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RESPONSE TO SELECTION IN TRIBOLIUM CASTANEUM

by

John Verne Moore

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Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	2
SOURCE AND COLLECTION OF DATA	10
METHODS OF ANALYSES	17
RESULTS	21
DISCUSSION	49
CONCLUSION	61
BIBLIOGRAPHY	63
ACKNOWLEDGMENTS	65

LIST OF TABLES

	Page
Table 1. Length of life cycle in days of <u>Tribolium castaneum</u> after Gray (1948) (G) and Park and Frank (1948) (P)	2
Table 2. Laboratory work schedule	14
Table 3. Numbers and means of unadjusted data by subclass for the least squares analyses employed to give constants	23
Table 4. Analyses of variance of pupation age, pupal weight and emergence age	26
Table 5. Least squares estimates of constants for pupation age, pupal weight and adult emergence age	27
Table 6. Analysis of variance of pupation age used to estimate heritabilities	29
Table 7. Analysis of variance of pupal weight used to estimate heritabilities	29
Table 8. Analysis of variance of emergence age used to estimate heritabilities	30
Table 9. Expected mean squares of analyses of variances presented in Tables 6, 7 and 8	30
Table 10. Estimates and standard errors of heritabilities of pupation age, pupal weight and emergence age computed from components of variance	31
Table 11. Genotypic and phenotypic correlations computed in random mating population. Genotypic correlations are above diagonal, and phenotypic correlations are below the diagonal	32
Table 12. Analyses of variance of pupation age, pupal weight and adult emergence age between and within generations within populations	33
Table 13. Numbers, adjusted means and standard deviations of traits by population and generation	35
Table 14. Fractions of males and females selected and standardized selection differentials in the Weight population	39

LIST OF TABLES (continued)

	Page
Table 15. Expected and actual responses to selection in the Weight population	39
Table 16. Fractions of males and females selected and standardized selection differentials in the Early Pupation population	40
Table 17. Expected and actual responses to selection in the Early Pupation population	41
Table 18. Deviations of average pupation age of selected males and females in the Control population from generation averages	42
Table 19. Deviations of average pupal weight of selected males and females in the Control population from generation averages	43
Table 20. Deviations of average emergence age of selected males and females in the Control population from generation averages	44
Table 21. Correlations of expected and actual average responses to selection	48
Table 22. Expected components of variances from Dickerson (1960)	53

LIST OF FIGURES

	Page
Figure 1. Average pupation age by generation for three populations	45
Figure 2. Average pupal weight by generation for three populations	46
Figure 3. Average emergence age by generation for three populations	47

INTRODUCTION

The creation and accurate measurement of genotypic and phenotypic changes in quantitative traits having economic value have always been among the goals of animal improvement. Selection is one of the primary ways to create such changes. The measurements of direct and indirect responses to selection provide estimates of the changes.

Difficulties in accurately measuring responses to selection have frequently occurred, particularly in traits associated with reproduction and fitness. Much time is required to accumulate data over several generations of selection. Large environmental changes may occur over time. The amount of space required to maintain optimum population sizes has also been a limiting factor in measuring responses to selection. Thus, the use of a laboratory animal which has a short generation interval and can be reared in sufficient numbers in a constant environment would be beneficial in elucidating responses to selection. Nevertheless, caution must be taken in extrapolating directly from the results obtained from such investigations to the improvement of traits in other organisms in which distinct physiological and environmental differences may exist.

The purposes of this investigation were to estimate the effects of day of weighing during the pupal stage, size of creamer bottle, sex, and full sib family size on pupation age, pupal weight and adult emergence age in Tribolium castaneum. Estimates of phenotypic and genotypic parameters were also obtained from random mating populations. Response to mass selection for heavy pupal weight was measured. In another population response to mass selection for early age at time of pupation was investigated. Indirect responses to selection were also considered in each population.

REVIEW OF LITERATURE

The flour beetle, Tribolium castaneum, has been used extensively in ecological and genetic investigations. However, only in recent years has the organism become a relatively common pilot research animal in the areas of population genetics and animal breeding. The organism is easily maintained on an uncomplicated diet in relatively inexpensive facilities. It has a high rate of reproduction, short life cycle and a long life span. Stages in the life cycle are easily identified, and sex can be determined before sexual maturity. There is equal crossing over in males and females. The organism is subject to parasitism and will exhibit cannibalistic tendencies.

The life cycle consists of the egg, larva, pupa and adult. The length of the life cycle at different temperatures is shown in Table 1. Five to eight days should be added to the totals to allow for time required to reach sexual maturity.

Table 1. Length of life cycle in days of Tribolium castaneum after Gray (1948) (G) and Park and Frank (1948) (P)

Stage	Temperature (Centigrade)					
	P	G	P	G	P	G
	24.0°	25.0°	27.2°	29.0°	32.8°	34.0°
Egg	7	6	5	4	3	3
Larval	33	34	24	17	16	16
Pupal	<u>10</u>	<u>10</u>	<u>8</u>	<u>5</u>	<u>4</u>	<u>4</u>
Total	50	50	37	26	23	23

Parameter Estimation

Bartlett et al. (1966) and Bartlett and Bell (1962) reported estimates of heritability of pupal weight by pooled regression of offspring on mid-parent. The progeny were raised in four gm. of standard media and were obtained from a 48-hour egg collection. The environmental conditions were $33^{\circ} \pm 1^{\circ}\text{C}$. and $70 \pm 2\%$ relative humidity. The estimates of heritability were $0.44 \pm .05$ in a random mating foundation strain which averaged approximately $2,100\mu\text{g}$ in pupal weight and $0.34 \pm .08$ in a population which had been under selection for heavy pupal weight for 44 generations. The average weight in the selected strain was $5,500\mu\text{g}$. Bell and Moore (1958) reported higher heritability estimates (0.60 - 0.80) for pupal weight in a random mating population. Male pupal weights averaged $2,100\mu\text{g}$, and female pupal weights averaged $2,300\mu\text{g}$. The phenotypic standard deviation of pupal weight was $250\mu\text{g}$.

Bell and McNary (1963) found no significant difference between the heritability estimates of pupal weight in 70% relative humidity and in 40% relative humidity. The estimates were 0.48 in the wet environment and 0.55 in the dry environment. However, the phenotypic variation under dry conditions was nearly double that variation under wet conditions. Scheinberg et al. (1967) indicated a much higher estimate of $0.80 \pm .06$ in a random mating population. Average pupal weights ranged from $2,037\mu\text{g}$ to $2,166\mu\text{g}$.

Wilson et al. (1963) reported low realized heritability estimates of pupal weight ranging from 0.28 to 0.35. Enfield et al. (1966) reported realized heritability estimates for pupal weight of $0.27 \pm .03$ and $0.34 \pm .02$ for pupal weight in two replicates over 12 generations of selection for heavy pupal weight. From each generation the

heaviest male and the two heaviest females were selected from each half sib family. Matings among selected individuals were random except that full sib matings were avoided. The estimates of heritabilities of pupal weight from sire component analyses were $0.36 \pm .04$ and $0.27 \pm .05$ for males and females, respectively. Estimates of heritability of pupal weight from sire-female offspring regression were $0.36 \pm .05$, from sire-male offspring regression, $0.34 \pm .05$, from dam-male offspring regression, $0.57 \pm .05$ and from dam-female offspring regression, $0.53 \pm .05$. In the data of Enfield et al. (1966) average pupal weights were $2,504 \pm 27\mu\text{g}$ and $2,558 \pm 59\mu\text{g}$ for males and females, respectively, from progeny resulting from crossing two highly inbred lines. Such crossbred progeny formed the base population.

Howe (1956) investigated the effects of temperature and humidity on developmental rate in Tribolium castaneum. Eggs did not hatch at any levels of humidity below 17.5°C . or at 10% relative humidity at 40°C . Humidity did not appear to affect the length of the egg stage. However, larvae failed to develop into normal adults at 20°C . and 90% relative humidity and at 40°C . and 30% relative humidity. At 70% relative humidity and 20°C . pupae developed but did not emerge into normal adults. Length of pupal period was not affected by humidity. The ratio of number of males to the number of females approached unity under all environmental conditions, and no sex differences in developmental rate were observed.

Englert and Bell (1964) selected for early and late pupation time for six generations. The estimates of realized heritability were $0.38 \pm .03$ for early pupation and $0.26 \pm .03$ for late pupation. Dawson (1964b) reported a low estimate of heritability for age at time of pupation of less

than 0.20 based on regression of offspring on sire analyses. Use of the dam record as parent record yielded estimates ranging from 0.26 to 0.42. Regression of offspring on midparent values indicated an estimate for heritability of approximately $0.25 \pm .20$. Dawson (1964a) found that the proportion of variance attributable to maternal effects was about half as large as the proportion due to additive genetic effects in developmental time. Dawson (1964a) surmised that there were differences in substances deposited in eggs among females. Advantageous utilization of such substances occurred during the early stages of development, and later the progenies own genotypes assumed more importance. Dawson (1965) also reported relatively older ages at the time of pupation. All members of a full sib family remained together until they had become pupae. Average age at time of pupation was 23.29 days. The phenotypic variance was $5.70 (\text{da.})^2$. A total of 10,929 individuals was observed. The regression of developmental time on family size was linear and significant.

Dawson's (1965) estimates of realized heritability for pupation age were $0.14 \pm .01$ and $0.14 \pm .02$ in fast and slow selections, respectively. Selection continued for seven generations. Other estimates for realized heritability for pupation age of $0.11 \pm .01$ and $0.32 \pm .04$ for fast and slow selections, respectively, were also given. Selection has been practiced in the latter populations for 13 generations.

Scheinberg et al. (1967) used more refined laboratory procedures of collecting data and reported an estimate of heritability for pupation age of $0.43 \pm .06$. The individuals were obtained from an 18-hour egg collection and were observed every twelve hours for stage changes beginning at 13.5 days of age. Average age at time of pupation in each of three

generations of random mating were $16.46 \pm .04$, $17.19 \pm .03$ and $16.19 \pm .02$ days. Englert and Bell (1963) reported average age at pupation of 25.5 and 21.5 days in two inbred lines and 19.0 and 20.8 days in the progeny resulting from the two reciprocal crosses. The temperature was 33°C and 40% relative humidity.

Age at time of adult emergence has not been extensively reported. Howe (1956) reported average ages of 20.3 days and 27.2 days at 70% relative humidity and at 35°C . and 30°C ., respectively. Howe (1956) reported little variation in the length of the pupal period under relatively similar environments.

Scheinberg et al. (1967) reported estimates of $-0.31 \pm .08$ and $-0.20 \pm .03$ for genotypic and phenotypic correlations, respectively, between age at pupation and pupal weight. Estimates of phenotypic and genotypic variances of age at pupation were $0.3963 (\text{da.})^2$ and $0.1703 (\text{da.})^2$, respectively. Phenotypic and genotypic variances for pupal weight were $39,186 (\mu\text{g})^2$ and $31,186 (\mu\text{g})^2$, respectively. Englert (1964) calculated an average estimate of the genotypic correlation between pupal weight and age at time of pupation of 0.50 in a population selected for early pupation and -0.12 in a population selected for late pupation. Both estimates were based on deviations from a control population.

Responses to Selection

Enfield et al. (1966) selected for heavy pupal weights within half-sib families for 12 generations. Regressions of the differences in mean pupal weight between the selected populations and corresponding random mating control populations on generation time were 60 ± 5 and $62 \pm 5 \mu\text{g}$

for two replicates. The regressions of average pupal weight on generation time were -3.2 ± 4 and $-6 \pm 3 \mu\text{g}$ in the two control populations. No significant changes during the experiment in the heritability estimate of pupal weight were found. Total phenotypic variation in pupal weight appeared to have slightly increased as pupal weight increased. Response to selection in the two sexes was similar and an estimate of the correlation between male and female pupal weight of $0.97 \pm .13$ did not indicate any sex-genotype interaction. No significant effects of family density on pupal weights were found.

Bell and McNary (1963) investigated direct and correlated responses to selection of heavy pupal weight in a wet (70% relative humidity) and in a dry (40% relative humidity) environment. Good agreement between the predicted and observed response was observed over nine generations of selection in both environments. The average observed correlated responses in the two environments were accurately predicted. The ratio of predicted correlated response to the observed correlated response was $147.5/141.5$ and $139.5/138.5 \mu\text{g}$ per generation in two replicates. However, in each replicate the observed correlated response for the population selected in dry conditions was approximately twice the predicted response, while the observed correlated response for the population selected in humid conditions was only about half of the predicted value. The asymmetry in response was attributed to the differences in the two effective genetic correlations in the two environments. The estimates of effective genetic correlations were 0.64 and 0.67 for the two replicates in 70% relative humidity and 0.93 and 1.12 for the 40% relative humidity environment.

Englert and Bell (1964) selected for age at pupation in two directions.

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Asymmetrical response was observed and was thought to be due to a large difference in the selection differentials. Selection for a late age at pupation resulted in an increase in variance, while selection for an early age at the time of pupation resulted in decreased variance. The relationships between age at time of pupation and pupal weight were inconsistent.

Burris and Bell (1965) selected in two directions in each of two traits, larval weight and pupal weight, for seven generations. By the fifth generation, the selected populations ranged from 80% to 120% of the pupal weight in the control population. The selection differentials were greatest in those populations selected for heavy weights in both traits or for light weights in both traits. Selection for heavy larval weights decreased age at time of pupation about one-half day, while selection for light larval weights resulted in an increase in age at time of pupation approximately one day. The time of adult emergence was highly associated with age at time of pupation.

In general, body weight of Tribolium castaneum has been more frequently used as a trait in population and selection investigations. Pupal weight appears to be a desirable trait because it is less affected by changes in environment. The heritability estimates of pupal weight are fairly consistent, provided the same procedures in obtaining the estimates are employed. Direct response in pupal weight as a result of selection for pupal weight has been predictable.

The stage traits, age at time of pupation and age at time of adult emergence, appeared to be more variable in reports. Such variations are probably due to significant effects of changes in temperature and humidity, unsatisfactory methods of accurately measuring age at the time a stage

change occurred, unknown complex physiological processes which may somehow become altered and possible interaction of the above causes. Estimates of heritabilities of age at time of stage changes are abundant but variable. The egg collections have generally been taken over a 24-hour or longer period and frequently the individuals have only been checked once a day for stage change. Thus, there is probably considerable error in establishing the exact chronological age at which the stage change did occur, regardless whether the individuals remained with their sibs in a common bottle until a stage change occurred, or whether individuals were placed in separate bottles early in the life cycle. Perhaps some of the above statements are partial reasons for correlated responses in stage traits failing to agree with predicted correlated responses when direct selection for pupal weight was practiced, even though significant genotypic and phenotypic correlations among the traits apparently do exist.

SOURCE AND COLLECTION OF DATA

A random mating foundation pearl stock of Tribolium castaneum was established in 1960 at Purdue University. Four unrelated stocks, one of which was homozygous for the recessive autosomal eye color mutation, pearl, were systematically crossed. The stock was then propagated by the mass transfer and random mating of 250 unsexed pearl adults. The frequency of transfer was approximately three months. The stock was maintained in an environmentally controlled chamber at approximately 22.2°C. and 61.5% relative humidity.

A sample of the Purdue University pearl stock was obtained by Iowa State University in October, 1966. Thereafter, the sample at Iowa State University was maintained by random selection, transfer to fresh media and subsequent mating of approximately 200 unsexed pupae every 28 days. The population was maintained in a one-half pint milk bottle which contained approximately 120 gm. of media in a modified Jamesway incubator. The temperature was approximately 32.8°C. and the relative humidity was approximately 65%. The media was composed of 95% whole wheat flour and 5% dried brewers yeast sifted through a 60 mesh sieve, thoroughly mixed and sterilized at an internal temperature of 50°C. for one hour.

In October, 1967, 90 pairs of pupae from generation 12 of the random mating pearl population at Iowa State University were randomly selected and mated. Each pair was placed in a 3/4 oz. standard creamer bottle which contained 1.5 gm. of media. Standard cardboard caps with a side tip were used. The same Jamesway incubator was used for storage. A ten-day period allowed for adult emergence, sexual maturity and subsequent mating. A

29-hour egg lay collection was obtained from each live female in a clean 3/4 oz. creamer bottle which contained 3 gm. of media. At an age of 12 days, counting the day on which the parents were removed from the media as the first day, each full sib family was removed from the media. Individuals were removed from the media by sifting the media through a fine screen into a household vacuum cleaner. The entire family was then placed on numbered spot plates, and family size was recorded. Four larvae were chosen by use of a set of random numbers for a given family size. The four larvae were placed into individual 3/4 oz. creamer bottles each of which contained 0.2 gm. of media. The remainder of the family was discarded. Family size, age at time of pupation, pupal weight and adult emergence age were obtained on each individual. Twenty-four adult males and 72 adult females were randomly chosen and randomly mated in 3/4 oz. creamer bottles containing 1.5 gm. of media. An effort was made to select at least one individual from each full sib family. Each male was mated to three females.

Three 24-hour egg lay collections were obtained within a two week period from each of the females in the 24 base population sire groups. The first egg lay collection formed the population which was subsequently selected on the basis of heavy pupal weight and was designated as the Weight population. The individuals from the second egg lay collection formed the designated Control population in which the selection was random. The third egg lay collection produced the population which was selected for early age at the time of pupation and was designated as the Early Pupation population.

In the Weight, Control and Early Pupation populations, family sizes at 14 days were recorded. Eight larvae were randomly selected from each

family on day 14 by use of random numbers and placed in individual creamer bottles. The remainder of the family was discarded. Age at time of pupation, pupal weight at 18, 19 or 20 days and age at the time of adult emergence were recorded for each individual. In subsequent generations, 24 males and 72 females were selected according to the type of population and were randomly mated to produce the next generation. Each male was mated to three females. The individual identity of females within a mating group was lost once the mating group was set up. Matings among the selected individuals were random with the exception that full sib matings were avoided. In each population, a maximum of four full sibs from a family could be selected if they were candidates for selection. Within each population within a generation an attempt was made to have a population derived from 20 sire groups, each of which was composed of three full sib families. Each full sib family was required to have at least eight members on day 14 before any of the individuals in the family could become candidates for selection. Thus, each population contained 480 individuals each generation. The Weight population underwent selection for five generations and the Control and Early Pupation populations continued for six generations.

The original 24 base population sires plus some additional males were retained, and each of 20 of these males was mated to two randomly chosen females from generations four and six in the Weight, Control and Early Pupation populations. The dams which were used in the backcross matings were chosen at random from generations four and six from a subsample which consisted of two additional larvae randomly selected from each full sib family on day 14. Similar mating periods, random selection of eight progeny per full sib family on day 14, and the measurements of the same

traits were employed in the backcross progeny. None of the backcross progeny was used in matings in succeeding generations. Thus, the fourth and sixth generations were composed of 600 individuals in each population. Each population contained 800 individuals in generation five. However, in generation seven only the Control population and the Early Pupation population contained 800 individuals; the Weight population of generation seven contained only the backcross progeny, or approximately 320 individuals.

The generation interval was 35 days in each population. A detailed laboratory work schedule for a single population for one generation is shown in Table 2. The Control population followed the Weight population one week later, and the Early Pupation followed the Weight population two weeks later. Within a population and within a generation the two days for obtaining pupal weights were determined by the rate of pupation. The reasons were to minimize the number of pupae which had emerged into young adults by the second day of weighing, and as a consequence would have an adult weight rather than a pupal weight, and to minimize the number of individuals which were still in the larval stage.

Although only two days of weighing are shown in Table 2, pupal weights were obtained on either days 18 and 19, or on days 19 and 20. At the beginning of the investigation only one person obtained pupal weights. The rate of sexing and obtaining individual weights was approximately 45 per hour. Later in the investigation two people obtained weights. One person removed the pupae from the creamer bottles and determined the sex. The second person obtained the pupal weight. The rate of weighing was approximately 90 per hour with two people. The mating groups were transferred into fresh media on day 34 in an effort to avoid the presence of

Table 2. Laboratory work schedule

Generation day	Weekday	Item
35-1	Thursday-Friday	Twenty-four hour egg lay collection in 3/4 oz. creamer bottles containing 3 gm. of media from each of 72 females.
14	Thursday	Count family numbers and randomly select eight full sib larvae from each of 60 families. Four full sibs are placed individually into 3/4 oz. creamer bottles and the other four larvae are placed individually into 1/2 oz. creamer bottles. Each bottle contains approximately 0.2 gm. of media. Total 14-day family size is recorded, and the remainder of each family is discarded.
15-18	Friday-Monday	Observe 480 individuals daily at approximately the same time each day for stage change. Record date of stage change on bottle cap.
19	Tuesday	Randomly select, sex and weigh to the nearest μ g two of the four full sib pupae in 3/4 oz. creamer bottles and two of the four full sib pupae in 1/2 oz. creamer bottles within each full sib family. Observe remaining four in each full sib family for stage change and record date of stage change on bottle cap.
20	Wednesday	Sex and weigh to the nearest μ g the remaining four individuals in each full sib family. Observe and record any stage changes.
21	Thursday	Observe and record any stage changes.
22	Friday	Observe remainder of population for stage change. Record pupation and adult emergence dates on data sheets. Make selections on paper for the following generation.
23-24	Saturday-Monday	Observe and record any stage changes in remainder of population.
25	Monday	Observe any remaining individuals for stage changes and record any

data on the data sheets not previously recorded on day 22. Make selections. Discard unselected individuals and wash bottles.

27	Wednesday	Mate selected individuals in 3/4 oz. creamer bottles containing 1.5 gm. of media.
34	Wednesday	Transfer mating groups to 3/4 oz. creamer bottles containing 1.5 gm. of fresh media. Discard old media.
35-1	Thursday-Friday	Twenty-four hour egg lay collection in 3/4 oz. creamer bottles containing 3 gm. of media from each of 72 females.

obviously older offspring in the egg lay collections.

In April, 1968, approximately 70 fertile matings each of which consisted of two females per male were made. The individuals entering the mating groups were randomly chosen from generation 18 of the random mating pearl stock which was continuously maintained in the laboratory. Females were 35 days of age at the time the egg collection period was initiated. Laboratory procedures for management and data collection in the offspring were exactly like those procedures employed in the previously described populations. The purpose for the formation of the additional group was to provide additional sire group data which could be added to the sire group data in the entire Control population. Combination and analyses of the pooled data provided estimates of phenotypic and genotypic parameters.

METHODS OF ANALYSES

Fourteen day family size, pupation age, pupal weight and adult emergence age were recorded from 13,062 individuals. Mass selection for heavy pupal weight was practiced for five generations. Mass selection on the basis of early age at time of pupation continued in another population for six generations. A random mating control population was also maintained for six generations. Another random mating group provided additional data which was used in estimation of parameters.

Environmental Factor Analysis

In order to obtain estimates of certain environmental factors, the following model was assumed for each of three traits, age at time of pupation, pupal weight and age at time of adult emergence.

$$Y_{ijkl} = \mu + d_i + b_j + s_k + (db)_{ij} + (ds)_{ik} + (bs)_{jk} + (dbs)_{ijk} + b_1 F_{ijk} + e_{ijkl}$$

Y_{ijkl} = a particular trait

μ = intercept

d_i = effect of i^{th} day of weighing, $i = 1, 3$

b_j = effect of j^{th} bottle size, $j = 1, 2$

s_k = effect of k^{th} sex, $k = 1, 2$

$(db)_{ij}$ = effect of the interaction of the i^{th} day of weighing and the j^{th} bottle size

$(ds)_{ik}$ = effect of the interaction of the i^{th} day of weighing and the k^{th} sex

$(bs)_{jk}$ = effect of the interaction of the j^{th} bottle size and the k^{th} sex

$(dbs)_{ijk}$ = effect of the interaction among the i^{th} day of weighing,
the j^{th} bottle size and the k^{th} sex

b_1 = regression of particular trait on 14-day family size

F_{ijk} = 14-day family size

e_{ijkl} = random error associated with a particular observation

Ages at time of stage changes were recorded in whole days, and pupal weights were recorded to the nearest μ g. Only individuals which had measurements of all three traits were included in the analyses.

Procedures given by Harvey (1960) were followed to obtain analyses of variance and estimates of the various effects with unequal subclass numbers. The effects of day of weighing, bottle size, sex and all possible interactions of the three factors were removed additively from each of the three traits. The resulting measurements were then adjusted to the average 14 day family size, 22 individuals. Remaining analyses were performed on the adjusted data.

Parameter Estimates

Estimates of heritabilities were obtained by analyses of variance of each of the three adjusted traits in the random mating Control population and the additional random mating group set up specifically to increase the numbers of individuals for the parameter estimation.

The mathematic model used to estimate heritabilities was:

$$Y_{ijkl} = \mu + p_i + s_{ij} + d_{ijk} + e_{ijkl}$$

Y_{ijkl} = the adjusted trait

μ = overall mean

p_i = effect of the i^{th} population, $i = 1, 2$

d_{ijk} = effect of the k^{th} dam within the j^{th} sire

o_{ijk1} = effect of the l^{th} offspring

The estimates of heritabilities and their standard errors were computed from component analyses according to procedures outlined by Dickerson (1960). All possible sums of any two of the three traits were also used as variables. Combination of the resulting analyses and analyses of single variables provided estimates of genetic correlations. Standard errors for the genetic correlations, estimated from the half-sib components of variance and covariance, were computed through the use of the formula given by Robertson (1959):

$$\sigma_{(r_A)} \approx \frac{1-r_A^2}{\sqrt{2}} \sqrt{\frac{\sigma_{(h_x^2)}^2}{h_x^2} \frac{\sigma_{(h_y^2)}^2}{h_y^2}}$$

where $\sigma_{(r_A)}$ is the standard error, r_A is the estimate of the genetic correlation between two traits, x and y , h_x^2 and h_y^2 are respective estimates of heritabilities of the two traits and $\sigma_{(h_x^2)}^2$ and $\sigma_{(h_y^2)}^2$ are the standard errors of the corresponding estimates of heritabilities. The approximation assumes that the heritability of the two traits is the same, the estimation of the genetic correlation is not close to one and that the genetic correlation and the correlation within subgroups are similar. Realized heritabilities for pupal weight and pupation age were calculated by dividing total response by the total selection differential within the appropriate population.

Responses to Selection

In order to determine whether any changes in the means of traits occurred with respect to time, analyses of variance between generations

and within generations were computed for each of the three adjusted traits in each of the three populations. Data from generation zero and from progeny which resulted from the backcrossing of generation four and six females to generation zero males were not included in the analyses. In order to determine the type of responses which occurred over time, the variance between generations was separated into linear, quadratic and cubic portions for each trait in each of the three populations. F tests were computed for each type of effect according to Steel and Torrie (1960).

The expected direct responses to selection for each generation in the Weight population and Early Pupation population were computed by the multiplication of the estimate of heritability of a given trait from the sire component analyses and the standardized selection differential within a generation. The resulting quantity was converted to actual units and added to the population mean from which the parents were selected. The expected correlated responses to selection in other traits were calculated according to the following formula:

$$CR_y = \frac{(\bar{x}_s - \bar{\bar{x}})}{\sigma_{P_x}} h_x h_y r_A \sigma_{P_y}$$

where CR_y is the expected correlated response in trait y, $(\bar{x}_s - \bar{\bar{x}})$ is the deviation from generation average of trait x in parents and h_x and h_y are the square roots of heritability estimates of the two traits. The phenotypic standard deviation of trait x and trait y are symbolized by σ_{P_x} and σ_{P_y} , respectively and r_A is the estimate of the genetic correlation between the two traits. Correlations between expected responses and actual responses were also calculated for all traits in the two selected populations.

RESULTS

The numbers of individuals and the unadjusted means of traits within a subclass which were included in the analyses to estimate constants for the various environmental effects are presented in Table 3. The analyses of variance of pupation age, pupal weight and emergence age are shown in Table 4. The proportion of the total variation which was attributable to a given environmental effect is also given for each source of variation in Table 4. The least-squares estimates for each effect on each of the three traits are shown in Table 5.

The analyses of the data from the random mating groups provided estimates of heritabilities of pupation age, pupal weight and emergence age. The analyses of variance are presented in Tables 6, 7, and 8 for pupation age, pupal weight and emergence age, respectively. Only those individuals which had all three traits measured were included in the analyses. Thus, the expected mean squares are the same for each trait. The expected mean squares are given in Table 9. The estimates of heritabilities of each trait from the components of variance are shown in Table 10. The estimates of the phenotypic and genetic correlations among the three traits are shown in Table 11.

Analyses of variance among and within generations within each population are shown in Table 12. Significance of linear, cubic and quadratic effects over time are also shown in Table 12. The numbers, adjusted means and standard deviations of each trait by population and generation are shown in Table 13.

The fractions of males and females saved and the standardized

selection differentials by generation for the Weight population are shown in Table 14. The expected and actual responses to selection for the Weight population are shown in Table 15. The fractions of males and females saved and the standardized selection differentials by generation for the Early Pupation population are shown in Table 16. The expected and actual responses to selection in the Early Pupation population are shown in Table 17. Although the selection was random in the Control population, the superiority or inferiority of trait means of the parents selected by generation can be seen in Tables 18, 19 and 20 for pupation age, pupal weight and emergence age, respectively. The generation averages of pupation age, pupal weight and emergence age by population are graphically illustrated in Figures 1, 2 and 3, respectively. The correlations of expected and actual responses to selection of each of the three traits in the Weight population and in the Early Pupation population are shown in Table 21.

Table 3. Numbers and means of unadjusted data by subclass for the least squares analyses employed to give constants

Subclass	n	14-day family size	Pupation age (da.)	Pupal weight (μ g)	Emergence age (da.)
Overall	13,062	22.2	17.00	2628	21.60
Day of weighing:					
18 da. (d_1)	3,964	21.8	16.72	2598	21.31
19 da. (d_2)	6,512	22.2	17.01	2631	21.61
20 da. (d_3)	2,586	22.8	17.41	2666	22.03
Creamer size:					
1/2 oz. creamer (b_1)	6,133	22.1	16.96	2639	21.56
3/4 oz. creamer (b_2)	6,929	22.3	17.04	2618	21.64
Sex:					
Males (s_1)	6,566	22.2	17.03	2577	21.64
Females (s_2)	6,496	22.2	16.97	2679	21.57
Interactions:					
d_1b_1	1,972	21.8	16.70	2602	21.28
d_1b_2	1,992	21.8	16.74	2594	21.33
d_2b_1	3,059	22.1	16.98	2641	21.58

Table 3. (Continued)

Subclass	n	14-day family size	Pupation age (da.)	Pupal weight (μ g)	Emergence age (da.)
d ₂ b ₂	3,453	22.3	17.04	2621	21.64
d ₃ b ₁	1,102	22.6	17.39	2699	22.01
d ₃ b ₂	1,484	22.9	17.43	2642	22.05
d ₁ s ₁	1,991	21.8	16.75	2549	21.34
d ₁ s ₂	1,973	21.8	16.69	2647	21.27
d ₂ s ₁	3,231	22.2	17.03	2577	21.63
d ₂ s ₂	3,281	22.2	17.00	2684	21.60
d ₃ s ₁	1,344	22.8	17.47	2619	22.08
d ₃ s ₂	1,242	22.6	17.35	2718	21.98
b ₁ s ₁	3,072	22.0	16.99	2588	21.59
b ₁ s ₂	3,061	22.2	16.93	2690	21.54
b ₂ s ₁	3,494	22.4	17.07	2568	21.68
b ₂ s ₂	3,435	22.1	17.01	2669	21.60
d ₁ b ₁ s ₁	992	21.5	16.74	2553	21.34
d ₁ b ₁ s ₂	980	22.1	16.67	2652	21.23
d ₁ b ₂ s ₁	999	22.1	16.76	2545	21.35

Table 3. (Continued)

Subclass	n	14-day family size	Pupation age (da.)	Pupal weight (μ g)	Emergence age (da.)
d ₁ b ₂ s ₂	993	21.5	16.72	2643	21.31
d ₂ b ₁ s ₁	1,506	22.1	16.98	2590	21.57
d ₂ b ₁ s ₂	1,553	22.0	16.97	2691	21.59
d ₂ b ₂ s ₁	1,725	22.3	17.06	2565	21.68
d ₂ b ₂ s ₂	1,728	22.3	17.02	2678	21.61
d ₃ b ₁ s ₁	574	22.6	17.46	2641	22.07
d ₃ b ₁ s ₂	528	22.6	17.31	2762	21.94
d ₃ b ₂ s ₁	770	23.0	17.48	2603	22.09
d ₃ b ₂ s ₂	714	22.7	17.37	2685	22.00

Table 4. Analyses of variance of pupation age, pupal weight and emergence age

Source	d.f.	Trait					
		Pupation age		Pupal weight		Emergence age	
		Mean square	Percent ^a of total variation	Mean square	Percent ^a of total variation	Mean square	Percent ^a of total variation
Total	13,062						
Q	1	3,768,177		89,250,805,424		6,088,047	
Day of weighing (d)	2	361.9084**	12.99	4,062,733**	1.24	395.4080**	13.38
Creamer size (b)	1	5.6076**	0.01	2,297,087**	0.50	6.9601**	0.01
Sex (s)	1	13.5343**	0.03	29,369,829**	6.53	13,4107**	0.03
d x b	2	0.3598		500,566**	0.26	0.1613	
d x s	2	2.1683	0.01	18,800		1.4166	0.04
b x s	1	0.1606		44,921		0.0051	
d x b x s	2	0.4797		141,492		1.9552	0.07
Family size	1	0.7703		2,313,470**		1.2311	
Error	13,049	0.6070	86.96	73,171	91.45	0.6357	86.47

^aPercent of total variation was computed from variance components.

**P < .01.

Table 5. Least squares estimates of constants for pupation age, pupal weight and adult emergence age

Effect	Pupation age (da.)	Pupal weight (μ g)	Emergence age (da.)
Cl	17.0153	2581	21.6101
Day of weighing (d):			
18 da. (d_1)	-0.3248	-35	-0.3400
19 da. (d_2)	-0.0356	- 3	-0.0361
20 da. (d_3)	0.3604	38	0.3761
Creamer size (b):			
1/2 oz. (b_1)	-0.0224	+14	-0.0249
3/4 oz. (b_2)	0.0224	-14	0.0249
Sex (s):			
Males (s_1)	0.0347	-51	0.0346
Females (s_2)	-0.0347	51	-0.0346
Interactions:			
d_1b_1	0.0052	-10	0.0024
d_1b_2	-0.0052	10	-0.0024
d_2b_1	-0.0099	- 4	-0.0067
d_2b_2	0.0099	4	0.0067
d_3b_1	0.0047	15	0.0044
d_3b_2	-0.0047	-15	-0.0044
d_1s_1	-0.0067	2	0.0017
d_1s_2	0.0067	- 2	-0.0017
d_2s_1	-0.0210	- 2	-0.0194
d_2s_2	0.0210	2	0.0194

Table 5. (Continued)

Effect	Pupation age (da.)	Pupal weight (μ g)	Emergence age (da.)
d_3s_1	0.0278	0	0.0177
d_3s_2	-0.0278	0	-0.0177
b_1s_1	0.0038	- 2	0.0007
b_1s_2	-0.0038	2	-0.0007
b_2s_1	-0.0038	2	-0.0007
b_2s_2	0.0038	- 2	0.0007
$d_1b_1s_1$	0.0033	2	0.0140
$d_1b_1s_2$	-0.0033	- 2	-0.0140
$d_1b_2s_1$	-0.0033	2	-0.0140
$d_1b_2s_2$	0.0033	- 2	0.0140
$d_2b_1s_1$	-0.0116	4	-0.0226
$d_2b_1s_2$	0.0116	- 4	0.0226
$d_2b_2s_1$	0.0116	- 4	0.0226
$d_2b_2s_2$	-0.0116	4	-0.0226
$d_3b_1s_1$	0.0082	- 7	0.0086
$d_3b_1s_2$	-0.0082	7	-0.0086
$d_3b_2s_1$	-0.0082	7	-0.0086
$d_3b_2s_2$	0.0082	- 7	0.0086
Regression	0.0014	2.3615	0.0017

Table 6. Analysis of variance of pupation age used to estimate heritabilities

Source	d.f.	Mean square	Component	Percent of variation
Population	1	0.0005	-0.0012	0
Sires	216	2.3434	0.0581	10.9
Dams/Sires	419	0.9859	0.0748	14.0
Progeny/Dams	4,394	0.4005	0.4005	75.1
Total	5,030	0.5326	0.5334	100.0

Table 7. Analysis of variance of pupal weight used to estimate heritabilities

Source	d.f.	Mean square	Component	Percent of variation
Population	1	3,359,509	1,870	4.2
Sires	216	230,743	4,825	10.8
Dams/Sires	419	117,057	11,580	25.9
Progeny/Dams	4394	26,388	26,388	59.1
Total	5030	43,378	44,663	100.0

Table 8. Analysis of variance of emergence age used to estimate heritabilities

Source	d.f.	Mean square	Component	Percent of variation
Population	1	9.1989	0.0040	0.7
Sires	216	2.7052	0.0700	11.9
Dams/Sires	419	1.0727	0.0815	13.8
Progeny/Dams	4,394	0.4348	0.4348	73.6
Total	5,030	0.5872	0.5904	100.0

Table 9. Expected mean squares of analyses of variances presented in Tables 6, 7 and 8

Source	d.f.	Expected mean square
Population	1	$\sigma^2 + 7.8893\sigma_D^2 + 18.5375\sigma_S^2 + 1686.0107\sigma_P^2$
Sires	216	$\sigma^2 + 8.0299\sigma_D^2 + 23.0796\sigma_S^2$
Dams/Sires	419	$\sigma^2 + 7.8295\sigma_D^2$
Progeny/Dams	4,394	σ^2

Table 10. Estimates and standard errors of heritabilities of pupation age, pupal weight and emergence age computed from components of variance

Method of computation	Pupation age		Pupal weight		Emergence age	
	Heritability estimate	Standard error	Heritability estimate	Standard error	Heritability estimate	Standard error
$\frac{4\hat{\sigma}_S^2}{\hat{\sigma}_S^2 + \hat{\sigma}_D^2 + \hat{\sigma}^2}$	0.44	0.08	0.45	0.10	0.48	0.08
$\frac{4\hat{\sigma}_D^2}{\hat{\sigma}_S^2 + \hat{\sigma}_D^2 + \hat{\sigma}^2}$	0.56	0.06	1.08	0.10	0.56	0.06
$\frac{2(\hat{\sigma}_S^2 + \hat{\sigma}_D^2)}{\hat{\sigma}_S^2 + \hat{\sigma}_D^2 + \hat{\sigma}^2}$	0.50	0.04	0.77	0.05	0.52	0.04

Table 11. Genotypic and phenotypic correlations computed in random mating population. Genotypic correlations are above diagonal, and phenotypic correlations are below the diagonal

	Pupation age	Pupal weight	Emergence age
Pupation age	--	-0.28 (.126)*	0.97 (.001)*
Pupal weight	-0.08	--	-0.20 (.128)*
Emergence age	0.74	-0.07	--

*Standard error of genetic correlation.

Table 12. Analyses of variance of pupation age, pupal weight and adult emergence age between and within generations within populations

Within generations within populations								
Source within population	d.f.	k ₁ value	Pupation age		Pupal weight		Emergence age	
			Mean square	Percent of variance	Mean square	Percent of variance	Mean square	Percent of variance
Weight population:			456					
Generations	5		31.2055**	11	26,364,866**	55	17.1792**	6
Linear regression	1		57.7164		130,669,540**		36.4128	
Deviations from linear regression	4		24.5773		28,863		12.3711	
Second degree term	1		76.3688**		165,528		43.3856*	
Deviations from quadratic regression	3		7.3135		329,666		2.0329	
Third degree term	1		0.0669		303,085		1.2192	
Deviations from third degree regression	2		10.9368		342,957		2.4398	
Within generations	2,734		0.5439	89	47,700	45	0.5555	94
Control population:			448					
Generations	6		36.9358**	13	1,110,193**	5	45.3212**	15
Linear regression	1		10.4078		4,264,900*		31.6771	
Deviations from linear regression	5		42.2405		479,252		48.0510	
Second degree term	1		48.1974		68,315		39.7571	
Deviations from quadratic regression	4		40.7513		581,986		50.1245	

Third degree term	1	47.9580		687,734		11.0974	
Deviations from third degree regression	3	38.3490		546,737		63.1335	
Within generations	3,131	0.5318	87	43,963	95	0.5670	85
Early Pupation population	446						
Generations	6	55.9081**	18	862,703**	4	59.1424**	20
Linear regression	1	232.6517*		4,073,355**		232.0683*	
Deviations from linear regression	5	20.5590		220,571		24.5575	
Second degree term	1	49.2205		466,147		52.3226	
Deviations from quadratic regression	4	13.3936		159,178		17.6162	
Third degree term	1	18.9749		912		20.1391	
Deviations from third degree regression	3	11.5331		211,933		16.7753	
Within generations	3,255	0.5207	82	41,942	96	0.5174	80

*P < .05.

**P < .01.

Table 13. Numbers, adjusted means and standard deviations of traits by population and generation

Trait within population	Generation								
	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s
Weight population:									
Pupation age (da.)	--	--	--	465	17.1442	.8112	476	17.0703	.7203
Pupal weight (μ g)	--	--	--	465	2451	212	476	2615	195
Emergence age (da.)	--	--	--	465	21.7403	.7989	476	21.6769	.7523
Control population:									
Pupation age	330	17.3655	.8115	462	16.7801	.7753	438	17.6551	.7638
Pupal weight	330	2436	214	462	2438	200	438	2414	226
Emergence age	330	22.0643	.7896	462	21.4789	.7635	438	22.3084	.8164
Early pupation population:									
Pupation age	--	--	--	465	16.8763	.8446	444	17.0716	.7832
Pupal weight	--	--	--	465	2440	218	444	2466	211
Emergence age	--	--	--	465	21.4275	.8445	444	21.6756	.8349

Table 13. (Continued)

Trait within population	Generation								
	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s
Weight population:									
Pupation age (da.)	464	16.7809	.6872	477	17.1408	.6710	435	17.2155	.8280
Pupal weight (μ g)	464	2729	211	477	2885	210	435	2953	230
Emergence age (da.)	464	21.5580	.7134	477	21.7227	.6732	435	21.7687	.8211
Control population:									
Pupation age	462	17.2578	.8248	474	17.0093	.6661	402	17.2493	.7117
Pupal weight	462	2428	204	474	2551	204	402	2490	210
Emergence age	462	21.7113	.8524	474	21.5389	.6832	402	21.9918	.7416
Early pupation population:									
Pupation age	469	17.1865	.7124	476	16.6806	.6784	478	16.7569	.6243
Pupal weight	469	2510	214	476	2534	204	478	2510	194
Emergence age	469	21.8085	.7393	476	21.2061	.7145	478	21.2846	.6073

Table 13. (Continued)

Trait within population	Generation								
	5 ^a			6			7		
	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s
Weight population:									
Pupation age (da.)	319	17.2391	.7668	423	17.6058	.6966	--	--	--
Pupal weight (μg)	319	2631	206	423	3123	251	--	--	--
Emergence age (da.)	319	21.8092	.8210	423	22.1453	.7051	--	--	--
Control population:									
Pupation age	298	17.3630	.7519	456	17.0353	.6883	444	16.9268	.6564
Pupal weight	298	2471	194	456	2523	210	444	2516	214
Emergence age	298	22.0366	.8233	456	21.5983	.6980	444	21.3901	.7005
Early pupation population:									
Pupation age	308	16.9955	.6336	458	16.3662	.6902	472	16.2386	.7026
Pupal weight	308	2536	203	458	2564	179	472	2541	209
Emergence age	308	21.5463	.6750	458	21.0393	.6406	472	20.7780	.6226

^aOffspring produced from mating randomly selected generation 4 females to randomly selected generation 0 males.

Table 13. (Continued)

Trait within population	Generation 7 ^b		
	n	$\bar{x}$	s
Weight population:			
Pupation age (da.)	311	17.0518	.6962
Pupal weight (μ g)	311	2861	208
Emergence age (da.)	311	21.5805	.7392
Control population:			
Pupation age	302	16.8723	.7574
Pupal weight	302	2509	193
Emergence age	302	21.4637	.7320
Early pupation population:			
Pupation age	309	16.7023	.6745
Pupal weight	309	2525	204
Emergence age	309	21.2373	.6944

^bOffspring produced from mating randomly selected generation 6 females to randomly selected generation 0 males.

Table 14. Fractions of males and females selected and standardized selection differentials in the Weight population

Gener- ation	Fraction selected	Percent selected	Generation		Selected average	Standardized selection differential
			Average	Standard deviation		
Male:						
1	21/245	8.57	2448	215	2790	1.591
2	21/251	8.37	2601	193	2894	1.518
3	21/224	9.38	2714	208	3048	1.606
4	20/237	8.44	2886	214	3247	1.687
5	20/229	8.73	2966	224	3316	1.562
Females:						
1	59/220	26.82	2454	210	2656	0.962
2	59/225	26.22	2631	197	2823	0.975
3	59/240	24.58	2743	214	2970	1.061
4	60/240	25.00	2883	208	3104	1.062
5	60/206	29.13	2938	236	3157	0.928

Table 15. Expected and actual responses to selection in the Weight population

Generation	Pupation age		Pupal weight		Emergence age	
	Expected	Actual	Expected	Actual	Expected	Actual
1	17.0165	17.0703	2573	2615	21.6436	21.6769
2	16.9597	16.7809	2725	2729	21.5936	21.5580
3	16.6703	17.1408	2855	2885	21.4737	21.7227
4	17.0281	17.2155	3014	2953	21.6378	21.7687
5	17.0884	17.6058	3081	3123	21.6729	22.1453

Table 16. Fractions of males and females selected and standardized selection differentials in the Early Pupation population

Gener- ation	Fraction selected	Percent selected	Generation		Selected Average	Standardized selection differential
			Average	Standard deviation		
Male:						
1	20/233	8.58	16.8593	.7602	15.7730	-1.429
2	19/218	8.72	17.0983	.7428	16.3077	-1.064
3	20/234	8.55	17.2335	.6712	16.1954	-1.547
4	20/226	8.85	16.6925	.6742	16.0341	-0.976
5	21/243	8.23	16.7641	.6258	16.1781	-0.936
6	20/251	7.97	16.3618	.6871	15.1281	-1.796
Female:						
1	60/232	25.86	16.8934	.9923	16.0107	-0.890
2	57/226	25.22	17.0459	.8211	16.4597	-0.714
3	60/235	25.53	17.1397	.7497	16.3534	-1.009
4	60/250	24.00	16.6699	.6833	16.1031	-0.830
5	59/235	25.11	16.7496	.6240	16.1824	-0.909
6	60/207	28.98	16.3715	.6954	15.8066	-0.565

Table 17. Expected and actual responses to selection in the Early
Pupation population

Generation	<u>Pupation age</u>		<u>Pupal weight</u>		<u>Emergence age</u>	
	Expected	Actual	Expected	Actual	Expected	Actual
1	16.4660	17.0716	2472	2466	20.9833	21.6756
2	16.7936	17.1865	2489	2510	21.3483	21.8085
3	16.8053	16.6806	2543	2534	21.3915	21.2061
4	16.4333	16.7569	2556	2510	20.9238	21.2846
5	16.4766	16.3662	2532	2564	21.0371	21.0393
6	15.9633	16.2386	2593	2539	20.6740	20.7780

Table 18. Deviations of average pupation age of selected males and females in the Control population from generation averages

Gener- ation	Fraction saved	Percent saved	Generation		Selected Average	Deviation
			Average	Standard deviation		
Males:						
0	22/166	13.25	17.4547	.8430	17.2884	-.1663
1	20/230	8.70	16.7430	.8349	16.6655	-.0775
2	20/205	9.76	17.6625	.7509	17.3932	-.2693
3	20/241	8.30	17.2432	.7653	17.2191	-.0241
4	21/254	8.27	16.9912	.6456	16.9958	.0046
5	20/198	10.10	17.2427	.6306	17.2355	-.0072
6	20/214	9.34	16.9864	.7102	16.8748	-.1116
Sum						-.6514
Females:						
0	62/164	37.80	17.2751	.7703	17.2790	.0039
1	60/232	25.86	16.7432	.8349	16.7699	.0267
2	56/233	24.03	17.6485	.7765	17.6393	-.0092
3	59/221	26.70	17.2736	.8866	17.2404	-.0332
4	60/220	27.27	17.0301	.6990	17.0774	.0473
5	59/204	28.92	17.2557	.7840	17.4057	.1550
6	60/242	24.79	17.0408	.6687	16.9797	-.0611
Sum						.1294

Table 19. Deviations of average pupal weight of selected males and females in the Control population from generation averages

Gener- ation	Fraction saved	Percent saved	Generation		Selected Average	Deviation
			Average	Standard deviation		
Males:						
0	22/166	13.25	2430	203	2407	- 23
1	20/230	8.70	2455	208	2448	- 7
2	20/205	9.76	2413	237	2412	1
3	20/241	8.30	2441	182	2364	- 77
4	21/254	8.27	2519	204	2480	- 39
5	20/198	10.10	2493	204	2477	- 16
6	20/214	9.34	2524	219	2547	23
Sum						-138
Females:						
0	62/164	37.80	2442	225	2430	- 12
1	60/232	25.86	2421	192	2451	30
2	56/233	24.03	2414	216	2441	27
3	59/221	26.70	2414	215	2417	3
4	60/220	27.27	2544	205	2537	- 7
5	59/204	28.92	2487	216	2538	51
6	60/242	24.79	2522	201	2549	27
Sum						119

Table 20. Deviations of average emergence age of selected males and females in the Control population from generation averages

Gener- ation	Fraction saved	Percent saved	Generation		Selected Average	Deviation
			Average	Standard deviation		
Males:						
0	22/166	13.25	22.1028	.8160	21.9501	-.1527
1	20/230	8.70	21.4585	.8228	21.4558	-.0027
2	20/205	9.76	22.2532	.7883	22.0842	-.1620
3	20/241	8.30	21.7265	.8299	21.8604	.1339
4	21/254	8.27	21.5338	.6853	21.5649	.0311
5	20/198	10.10	21.9767	.6853	21.9831	.0064
6	20/214	9.34	21.5860	.7421	21.4200	-.1660
Sum						-.3190
Females:						
0	62/164	37.80	22.0233	.7625	22.0726	.0493
1	60/232	25.86	21.4981	.7010	21.3818	-.1163
2	56/233	24.03	22.3570	.8390	22.3530	-.0040
3	59/221	26.70	21.6956	.8778	21.7307	.0351
4	60/220	27.27	21.5447	.6822	21.5826	.0379
5	59/204	28.92	21.9474	.7938	22.1376	.1902
6	60/242	24.79	21.6093	.6580	21.6156	.0063
Sum						.1985

Figure 1. Average pupation age by generation for three populations

Figure 2. Average pupal weight by generation for three populations

Figure 3. Average emergence age by generation for three populations

Table 21. Correlations of expected and actual average responses to selection

Trait	Population	
	Weight	Early Pupation
Pupation age	0.488	0.654
Pupal weight	0.978**	0.638
Emergence age	0.457	0.565

** $P < .01$.

DISCUSSION

Environmental Factors

The analyses of variance and least squares estimates of the environmental effects are shown in Table 4 and Table 5, respectively. Day of weighing accounted for approximately 13% of the variation in each of the stage traits and 1% in pupal weight. Differences in sexes accounted for 6% of the variation in pupal weight. Effect of day of weighing was highly significant on pupation age, pupal weight and adult emergence age. Significant effects on pupation age were expected, since the two days on which weighing occurred, either days 18 and 19, or days 19 and 20, were determined by the pupation rate within a population within a generation. Since there was apparently little variation in the length of pupal stage, similar effects of day of weighing on adult emergence age were also expected. Pupal weights were statistically different among days of weighing. Weights increased as age at time of weighing increased. The second day's weighing within any generation within a population always included the individuals which had pupated late. Pupal weight is heaviest immediately following the pupation process (Englert and Bell, 1963), and decreases during the pupal stage. The individuals which were late in pupation time would have also had more opportunity to consume larger amounts of media during the larval stage.

The differences between the size of the creamer bottles were small, but were significant. The individuals which were raised in 1/2 oz. creamer bottles after day 14 tended to pupate and subsequently emerge into adults somewhat earlier than the individuals which were reared in 3/4 oz. creamer

bottles. The advantage of $28 \mu\text{g}$ in pupal weight of the individuals in the 1/2 oz. creamer bottles was significant. Perhaps the differences in volumes of the two sizes of containers were effective, but a more logical explanation is the fact that the cardboard caps did not fit as tightly on the 1/2 oz. creamer bottles as the caps on the 3/4 oz. creamer bottles. Thus, there was more air movement in the smaller creamer bottles. Any sudden changes in environmental conditions would tend to affect the space in the 1/2 oz. creamer bottles more quickly. On the other hand, recovery from sudden environmental conditions would be faster in the smaller creamer bottles. Although eight full sibs were randomly selected on day 14 from each family, there was undoubtedly a tendency to place any smaller larvae into individual creamer bottles first in order to avoid losing smaller individuals. The first four larvae were always placed in 3/4 oz. creamer bottles, and the remaining four were placed in 1/2 oz. creamer bottles.

Females tended to pupate and emerge into adults at slightly younger ages than males. Females were $102 \mu\text{g}$ heavier than males. The difference in weight was about half the sex difference reported by Bell and Moore (1958).

Family size at 14 days appeared to have a highly significant effect on pupal weight but did not influence pupation age or adult emergence age. The estimate of the regression of pupal weight on family size was $+2.3 \mu\text{g}$. Enfield et al. (1966) did not detect any effect of numbers of individuals contained in a family on individual weight. However, Enfield et al. (1966) had a three-day egg collection period. In the present study, an effort was made to have exactly a 24-hour egg collection from each female.

Family sizes did increase during the investigation. The data from the

Weight population are included in the analyses, thus a positive regression was expected. As will be shown later, the pupal weight means also increased over time. However, it was more likely that improved procedures in management of the female could have resulted in larger family sizes. The family sizes appeared large and might have been due to the fact that the male was not removed from a mating group until the initiation of the egg collection. Thus, the presence of the male and recent matings might have stimulated egg production in the female.

It is noted that the random effect of sires and dams were not absorbed in the computation of the least-squares estimates of the environmental effects. Thus, it is possible that some of the estimates are inflated from sire and dam effects.

Parameter Estimation

The analyses of variance of adjusted pupation age, pupal weight and emergence age which provided estimates of heritabilities and phenotypic and genetic correlations of the adjusted traits are shown in Tables 6, 7 and 8, respectively. The data which were included in the analyses were from the random mating Control population and from an additional random mating population. Each population originated from the random mating population which was maintained in the laboratory. Thus, the two groups were assumed to be representative of the same population. A small proportion of the total variation in each trait was attributed to differences in the two populations. The estimates of the heritabilities of the three traits, as shown in Table 10, indicated close agreement among the three methods of computation for heritabilities for the stage traits. The expected

composition of the variances is shown in Table 22.

A comparison of the composition of the sire component with the dam component estimates of heritability for the stage traits indicated that the dam component contained very little more than the variance from average effects of genes. Dawson (1964a) did not report similar results in his analyses of pupation age. Dawson (1964a) reported that the portion of variance in pupation age which was due to maternal effects was about one-half as large as the portion due to additive gene effects. Differences in egg content of different females were offered as a possible explanation.

The estimates of heritability of pupal weight appeared different among the three methods of computation, with the estimate from the dam component being extremely high. The expected composition of the dam component of variance, as shown in Table 22, indicates that one-fourth of the dominance variance is included in the dam component, as well as several other components. It is more likely that the high heritability for pupal weight was due to the fact that individuals in a full sib family remained together in a creamer bottle until day 14. Thus, there may have been a maternal effect and an environmental effect which was unique for each full sib family. After the eight full sib larvae were placed into individual creamer bottles on day 14, the full sib family still remained together throughout its life cycle. Thus, any effective environmental changes probably affected all individuals in a full sib family. Also, the four members of a full sib family were weighed in consecutive order on each of the two days.

It must be indicated that the estimates of the heritabilities were computed on the adjusted data. The additive adjustment for those observations in a given day of weighing-creamer bottle size-sex subclass increased

Table 22. Expected components of variances from Dickerson (1960)

Component of variance	σ^2_A	σ^2_D	σ^2_{AA}	σ^2_{AD}	σ^2_{DD}	σ^2_{AAA}	σ_{MG}	σ^2_M	σ^2_C	σ^2_{GE}
Sire, S	.25	0	>.0625	0	0	.016	0	0	0	<.25
Dam, D	.25	.25	>.1875	>.125	>.0625	>.109	1	1	1	<.25

σ^2_A = Variance from average effects of genes.

σ^2_D = Variance from dominance deviations from average gene effects.

σ^2_{AA} = Variance from two-loci interactions of average effects.

σ^2_{AD} = Variance from two-loci interaction of average effects with dominance deviations.

σ^2_{DD} = Variance from two-loci interaction of dominance deviations.

σ^2_{AAA} = Variance from three-loci interaction of average effects, etc.

σ^2_M = Variance from genetic variation in direct maternal effects.

σ_{MG} = Covariance between total genetic deviations in the transmitted and the direct maternal effects.

σ^2_C = Variance from environment that is alike for full sibs but differs between families from different dams.

σ^2_{GE} = Variance from interaction of genotypes with different samples of environments (i.e., years, locations, generations, etc.)

the intraclass correlation among individuals. Adjustment of the data for all of the effects may have introduced a correlation structure that is not accounted for in the hierarchical structure of sires, dams and progeny. Such correlations could have undetermined effects on the results. Also, the two days on which pupal weights were obtained were completely confounded with sires and dams in a generation in the Control population. The two days of weighing were uniformly distributed over generations in the Control population, but generation effects were not removed in the analyses. Thus, the sire and dam components of variance may be inflated by this procedure, but fitting the environmental effects without absorbing the random effects of sires and dams could have removed some sire and dam differences.

Responses to Selection

Analyses of variance between and within generations of the traits for each population are shown in Table 12. The generation means for the traits for each population are presented in Table 13. The same means are also shown graphically in Figures 1, 2 and 3. The F tests for generation means, shown in Table 12, were all significant. Since there were no replicates of generations, the variance within generations is not true experimental error. In order to establish the types of time trends in the means of the traits, polynomials were fitted to the means. The results are shown in Table 12. In the Weight population, the generation responses were obviously linear, whereas the quadratic effect on the stage traits was the only significant effect. In the Control population, the trend in pupal weights over generations was linear. The stage traits in the Control

population were not adequately explained by any effects up through the cubic effect. Ages at time of stage changes are apparently greatly affected by unknown environmental changes. All three traits in the Early Pupation population were adequately explained by linearity. The correlated response to the linear change in pupation age was linear in pupal weight and emergence age in the Early Pupation population. However, the correlated response to the linear change in pupal weight was quadratic in the stage traits in the Weight population. Thus, there may have been some asymmetrical responses to selection.

Age at time of the occurrence of a stage change was most variable. The actual chronological age at the time of stage change was not known, since no effort was made to establish the egg laying patterns of females. Also, age was recorded only in whole days. There was some variation in the laboratory and in the Jamesway incubator, but no adjustments for such variation were made, since the three populations were not at exactly the same phase of the life cycle when any fluctuations occurred. The temperature and humidity in the incubator were recorded only once a day unless there were fluctuations, and then the amount of elapsed time during which environmental changes prevailed was not known. Therefore, the designated Control population was not considered to be a control from which the other two populations could be deviated.

The numbers of individuals within a generation within a population deviated considerably from the expected number of 480 individuals. Only those individuals which had completed records for each of the three traits were included in the computations. Since the age at time of weighing was determined by the population trend within a generation, the data from

individuals which had not pupated, had emerged into adults before weighing or did not emerge into adults following weighing were excluded. The expected number of progeny from the matings of generation 4 and generation 6 females to generation 0 males was 320 in generations 5 and 7 within each population. Fertility of the generation 0 males was most satisfactory in both sets of backcross matings.

The direct and correlated responses to selection in the Weight population and in the Early Pupation population are shown in Tables 15 and 17, respectively. The correlations of average expected response and average response actually obtained are shown in Table 21. It is seen that only the response to selection for heavy pupal weight in the Weight population and the expected response are in agreement, statistically. All other correlations were not significant. Thus the response to selection for heavy pupal weight was the only response which could be accurately predicted.

The estimate of the realized heritability for pupal weight from the Weight population was 0.50 which was similar to the estimate of 0.45 from the sire component analyses in the random mating populations. Enfield *et al.* (1966) also had similar agreement in the two methods of estimating heritability of pupal weight.

Although the estimate of heritability of pupation age in the random mating populations was high, and large standardized selection differentials were imposed in the Early Pupation population, little realized progress was made in changing the age at the time of pupation. Some of the mean changes of pupation age were in the positive direction rather than a negative direction. The realized heritability of age at time of pupation was 0.14 which is smaller than the estimate of 0.44 from the sire component

analyses in the random mating populations. Englert and Bell (1964) reported estimates of realized heritability of age at time of pupation of 0.38 and 0.26 for early and late pupation, respectively. Dawson (1965) estimated the realized heritability of pupation age to be approximately 0.14 in both fast and in slow developing populations.

The larger estimate of heritability of pupation age from the sire component analysis may have been affected by the proximity of the individuals in a full sib family. Although the individuals were placed into individual creamer bottles on day 14, they were still maintained adjacently in the incubator. Thus, any environmental changes would have more likely affected a complete full sib family. Similarly, the range of error of one full day in the age of individuals at the time of stage change would have an influence, particularly if the egg production pattern of the female was not uniform or was not near the middle of the egg collection period. There was also additional error introduced in the measurement of age at time of stage change due to the fact that the individuals were observed only once a day for stage changes. Individuals were checked at approximately the same time each day even on the days of weighing. However, any individuals that had become pupae or adults between the time of checking on the second day of weighing and the time of weighing on the second day were excluded from the analyses. So, lack of precision in measurement could account for the lack of response in the Early Pupation population.

In the Early Pupation population, the actual responses to selection for an early age at time of selection were less than the expected responses. Changes in environmental conditions could have caused the variation in pupation age. Scheinberg et al. (1967) reported considerable differences in

average pupation age in three consecutive generations of a random mating population. The average pupation ages were $16.46 \pm .04$, $17.19 \pm .03$ and $16.19 \pm .02$ days for the three generations.

The correlated responses in pupal weights in the Early Pupation population were closer to the expected correlated responses than the analogous comparison in the Weight population. In the Weight population and the Early Pupation population, age at time of adult emergence appeared to be mainly a function of age at the time of pupation, at least from the manner in which ages were established in this study. Howe (1956) reported little variation in the length of the pupal stage.

The means of traits of the progeny that were produced by crossing randomly selected females from generations 4 and 6 to generation 0 males in each of the three populations are shown in Table 13. The backcross progeny in the Control population appeared almost identical in pupal weight with the regularly produced Control populations in both generations 5 and 7. Since there was close agreement in pupal weight, the unexplained increase in average pupal weight in generation 4 within the Control population might have been due to an environmental change which was maintained, rather than any genetic effect. Pupal weights through generation 3 were obtained by one person; thereafter, two people obtained weights. The rate of weighing was almost doubled, and consequently individuals were not out of the incubator conditions for as long a period when pupal weights were obtained. If the increase in pupal weight in the Control population had been completely genetic, the means of pupal weights in the backcross progeny would have regressed toward the original mean. The differences in the average pupation and adult emergence ages provide more indications of the amount of

variation in ages at time of stage changes. However, the individuals were observed for stage changes by two people following generation 3. Thus, the time required to observe an entire population for stage change was also reduced, and consequently, there could have been more or less environmental effects in removing the individuals from the incubator out into the laboratory conditions.

The backcross progeny in the Weight population were exceptionally close to the expected values for pupal weight. The comparable analogy in the Early Pupation population was not true, although the direction was similar. The pupal weights in the backcross progeny in the Early Pupation population were greater in generation 5 than any of the preceding generations. However, there was little difference in the two groups. There was little difference in ages at time of stage changes in the backcross and normal generation 5 progeny within the Weight population. Since there were no normal generation 7 progeny in the Weight population, there was no comparison with the backcross progeny.

Again, it is important to realize that pupal weights were obtained at a given calendar age rather than at a physiological age. Englert and Bell (1963) showed a decrease in pupal weight during the pupal stage, particularly during the latter portion of the pupal stage. Thus, the individuals which had pupated early would have a lighter pupal weight, even though some adjustment was made by changing the day of weighing. A design which would allow for adequate adjustment for the time within the pupal stage at which weight was obtained, or one which would allow pupal weights to be obtained at a certain point of time during the pupal stage would have merit. Although the extreme environmental differences between the incubator and

room conditions may be comparable to extremes for other species, such differences should be minimized in order to adequately investigate quantitative traits in Tribolium castaneum.

Pupal weight appeared to be nearly a classical quantitative trait, while ages at time of stage changes were less predictable. The latter situation was probably due to inadequate measurement of age and the effects of environmental fluctuations. Pupal weight, pupation age and adult emergence age appeared to be moderately heritable, and genetic correlations among them apparently existed. Only direct response to selection for heavy pupal weight was accurately predicted. However, the maintenance of a control population at the same stage of the life cycle would be most desirable if fitness traits such as ages at time of stage change are under consideration.

CONCLUSION

Day of weighing during the pupal stage, creamer bottle size and sex of individuals were all highly significant sources of variation in pupation age, pupal weight and adult emergence age. Family size at 14 days exhibited a significant positive influence on pupal weight. Family size did not statistically affect pupation age or adult emergence age. Only day of weighing accounted for any appreciable amount of variation in the two stage traits, whereas sex also accounted for some variation in pupal weight. The estimates of heritabilities of the three traits from component analyses of data collected from random mating populations indicated that all three traits had moderate heritabilities. The estimates of heritabilities from the sire and dam components were quite similar for the stage traits. The estimate of heritability of pupal weight from the dam component of variance was more than twice the estimate obtained from the sire component of variance.

Only the direct response to selection for heavy pupal weight was accurately predicted. The regressions of generation averages of the stage traits on generation number were positive, but not significant in the Weight population. The direct response to selection for early pupation age was not accurately predicted. However, the trend of average pupation age was negative and significant. The regressions of average pupal weight and of average emergence age on generation number were statistically significant in the Early Pupation population.

Although genetic correlations among the three traits existed, the correlated responses to selection were not adequately predicted. Apparently

ages at time of stage changes were influenced by slight environmental fluctuations and/or the ages were inaccurately established. More accurate knowledge of the time at which an egg was laid, the time at which a stage change occurred and greater control over the environment throughout the life cycle would have been desirable.

The Control population was maintained by random selection, yet significant changes occurred in all three traits during the seven generations. The three populations were separated in time. Although the three populations had the same origin, no effort was made to utilize the Control population performance as a base from which the other two populations could be deviated. The use of sires from the base generation indicated that males retained their fertility adequately for at least six generations after they were hatched.

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