Chapter 4

Selection Experiment

4.1 Introduction

Duration of development is an important fitness component (Dawson 1977; Lavie 1981; Lewontin 1965). The relationships between development time and other life-history characters, in particular life-span, have been the subject of much speculation, but whether life-span can respond to selection has been doubted because of apparent lack of genetic variation. However, in *Drosophila* longevity appears to be influenced by genes whose expression can be modified by the environment during development (Clare and Luckinbill 1985; Economos and Lints 1986b; Luckinbill, Arking, Clare, Cirocco and Buck 1984; Luckinbill and Clare 1985), and it has been hypothesised that stressful conditions for the developing organism may reveal additive genetic variation for longevity, previously hidden by developmental homeostasis (Clare and Arking 1986).

This decanalisation of the phenotype has been tested and demonstrated in *Drosophila*, using uncontrolled larval density as the environmental trigger (Luckinbill and Clare 1985), but not in any other species.

Divergent selection for developmental period in a population of *T. castaneum* in optimal conditions, followed by a life-history assay, should confirm whether changes in duration of development affect longevity in *Tribolium*. Correlated responses in length of life-span were not expected on the basis of results of the diallel analysis. The heritabilities of traits were low and the genetic correlations between indices of development and indices of life-span were very small and, in the case of paternal half-sisters, undefined.

If indirect selection for longevity was not successful, this selection experiment would not

discriminate between:

a) no correlated variation between development and longevity; or

b) developmental buffering systems suppressing the expression of genetic covariation.

Of course, since developmental stability does decrease due to selection (Leamy 1986), strong directional selection for development may itself be a stress which allows the expression of genetic variation for longevity. In this case, there could be correlated response for longevity. Selection for high dispersal in *Tribolium,* a trait associated with colonisers selected for early adult fitness, has produced beetles with faster developmental times and lower longevities than beetles selected for low dispersal (Lavie 1981; Lavie and Ritte 1978; Wu 1981).

Selection for the one fitness character, developmental duration, enabled the predictions based on the genetic parameters estimated from the base population to be tested. Results also were used to confirm or to put into question some of the conclusions concerning the evolutionary theories of senescence that were derived from the genetic variance-covariance matrix.

Any short-term constraints on response to natural selection also should have become apparent.

4.2 Materials and Methods

4.2.1 Base Population

The culturing conditions were the same as described in sections 2.3 and 3.2.1.

During the diallel analysis, the natural population was maintained so as to minimise random drift (Bray *et al.* 1962), i.e. the breeding population was not less than 50 males and 50 females, and mass mating was not used.

From the same generation that the parent beetles of the diallel were drawn, 108 males and 108 females were taken at random and set up as single pairs each in 1 g media. After 8 days, each single pair was transferred into 2 g of medium (day 0), from which they were removed after 24 hours and discarded. The 108 vials containing eggs were retained. From day 19 all vials were checked for pupae. Pupae were sexed and placed into one of two single-sex bottles. Since selection was to be for developmental period, which can be affected by handling (Kence 1973), vials were checked for pupae only once every 72 hours. This allowed

pupae to be in contact with larvae for substantial amounts of time, and cannibalism may have exerted a selection pressure for slow development. This selection pressure should have been slight, as there was separate culturing of each family and food medium was in excess. As time to adult emergence did not increase (Appendix 4, Tables 1, 2 and 3), it was assumed that cannibalism did not exert undue selection pressure.

Daily checks for pupae continued until all larvae pupated or only larve were left that were extremely small and showed no propensity to pupate. From day 19, the two single-sex bottles of pupae were examined for the presence of adults and adult emergence was recorded.

Once all adults had emerged, 108 males and 108 females were randomly selected and set up as single pairs in 1 g media each. Eight days later the single pairs were transferred into 2 g media each for 24 hours and then discarded. The retained eggs were the basis for the next generation.

Three generations were maintained in this fashion (Appendix 4): Generations 3, 4 and 5 in the laboratory.

4.2.2 The Selection Program

Figure 4.1 is a diagrammatic presentation of the experimental procedure, which is detailed in the text.

Generation 5 provided the parents for the first generation of selection. Once all adults had emerged in Generation 5, 150 males and 150 females were taken at random and placed en masse into 150 g media for 8 days. The adults then were transferred at 24 hour intervals into 150 g of fresh media to obtain three successive 24 hour egg collections. After each egg collection, the eggs were counted and three sets of 400 eggs were each transferred into 200 g media in plastic containers. Each set of eggs was randomly assigned to be either the control line, the line selected for short duration of development or the line selected for long duration of development. Thus each successive egg collection provided one replicate of each line. To minimise environmental variation, all replicate egg collections were placed on one shelf in the incubator and lines were randomised within each replicate.

Procedures for checking for pupae and adult emergence in each line were the same as for the previous generations, except that larvae were kept *en masse.* Once all adults of all the lines in one replicate had emerged 20 males and 40 females were chosen as parents for the next generation of each line. The fastest 20 males and 40 females were selected

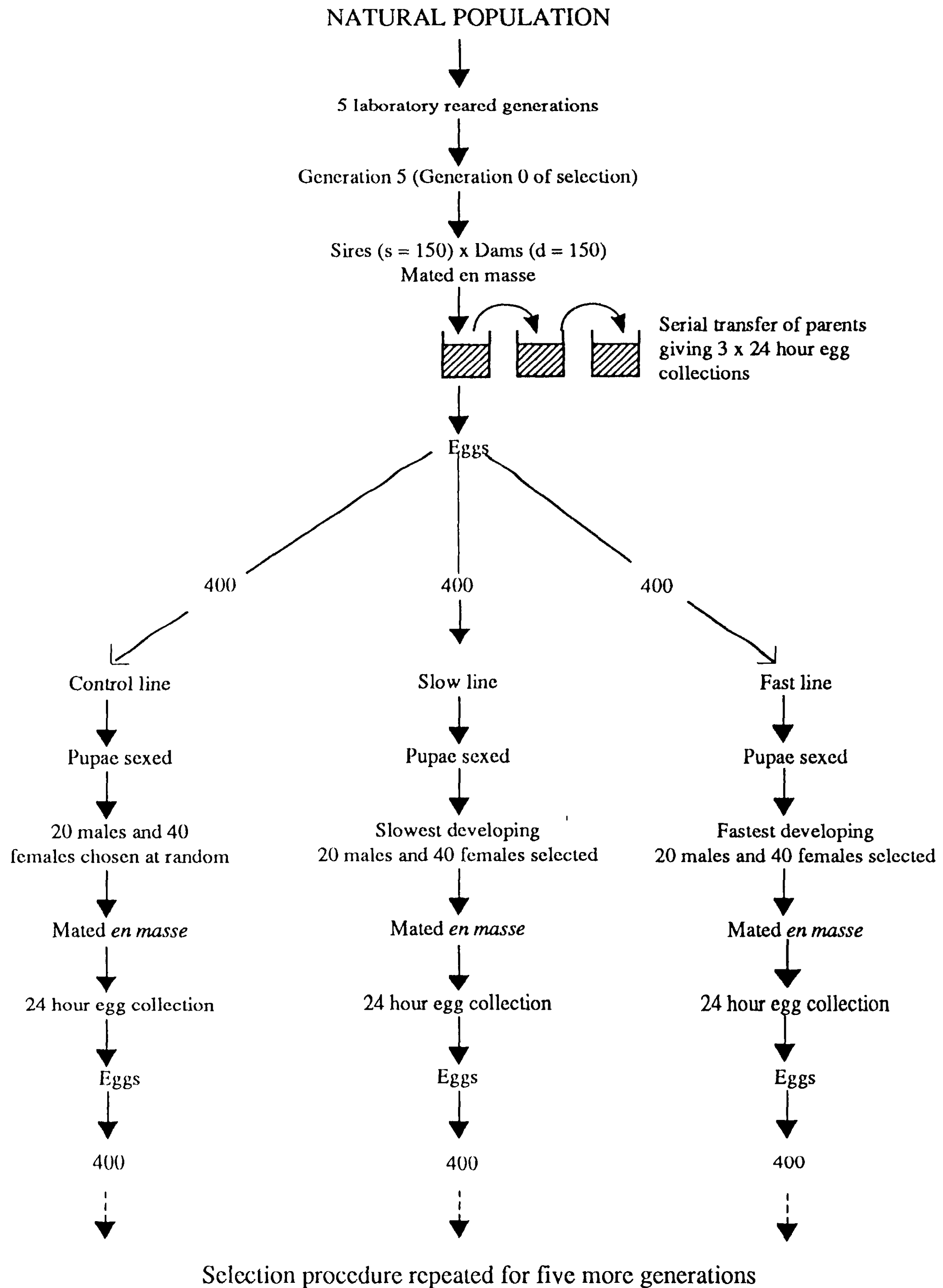

Figure 4.1: Schematic presentation of the Selection experiment

as parents for the Fast line, the slowest 20 males and 40 females for the Slow line and a random selection for the Control. Mass selection was the selection method chosen as it was the simplest method to put into practice (Falconer 1981). After 8 days, once the youngest beetle had matured, the parents of each line were placed into 40 g media for 24 hours and then discarded. The number of eggs laid were counted, recorded (Appendix 5) and 400 from each line were retained and seeded into 200 g medium. Within lines, parental beetles differed by less than three weeks of age, thus there should have been no parental age effect on progeny traits (Dawson 1965b; Soliman and Lints 1975). Comparisons between the productivity of each line as a measure of reproductive fitness was not feasible because on average, parental beetles in different lines were at different stages of their reproductive schedule when eggs were collected.

This selection procedure was repeated for five more generations. The large number of parents, 20 males and 40 females, selected to found the new generation of each line was used to minimise the variance of the realised heritability estimate. The proportion of adults selected from each line (p) should be between 15-20% to minimise the variance of the realized heritability estimate (Soller and Genizi 1967). For 400 eggs, p = 60/400 = 0.15. If there were 25% mortality, far higher than actual mortality rates, p = 60/300 = 0.20. Four hundred eggs per generation were guaranteed by the use of forty females. Twenty males insured that all females would be successfully fertilised, and that inbreeding would not be excessive.

After six generations of selection there was a significant response to selection and work on the diallel experiment was significantly reduced.

In generation 7 of selection, which was the parental generation of the beetles in the life-history assay, selection was relaxed and parents were chosen at random from each line. The relaxation of selection was to make certain that there were no cumulative parental age effects which could have affected the assays (Mertz 1975).

4.2.3 The Life-history Assay

The 20 males and 40 females of each line at generation 7 were transferred into 40 g media for 24 hours (day 0). The adults were then discarded. The eggs were counted and 216 eggs were retained for each line and transferred into 225 g media. For each line, a maximum of 108 females were to be assayed; a number determined by space and laboratory restrictions. The density of developing larvae was less than one per gram media, so duration of development

should have been unaffected by crowding.

From day 19, lines were checked every three days for pupae. Pupae were sexed and placed in single sex vials. Adult emergence was checked daily and all emergent adults had their day of emergence and weight recorded. Males were discarded but each female was placed in a vial with 1 g medium and a male of a black mutant strain of *T. castaneum.*

The black strain, derived from the same stock as used in the diallel experiment, was used to facilitate the sex identification of dead beetles.

Echarina males were discarded because they entailed too much extra work to set up separately with unrelated females to obtain longevity data.

Vials of one line of one replicate were kept together. As before all replicates were placed on one shelf in the incubator and lines were randomised within each replicate. Egg production of each female was assayed by two consecutive 48 hour egg collections seven days after adult emergence. As well each female's egg production was measured and recorded between days 46 and 48, and days 53 and 55 (taken from day 0). Subsequently, eggs were collected over a 48 hour period at 12 day intervals.

Eggs laid by individual females between days 53 and 55 were counted, retained and placed into individual vials containing 2 g medium each. Twenty days later, the numbers of larvae and pupae in each vial were counted and recorded. By mating females with unrelated males, the survival of progeny of pairs could be determined. This index, called productivity, is an important component of population fitness, as it is a good estimate of the potential rate of increase (Sokoloff 1977). It may have been affected differentially by selection pressures in opposing directions.

4.2.4 Statistical Analyses

4.2.4.1 Selection Response

The main purpose of the selection program was to obtain a significant deviation of the mean phenotypic value of developmental duration of selected lines from control lines.

The response to selection also was used to estimate the realized heritability of developmental time, for comparison with the heritability estimate in the base population. Realized heritability was estimated as the regression coefficient of the generation means on cumulated selection differential (Hill 1971, 1972a,b).

The selection differential is the mean phenotypic value of the individuals selected as

parents expressed as a deviation from the mean phenotypic value of all the individuals in that generation. The cumulated selection differential is the summation of selection differentials over successive generations. Selection differentials may be weighted (effective selection differentials) by the proportionate contribution of individual parents to the individuals that are measured in the next generation. The effective selection differential allows differences in fertility, and thus the possible operation of natural selection through this mechanism, to be taken into account. Since the numbers of offspring per male or female parent were not counted, the selection differentials could not be weighted. If natural selection were important as a factor influencing the response, then heritabilities were somewhat underestimated for duration of development assuming that natural selection opposes artificial selection for development time in either direction.

The selection differentials were calculated separately for males and females, then averaged i.e. $S = \frac{(S_m + S_f)}{2}$. This is because though the sexes differed in the numbers used as parents, half the genes in the offspring came from each parental sex (Falconer 1981).

For the calculation of realized heritabilities, responses of selection lines were expressed in terms of deviations of their generation means from the pertinent control lines (Englert and Bell 1970). This was to eliminate environmental fluctuations as a cause of variation between generation means, enabling a more accurate measurement of response. Divergent selected lines can act as "controls" for each other, but are less useful because of the likelihood of asymmetry of response (Hill 1980). Many studies have demonstrated asymmetrical responses to selection in opposite directions for developmental rate in *Tribolium castaneum* (Dawson 1965a; Englert and Bell 1970; Soliman 1982). The unselected control populations also were used to reveal if inbreeding depression (Falconer 1981) could be responsible for any asymmetry of response.

Realized heritabilities were calculated for each line of each replicate separately. The response of each selected line of a particular replicate was "corrected" by the Control line of the same replicate, as the Control was thought to be the best estimate of environmental trends experienced by the selected line.

The arithmetic mean of the regression coefficients was also calculated, and was a better estimate of realized heritability than those calculated from single replicates because of the larger numbers of selected parents and measured progeny involved.

The replicated selected lines allowed estimation of the sampling variance of the realized heritability. The standard error of the regression coefficient underestimates the standard

error of the heritability because the cumulative nature of the changes due to random genetic drift are not accounted for by the deviations of each replicate about its own regression line (Falconer 1973,1981; Hill 1971). Differences between replicates of selection lines, if not biased by sampling, are due to genetic drift (Falconer 1981). The empirical standard error, calculated from the actual variation of regression coefficients between replicates, is the valid standard error of the realized heritability for each line (Falconer 1973).

Coefficients of variation were checked for constancy at generation seven. Scale effects were present (Appendix 6), but realized heritability estimates were calculated from the untransformed data since they are little influenced by scale effects (Falconer 1954). Plotting generation means against the cumulated selection differentials removed scale effects which may have produced asymmetries in response (Falconer 1981).

4.2.4.2 Phenotypic Assay

Male traits assayed for responses to selection for developmental time in generation 8 were:

1) ADULT = time interval between day 0 and adult eclosion (days).
2) WT = adult body weight on day of adult emergence (mg).
3) GR = WT/ADULT (mg/day).

Many of the female reproductive indices were derived from the data in the same manner as for the diallel analysis of the base population (see section 3.2.2). Female traits assayed for responses to selection for developmental time in generation 8 were:

1) ADULT = time interval between day 0 and adult eclosion (days).
2) WT = adult body weight on day of adult emergence (mg).
3) GR = WT/ADULT (mg/day).
4) DEATH = time interval between day 0 and day of death (days).
5) ALS = time interval between eclosion and DEATH i.e. adult life- span (days).
6) EGGTOT = the total number of eggs produced in the lifetime of each female (eggs).
7) MAXEGG = the maximum number of eggs laid by a female in a 48 hour period (egg/day).
8) MAXDAY = day of peak egg production i.e. MAXEGG (day).

9) LASEGG = last egg collection from which eggs were collected (day).
10) RLS = time interval between eclosion and LASEGG i.e. reproductive life-span (days).
11) DAY711 = total number of eggs laid in the time interval 7 and 11 days following eclosion (eggs).
12) EGG54 = total number of eggs laid during the 48 hour period between days 53 and 55 (egg/day).
13) Q1R = total number of eggs laid in the first quarter of RLS (eggs).
14) Q2R = total number of eggs laid in the second quarter of RLS (eggs).
15) Q3R = total number of eggs laid in the third quarter of RLS (eggs).
16) Q4R = total number of eggs laid in the fourth quarter of RLS (eggs).
17) ADFR = average daily fecundity for RLS (egg/day).
18) ADFQ1R = average daily fecundity for Q1R (egg/day).
19) ADFQ2R = average daily fecundity for Q2R (egg/day).
20) ADFQ3R = average daily fecundity for Q3R (egg/day).
21) ADFQ4R = average daily fecundity for Q4R (egg/day).
22) Q1L = total number of eggs laid in the first quarter of ALS (eggs).
23) Q2L = total number of eggs laid in the second quarter of ALS (eggs).
24) Q3L = total number of eggs laid in the third quarter of ALS (eggs).
25) Q4L = total number of eggs laid in the fourth quarter of ALS (eggs).
26) ADFL = average daily fecundity for ALS (egg/day).
27) ADFQ1L = average daily fecundity for Q1L (egg/day).
28) ADFQ2L = average daily fecundity for Q2L (egg/day).
29) ADFQ3L = average daily fecundity for Q3L (egg/day).
30) ADFQ4L = average daily fecundity for Q4L (egg/day).

31) PROD = productivity = percentage of live progeny 20 days after egg collection (day 53-55 of parental generation).

The variable PROD was filtered by the analysis program Reg87 to exclude individuals with missing values. This filtering effectively excluded all beetles from the analysis who died before day 54 and probably died from non-senescent causes. Fifty-three beetles died between adult emergence and day 54 and, of these, only five laid eggs.

Apart from this difference in cut-off points for non-senescent deaths between the two experiments, there are other reasons why the mean values for variables in this assay may not be comparable with those estimated in the diallel analysis. Many *Tribolium* traits are sensitive to environmental conditions. Small changes in the environment can elicit large changes in traits such as developmental rate, weight and oviposition (Sokoloff 1977). Beetles of the diallel analysis were far more frequently handled as larvae and pupae than beetles of the phenotypic assay. The density of larvae also was greater. The developmental periods of the two groups were probably differentially affected, and this may have had far-reaching effects on other life-history traits for both groups. Furthermore, beetles of the phenotypic assay in the first seven days following adult emergence did not experience the stimulatory effect on oviposition of fresh media every 48 hours (Sokoloff, Shrode and Bywaters 1965), as did beetles of the diallel analysis. This difference in handling technique may have affected oviposition between days 7 and 11, and, because of the phenomenon of reproductive compensation (Boyer 1978) may have also affected rates of late life oviposition.

For these reasons comparisons were not drawn between results of the diallel analysis and the phenotypic assay, but restricted to between lines of generation 8. Consistent bidirectional changes in selected lines relative to their unselected Controls for unselected traits were assumed due to the effects of selection (Lynch 1980), and thus show genetic covariance. Though traits were only measured in one generation at the end of selection, replication of selected lines indicated whether changes were due to the effects of selection or merely the result of fluctuating generation means.

Differences between lines were identified by analyses of variance for each trait. The computer program Reg87 was used for the analysis. It uses the regression approach, so lines were adjusted for blocks.

The same variables as in the diallel analysis were square-root transformed to more closely approximate normality i.e. MAXDAY, Q1R and ADFQ1R. Productivity required an

inverse sine transformation. This transformation improved its normality slightly, reducing the positive kurtosis and negative skewness.

The experiment was analysed as a randomised complete block, as each line of selection was represented in each replicate or block, which was a homogeneous unit within the incubator. No interaction was expected between lines and blocks so the linear statistical model assumed was a fixed effects model without interaction and with sampling(Steel and Torrie 1981):

$$Y_{ijk} = \mu + L_i + B_j + E_{ij} + \gamma_{ijk}$$

where			
	Y_{ijk}	=	the phenotypic value of the kth beetle;
	μ	=	the value of the overall mean;
	B_j	=	the fixed effect of the jth block;
	L_i	=	the fixed effect of the ith line;
	E_{ij}	=	experimental error; and
	γ_{ijk}	=	sampling error.

For a balanced design:

Source	**d.f.**	**Expected Mean Square**
BLOCK	(b-1)	$\sigma^2 + s\sigma_E^2 + sl\Sigma B_j^2/(b-1)$
LINE	(l-1)	$\sigma^2 + s\sigma_E^2 + sb\Sigma L_i^2/(l-1)$
EXPERIMENTAL ERROR	(b-1)(l-1)	$\sigma^2 + s\sigma_E^2$
SAMPLING ERROR	lb(s-1)	σ^2
TOTAL	bls-1	

where			
	b	=	number of blocks;
	l	=	number of lines; and
	s	=	number of individuals per line within a block.

The appropriate error term for testing hypotheses concerning lines was the experimental error term.

In practice, testing against the experimental error term was only an approximation, as the actual design was unbalanced, and thus the Expected Mean Squares probably varied from above. Significance levels should be treated with caution.

The non-orthogonality of the design also meant that the summation of Block SS, Line SS and Experimental Error SS did not give the full model SS.

When the analysis of variance detected differences between the means of lines, comparisons between line means were made by the method of least significant difference (lsd) (Steel and Torrie 1981). Line means were the weighted means from the three blocks.

4.3 Results

4.3.1 Response to Selection

Figures 4.2 and 4.3 show the results of 6 generations of bidirectional selection for duration of development for males and females respectively. Selection appears to have been successful in both directions, though response was asymmetrical in favour of longer developmental time.

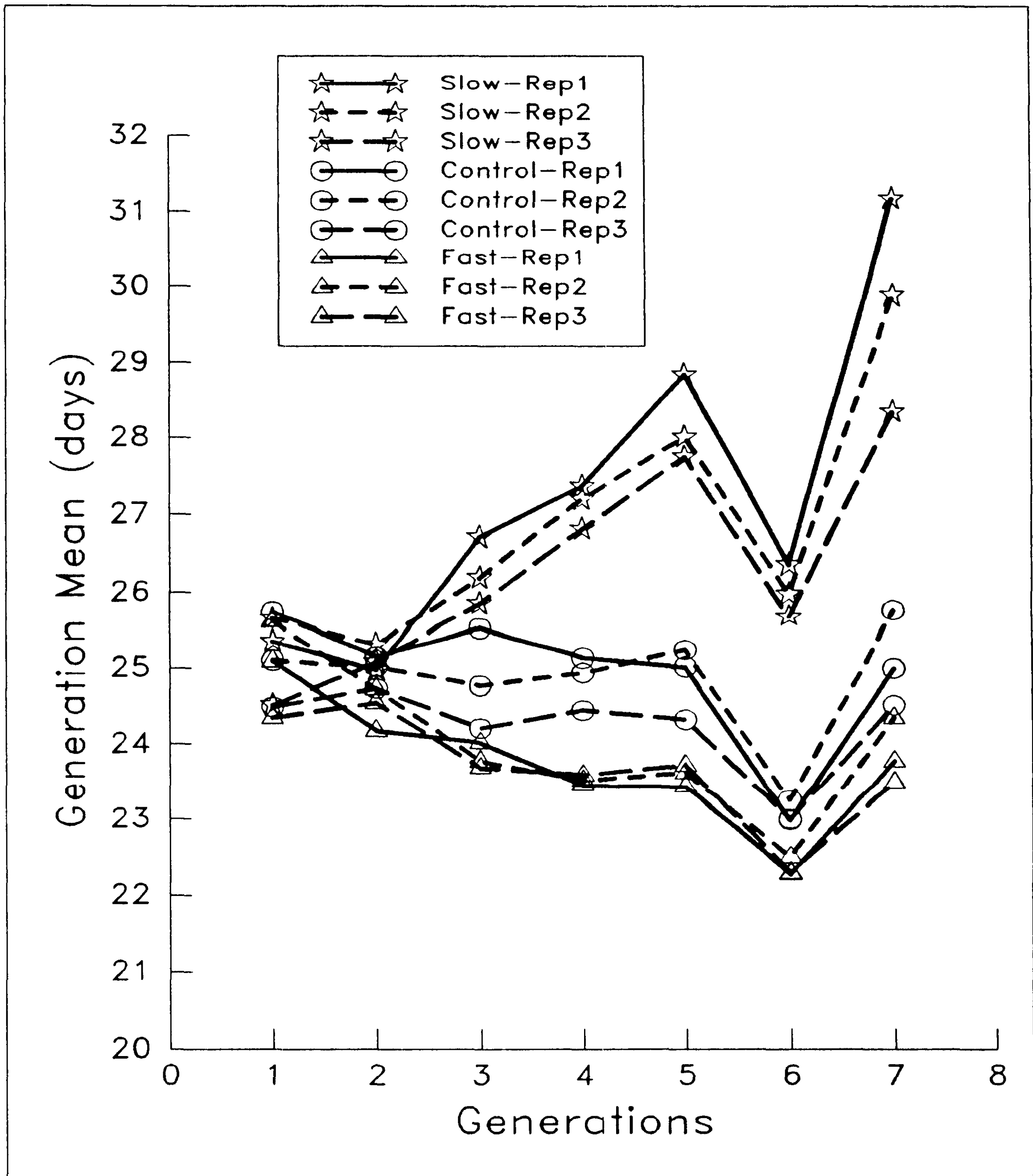

Figure 4.2: Mean time to adult emergence (generation mean) of males of the three replicates in each line, Fast, Control and Slow developing.

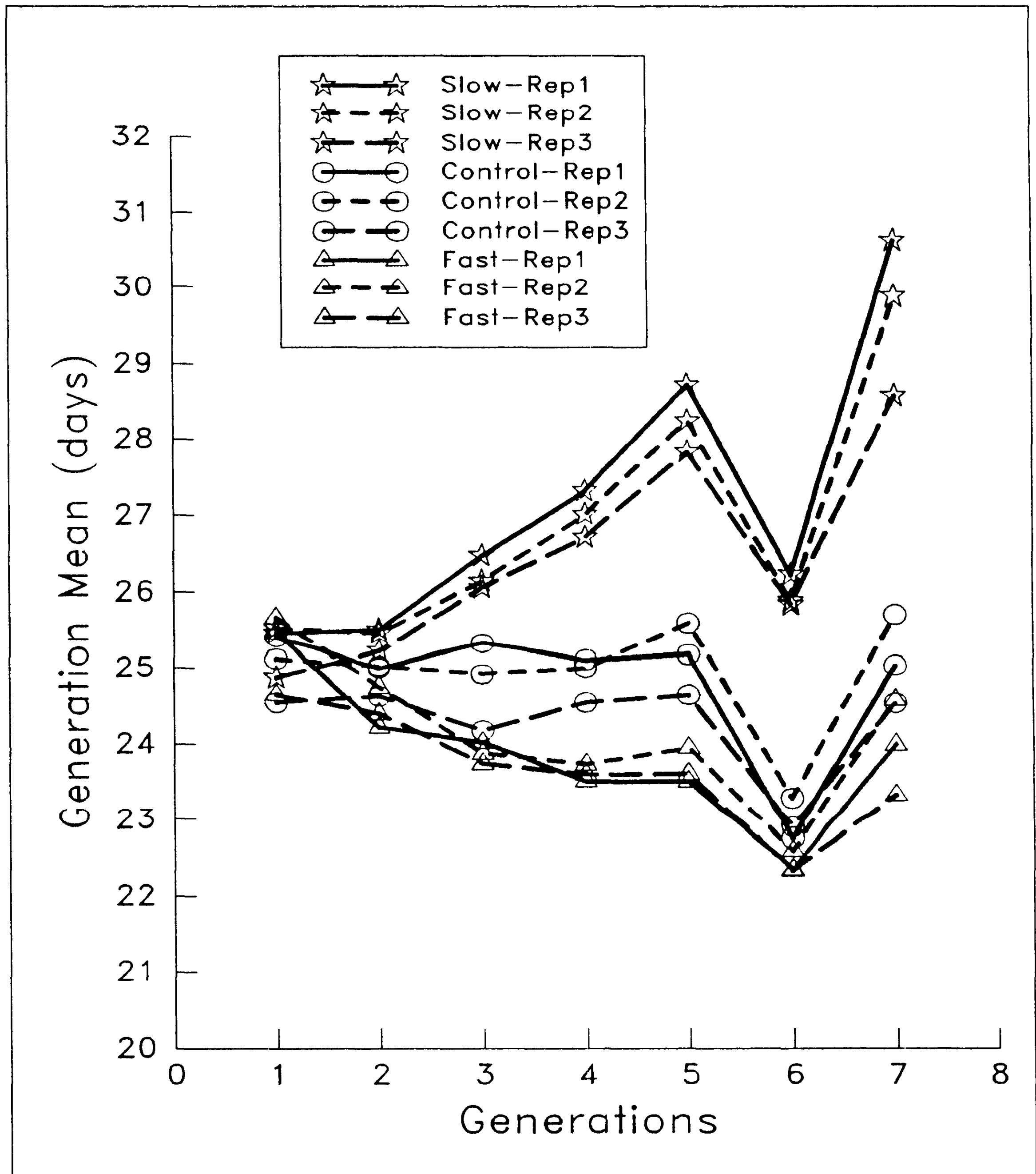

Figure 4.3: Mean time to adult emergence (generation mean) of females of the three replicates in each line, Fast, Control and Slow developing.

A sudden decrease in duration of development occurred in generation six for all replicates in each line. As this decrease was general for all replicates and all lines, a common environmental effect must be assumed. On day 5, 4 and 3 of replicates 1, 2 and 3 respectively of generation 6, the electricity supply was cut off for six hours with a resultant drop in the temperature of the incubator. This was the only difference in experimental conditions for generation six and it can only be assumed that this temperature change was responsible for the faster developmental rates. Duration of development of *Tribolium castaneum* is sensitive to temperature, usually increasing with decreasing temperatures below 35°C (Howe 1956), so the faster development is unexpected. It would be interesting to investigate whether a short sharp decrease in temperature a few days after hatching, always results in a shorter duration of development and whether it has any effects on adult life-history.

The relative constancy of the developmental rates of the control lines, apart from generation six, indicates inbreeding depression did not affect the character. This was not unexpected as the rate of inbreeding was less than 1% per generation. The maintenance of control lines was obviously necessary, as they did enable a correction of the response of selection lines for the deviations at generation 6.

The responses for the two sexes paralleled each other closely, so henceforth in all subsequent figures, the generation means are the average of the two sexes.

Figures 4.4, 4.5 and 4.6 show the individual responses of each replicate.

Realized heritabilities and their standard errors are given in Table 4.1.

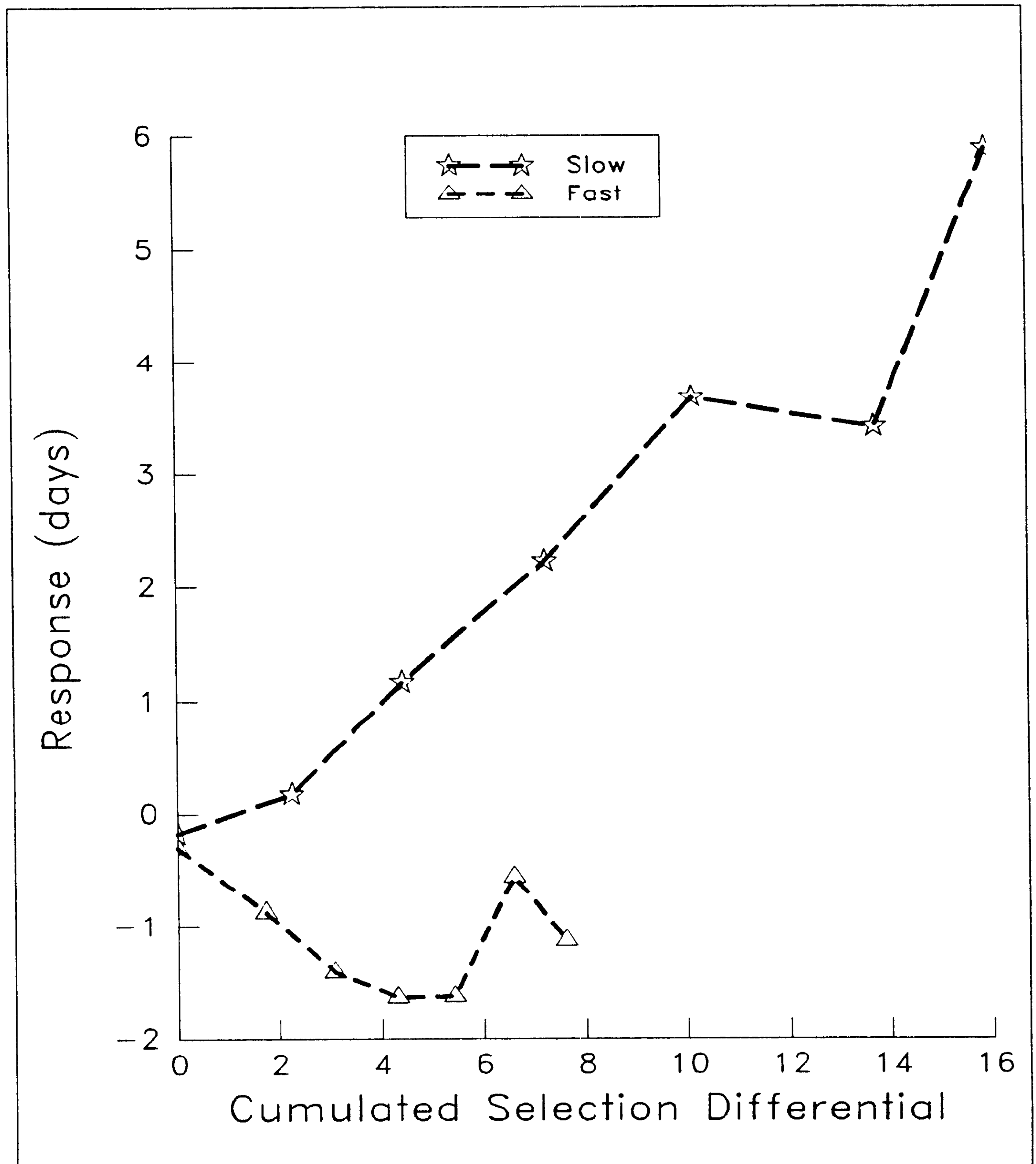

Figure 4.4: Responses to selection for replicate 1, expressed as deviations from the Control, plotted against cumulated selection differentials.

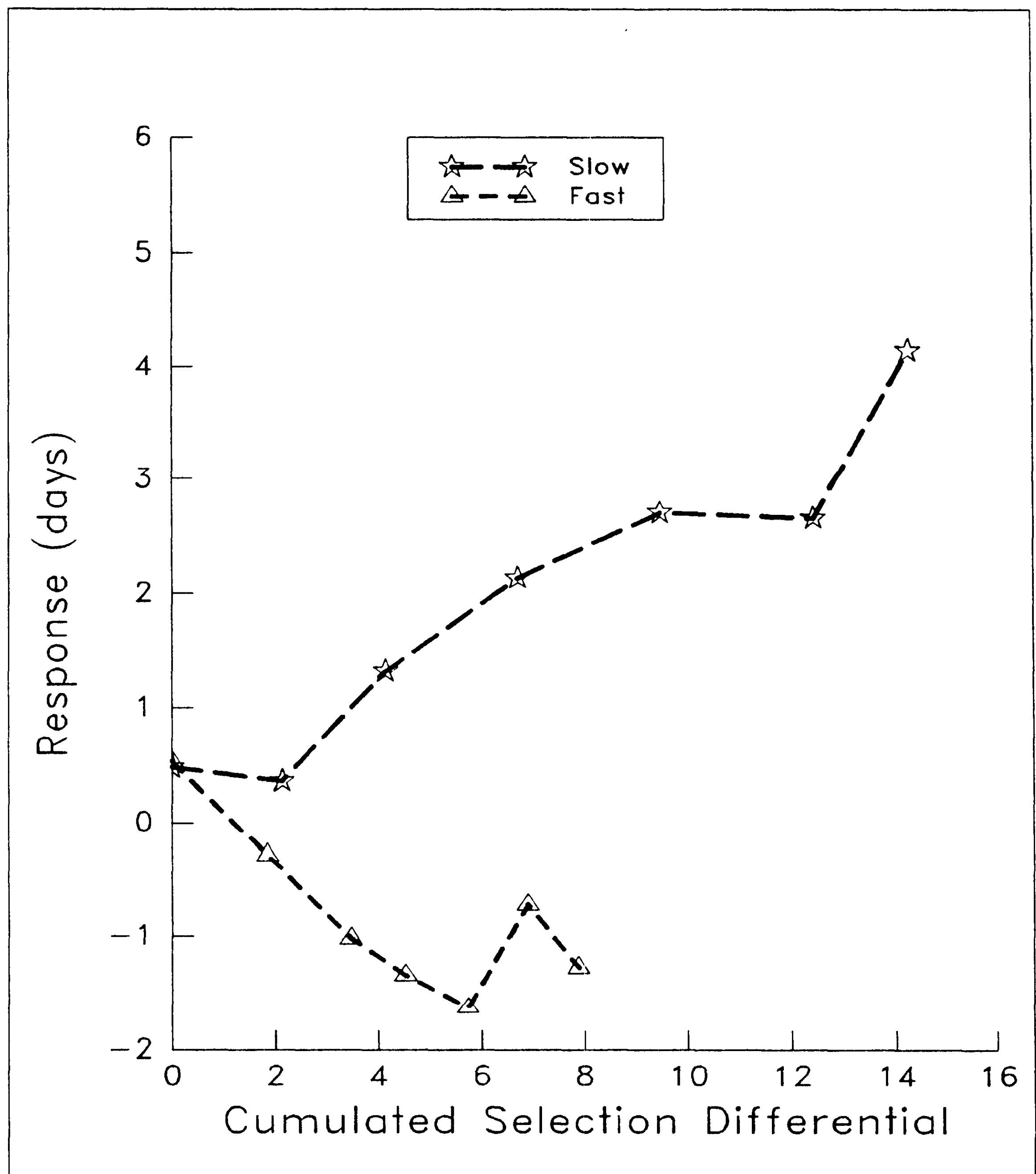

Figure 4.5: Responses to selection for replicate 2, expressed as deviations from the Control, plotted against cumulated selection differentials.

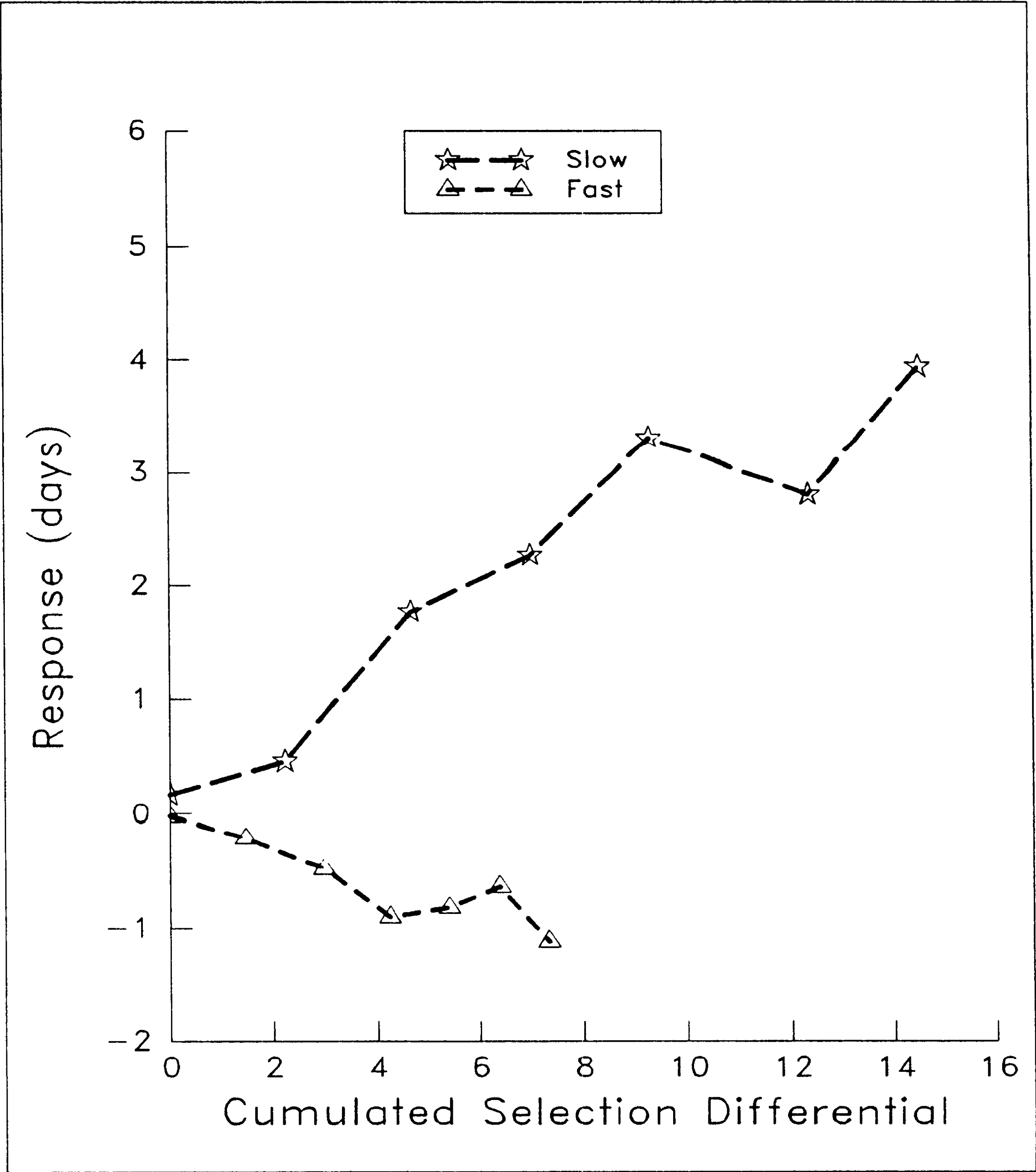

Figure 4.6: Responses to selection for replicate 3, expressed as deviations from the Control, plotted against cumulated selection differentials.

Table 4.1: Realized heritabilities for the separate replicates and for the means of all Fast and Slow developing lines of *Tribolium castaneum.* Regression coefficients (b) are presented with standard errors (± SE).

Replicate	**Fast**		**Slow**	
	b	**SE**	**b**	**SE**
1	0.070 ±	0.081	0.353±	0.042
2	0.205±	0.075	0.245±	0.032
3	0.132±	0.028	0.253±	0.037
*Replicate Average**	0.136±	0.039	0.284±	0.035

* Arithmetic mean of b's with empirical standard error based on variance of b between replicates.

The mean realized heritabilities were 13.6% and 28.4% for the Fast and Slow lines respectively, and were both significantly different from zero at 0.01 level of probability (one-tailed t-test).

There was a marked asymmetry in response, with selection for increased duration of development more than twice as effective as selection for decreased duration. The realized heritability for increased duration of development was comparable with the paternal half-brother estimate (26.4 ± 10.1%) and the paternal half-sister estimate (22.8 ± 11.7%) in the base population. On the other hand, the realized heritability estimate for decreased duration was markedly lower than either estimate.

Contrary to predictions of Hill (1971) and Falconer (1973, 1981) the standard errors calculated from the actual variation between replicates were not substantially greater than the standard errors of the regression coefficients. Thus over the seven generations, random genetic drift was not an important factor in the differentiation of replicate lines.

4.3.2 Phenotypic Assay

4.3.2.1 Means and Standard Deviations

Means and standard deviations of male traits for the Fast, Control and Slow developing lines are presented in Tables 4.2, 4.3 and 4.4 respectively. Values for female traits of Fast,

Table 4.2: Means and standard deviations of male traits for replicates of the Fast developing lines in the phenotypic assay.

Traits	Replicate 1*	Replicate 2**	Replicate 3***
ADULT	21.989 ± 0.913	22.461 ± 2.151	22.064 ± 1.134
WT	1.576 ± 0.212	1.616 ± 0.176	1.718 ± 0.183
GR	0.072 ± 0.009	0.072 ± 0.009	0.078 ± 0.009

* n = 91, ** n = 102, *** n = 94

Table 4.3: Means and standard deviations of male traits for replicates of the Control lines in the phenotypic assay.

Traits	Replicate 1*	Replicate 2**	Replicate 3***
ADULT	22.654 ± 0.760	23.517 ± 1.680	22.844 ± 1.249
WT	1.628 ± 0.150	1.712 ± 0.262	1.814 ± 0.203
GR	0.072 ± 0.007	0.073 ± 0.009	0.080 ± 0.009

* n = 104, ** n = 89, *** n = 109

Control and Slow lines are given in Tables 4.5, 4.6 and 4.7.

There was a slight increase in variance with an increase in developmental duration of males and females of slow lines. Examination of coefficients of variation for ADULT though for each of the lines showed that the increase was probably a scale effect, as the coefficients were all very similar (Table 4.8).

Table 4.4: Means and standard deviations of male traits for replicates of the Slow developing lines in the phenotypic assay.

Traits	**Replicate 1***	**Replicate 2****	**Replicate 3*****
ADULT	27.517 ± 2.749	26.786 ± 2.570	26.548 ± 1.908
WT	1.678 ± 0.240	1.727 ± 0.201	1.655 ± 0.180
GR	0.062 ± 0.012	0.065 ± 0.009	0.063 ± 0.008

* n = 87, ** n = 84, *** n = 93

Table 4.5: Means and standard deviations of female traits for replicates of the Fast developing lines in the phenotypic assay.

Traits	Replicate 1*	Replicate 2**	Replicate 3***
ADULT	22.032 ± 0.714	22.258 ± 0.927	22.148 ± 1.226
WT	1.712 ± 0.216	1.690 ± 0.168	1.886 ± 0.196
GR	0.078 ± 0.010	0.076 ± 0.007	0.085 ± 0.009
DEATH	158.237 ± 65.065	152.412 ± 58.722	162.840 ± 55.501
ALS	136.204 ± 65.027	130.155 ± 58.649	140.691 ± 55.436
EGGTOT	1204.032 ± 663.977	1350.771 ± 668.095	1614.040 ± 616.087
MAXEGG	16.070 ± 5.271	17.119 ± 5.247	19.457 ± 4.378
MAXDAY	41.390 ± 18.399	41.863 ± 16.735	40.694 ± 15.774
LASEGG	140.118 ± 57.084	142.423 ± 56.147	154.124 ± 53.884
RLS	118.086 ± 56.983	120.165 ± 56.104	131.975 ± 53.789
DAY711	60.659 ± 16.109	67.156 ± 12.537	73.138 ± 15.374
EGG54	13.635 ± 5.094	15.483 ± 4.484	17.595 ± 3.849
Q1R	372.154 ± 214.717	417.875 ± 221.219	499.562 ± 222.953
Q2R	363.405 ± 201.244	413.950 ± 207.757	491.117 ± 193.879
Q3R	309.360 ± 184.785	350.061 ± 183.379	412.104 ± 162.620
Q4R	159.113 ± 119.768	168.884 ± 114.933	211.256 ± 117.270
ADFR	9.533 ± 3.874	10.506 ± 3.856	12.096 ± 3.088
ADFQ1R	11.492 ± 4.270	12.612 ± 4.373	14.501 ± 3.683
ADFQ2R	11.662 ± 4.801	13.020 ± 4.822	14.780 ± 4.094
ADFQ3R	9.861 ± 4.629	11.034 ± 4.701	12.590 ± 3.925
ADFQ4R	5.117 ± 3.435	5.357 ± 3.507	6.511 ± 3.227
Q1L	416.102 ± 222.665	450.870 ± 233.447	534.783 ± 237.741
Q2L	379.518 ± 214.580	427.031 ± 216.138	507.912 ± 198.765
Q3L	282.823 ± 205.083	333.990 ± 200.514	395.921 ± 189.804
Q4L	125.589 ± 126.033	138.879 ± 115.373	175.424 ± 116.439
ADFL	8.725 ± 4.178	9.863 ± 3.969	11.431 ± 3.172
ADFQ1L	11.472 ± 4.331	12.702 ± 4.375	14.577 ± 3.687
ADFQ2L	11.030 ± 5.206	12.581 ± 5.031	14.410 ± 4.101
ADFQ3L	8.516 ± 5.505	9.931 ± 5.303	11.515 ± 4.811
ADFQ4L	3.880 ± 3.788	4.237 ± 3.672	5.221 ± 3.301
PROD	75.928 ± 30.390	86.962 ± 24.970	90.948 ± 17.329

* n = 85, ** n = 89, *** n = 80

Table 4.6: Means and standard deviations of female traits for replicates of the Control lines in the phenotypic assay.

Traits	Replicate 1*	Replicate 2**	Replicate 3***
ADULT	23.159 ± 1.347	23.416 ± 1.160	23.181 ± 2.063
WT	1.685 ± 0.186	1.867 ± 0.267	1.923 ± 0.221
GR	0.073 ± 0.008	0.080 ± 0.010	0.083 ± 0.009
DEATH	168.375 ± 65.600	154.614 ± 61.703	148.734 ± 54.633
ALS	145.216 ± 65.760	131.198 ± 61.771	125.553 ± 55.202
EGGTOT	1364.622 ± 772.165	1322.010 ± 705.609	1378.412 ± 619.620
MAXEGG	15.818 ± 5.814	17.951 ± 6.292	19.160 ± 4.607
MAXDAY	45.324 ± 18.194	42.590 ± 18.100	44.508 ± 20.314
LASEGG	147.773 ± 59.279	140.109 ± 54.351	138.266 ± 48.812
RLS	124.614 ± 59.435	116.693 ± 54.369	115.085 ± 49.477
DAY711	62.100 ± 15.607	69.309 ± 19.172	68.978 ± 18.941
EGG54	14.544 ± 4.856	16.048 ± 5.021	16.440 ± 4.680
Q1R	419.088 ± 239.405	418.846 ± 235.127	409.782 ± 214.460
Q2R	429.915 ± 238.117	411.895 ± 211.246	430.534 ± 199.916
Q3R	351.909 ± 216.217	332.119 ± 197.920	366.046 ± 164.807
Q4R	163.711 ± 137.575	159.151 ± 115.989	172.050 ± 105.130
ADFR	9.708 ± 4.112	10.572 ± 4.296	11.675 ± 3.300
ADFQ1R	11.817 ± 4.849	12.981 ± 5.131	13.463 ± 4.073
ADFQ2R	12.304 ± 5.070	13.305 ± 5.474	14.547 ± 4.338
ADFQ3R	10.055 ± 4.937	10.789 ± 5.212	12.641 ± 4.245
ADFQ4R	4.654 ± 3.421	5.214 ± 3.237	6.050 ± 3.367
Q1L	479.431 ± 261.571	460.689 ± 250.674	448.359 ± 248.043
Q2L	450.936 ± 256.524	432.296 ± 226.000	453.391 ± 217.824
Q3L	306.010 ± 234.309	308.502 ± 205.145	334.526 ± 174.495
Q4L	128.245 ± 148.846	120.523 ± 123.302	142.137 ± 118.691
ADFL	8.768 ± 4.316	9.814 ± 4.427	10.968 ± 3.504
ADFQ1L	11.863 ± 4.914	13.012 ± 5.118	13.502 ± 4.080
ADFQ2L	11.524 ± 5.503	12.866 ± 5.715	14.244 ± 4.495
ADFQ3L	8.143 ± 5.622	9.501 ± 5.623	11.149 ± 5.204
ADFQ4L	3.540 ± 3.783	3.874 ± 3.604	4.976 ± 3.985
PROD	83.158 ± 23.332	80.774 ± 29.931	87.402 ± 24.223

* n = 80, ** n = 93, *** n = 91

Table 4.7: Means and standard deviations of female traits for replicates of the Slow developing lines in the phenotypic assay.

Traits	Replicate 1*	Replicate 2**	Replicate 3***
ADULT	26.914 ± 1.915	26.515 ± 1.993	26.609 ± 2.224
WT	1.787 ± 0.232	1.799 ± 0.182	1.761 ± 0.183
GR	0.067 ± 0.010	0.068 ± 0.008	0.067 ± 0.008
DEATH	161.462 ± 52.838	159.594 ± 59.749	154.728 ± 55.350
ALS	134.548 ± 53.252	133.079 ± 59.777	128.120 ± 55.808
EGGTOT	1304.005 ± 653.318	1209.203 ± 668.878	1192.296 ± 570.912
MAXEGG	17.366 ± 5.016	16.470 ± 5.552	15.913 ± 5.242
MAXDAY	43.006 ± 13.221	43.013 ± 13.011	42.727 ± 11.891
LASEGG	152.957 ± 50.362	147.010 ± 60.037	143.739 ± 49.232
RLS	126.043 ± 50.739	120.495 ± 60.134	117.130 ± 49.694
DAY711	65.112 ± 16.090	64.851 ± 15.502	62.198 ± 12.892
EGG54	14.882 ± 4.531	14.851 ± 4.242	14.704 ± 3.629
Q1R	439.088 ± 235.818	409.859 ± 242.001	389.657 ± 196.617
Q2R	408.588 ± 212.618	386.377 ± 213.341	375.909 ± 178.310
Q3R	313.286 ± 174.478	282.625 ± 168.326	294.075 ± 160.129
Q4R	143.044 ± 89.047	130.342 ± 86.819	132.655 ± 85.935
ADFR	9.819 ± 3.449	9.330 ± 3.705	9.514 ± 3.484
ADFQ1R	12.841 ± 4.256	12.225 ± 4.622	12.128 ± 4.160
ADFQ2R	12.461 ± 4.849	12.011 ± 4.764	12.087 ± 4.455
ADFQ3R	9.531 ± 4.303	8.858 ± 4.374	9.498 ± 4.372
ADFQ4R	4.445 ± 2.500	4.226 ± 2.864	4.342 ± 2.755
Q1L	466.903 ± 241.617	448.328 ± 250.139	427.161 ± 222.145
Q2L	419.715 ± 220.420	398.381 ± 223.128	390.463 ± 189.642
Q3L	302.492 ± 177.038	259.352 ± 176.902	270.002 ± 165.643
Q4L	114.896 ± 95.603	103.143 ± 93.979	104.671 ± 90.976
ADFL	9.291 ± 3.465	8.583 ± 3.812	8.896 ± 3.498
ADFQ1L	12.928 ± 4.272	12.333 ± 4.617	12.183 ± 4.155
ADFQ2L	12.023 ± 4.813	11.299 ± 4.983	11.639 ± 4.481
ADFQ3L	8.783 ± 4.478	7.517 ± 4.830	8.379 ± 4.745
ADFQ4L	3.431 ± 2.809	3.185 ± 3.020	3.383 ± 2.969
PROD	87.010 ± 21.186	83.911 ± 19.974	84.407 ± 20.645

* n = 89, ** n = 94, *** n = 86

Table 4.8: Coefficients of Variation (%) for ADULT of males and females for selected and control lines.

Line	Replicate 1	Replicate 2	Replicate 3
Male			
Fast	4.15	9.58	5.14
Control	3.35	7.14	5.47
Slow	9.99	9.59	7.19
Female			
Fast	3.24	4.16	5.54
Control	5.82	4.95	8.90
Slow	7.12	7.52	8.36

4.3.2.2 Analyses of Variance

F-ratios and significance levels from the analyses of variance (ANOVAs) for male traits are presented in Table 4.9. The full anova are contained in Appendix 9 for male traits and Appendix 10 for female.

Table 4.9: F-ratios and significance levels from the ANOVAs for male traits.

Traits	F	P
ADULT	103.0025	***
WT	1.3604	NS
GR	18.4555	**

** $P \leq 0.01$, *** $P \leq 0.001$, NS = non-significant

As expected there were significant differences between lines for developmental time. The weighted means ($\overline{Y}_W$) of ADULT were $\overline{Y}_W = 22.181$ for Fast lines, $\overline{Y}_W = 22.977$ for Control lines and $\overline{Y}_W = 26.943$ for Slow lines. The Fast and Control lines were not significantly different from each other but both differed significantly at the 1% level from the Slow line.

There was no significant correlated change in body weight, but growth rate did change. The weighted means of GR were $\overline{Y}_W = 0.074$ for Fast lines, $\overline{Y}_W = 0.075$ for Control lines and $\overline{Y}_W = 0.063$ for Slow lines. There was no significant difference between Fast and

Control lines for growth rate but once again, both differed significantly at the 1% level from the Slow line.

Changes in growth rate were obviously directly due to changes in adult emergence, as adult emergence was a component in the derivation of growth rate.

Table 4.10 presents the F-ratios and significance levels from the ANOVAs for female traits.

There were no significant differences between lines for female traits except ADULT and GR. The weighted means ($\overline{Y}_W$) of ADULT were $\overline{Y}_W = 22.148$ for Fast lines, $\overline{Y}_W = 23.257$ for Control lines and $\overline{Y}_W = 26.677$ for Slow lines. All lines differed significantly from each other at the 1% level, in contrast to males, where no significant differences were found between Control and Fast lines. The results are suggestive that females retain higher levels of additive genetic variation for rapid development than males. Growth rate of the Slow lines ($\overline{Y}_W = 0.067$) only differed at the 5% level from Fast lines ($\overline{Y}_W = 0.080$) and Control lines ($\overline{Y}_W = 0.079$) and, as in males, no significant difference was found between Control and Fast lines.

The F-ratios of three traits did approach significance at the 0.05 level: Q4L, ADFQ4L and ADFQ3R. Tables 4.5, 4.6 and 4.7 show that for ADFQ4L, within each replicate, Fast developing lines produced more eggs late in life than Control or Slow developing lines. Control lines produced more eggs than slow developing lines. This pattern was repeated for Q4L except in Replicate 1, where the value for the Fast line was roughly equivalent to that of the Control line. The values of ADFQ3R for Control lines were always greater than those of Slow developing lines. In conclusion slow developing lines appeared to produce less eggs late in life than Control and Fast developing lines. This difference was not significant but may have become so if selection had been continued for more generations.

The differences between Control and Fast developing lines for late life egg lay rate were not as consistent. This may have been due to the much greater response to selection for longer development time than shorter development time, and thus a smaller correlated response to selection for shorter development in correlated traits such as late life egg lay. On the other hand the results may indicate a lack of genetic correlation between developmental rate and the number of eggs laid late in life. In this case, the depression of late life egg lay in Slow developing lines may have been due to a breakdown in developmental homeostasis i.e. developmental disruption. The decrease was highly unlikely to be due to inbreeding depression as neither the Control or Fast developing lines were adversely affected.

Table 4.10: F-ratios and significance levels from the ANOVAs for female traits.

Traits	F	P
ADULT	460.2675	***
WT	0.4133	NS
GR	11.1092	*
DEATH	0.0331	NS
ALS	0.2474	NS
EGGTPT	1.1204	NS
MAXEGG	0.5265	NS
MAXDAY	0.6804	NS
LASEGG	0.6032	NS
RLS	0.3660	NS
DAY711	0.4079	NS
EGG54	0.2540	NS
Q1R	0.1167	NS
Q2R	0.6494	NS
Q3R	3.3832	NS
Q4R	3.3282	NS
ADFR	2.2874	NS
ADFQ1R	0.2006	NS
ADFQ2R	1.5310	NS
ADFFQ3R	5.7151	NS
ADFQ4R	3.5165	NS
Q1L	0.1684	NS
Q2L	0.9502	NS
Q3L	2.1038	NS
Q4L	5.1336	NS
ADFL	1.4890	NS
ADFQ1L	0.1592	NS
ADFQ2L	1.2952	NS
ADFQ3L	2.4394	NS
ADFQ4L	5.7501	NS
PROD	0.0695	NS

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, NS = non-significant

4.4 Discussion

4.4.1 Additive Genetic Variation in Adult Emergence

The realized heritability for increased development time corresponded closely to the paternal half-brother estimate of heritability from the diallel analysis, which was unbiased by sex-linkage or maternal effects. The estimate of 0.284 is comparable to those obtained by previous workers: 0.26 (Englert and Bell 1970); 0.324 (Soliman 1982); 0.32 (Dawson 1965a) and 0.262 (Englert 1964). These values are for development time from egg to pupa, but time to pupation and time to adult emergence appear to have much the same genetic variance and covariance matrices (see sections 3.3.2, 3.3.3, 3.3.4 and 3.3.5; and Bell and Burris 1973).

The realized heritability for decreased duration of development was much lower at 0.136. Previous estimates for fast development time range from about 0.11 (Dawson 1965a) to 0.38 (Englert and Bell 1970). Differences in estimates were most likely due to the different base populations. Dawson (1975) has shown that standard husbandry procedures for laboratory strains, which allow cannibalism of early pupae by larvae, subject populations to stabilising selection for intermediate developmental time. Natural populations are probably subjected to directional selection for rapid development. Thus artificial selection for fast development is successful in long-established laboratory populations of *Tribolium,* but not in strains recently derived from natural populations. Laboratory husbandry procedures may have differed between Dawson's and Bell's laboratories to give such divergent estimates of heritability.

The asymmetrical response of developmental time to divergent selection confirmed the expectation of low levels of additive genetic variation for rapid development in a colonising species (Lewontin 1965). Females appeared to have retained higher levels of additive genetic variation for rapid development than males, as they responded to selection for faster development whereas males did not. Either some additive genetic variation remained for rapid development for females in the wild, the "new" environment in the laboratory exposed fresh genetic variation for females but not for males or laboratory husbandry procedures had already selected for a slower development time in females but not in males. This last hypothesis is probably not pertinent as the generations before selection showed no increase in duration of development (Appendices 1, 2 and 4), nor did the control populations during the generations of selection (Figures 4.2 and 4.3). Both latter hypotheses postulate a difference in male and female responses to a change in environmental conditions, an assumption

which cannot be justified as yet. The simplest hypothesis is that females maintain higher levels of additive genetic variation for rapid development in the wild than males, which suggests that males experience stronger selection pressures for rapid development. This may be due to sexual selection in males for early eclosion and mating first.

The asymmetrical response demonstrates that genetic parameters from an analysis of a base population may be misleading. Genetic variation may be present for a trait but only in one direction. In such cases predictions of direct and correlated responses to divergent selection based on the measure of additive genetic variation, heritability, may be erroneous for one direction of selection.

4.4.2 Correlated Responses

4.4.2.1 Longevity

There was no obvious connection between duration of development and life-span, reproductive or otherwise, in females. This was expected from the results of the diallel analysis. Even though there were significant changes in duration of development and growth rate, no concomitant changes occurred in life-span. These results confirm that senescence is not a completely "epigenetically-controlled" trait in *Tribolium castaneum.* This disagrees fundamentally with the conclusions drawn by Soliman and Lints (1982), who found that longevity was directly related to the pre-imaginal growth rate in populations of *T. castaneum.* However, their conclusions were doubtful (see section 1.3.3.1) and Lints, the main proponent of the developmental hypothesis of senescence, now rejects the general existence of a direct relationship between growth rate and life-span (Economos and Lints 1986a,b; Lints 1988a).

Further, the results do not support the Unitary hypothesis of senescence. Although selection for fast development did not shorten life-span, neither did selection for slow development, and changes in developmental rate did not affect all other life-history traits (section 1.3.3.3). Life-history traits are not completely interdependent as postulated by the hypothesis.

4.4.2.2 Bodyweight

The lack of correlated response in weight was not expected from the base population genetic parameters. From the diallel design, the heritability of WT70 was 0.556 $\pm$ 0.089 for males and 0.540 $\pm$ 0.103 for females. The heritability of ADULT was 0.264 $\pm$ 0.101 for males and

0.228 ± 0.117 for females. The genetic correlation between ADULT and WT70 was 0.285 ±0.133 for males and 0.414 ± 0.234 for females. Neither sex response even approached signficance for a correlated change in weight. WT70 and WT are different indices, but one would expect weight on day of adult emergence to be more closely related to time to adult emergence, than a measurement of weight taken 40 to 50 days after adult emergence. Englert and Bell (1970) after selection for six generations for early and late pupation in a laboratory population of *T. castaneum* also found no consistent or statistically significant correlated response in pupal weight. However, using the same population and selecting for larval and pupal body weight, Bell and Burris (1973) produced significant changes in adult emergence time and adult weight after eight generations of selection. Interestingly, in this population there was a negative genetic correlation between body weight and developmental time, though in other populations positive genetic correlations have been found and are not uncommon (Sokoloff 1977). The fact that the correlated response of bodyweight to selection for developmental time was less than the correlated response of developmental time to selection for bodyweight was probably due to the higher heritability of body weight as compared with that of developmental time. It would seem probable that direct divergent selection for body weight may have been more effective than selection for developmental rate in eliciting correlated responses from other traits, as bodyweight has a higher heritability and larger significant genetic correlations with reproductive traits than development time (see sections 3.3.4 and 3.3.5). Also, bodyweight responds in a practically symmetrical fashion to selection (Sokoloff 1977), thus changes in correlated traits may have been more obvious.

4.4.2.3 Fecundity

As discussed in the results, late life egg lay did seem to be affected, though not significantly, by change in time to adult emergence. If it can be assumed that the correlated response would have been significant if selection had continued for more generations, depression in late life egg lay in Slow developing lines may have been due either to a negative genetic correlation between late life fecundity and ADULT, or developmental disruption. Slow development has been linked to a decrease in fecundity in *Tribolium castaneum,* though there is no increase in egg production when the speed of development is increased (Dawson 1966). This response then is not an aberration.

ADFQ4L had no heritable genetic variation but the paternal genetic correlation from

the diallel analysis between ADFQ3R and ADULT was 0.143 ± 0.374. Thus any correlated response to selection for slow development should have been an increase in late life egg lay. It is difficult to explain why later life fecundity traits with very low to non-existent genetic variation would respond to indirect selection but not a more heritable trait such as bodyweight ($h^2_{S+D} = 0.540 \pm 0.103$), which has a much higher genetic correlation with ADULT ($r_S = 0.414 \pm 0.234$), though admittedly still not significant.

Based on the above, it seems likely that the drop in egg numbers was due to a breakdown in developmental homeostasis. Selection for fast development did not have the same effect. This may have been because response by the character in this direction was small and thus so were changes within the internal environment.

The argument against developmental disruption is the lack of any depression in early fecundity. Of course, it is likely that early life history characters are more canalised than late life characters as they experience stronger selection pressures. The depression of late fecundity may be indicative of developmentally linked senescent processes whose presence was revealed by destabilising developmental processes. This is reminiscent of Luckinbill and Clare's (1985) success in revealing genetic variation for longevity in *D. melanogaster* after stressing populations with crowded larval conditions. It would have been interesting to continue selection for slow development to determine if the depression in late fecundity became significant. If so, then it would have been possible to commence selection for early and late reproduction in the slow developing lines to determine if longevity could be increased in *Tribolium*, as shown by Luckinbill *et al.* (1984) and Clare and Luckinbill (1985) in *Drosophila*.

The fact that changes in late reproduction did occur without affecting early fecundity does show that the reproductive schedule can be moulded i.e. adjustments can be made in age-specific reproduction.

4.4.3 Conclusions

Some general conclusions, and recommendations for further investigations, can be drawn from this experiment.

In this instance, the variance-covariance matrix was not particularly successful in predicting outcomes of selection. It did not predict a lack of genetic variation in one direction of selection, although examination of the phenotypic distribution of the trait indicated that this was likely. The positively skewed distribution of developmental time indicates

that selection had been more intense for fast development than slow development and gene frequencies were probably well above the symmetrical point (Falconer 1981).

Another problem was that the variance-covariance matrix provided no measure of developmental homeostasis or the effects on life-history if development was disrupted by selection. Developmental homeostasis is expected to be an important feature of life-history variation. Although the evidence was slight, there was some indication that developmental homeostasis may have been a feature of the genetic variation of fecundity. The selection program probably needed to be continued for more generations to have obtained conclusive results.

Future work might be best not to use developmental rate, but select for adult body weight. It has a higher heritability than duration of development and has substantial genetic correlations with various indices of fecundity. Correlated responses to selection should be substantial and appear within a few generations. As mentioned before, body weight responds in a practically symmetrical fashion to selection, so divergent selection should show up developmental disruption in traits. Furthermore, changes in weight may affect developmental processes more than changes in duration of development. A developmental link is important if the goal of a selection experiment is to cause developmental disruption within organisms, and observe if genetic variation is revealed for longevity. Strong links between developmental processes and body weight have been demonstrated in other organisms (Gilbert 1984c). Such links may explain why body size or weight seems to be so important in life-history evolution (Dunham and Miles 1985; Gilbert 1984c; Koufopanou and Bell 1984; Mousseau and Roff 1987; Palenzona, Rocchetta and Jacuzzi 1972; Reznick 1983; Tinkle, Wilbur and Tilley 1970). It would be interesting to see if divergent long-term selection for body weight did cause developmental disruption in *Tribolium castaneum*, or if predictions from the variance-covariance matrix of the population would be met.

Chapter 5

General Conclusions

5.1 The Cost of Reproduction

Survival costs were present in the Echarina population. Negative genetic correlations were found between various fecundity indices and reproductive life-span. Genetic correlations between early and late life fecundity indices were high and positive so fecundity costs were not present. Survival costs have also been found by McRae (1988) in two other populations of *Tribolium castaneum*, when tested in two different environments. Survival costs may be a universal feature of *Tribolium castaneum* life-history strategy.

Early and late fecundity are probably influenced by many of the same genes, but genetic correlations of less than unity and results from the selection experiments are suggestive that the two traits are partially independent. It is possible to change the expression of one without affecting the other e.g. depress late fecundity but not change levels of early fecundity. Moulding of the reproductive schedule is thus possible by natural selection pressures.

5.2 The Evolution of Senescence

Most of the evolutionary hypotheses of senescence (see section 1.3.3) have been tested and found wanting.

There was no genetic connection between duration of development and/or growth rate and life-span in either the diallel or selection experiment. The Developmental hypothesis is predicated on a relationship between development and longevity.

Neither did predictions of the Unitary hypothesis match actual results. Substantial

changes in developmental rate did not affect all other life-history traits, and a number of quite high genetic correlations between life-history traits were found. These results do not support the concept of universal pleiotropy of all life-history characters, bound by positive correlations with fitness. The negative genetic correlations were between early fecundity indices and reproductive life-span.

Negative genetic correlations between early and late life-history traits are not expected by the Deleterious Mutation Accumulation hypothesis, which postulates senescence and death as resulting from the accumulation of mutations with deleterious effects in old age. Another problem with the Deleterious Mutation Accumulation theorem is that there was no increase in additive genetic variation of fecundity with age.

Antagonistic pleiotropy, that is genes which maximise early reproduction and viability but have deleterious effects later on in the individual's life-span, is also unlikely to be primarily responsible for the maintenance of senescence as early and late life reproduction were not negatively correlated.

Only the Running-out-of-Program (RP) evolutionary theory of ageing was not undermined by the results of this study. This theorem proposes that senescence is an inherent feature of organisms, there is natural selection for longer life and this is mediated by positive genetically controlled mechanisms (Cutler 1978,1980,1982; Sacher 1978a,1982). Negative genetic correlations were found between reproduction and life-span, whilst there was some suggestive evidence that longevity experienced strong directional selection. Both are correlates of the RP hypothesis. None of this evidence 'proves' the validity of the RP hypothesis and further research is required for verification. As far as whether there are developmentally linked ageing processes, a postulate which distinguishes Cutler's from Sacher's hypothesis, this study did not address that question and this point also needs resolution.

It should be recognised that the RP theory does not exclude the operation of genetic mechanisms of senescence, such as mutation accumulation and antagonistic pleiotropy. Since, in its simplest version (Sacher 1978a), it merely envisions that senescence is due to an accumulation of damage and that longer life can be achieved by selection for genes that regulate, protect and repair the organism at all levels, it can embrace many mechanisms of ageing. For example, the accumulation of mutations could be fully or partially responsible for senescence in an organism, but the timing of senescence could be due to the efficiency of the DNA repair system. Physiological constraints can also lead to antagonistic pleiotropy being a feature of the senescent process. For example, in *Drosophila* there is a

trade-off between lipid accumulation, which improves starvation resistance, and early-age fecundity (Service 1987).

Examining why senescence has evolved or is maintained in populations should never be confused with the identification of genetic mechanisms of ageing.

5.3 Genetic Variation and Life-History Evolution

The life-history strategy of a *T. castaneum* population exhibits many of the features expected in a colonising species. Rapid development in the Echarina population had very low levels of heritable genetic variation, though some significant variation did remain for females. It may be that selection for rapid development is stronger in males than females. Genetic variation for short duration of development is expected to be minimal in a colonising species, as they should experience directional selection for rapid development. Also, as predicted by Lewontin (1965) for colonising species, there were relatively higher amounts of additive genetic variation for fecundity than developmental rate.

There were no fecundity costs, that is, beetles which had high rates of egg lay early in life maintained this advantage throughout their life-span. The total number of eggs produced by such beetles was greater than for beetles with relatively low rates of egg lay, but high producers paid a cost in that their reproductive life-spans were shorter than those of low producers. The negative genetic correlation between fecundity and reproductive life-span is possibly the result of selection acting to maximise both fecundity and reproductive life-span. A long reproductive life-span is favoured because the ability to survive overwinter is extremely important for *T. castaneum.* Temperatures during winter are too low for too long for females to lay eggs or for eggs to be viable. Individuals that can survive winter and then still reproduce have a distinct advantage over those which do not survive and/or are unable to then produce eggs.

The genetic variation of longevity may be suppressed in optimal conditions by developmental buffering systems in *T. castaneum* and longevity may be more developmentally buffered for females than for males. It would not be surprising if it was, as the need for overwintering capacity ensures that longevity is an important trait in the life-history strategy of *T. castaneum.* The genetic architecture of longevity (i.e. low additive genetic variation and possibly high dominance variation) also was suggestive that the trait had experienced strong directional selection and thus was an important component of fitness.

Another trait which had extremely high levels of dominance was the time to reach peak fecundity (MAXDAY). The earlier a coloniser, such as *T. castaneum* with a platykurtic reproduction schedule, attains peak fecundity, the greater the likelihood of a successful colonising episode. Genotypes which are able to concentrate a large part of their reproduction at a young age, are also able with their offspring to take advantage of the (usually) plentiful resources following founding of a colony, and so rapidly increase in numbers (Dawson 1977).

Mertz (1971) has suggested "that *T. castaneum* is adapted to a combination of colonising life with rapid increase in numbers followed by a period of stabilization or decline". Rapid development and a concentrated reproduction at a young age suit a colonising life style, whereas long life, slow development and delayed reproduction are highly advantageous in a stable or decreasing population (Dawson 1977). Natural selection would have favoured both long life, early reproduction and a long reproductive period in *Tribolium* populations, as beetles do tend to migrate from overcrowded conditions. Both length of life and timing of peak reproduction would be expected to experience strong directional selection pressures that would deplete additive genetic variation of the two characters, as found by this study.

Early reproduction would be expected to be more important than late reproduction in such a life-history strategy and results from the Echarina population suggest that this is true. Late fecundity was perhaps less developmentally buffered than early fecundity, as late reproduction was more variable and more affected by changes in duration of development than early fecundity. Fecundity indices, particularly early and mid life, had moderate levels of additive genetic variation. The maintenance of variation for fecundity may have been due to the antagonistic selection pressures on the reproductive schedule and/or antagonistic pleiotropy between fecundity and reproductive life-span.

Bodysize had the largest heritable variation of fitness components and low levels of nonadditive genetic variation. It was highly and positively correlated with many fecundity indices. It is possibly not a very important fitness trait for *T. castaneum* as it does not seem to have experienced the same intensity of selection as fecundity. On the other hand, because of strong genetic correlations between it and other life-history traits any large change in bodyweight through selection has far-reaching effects on other life-history characters and the fitness of the individual. Though positively correlated with fecundity, it was negatively correlated with developmental rate. A decrease in either fecundity or developmental rate would detrimentally affect fitness.

Some general comments can be made about the possible genetic constraints acting on life-history evolution in *Tribolium.*

Most life-history traits exhibited low to moderate heritabilities and therefore would respond to selection. A few traits had very low to no apparent additive genetic variation, in particular longevity and MAXDAY, and thus would probably not be able to respond. However, the genetic architecture of both these traits was suggestive that their additive genetic variation was suppressed by developmental buffering. It is known that stress can destabilise developmental processes and reveal additive genetic variation. It is unlikely that lack of genetic variation would constrain responses to selection by most life-history traits.

It has already been noted that the genetic correlation matrix of the Echarina population may constrain change in a number of characters. As mentioned before though, any quantitative genetic analysis is specific to a particular population in a particular environment at a particular time (Barker and Thomas 1987). The genetic correlation matrix can change in different environments and thus so can constraints. Fundamental constraints, such as the negative genetic correlation between reproduction and survival, may operate in all "realistic" environments (McRae 1988).

Changes in developmental rate did not significantly affect longevity, fecundity and/or weight. Therefore there appear to be no major constraints operating through developmental homeostasis in the short-term. The lack of additive genetic variation for rapid development in males is a sign of a developmental constraint, as developmental duration in *Tribolium* appears to be unable to be shortened any further i.e. a developmental constraint on the production of future variant phenotypes. There were also some indications of developmental disruption in late fecundity after selection for slow development. Only continued selection would have revealed if there were such a phenomenon. Developmental buffering of genetic variation may be important for a number of life-history traits (e.g. longevity and MAXDAY), and only long-term selection may be able to reveal the extent and consequences of such developmental constraints.

5.4 Direction of Future Work

When proposing what direction future work in the field of the genetics of life-history evolution should take, the suitability of quantitative genetic analysis as a methodology needs examination.

It is well known that genetic correlation estimates are dependent upon environmental variance, environmental covariance and genotype x environment interactions (Clark 1987a). Quantitative genetic experiments using natural populations usually involve introducing populations to novel environments i.e. transferring a wild population into the laboratory. This must naturally limit the understanding of phenotypic evolution in nature, as laboratory estimates of genetic correlations may have little to do with genetic correlations realised in nature (Clark 1987a,b). It also gives little idea of the selection pressures wild populations experience.

But, if we acknowledge these limitations on our understanding of phenotypic evolution, I believe that quantitative genetic analysis can improve our understanding of genetic constraints on life-history evolution. In particular, estimates of genetic variance and covariance combined with long-term selection experiments can reveal what type of constraints are likely to be important. This information will be more useful if one repeats the experiment in a number of "natural" environments in the laboratory. *Tribolium* populations are particularly useful as:

a) it is easy to sample a large number of individuals from a natural population, and sample many natural populations from the wild;

b) *Tribolium* have a colonising life-history strategy, so are adapted to encountering novel environments;

c) the laboratory conditions are very similar to conditions *Tribolium* experience in the wild; and

d) *Tribolium* have a large number of linkage groups, so linkage disequilibrium should not confuse results.

Though it would be impossible to replicate all the combinations of environmental variables an organism meets in nature, and fully map life-history strategies, genetic constraints on life-history evolution should become apparent. Such experiments should show up fundamental constraints.

It may be possible to extrapolate results of such extensive experiments from the laboratory to nature. To justify this, some investigation is needed into the effects of transferring wild populations into laboratories on genetic variance and covariance matrices.

The usefulness of quantitative genetic analyses may be further expanded, if we understand the connections between genotypes and quantitative trait phenotypes i.e. gene structure, control and regulation; gene action and interaction; and, physiology, biochemistry and development of the organism (Barker and Thomas 1987).

As far as future work is concerned, it would be interesting to repeat the diallel analysis using greater numbers. Accuracy and precision of estimates would be improved. This was impossible in this study because of the work involved, but it would be possible to increase numbers without increasing workload, by not measuring all the indices used in this study. High genetic correlation estimates between traits measured by the diallel analysis, make the measurement of some traits redundant. For example the strong correlation between day of pupation and day of adult emergence, makes the measurement of both traits a useless repetition. Measurement of total lifetime fecundity may also be unnecessary, as indices of early fecundity can give quite good estimates of relative total lifetime fecundity.

The selection experiment also should be repeated but continued for many more generations in order to get some conclusive changes in correlated characters and, perhaps, definite proof of developmental disruption. Rather than duration of development, bodyweight may be more suitable for direct selection. It has strong links with development, a higher heritability than duration of development, substantial genetic correlations with various indices of fecundity and responds in a practically symmetrical fashion to selection.

To test the existence of costs to reproduction, reproductive cost needs to be confirmed in other species. Sib analyses and selection experiments should be done on other organisms in a number of different environmental conditions. Selection experiments should directly select for increased early fecundity, late fecundity or longevity and measure the response in the other two traits. Selection for late fecundity can inadvertantly select for long life, thus it may be difficult to interpret results of such a selection experiment. Selection for decreased early fecundity, late fecundity or longevity can also be misleading, as one may be increasing the frequency of deleterious mutations that are not usually important in the determination of the characters.

Future experimental work could show that reproductive costs are manifested differently in different species, that is, either as survival or fecundity costs. It may well be that species with a burst of high early reproduction and short life-span may exhibit fecundity costs, in contrast to the long-lived *T. castaneum*.

I believe one very promising area of research is the RP hypothesis concerning the evolution of senescence. One of its premises is that it should be possible to get a response to direct selection for increased longevity. In a long-lived organism such as *Tribolium,* it may be best to use the approach of Rose *et al.* (1987) and select for subsidiary characters related to longevity. Such useful subsidiary characters need to be identified in *Tribolium.* In view of the work of Luckinbill *et al.* (1984) and Clare and Luckinbill (1985) with *Drosophila,* it may be necessary to disrupt developmental processes in organisms and then select for increased life-span. Intense divergent selection for bodyweight may be a stress that would reveal additive genetic variation for longevity. Otherwise one could use environmental stresses such as population density (Luckinbill *et al.* 1984; Clare and Luckinbill 1985). It would be best to use an environmental stress such as density, that could well act as a cue for changing selection pressures in the wild for natural populations. If an increase in longevity is obtained then an investigation into how it is obtained should be done. This would involve an examination of the physiology, biochemistry and development of the organism in order to affirm that selection has been for genes that regulate, protect and/or repair the organism.