ± 22	2723 ± 22
LH	2593 ± 17	2722 ± 16
LL	2079 ± 18	2331 ± 24
Midparent	2378 ± 15	2604 ± 19
Average of F_1	2448 ± 14	2722 ± 14
Heterosis	70 ± 10	118 ± 12
% Heterosis	2.9%	4.5%

Reciprocal Crosses. A summary of the data collected in the reciprocal crosses is shown in Table 6. Heterosis was calculated as the difference between the average of the F_1 and the midparent value, and then expressed as a percentage of the midparent value. The quantity $\frac{HH + LL}{2}$ was used as the mean parent value. Boylan and Wong (1965) and Enfield *et al.* (1966) reported more heterosis in the heterogametic sex (male) than in the homogametic sex. The data in Table 6 do not agree with this. In both sexes the average of the reciprocal crosses is significantly larger ($P < .01$) than the midparent values.

Heterosis in a cross of two populations requires different average gene frequencies in the populations, as well as some level of dominance. Thus, the presence of heterosis may be interpreted as evidence that selection has been effective in changing gene frequency (although genetic drift could have the same effect).

In the male progeny there is a distinct maternal influence. In the LH cross, in which the dam is from the heavier line, the male progeny are significantly heavier than in the reciprocal cross, HL. There is no similar maternal influence observed in the female progeny.

The means in the reciprocal crosses were analyzed under the assumption of the following linear model:

$$Y_{ijk} = u + s_i + d_j + s\,d_{ij} + r_k + e_{ijk}$$

where Y_{ijk} is the mean of the progeny in the k^{th} replication, resulting from the mating of sires from the i^{th} line with dams from the j^{th} line. The effects due to replications (r_k) were considered fixed, while the effects due to gametes from sires of the i^{th} line (si) and effects due to gametes from dams of the j^{th} line (d_j) were considered random. The data for each set of progeny consist of two levels of two different treatments with five replications. The analysis of variance takes the form of a conventional 2×2 factorial experiment with randomized block design.

The results of the analyses of variance performed on the data are shown for the male and female progeny in Tables 7 and 8, respectively. There is no significant variation due to either (a) differences between replications, or (b) interaction between the dam and sire gametes. This indicates that the gametes are acting independently and in a similar manner in all replications.

Table 7. *2 × 2 factorial ANOVA of the mean pupa weight of the male reciprocal cross progeny*

S.V.	*df*	*M.S.*	*F*
Repl.	4	23,738	2.52 n. s.
Trt.	3	373,799	39.79**
Female gametes	1	960,973	102.30**
Male gametes	1	131,544	14.00**
Male × Female	1	28,880	3.07 n. s.
Res	12	9,394	

** significant at the .01 level
n. s. nonsignificant at the .05 level

Table 8. *2×2 factorial ANOVA of the mean pupa weight of the female reciprocal cross progeny*

S.V.	*df*	*M.S.*	*F*
Repl.	4	22,171	1
Trt.	3	282,481	12.73**
Female gametes	1	391,160	17.63**
Male gametes	1	383,922	17.30**
Male × Female	1	72,362	3.26 n. s.
Res.	12	12,185	

** significant at the .01 level
n. s. nonsignificant at the .05 level

The main effects due to sire gametes and dam gametes are shown in Table 9. The contribution of the dam's gamete to the pupa weight of the male progeny is more than twice that of the sire's gamete, while the gametes from the sire and dam contribute equally to the weight of the female progeny.

Table 9. *The main effects due to dam and sire gametes (from the 2 × 2 factorial ANOVA)*

	Progeny	
	Male	Female
Sire gametes	815	2770
Dam gametes	2189	2800

The presence of a maternal influence in the male and not in the female progeny does not fit the pattern normally associated with extrachromosomal genes. Thus if this maternal influence has a genetic basis, there is no reason to doubt that the genes are located on one of the chromosomes. The consistency with which this maternal influence is expressed is substantial evidence that it does come under the control of genes. Since the male receives a Y chromosome from his sire and an X chromosome from his dam, genes located on the X chromosome are expected to have this type of effect.

The expected mean square for the 2 × 2 factorial ANOVA can be utilized to obtain estimates of the genetic variance contributed by the female gametes (σ_d^2) and the genetic variance contributed by the male gametes (σ_s^2). In the male progeny the ratio,

$$\frac{\sigma_d^2 - \sigma_s^2}{\sigma_d^2 + \sigma_s^2} = .80.$$

This ratio gives an estimate of the proportion of genetic variance associated with the X chromosome.

Random Breeding Populations. The averages of the mean 21-day pupa weights for the two replicates of the random breeding populations, along with the means in generations 0 and 17 of the 10-N lines, are shown in Table 10.

The HH and LL populations in generation 1 show very good agreement with generation 17 of the 10-N High and 10-N Low lines, respectively. During the 13 generations of random mating, the mean of the HH line regressed to a value very close to that of the unselected 10-N High line. The mean of the LL line did not change appreciably during this period. Mutation or the breakdown of epistatic blocks of genes are remotely possible explanations for the decline in the HH population. However, it is unlikely that either of these account for much of the decline, since, they would be expected to have similar effects in the LL population.

The SY populations established with equal numbers of males and females from the High and Low lines would also be affected by mutation and epistasis. However, the means in the SY population in generation 1 are approximately equal to the means of the unselected 10-N population. The mean in the SY population does not change significantly during the period of random breeding. In view of this, it appears more likely that the means of the HH population have declined as a result of a negative genetic correlation between fitness and 21-day pupa weight.

Table 10. *Mean 21-day pupa weight in generation 1 and generation 13 of the random breeding populations and in generation 0 and generation 17 of 10-N High and Low lines*

Gen.	Line	Males	Females
0	10-N High	2389 ± 30	2579 ± 33
17	10-N High	2792 ± 36	3014 ± 40
1	HH	2794 ± 82	3044 ± 105
13	HH	2308 ± 45	2562 ± 61
0	10-N Low	2455 ± 30	2669 ± 35
17	10-N Low	1949 ± 69	2002 ± 45
1	LL	1976 ± 22	2126 ± 55
13	LL	2008 ± 64	2100 ± 40
1	HL	2243 ± 42	2774 ± 48
13	HL	2212 ± 77	2332 ± 66
1	LH	2534 ± 42	2761 ± 54
13	LH	2247 ± 33	2371 ± 46
1	SY	2444 ± 57	2577 ± 71
13	SY	2301 ± 55	2450 ± 54

Additional evidence to support this hypothesis is seen in the means of the HL and LH populations. In generation 13, the means of the males in these populations are 2222 ± 77 and 2247 ± 33, respectively. These are not significantly different from the means of the males in generation 13 of the HH and SY populations. The observation that in two of these populations (HH and LH) the means declined during the period of random mating while the means of the other two populations did not change significantly is a clear indication that the means of these populations (HH, HL, LH, SY) are near the optimum value for fitness with respect to male pupa weight. The means of the females in the HL and LH populations changed from 2774 ± 48 and 2761 ± 54 to 2332 ± 66 and 2371 ± 46, respectively. These means are not significantly different from the mean of the females in generation 13 of the SY population. It is probable that these means are very close to the optimum

Table 11. *Estimates of the number of segregating loci (n_3) obtained from ratios of the response squared to additive genetic variance (σ_g^2)*

	n_3		
10 males	5.7	< 7.2	<10.3
10 females	6.8	< 8.7	<12.3
10-N males	11.3	<13.6	<17.1
10-N females	16.1	<19.3	<24.3

fitness values for female pupa weight in these populations.

After random mating the means of the males and females in the HL, LH and SY populations are lower than the respective means in the unselected 10-N lines. The differences are significant in the case of the HL and LH populations. Either mutation or the loss of alleles from the gene pool through random sampling might be responsible for this. The observations relating to the HH line indicate that any such changes to the gene pool occurred in the 10-N Low line. Overall, these data suggest the change to the gene pool is rather small.

Discussion

These experiments indicate that CSI-10 inbred line possessed a moderate amount of genetic variance for 21-day pupa weight. Furthermore, they suggest that as much as 80% of this variation may be associated with the sex chromosome.

The presence of genetic variation in a highly inbred line may be the result of either recurrent mutation or natural selection acting in some manner to preserve the heterozygous condition. While there is no direct evidence to establish which of these phenomena is the one with major importance in the case of the CSI-10 line, there are a number of implications worth noting.

Haldane (1936) demonstrated that in a highly inbred line resulting from full-sib matings the frequency of heterozygotes at a locus as a result of mutation is 12 u for autosomal neutral genes and only 9 u for sex linked neutral genes, where u represents the mutation rate per locus. Since 80% of the variation is associated with sex linked genes, these sex linked genes must have a much larger effect than the autosomal genes if the presence of the polymorphic loci are to be attributed to mutation.

Furthermore, Wright (1931) demonstrated that the number of unfixed loci (L) which a given mutation rate per individual will support can be found using the expression:

$$L = 2\,NU \log 3.6\,N,$$

where N is the effective population size and U is the mutation rate per individual. It is clear from this that very few polymorphic loci can be maintained (with N = 30) unless mutation rates are unusually high. But unusually high mutation rates are not likely since it was observed that 1) the means of the random breeding populations returned to values very close to the means of the unselected lines, 2) similar responses and estimates of heritability were observed in the 10 and the 10-N populations and 3) no appreciable genetic variance was present in the CSI-5 line.

It is possible that only two or three loci are contributing to the observed genetic variance. In this case, mutation could be responsible for their heterozygous condition. The number of loci involved may be estimated using logic similar to that of Wright (1952) and (1968) and Comstock (1969). These estimates may be biased if an incorrect genetic model is used. However, it has been shown by Wright (1968) and Comstock (1969) that minimum estimates may result when it is assumed that there is 1) no dominance, 2) no variation from locus to locus in the effects of genes, 3) no epistasis and 4) linkage equilibrium exists.

With this genetic model it is easily demonstrated that the ratio of the response squared to twice the additive genetic variance is a function of effective population size, the number of segregating loci, and the average gene frequency in the initial population. While the average gene frequencies are not known it can be assumed they are equal in the random samples used to form the High and Low lines in each population. Thus, estimates of the number segregating loci can be obtained by solving these functions from the High and Low lines simultaneously. The estimates obtained in this manner are presented in Table 11.

The highest and lowest estimates of n_3 were obtained using $\sigma_g^2 - 2$ S. D. and $\sigma_g^2 + 2$ S. D., respectively (S. D. refers to standard deviation of estimate). These estimates are biased downward since it has been assumed that selection resulted in complete fixation at all pertinent loci while there was no indication that a plateau had been reached.

Accepting 15, as a conservative estimate of the number of loci contributing to the genetic variance, implies that no fewer than 12 loci on the X chromosome are polymorphic. While recurrent mutation might be responsible for a few unfixed loci it is highly unlikely that mutation could support this many sex linked polymorphic loci that affect pupa weight. Thus, a more plausible suggestion is that natural selection has preserved the polymorphic condition in spite of the intense inbreeding.

As to the actual mechanisms, we can only point out that Reeve and Gower (1958) reported that selection between lines can effectively reduce the rate of approach to homozygosity. An examination of the pedigree data has revealed that the present CSI-10 population can be traced back to two of the original 15 matings. It may be that homozygosity above a certain level has caused sterility, making it necessary to use individuals with a higher level of heterozygosity to propagate the lines.

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