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The importance of genotype by environment interaction with reference to control populations*

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INTRODUCTION

A basic problem in any experiment involving the use of genetic material has been the separation of environmental and genetic effects. Meaningful comparisons within generation may be made between treatments without the use of a control when the treatments are applied to the same population and all of the material is grown in the same environment. When the experiment involves several generations, genetic treatments cannot be related to the original generation without assuming that the environment has remained constant. Other kinds of treatment comparisons cannot be related to the original generation without further assuming that the genetic differences occur randomly over treatments or that drift is not a source of bias. Some method of maintaining genetic material for comparison with later generations is necessary for population studies spanning several generations.

The value of control populations has been recognized by workers who have used controls as an aid in evaluating response to selection (Robertson & Reeve, 1952; Bell *et al.*, 1955).

Two general types of controls other than inbred lines have been suggested. Random-bred control populations constitute one general type of control. These have been utilized in poultry (King *et al.*, 1959; Gowe *et al.*, 1959*b*). A second general type of control is the method of repeat matings. Repeat mating systems have been suggested for both dairy cattle (Hickman, 1958), and poultry (Goodwin *et al.*, 1960).

Control populations can be used to answer two distinct questions in selection studies:

(1) The separation of genetic and environmental effects in a selected line.
(2) The response of the original genotype from which the selected line was drawn to new environments which may be encountered in later generations.

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A control which is to be used to determine the genetic progress of a selected line must accurately measure changes in the environment as they affect the selected population of interest. When definable environmental changes differentially affect a selected line and a control then comparisons between them are biased to the extent of the genotype by environment interactions.

If the genetic changes which occur in selected lines yield genotypes which are unchanged in their response to environmental differences, then the random-bred controls will answer both questions. If, however, the genetic changes in the selected lines yield genotypes which give new responses to environmental shifts then a repeat system is required to answer the first question while a random-bred method of reproducing the original population is required to answer the second.

In any given situation an experimenter may be satisfied, on the basis of preliminary tests, that no genotype by environment interaction exists with respect to his selected line and a random-bred control. However, such tests cannot be certain of supplying the precise environments to be encountered in future generations, nor can they entirely predict what effect future selection may have on the selected line. The probability of encountering interactions in future generations is of considerable importance.

Griffing (1954) found that heterosis may be exhibited by a specific cross in a specific environment. He concluded that changes in genetic parameters may occur in varying degrees in different environments.

Dobzhansky (1948) reported that differences in fitness between certain chromosomal types in *Drosophila* are observed at 25°C., while at 16°C. the adaptive value of these types are more nearly similar or even identical. Evidence of the effect of environment on body size is seen in the data presented by Pantelouris (1957) who observed larger differences between lines of *Drosophila* at lower temperatures.

The general problem of interaction between environment and heredity in animals has been reviewed by McBride (1958).

It is clear from the literature that genotype by environment interaction may be encountered under a wide variety of situations. In general, it would seem advisable to take whatever precautions are necessary to detect its presence in selection studies.

In the absence of an interaction base controls can be used to measure selection response and in any event may be expected to measure environmental changes with respect to their own genotype.

Theoretical objections have been raised to the use of inbred lines and their crosses, Gowe *et al.* (1959*a*), King *et al.* (1959). The major difficulty is that inbreds and inbred crosses represent at best only a narrow range of genotypes so that their response to environmental shifts is likely to be specific to the genotypes which they represent. Individual inbred lines cannot be truly representative of any original outbred population from which selected lines may be drawn and should not be expected to be good indicators of how environmental shifts affect other genotypes. They should give highly repeatable results with respect to how such changes affect their own genotype.

Random mating may be carried out by mass mating, multiple matings which may

involve a nested design, or single pair matings. Bell and Moore (1958) found that small mass matings varied considerably, but that the average of several such lines tended to be quite stable. The populations described by King *et al.* (1959) are based on a nested design involving 50 males and 250 females. Two restrictions are used, one is that no full or half sib matings are allowed. The other is that each sire may leave only one son and each dam only one daughter in the next generation. Four methods of random breeding may be devised according to whether none, one, or both of these restrictions are used.

Still another way of reproducing the base population is the stabilized selection method. In this instance selection for the mean value is practised each generation.

A possible compromise between the random-bred and the repeated methods is the relaxed selection technique. Relaxed lines may be taken from the selected lines at intervals throughout a selection experiment. These lines will be closely related to the selected line during the first few generations following their formation. Provided that they are formed at frequent intervals relaxed selection lines should give similar results to the repeat mating technique in the presence of a genotype by environment interaction.

This experiment was designed to test:

(1) The relative usefulness of inbreds, inbred crosses, stabilized selected, and random-breds as base controls;
(2) The effectiveness of two restrictions which are being used in maintaining random-bred control populations;
(3) The relative usefulness of base control methods compared to repeated and relaxed methods of maintaining controls for use in measuring environmental effects as they affect selected lines.

MATERIALS AND METHODS

Tribolium castaneum is a remarkably useful animal for the investigation of methods of maintaining control populations. These beetles have a life expectancy of about six months which is equivalent to six or more generation intervals. They have ten pairs of chromosomes and are easily cultured under laboratory conditions.

Single-pair matings in this study were maintained in $\frac{3}{4}$ oz. glass creamers with mass matings being cultured in $6\frac{1}{2} \times 4\frac{1}{2} \times 2\frac{1}{2}$ in. plastic containers. Culturing media consisted of whole wheat flour with the addition of 5% dried brewer's yeast.

Fifteen methods of maintaining control populations were reproduced along with two directionally selected lines in a replicated experiment spanning eight generations. Twelve of the control populations and the selected lines were initiated from a foundation stock which has been maintained since its formation in 1954 as a closed population at Purdue University. In that year, eight non-inbred laboratory stocks from widely diverse sources in the United States were systematically combined to form a random breeding population which was perpetuated by a mass transfer of 200 individuals each generation. The three remaining control populations consisting of two inbred lines and their cross were unrelated to the above foundation population.

Each of the two replications consisted of these seventeen lines. The two replicates were initiated by collecting large samples of eggs from the same mass matings in two successive weeks. Thus, Replication I proceeded with generations cycling one week ahead of Replication II.

Body-weight, a highly heritable trait, was chosen for this study with measurement being made at the pupal stage of development. Weighings were made on a Mettler 'Micro-Gramatic' balance and recorded in tens of μg.

Types of controls used

The various control populations and selected lines with a brief description of each are listed in Table 1. A more detailed consideration of each follows.

Table 1. *Description of the various populations*

Symbol	Title	Mating*	Comments
C_1	Master	mass	9 samples of base stored at 18° C.
C_2	Master	single pair	base matings repeated each generation
I_1	Inbred 1	mass	
I_2	Inbred 2	mass	
I_{12}	Inbred cross	mass	
M	Mass	mass	
R_o	No restrictions	single pair	
R_s	No sib matings	single pair	no full sib matings allowed
R_n	Equal numbers	single pair	1 ♂ and 1 ♀ from each family
R_{sn}	Equal numbers and no sib matings	single pair	both restrictions imposed
L	Selected large	single pair	5 males and 5 females chosen from each of 10 families in each line
S	Selected small	single pair	
Z	Selected stabilized	single pair	
TL	Repeated large	single pair	selected parents held 1 generation interval and repeated
TS	Repeated small	single pair	
XL	Relaxed large	single pair	mated R_{sn} after generation 4
XS	Relaxed small	single pair	mated R_{sn} after generation 4

*50 males and 50 females mated each generation for each population except C_2.

A *master control*, C_1, was obtained by storing nine random samples of the base population at temperatures at which they were just barely active (about 18°C.). Each sample consisted of 50 males and 50 females. One sample was withdrawn from storage each generation during the experiment and offspring were cultured under the same environment as the experimental populations. Very little mortality occurred as a result of this treatment, the maximum for any one sample being less than 10%. When one assumes that such storage of the parents has little effect on the offspring, then the only variation represented here is sampling variation in initially choosing the samples.

Because of the longevity of this insect it is possible to continue to sample offspring each generation from the 100 single-pair matings serving as parents of the initial generation of the selected lines (L, S and Z). Full sibs of the initial generation were

thus grown in each of the subsequent generations. This method, C_2, may also be considered a *master control* if one can assume that the age of the parents does not affect the pupa weight of the offspring. Other evidence in this laboratory has indicated this to be the case. Of course, some mortality occurred as time progressed so that the later generations may not be determined as accurately as the earlier ones. Provided that there is no correlation between pupa weight and longevity this is not a serious objection.

The most homogeneous material which can be readily obtained in animals is an inbred line. It was thought that such material, which is relatively constant, would provide a good measure of environmental variation from generation to generation. Inbred line 1, I_1, and inbred line 2, I_2, had resulted from 27 and 38 generations of full sibbing, respectively. A wider genotypic base, still of a repeatable nature, was obtained in the cross, I_{12}, between these two inbred lines.

One of the recommended procedures in reproducing control populations has been to place restrictions on both sib mating and the number of progeny left per parent in an attempt to control genetic drift. In this experiment two control populations were reproduced without any restrictions. In one case, M, 50 males and 50 females were randomly chosen each generation and mass mated in a plastic container. In the other case, R_o, 50 single-pair matings were made each generation and pupae from these matings were pooled from which 50 males and 50 females were randomly paired to produce the next generation. A restriction of no full sib matings was placed on the R_s control population. Again 50 single-pair matings were made and a table of random numbers was used in determining the number of offspring each mating would contribute to the matings for the next generation. The procedure was much simpler for the R_n population. The parents for this line were randomly obtained, one male and one female from each family, and randomly mated with no restriction on brother-sister matings. The parents for population R_{sn} were chosen in the same manner as those of R_n; however, when mating these, care was taken to ensure that no full sib matings occurred. When any family failed to produce progeny in each of these last two lines, additional pupae were drawn from the adjacent family in order to make up the same number of matings each generation. Of the 100 individuals required for replacement an average of seven were drawn from adjacent families each generation, the range being 1–16.

A family selection scheme was used. One hundred single-pair matings of randomly chosen pupae from the foundation stock were made to serve as the origin of the three selected lines: large, small and stabilized. The ten families which had the largest average pupa weight were chosen from the 100 pairs in the initial generation to begin the large line. Likewise the ten closest to the mean pupa weight initiated the stabilized line and the ten which had the smallest average pupa weight started the small line. Thereafter, ten full sib families were selected on the basis of the total weight of four males and four females randomly taken from each of 50 families each generation for each of the selected lines. Five males and five females were chosen from each of the selected families. In choosing these individuals within the selected families for the large and small lines, the larger and smaller pupae, respectively, were

visually selected. The 50 males and 50 females so obtained were then mated, avoiding full sib matings.

Three types of controls which are closely associated with selection were maintained. Stabilized selection, Z, or selection toward the mean, is a possible way of maintaining a base control. Repeated lines, TL and TS, are more closely associated with the selected lines in that they are initiated every generation. Offspring from the directionally selected parents of the preceding generation were grown with the selected groups of the current generation.

Relaxed lines XL and XS, are more closely associated with the selected lines than base methods such as stabilized or any of the five (M, R_o, R_s, R_n, R_{sn}) previously discussed. One relaxed line was initiated from each directionally selected line at the fourth generation.

Measurement of pupa weight

When the mated pairs had reached maximum fertility at approximately 10 days of age, each pair was transferred to a new creamer containing 2 g. of culture media. After 48 hours the parents were removed. The resulting cultures including eggs were incubated at 32·8°C. with specified humidity conditions as described later.

Pupae were weighed when a majority of the individuals in all lines had reached the desired stage of development. In the selected lines (L, S, Z, TL, and TS) and the repeated original line (C_2) weights were recorded by family for groups of eight pupae, when available, equalized for sex. The remaining or residual pupae from all 50 matings in each line were weighed as a single group. Both types of data were used to determine the line means. After selections were made, the pupae selected to be parents of the next generation were weighed in pairs. The unselected pupae from selected families were weighed as a group.

For all other lines, after the individuals necessary for reproducing the line were chosen, the residuals were weighed as a group. For those lines for which it was not necessary to retain family identification in order to avoid sib mating, the parents were weighed by sex, one group for each sex. Otherwise single-pair weighings were made.

In order to simulate changing environmental conditions as are often encountered with economic species (e.g. years and generations confounded in poultry), two controlled environments were utilized which differed only in their relative humidities. One incubator was maintained at 70% while the other was controlled at 40% with fluctuations seldom exceeding 2%. All lines were grown in one environment for two succeeding generations, and then in the other for two generations. Preliminary studies had revealed that *T. castaneum* reared under wet conditions were about 10% heavier than those reared in the dry environment. This difference is reflected in the expected response projected in Fig. 1 over 8 generations of alternating wet (W) and dry (D) environments. If one assumes that the absolute response to the environment will not change, then the results indicated by the solid lines should be obtained. Also, the dotted lines drawn within each environment for each direction would be parallel. The slope of the lines in this illustration was taken to be the average observed response in this study.

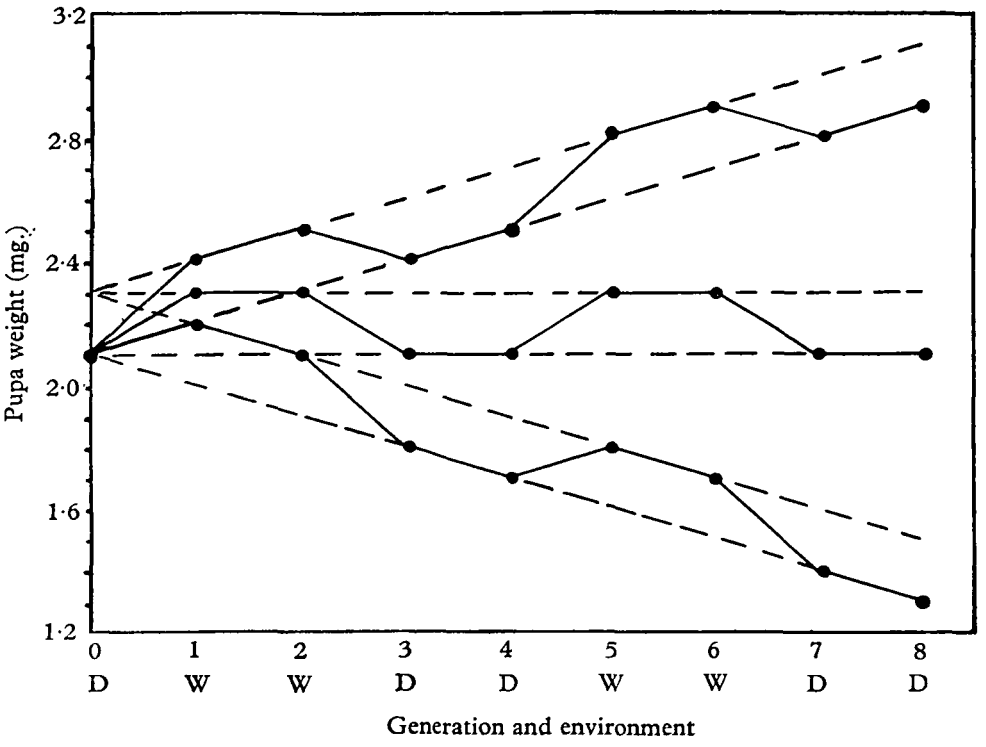

Fig. 1. Expected response under high, low and no selection.

RESULTS AND DISCUSSION

The mean pupa weights for each line and generation for Replication I and Replication II are recorded in Tables 2 and 3, respectively. Linear regressions of mean pupa weight on generation number were computed. For each of the lines which were observed from generation 0 through 8 inclusive, a 9-point regression was computed. Eight-point regressions (generations 1–8) were computed for repeated selected, directionally selected, and the average master control lines. Five-point regressions (generations 4–8) were computed for relaxed selected, directionally selected, and the average master control lines. Estimates (b) of the slopes (β) are tabulated together with their standard errors in Table 4 and will be referred to throughout this section. Any significant b value observed in unselected lines indicates that random drift has occurred in that line.

The lack of drift for methods C_1 and C_2 is evidence that there has been no consistent effect on the offspring due to ageing of the parents in either C_1, cold storage of different parental samples, or C_2, repeated samples from the same parents. Therefore, considerable confidence can be placed in these two methods of estimating the performance level each generation and the best biological indicator of the performance level is the average of these two master controls. The average value of these two lines, $\overline{C_1 C_2}$, has been used as a standard for comparison in this experiment.

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Table 2. *Mean pupa weight of offspring in tens of micrograms by line and generation, Replication I*

	Generation and environment								
	0	1	2	3	4	5	6	7	8
Line	Dry	Wet	Wet	Dry	Dry	Wet	Wet	Dry	Dry
C_1	220·0	226·0	223·9	221·6	221·2	225·4	217·5	226·8	224·4*
C_2	216·9	228·1	224·3	221·5	213·7	227·4	226·2	223·1	220·3
$\overline{C_1C_2}$	218·4	227·0	224·1	221·6	217·4	226·4	221·8	225·0	222·4
L	216·9	234·6	243·7	251·7	268·8	265·6	270·1	306·1	307·9
TL	—	228·1	235·8	244·4	246·5	266·4	267·8	288·3	299·0
XL	—	—	—	—	268·8	257·7	253·8	263·8	259·5
S	216·9	215·9	198·0	193·4	177·1	190·2	171·4	150·5	136·7
TS	—	228·1	217·9	190·2	188·2	195·8	192·0	154·4	142·6
XS	—	—	—	—	177·1	191·3	192·1	180·6	180·7
Z	216·9	226·6	216·8	225·0	213·3	222·0	215·9	222·0	214·2
M	223·7	224·3	224·9	235·6	230·9	227·4	232·1	235·8	234·1
R_o	223·6	222·5	223·8	218·8	207·6	229·5	222·8	215·9	211·5
R_s	220·0	228·0	221·3	226·1	220·9	225·4	226·0	230·7	225·4
R_n	228·0	223·0	221·9	222·9	221·5	232·1	228·2	231·8	219·1
R_{sn}	217·4	226·2	219·9	221·9	217·1	227·9	225·6	219·0	214·6
I_1	198·4	216·2†	214·5	213·9	220·0	201·2	205·0	221·5	220·4
I_2	181·5	191·4†	184·5	190·5	188·3	187·1	191·6	188·1	190·4
I_{12}	214·8	197·1†	202·9	211·7	221·9	190·2	193·6	217·6	211·3

*Mean of two groups.

†Parents of both replications were pooled to produce these offspring.

Table 3. *Mean pupa weight of offspring in tens of micrograms by line and generation, Replication II*

	Generation and environment								
	0	1	2	3	4	5	6	7	8
Line	Dry	Wet	Wet	Dry	Dry	Wet	Wet	Dry	Dry
C_1	215·3	229·4	229·2	220·6	214·9	227·1	222·8	223·6	216·0*
C_2	212·1	228·7	226·2	211·3	206·3	226·2	228·8	213·1	204·7
$\overline{C_1C_2}$	213·7	229·0	227·7	216·0	210·6	226·6	225·8	218·4	210·4
L	212·1	231·6	244·4	248·1	264·0	264·4	262·1	294·7	292·5
TL	—	228·7	236·8	237·8	240·8	260·3	266·2	287·2	291·8
XL	—	—	—	—	264·0	252·7	250·4	273·7	265·4
S	212·1	212·0	201·7	167·8	161·0	177·5	168·6	131·8	126·0
TS	—	228·7	215·3	185·6	171·9	189·0	177·8	144·6	130·8
XS	—	—	—	—	161·0	191·4	187·0	164·8	164·8
Z	212·1	230·2	227·7	207·0	209·2	229·9	223·2	208·9	205·1
M	219·3	219·3	228·4	218·8	221·9	231·9	230·6	227·8	224·4
R_o	217·0	227·2	224·4	214·3	215·3	227·2	229·2	225·5	221·5
R_s	218·1	229·5	227·2	223·2	227·1	228·0	226·2	232·4	221·4
R_n	217·8	222·7	224·2	218·1	221·8	224·6	222·9	229·4	222·7
R_{sn}	215·4	225·9	221·1	212·6	218·9	228·4	223·4	224·7	216·7
I_1	215·7†	212·6	217·3	232·2	228·6	224·8	225·5	239·2	246·0
I_2	191·7†	182·7	186·1	192·8	193·7	187·1	193·6	191·9	192·0
I_{12}	206·5	192·1	197·9	211·2	219·0	206·2	206·7	226·4	229·0

*Mean of two groups.

†Parents of both replications were pooled to produce these offspring.

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Table 4. *Estimates of β computed from regression of pupa weight on generation number in tens of micrograms per generation*

	9 points replication				8 points replication		5 points replication	
Line	I		II		I	II	I	II
C_1	0·18	(0·420)	−0·35	(0·791)				
C_2	0·14	(0·666)	−0·94	(1·316)				
L	10·75		9·38		10·44	8·57	11·87	8·73
TL					10·10	9·50		
XL							−1·25 (1·981)	2·38 (3·234)
S	−9·56		−10·69		−10·06	−11·10	−12·05	−11·57
TS					−10·75	−12·44		
XS							−0·35 (2·499)	−1·90 (5·079)
Z	−0·29	(0·639)	−1·30	(1·366)				
M	1·37*	(0·432)	1·06	(0·585)				
R_o	−0·99	(0·867)	0·59	(0·742)				
R_s	0·64	(0·421)	0·41	(0·579)				
R_n	0·21	(0·649)	0·73	(0·395)				
R_{sn}	−0·26	(0·631)	0·37	(0·741)				
I_1	1·20	(1·107)	3·50†	(0·757)				
I_2	0·61	(0·407)	0·64	(0·482)				
I_{12}	0·12	(1·550)	3·42*	(1·102)				

Values in parentheses are standard errors of the regression coefficients.
*Significant at $p = 0·05$.
†Significant at $p = 0·01$.

The mean values by environment are given for $\overline{C_1C_2}$ for each replication in Table 5. The analysis of variance shows that the environmental difference is significant in both Replication I and in Replication II. The magnitude of the difference is considerably less in Replication I, which suggests that the humidity

Table 5. *Environmental main effects by line and replication*

		Mean pupa weight in tens of μg		Analysis of variance mean squares	
Line	Rep	Wet	Dry	Environment	Within
$\overline{C_1C_2}$	I	224·8	221·0	33·20*	7·84
	II	227·3	213·8	402·30‡	7·66
I_1	I	209·2	219·0	189·15*	32·32
	II	220·0	236·5	541·20†	48·96
I_2	I	188·6	189·3	0·91	6·83
	II	187·4	192·6	54·60*	10·74
I_{12}	I	196·0	215·6	774·21‡	27·60
	II	200·7	221·4	854·91‡	56·77

*Significant at $p = 0·10$.
†Significant at $p = 0·05$.
‡Significant at $p = 0·01$.

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effect was not as predictable as the preliminary work had indicated. The lack of replication consistency on this point does not affect comparisons between methods within replications and simply indicates that a considerable amount of variation remains uncontrolled from generation to generation.

Base controls

A relatively small number of pupae were obtained in generation 0 from the inbred lines and their cross. In each case the number was about one-third of that obtained in later generations. Because of the relative imprecision of these generation 0 estimates, they have been omitted in summarizing the results of the inbred lines and their cross (Table 5).

From Table 5 it can be seen that I_1 and I_{12} were significantly heavier in the dry than the wet environment. There is some indication that I_2 behaved in a similar fashion in one replication. The response to the humidity difference as exhibited by these inbreds and their cross is opposite in direction to the response of the foundation population as indicated by $\overline{C_1 C_2}$. The inbred lines were chosen by chance without regard for their response to humidity differences. The fact that they behave in an opposite fashion to the foundation stock points to inherent differences between strains in their response to environmental differences. Such a result should serve as a caution against attempting to use genetic material from one source to separate environmental effects from genetic material derived from another source. This result illustrates that the restricted genotypes of inbred lines may give specific responses to environmental shifts which are not necessarily the same as those of unrelated stocks.

A further interesting observation may be made relative to the inbred lines. When the mid-parent value is subtracted from the mean of I_{12} each generation and the mean of these differences is computed within environment, the results given in Table 6 are obtained. The cross is significantly heavier than the mid-parent in the dry environment and consistently lighter in the wet environment. This can be interpreted as conditioning of the expression of dominance effects by the environment. This result is similar to those obtained by Griffing (1954) who observed that the expression of heterosis was influenced by the environment.

Table 6. *Comparison of body-weight of the inbred cross with mid-parent values when summarized by replication and environment*

	Mean difference in μg	
Rep	Wet	Dry
I	−30	141†
II	−30	60*

*Significant at $p = 0{\cdot}10$.
†Significant at $p = 0{\cdot}01$.

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Each of the base controls which originated from the foundation population was compared to the average master control, $\overline{C_1C_2}$. In each case $\overline{C_1C_2}$ was subtracted from the mean of the base control for each generation within each replication. Paired comparisons of this type provide an estimate of bias for each method. An ideal control would be one which did not show genetic drift, as indicated by the lack of a significant regression (Table 4), and which was not biased, as indicated by the lack of significant differences when compared to $\overline{C_1C_2}$. The mean differences are summarized by environment and replication in Table 7.

Table 7. *Comparison in both environments of several base controls with the average master control*

		Mean difference in μg	
Comparison	Replication	Wet	Dry
$Z-\overline{C_1C_2}$	I	−45*	−27
	II	+5	−54*
$M-\overline{C_1C_2}$	I	+24	+111‡
	II	+3	+86†
$R_0-\overline{C_1C_2}$	I	−2	−55*
	II	−3	+49*
$R_s-\overline{C_1C_2}$	I	+4	+37
	II	+4	+107‡
$R_n-\overline{C_1C_2}$	I	+15	+37
	II	−37*	+81‡
$R_{sn}-\overline{C_1C_2}$	I	+1	−30
	II	−26	+38§

§ Significant at $p = 0{\cdot}10$.
* Significant at $p = 0{\cdot}05$.
† Significant at $p = 0{\cdot}01$.
‡ Significant at $p = 0{\cdot}001$.

While the stabilized selected line gave no indication of drift (Table 4), it does deviate significantly from $\overline{C_1C_2}$ in the dry environment in one replication, but so do all the other base controls shown in Table 7. It is, however, one of only two lines which give significant deviations in the wet environment. Not much importance can be attached to these differences since they are inconsistent. These results are similar to those reported by Falconer (1957) in that he did not find any change in phenotypic variance after thirteen generations of selection for phenotypic intermediates in *Drosophila*.

The significant regression observed in Table 4 for Replication I of the mass mated random-bred line (M) is reflected in Table 7 by the large deviation of M from $\overline{C_1C_2}$. A similarly large deviation was observed in Replication II in the dry environment although the overall regression for this replication was not found to be significant (Table 4). Since the inbred lines were maintained by mass matings, the significant regressions for I_1 and I_{12} are of particular interest. Apparently, some drift occurred

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in inbred I_1 which not only affected its mean body-weight but also that of the cross I_{12}. These results indicate that the mass mating method is more subject to drift than other systems of random breeding which make use of single-pair matings.

None of the four single-pair methods of random breeding gave any indication of drift by regression analysis (Table 4). They all show in Table 7 some significant deviations from the average master control, but the superiority of any one method over the others is not obvious. Neither of the two criteria utilized here have differentiated between the four single-pair mated random-bred controls.

Effectiveness of restrictions

The lack of drift and consistent bias due to any of the four methods which were included to test the two restrictions described by King *et al.* (1959) deserves some comment.

The theory of chance fluctuations in gene frequency has been developed over the past forty years. Wright (1921 and 1931) considered both regular systems of inbreeding and random mating. Crow (1954) summarized the work to that time and presented the following formula, the validity of which was demonstrated by Crow & Morton (1955):

$$V_{\delta q} = \frac{q(1-q)}{4N}\left[1-F'+(1+F')\frac{V_k}{\mu_k}\right]$$

where $V_{\delta q}$ is the variance in gene frequency change in one generation due to dispersive factors, q is the frequency of the allele under discussion, N is the total number of offspring, μ_k and V_k are the mean and variance of the number of surviving offspring per parent, and F' is Wright's coefficient of inbreeding, used as a measure of the departure from random mating zygote proportions among the parents.

Gowe *et al.* (1959) presented an alternative formula for the variance of the change in gene frequency which does not contain F' since they considered truly random breeding populations. Their general approach is considered in detail by Latter (1959).

It has been assumed that the distribution of progeny number is Poisson such that $V_k = \mu_k$. The portion of the variance of δq due to the unequal distribution of progeny number can be theoretically eliminated by equalizing the number of individuals drawn from each parent such that $V_k = 0$. The total variance due to genetic drift is then one-half of that derived when the Poisson distribution is assumed (Crow, 1954; Gowe *et al.*, 1959). It is expected that such an effect will result from the equal number restriction imposed by King *et al.* (1959).

The other restriction utilized by King *et al.* (1959) will have its effect on F' in the formula given by Crow & Morton (1955). In relatively large populations (50 males and 50 females or more) the effect in any given generation will be small and can be estimated by the following formula given by Wright (1931):

$$\left(\frac{1}{8Nm}+\frac{1}{8Nf}\right)\left(1-\frac{1}{8Nm}-\frac{1}{8Nf}\right).$$

This restriction is not the same as consistently avoiding matings of related animals by the use of pedigrees which go back to the base population. If one only restricts against sib mating within each generation with no reference to the complete pedigree structure the relationship of individuals within that generation is only randomly influenced by the relationship of individuals within the base population. The major effect of this restriction is to prevent the union of gamates bearing genes identical by descent in the second generation. The *rate of inbreeding* is the same whether this restriction is imposed or not even though the *inbreeding coefficient* in any single generation with respect to the previous generation is slightly lower and is more uniform between individuals (Falconer, 1960). Since the restriction can only have a small effect on a factor (F') which itself has only a small effect on $V_{\delta q}$, it cannot be expected to be of major importance in reducing drift variance. It is expected that the first of these two restrictions would be considerably more effective than the second.

Since the number of breeding parents was kept fairly large in this experiment we were working with drift variances which were relatively small. It is expected that these restrictions would be more important in smaller populations.

Base controls vs. repeat or relaxed controls

As selection progresses the genotypes of the selected groups change. From the standpoint of describing genetic gain in response to selection, a good control is one which measures environmental changes as they affect the selected line. It follows that the selected lines are the best standards for comparison amongst controls for this purpose.

Environmental changes between succeeding generations were computed for each of: $\overline{C_1 C_2}$, TL, TS, XL, XS, and the average of the four single-pair random-bred base controls. For all of these lines except TL and TS the change in mean pupa weight from one generation to the next was obtained by performing subtractions of the type $\bar{M}_n - \bar{M}_{n-1}$, where $\bar{M}_n$ is the mean pupa weight for a particular method in the nth generation. For the repeated lines the selected mean for the $n-1$ generation was used with the repeated mean for the nth generation. Since these are two samples of offspring one generation apart from the same parents, this method provides an estimate of the environmental change.

Phenotypic gain by generation was obtained by subtraction: $\bar{S}_n - \bar{S}_{n-1}$, where $\bar{S}_n$ is the mean pupa weight for a selected line for the nth generation. The environmental changes as indicated by the various controls were then used to compute genetic gain for each generation in each of the large and small lines as follows:

$$\text{genetic gain} = \text{phenotypic gain} - \text{environmental change}$$

The values so obtained are given in Table 8. All methods of computing genetic gain indicate a response to selection in the direction in which selection was applied. However, there is considerable variability in the consistency of the response indicated by two of the methods. The best control to use for calculating genetic gain is the one which gives the most consistent result. Variances were computed for each method

Table 8. *Genetic gains in micrograms by generation with variances among the first four and among the last four estimates of gain*

		Generation and environment								Variance	
		W–D	*W–W*	*D–W*	*D–D*	*W–D*	*W–W*	*D–W*	*D–D*	1st	2nd
Comparison	Rep.	1–0	2–1	3–2	4–3	5–4	6–5	7–6	8–7	4	4
L–$\overline{C_1C_2}$	I	+91	+120	+105	+213	−122	+91	+328	+44	3,038	34,538
	II	+42	+141	+154	+213	−156	−15	+400	+58	5,035	55,778
L–Av. 4 Rs	I	+150	+123	+73	+227	−151	+76	+372	+86	4,135	45,895
	II	+103	+149	+109	+121	−58	−7	+300	+52	417	25,175
L–*TL*	I	+65	+79	+73	+223	−8	+23	+178	+89	5,708	6,772
	II	+29	+76	+103	+232	+41	−41	+75	+7	7,550	2,452
L–*XL*	I	—	—	—	—	+79	+84	+260	+61	—	8,685
	II	—	—	—	—	+117	0	+93	+61	—	2,566
S–$\overline{C_1C_2}$	I	−96	−150	−21	−121	+41	−142	−241	−112	3,054	13,647
	II	−154	−90	−222	−14	+5	−81	−294	+22	7,899	21,077
S–Av. 4 Rs	I	−37	−147	−53	−107	+12	−157	−197	−70	2,551	8,689
	II	−93	−82	−267	−106	+103	−73	−394	+16	7,607	47,051
S–*TS*	I	−122	−199	+32	−111	−56	−206	−39	−59	9,277	6,058
	II	−167	−136	−178	−109	−115	−92	−128	−48	975	1,235
S–*XS*	I	—	—	—	—	−11	−196	−94	−139	—	6,098
	II	—	—	—	—	−139	−45	−146	−58	—	2,797

within replication. The experimental period was divided in half leaving four estimates of gain in each half so that each variance describes the variability of either the first or the last four consecutive estimates of genetic gain (Table 8). The variances for the first half of the experiment indicate that there were no differences between the three control methods regardless of the direction of selection.

In the second half of the experiment, however, large variances are found for $\overline{C_1C_2}$ and for the average of the four random-breds. These methods do not give consistent estimates of genetic gain in the latter half of the experiment. Variances obtained from the TL and XL methods are similar to those obtained in the first half. The Bartlett test for homogeneity of variances indicates that the eight values from $\overline{C_1C_2}$ and the average of four random-breds in the second half are not different from each other but are different from the remaining 20 variances in Table 8 ($P = 0{\cdot}01$). The other 20 variances are also not significantly different from each other.

In the case of the relaxed lines drift is a second criteria of interest. No significant b values were obtained from the relaxed lines (Table 4).

These results demonstrate that a random-bred control can be used to measure environmental changes with respect to a selected line during the early generations of a selection experiment. As the genotypes of the selected lines change under selection, different responses to environmental changes may be expected. In this experiment $\overline{C_1C_2}$ indicated that generations 5 and 6 were heavier than generations 7 and 8. The repeated large line indicated that the opposite was true (Tables 2 and 3). The large line changed its response to the environmental difference so that the selected large line weighed more in dry than in wet in later generations. A similar change in response to environmental differences occurred in the selected small line. This line became more sensitive to the environmental difference after selection although the direction of the response was not altered.

Symmetrical response to selection

It is obvious that selection in opposite directions produced response in each direction. It was the object of this test to determine whether or not this response was symmetrical. If the b values as given for large, small and their repeated lines (TL and TS) in Table 4 were considered as the variable, a large direction effect would be detected. The question of much greater significance is that of the relative magnitude of these values. The negative values obtained from the small direction were considered as positive response to selection in that direction and so were rescaled. If one uses the rescaled value of these b's and still detects a significant effect for direction, then the response to selection in two directions has not been symmetrical. On the other hand, a lack of significance of such a direction effect indicates symmetry of response. Neither of the main effects nor the interaction were significant. It was concluded that the response to selection was symmetrical.

Differential response to selection within environments

In order to examine the effect of each environment separately on the two directionally selected lines, within environment regressions were computed. These values are shown in Table 9, where the 2×2 factorial arrangement of selected lines with

environment is apparent. The use of the rescaled values of the regressions make this analysis a test of symmetrical response to selection similar to that discussed in the previous section. If a significant line effect cannot be detected, then it may be concluded that the response to selection has been symmetrical on both environments provided the interaction was also not significant.

Table 9. *Values of* b *within environments, tens of micrograms per generation*

Environment	Replication	Selected line: Large	Selected line: Small	Means of rescaled totals	$\overline{C_1 C_2}$ for comparison
Wet	I	7·15	−7·24	7·29	−0·56
	II	6·25	−8·52		−0·56
Dry	I	11·95	−10·06	10·80	0·67
	II	10·56	−10·64		−0·04
Average				9·05	−0·12

The resulting analysis uncovered only one significant effect, environments. Neither the interaction nor the main effect for lines was significant and it was concluded that symmetrical response was obtained. Regardless of the direction of selection, more rapid progress has resulted where observations were recorded in the dry environment than in the wet. Perhaps this divergence would taper off if selection of this nature were continued. The within environment regression lines are shown in Fig. 2.

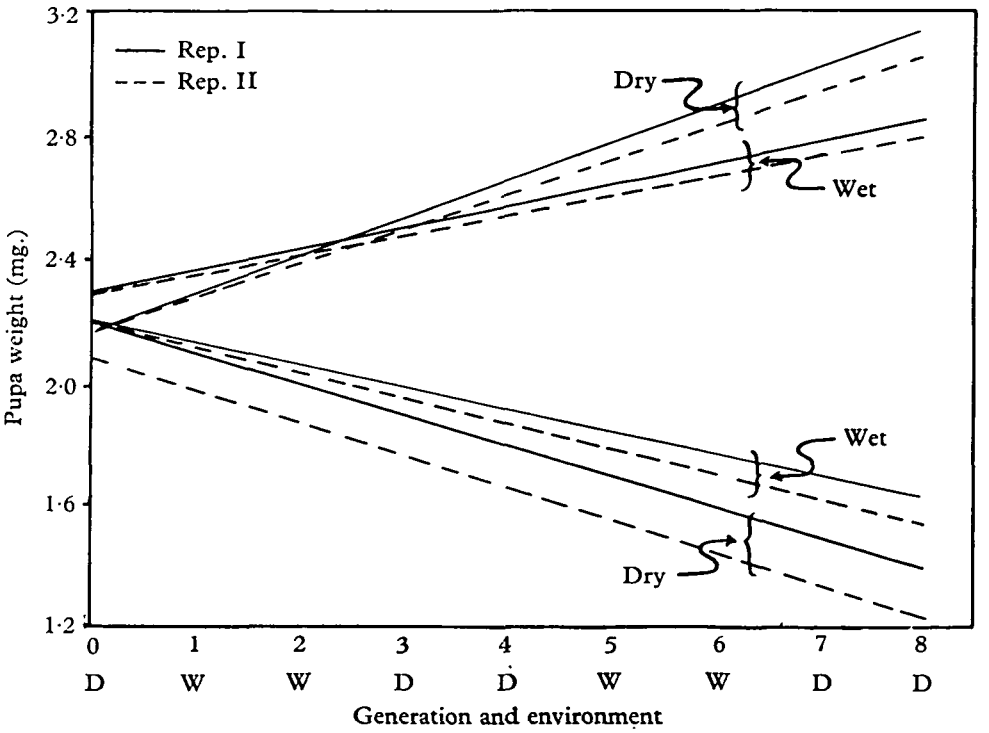

Fig. 2. Regression lines within environment showing symmetrical response.

x

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This result describes the change in response of the selected lines to shifts in the environment. Evidence for intra-strain interaction in the base population will be presented in a later section. The authors suggest that selection for large size in this experiment resulted in the selection of families which produced heavier offspring in the dry environment than in the wet environment. Figure 2 illustrates that an interaction of equal magnitude existed in the down direction as well as indicated by the divergence of those lines. In this instance the authors suggest that this selection scheme has resulted in the selection of families which produced heavier offspring in the wet environment than in the dry. It should be noted that the slopes of change in weight in either direction are essentially the same for the same environment.

Estimation of variance dependent on the environment

A differential response to selection dependent on the environment has been detected. The interpretation of this result from a selection point of view is difficult since the two environments have been applied in an alternating manner. Nevertheless, the data do suggest that selection for larger or smaller pupa weight in the dry environment has been more effective than in the wet.

McNary (1960) using stock from the same foundation population in the same environments has indicated that differential heritability will not explain this result. He determined heritability of pupa weight to be approximately 0·55 in both environments. He also reported the phenotypic variance of pupa weight for pupae grown in the low humidity to be approximately double that observed in the high humidity.

Although no direct estimates of individual variance were considered in the design of this experiment a method of estimating it is available. Assuming no maternal effects, no sex linkage, and the within male variance to be equal to the within female variance, it can be shown that the individual within sex variance is equal to twice the variance of the mean of a pair (one male and one female, each chosen at random).

Pair estimates from methods R_s and R_{sn} are valid estimates since no selection was applied to these lines. The pair estimates were adjusted to an individual basis and the pooled values are shown in Table 10. They show that the variance of pupae grown in the dry environment is consistently greater than the variance of pupae grown in the wet environment by at least a factor of two.

Table 10. *Pooled estimates of individual variance in (tens of μg)^2*

		Environment and generation				
		Dry	Wet	Dry	Wet	Dry
Rep.	Line	0	1, 2	3, 4	5, 6	7, 8
I	R_s	814	284	829	305	700
	R_{sn}	678	332	876	321	990
II	R_s	884	260	713	306	650
	R_{sn}	884	311	683	144	792

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The data indicate that the more rapid response to selection in the dry environment may be associated with the greater phenotypic variance in that environment. Although the hypothesis cannot be tested with these data, since the experiment was not designed to test this point, the following explanation is suggested.

The usual equation for genetic gain may be given in the following form:

$$\Delta \bar{G}_g = h^2 \bar{\imath} \sigma_P$$

Where $\Delta \bar{G}_g$ is the expected change in the genetic mean of the population, h^2 is heritability in the narrow sense and may be expressed as the ratio of additive genetic variance to phenotypic variance, σ_G^2/σ_P^2, $\bar{\imath}$ is the selection differential expressed in standard deviations and σ_P is the phenotypic standard deviation.

It follows that the equation can be written for each of the two environments:

dry: $$\Delta \bar{G}_{g_D} = h_D^2 \bar{\imath}_D \sigma_{P_D}$$

wet: $$\Delta \bar{G}_{g_W} = h_W^2 \bar{\imath}_W \sigma_{P_W}$$

As indicated previously, we may consider $h_D^2 = h_W^2$. Theoretically $E(\bar{\imath}_D) = E(\bar{\imath}_W)$ since the same numbers were required in each environment. If differential mortality had any effect, it was to lessen the selection intensity in the dry environment, since more reproductive difficulty was experienced in dry than in wet. Both these data and those of McNary (1960) indicate that $\sigma_{P_D}^2 = C\sigma_{P_W}^2$ where C is not less than one. Given these conditions, $\Delta \bar{G}_{g_D} > \Delta \bar{G}_{g_W}$ as indicated in Fig. 2. Since $\sigma_{P_D}^2 > \sigma_{P_W}^2$ while $h_D^2 = h_W^2$, we must conclude that $\sigma_{G_D}^2 > \sigma_{G_W}^2$. No critical test of this hypothesis is possible in an experiment of this type where generations and environments are confounded.

Evidence of intra-strain interaction in the base population

The differential variances discussed in the previous section provide a possible explanation for the observed differential rates of response to selection. This fact alone, however, does not fully account for the behaviour of the selected large line. It is possible that some families of the base population may have found the dry environment more favourable for large weight even though the population means showed that the wet environment favoured large weight.

It will be remembered that the original pairs from which the selected lines were derived (C_2) were repeated each generation. This method of reproducing the same full sib groups in time gives repeated observations on the two environments. Thus a factorial arrangement of pairs and environments exists. One major drawback of this approach is that a certain number of pairs die off each generation. Because of this the analysis was run for each replication on those pairs which produced a full sib group in generations 0 through 5. This procedure allows three observations within each environment for each pair.

The results are shown in Table 11. Both main effects and the interaction are significant at the 1% level.

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This analysis provided evidence that although the wet environment increased weight on a population basis, some pairs produced offspring which were heavier when grown in the dry environment.

Table 11. *Analysis of variance showing the interaction of pairs with environments with three observations per cell*

	Replication I		Replication II	
Source	d.f.	M.S.	d.f.	M.S.
Pairs	35	47,249·7*	23	41,303·8*
Environments	1	442,092·5*	1	832,352·1*
$P \times E$	35	23,997·2*	23	13,345·6*
Error	144	4,031·7	96	3,701·2
Total	215		143	

*Significant at the 1% level.

Implications with respect to animal breeding

The validity of transferring the results of an experiment involving the use of a pilot organism, of course, remains in question. Nevertheless, it seems apparent that once a genotype by environment interaction has been demonstrated to be associated with selection in any animal, animal breeders should take the necessary precautions to detect it.

The use of random-bred control strains of economic species to 'control' selected stocks from a different genetic origin is questionable. This does not detract from their usefulness in genetic studies. It does suggest, however, that such studies should be done using the control strain itself as a foundation stock. Should one desire information concerning the separation of environmental effects from the rate of improvement in some stock not related to such a control, then he should consider the maintenance of some control closely related in origin and time. It would be necessary to adopt both a base control method and one closely associated with the selected lines (repeated parent, or repeated origination of relaxed selected) if the detection of possible genotype by environment interactions were desired.

SUMMARY AND CONCLUSIONS

Fifteen methods of maintaining control populations have been studied over eight generations using the flour beetle, *Tribolium castaneum*. Populations were reproduced each generation from 50 males and 50 females. The methods were compared as to their ability to establish the level of the environment with respect to the base population and to separate environmental and genetic effects in two directionally selected lines.

It was demonstrated that a foundation stock of these beetles produced pupae which weighed about 10% more when grown in 70% relative humidity than when grown in 40% relative humidity. All lines were grown in one environment for two

generations and then in the other environment for the next two generations. The mean pupa weights of two master controls were averaged to give an average master value which served as a standard against which the other controls were compared.

Inbred lines derived from a separate source of stock responded differently to the environments than did the foundation stock from which selected lines were drawn.

Evidence of genetic drift in mass mated lines was presented. No differences among the other methods of maintaining the original population were observed. It was suggested that if a smaller number of families had been used, additional differences might have been detected.

Response to selection in both directions over eight generations was symmetrical. Symmetry was also demonstrated for within environment regressions and it appears that greater progress was made in the dry environment than in the wet.

Initially the foundation stock weighed more in the wet environment than in the dry environment. After selection, the large line weighed more in the dry than in the wet. It was shown that the original population contained the basis for this interaction of directionally selected lines with environments since some of the initial families weighed more in dry than in the wet environment. It was suggested that the apparent differential response to selection, dependent upon the environment, may have been due to the larger variance in the dry environment. This variance was shown to be about twice as large as that in wet.

Due to the presence of the interaction, the base control populations were ineffective in separating genetic and environmental effects in the later generations of the selected lines. Repeated and relaxed methods of maintaining controls more closely indicated how environmental shifts affected the later generations of the selected lines.

It is concluded that a control line must be closely related to the selected line in origin and time in order for its reactions to environmental shifts to be similar in nature to the selected line. If these conditions are not met, undetected genotype by environment interactions may contribute to faulty comparisons. In addition, some method of reproducing the original population must be followed if interactions with respect to the origin are to be detected.

It is further concluded that it is unnecessary to place restrictions on the method of mating base populations for the purpose of maintaining control stocks, provided that the size of the breeding population is not less than 50 males and 50 females and that mass mating is not used.

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