Chapter 3

The Nested Experiment

The aim of the Nested experiment was to compare genetic variation and covariation in several female life-history traits between two geographically distinct natural populations of *T. castaneum*. A nested (hierarchal) design was used to estimate heritabilities for single traits, and genetic correlations between different traits. The experiment was conducted under two constant laboratory temperatures (33° and 37°C) to investigate the importance of genotype-environment interactions.

3.1 Materials and Methods

3.1.1 The Experimental Populations

Two natural populations were sampled, one a sub-tropical population from Coalstoun Lakes in southern Queensland (25°37′ S and 151°53′ E), and the other a temperate population from Mangoplah in southern N.S.W. (35°22′ S and 147°13′ E).

The Coalstoun Lakes and Mangoplah samples consisted of 602 and 1188 live *T. castaneum* adults respectively, taken from similar sized grain silos in March 1984. Both populations were known to have infested their respective habitats for many generations (at least 5–10 years) and neither had any history of chemical treatment. Only grain from the same farm was stored in the silos. When small quantities of seed grain were introduced to the farms it was always chemically treated and insect free. This minimized immigration resulting from the movement of grain over long distances, and effectively restricted gene flow to insect flight. Each population sampled was confined to a silo or its immediate external surface. The populations were kept isolated after collection but treated in the same way.

The individuals sampled were classified according to species, placed in fresh culture medium (400 g), and incubated away from the experimental laboratory at 33°C and 70% relative humidity. After five days of acclimatization the wild caught adults were allowed to lay eggs *en masse* in fresh medium (400 g) for 48 hours. These eggs constituting the F_1 generation were counted (1889 for Mangoplah and 1928 for Coalstoun Lakes), then 1000 were randomly sampled and washed with a dilute solution of benzalkonium chloride (see General Materials and Methods). This large number of eggs was used to maintain large population sizes and allow for any viability problems associated with maternal effects (infertile eggs) or egg washing.

The 1000 washed eggs were placed in fresh medium (400 g), transferred to the experimental laboratory, incubated at 33°C and 70% relative humidity, and allowed to develop undisturbed until pupation. At this developmental stage, the pupae were removed daily (to minimize cannibalism by larvae) and sexed. After the emergence of the last imago, 200 of each sex were randomly sampled and mass mated in fresh medium (400 g) for seven days to establish fertility and reach peak egg lay (after Bhat and Bhat 1974b). A 24 hour egg sample was then taken in fresh medium (400 g) to establish the next generation (F_2). These eggs were counted, and 400 randomly sampled and placed in fresh medium (400 g). Again the eggs were allowed to develop undisturbed until pupation, at which time the pupae were removed and sexed daily. A random sample of the imagoes emerging from these pupae were used as parents (sires and dams) in the nested design.

Having put the wild caught populations through two generations of laboratory culturing to remove environmental effects associated with first-order developmental plasticity and maternal influences (after Stearns 1977) the nested stage of the experiment was set up.

3.1.2 Optimality of Design

The biometrical analysis of quantitative traits requires the rearing of individuals or groups of individuals of known parentage in environments that are as uniform as possible. Determining the best quantitative method and best design for estimating heritabilities, genetic correlations and genotype-environment interactions for life-history traits usually involves compromise. The nested method was chosen over the alternative parent-offspring regression procedure (see Falconer 1981) for several reasons. Firstly, the performance of F_2 descendents of wild caught beetles may be influenced by the original environment through

maternal effects. To overcome this problem in a parent-offspring regression design, "parents" would need to be F_3 descendents, making their progeny another generation removed (F_4) from the natural habitat. This would introduce the undesirable effect of another generation of possible drift and/or adaptation to the laboratory environment. Secondly, for a given number of animals measured, the estimate of heritability obtained from a half-sib analysis with optimal structure is more accurate than that from a parent-offspring regression if the heritability is less than 0.25 (Robertson 1959a). Based on the empirical literature for *Tribolium* and the assumption that fitness components might display low levels of additive genetic variance in natural populations, low or moderate heritabilities were expected for the life-history traits in question.

The optimum structure for the measurement of heritability is also the optimum for the measurement of the genetic correlation between two characters measured on the same individual (Robertson 1959a). However, the optimal design for estimating the genetic correlation between character states measured in different environments entails a larger group (family) size in each environment than is optimum for a heritability estimate (Guiard and Herrendörfer 1977; Robertson 1959b).

The problem of design optimality is to determine the most efficient number of sires (s), number of dams per sire (d) and the number of progeny per dam (n) to use in the hierarchical classification, given that the total number of beetles to be recorded is limited by time and space. The final decision was based on :

a) the biology of the organism;

b) the suitability of the culturing environment for replication and randomization;

c) the types of traits measured;

d) the statistical accuracy of estimates (see Falconer 1981; Guiard and Herrendörfer 1977; Klein 1974; Klein, DeFries and Finkbeiner 1973; Robertson 1959a,b);

e) the fact that large departures from the optimum design often involve only a small reduction in the amount of information per observation (Hill and Nicholas 1974); and

f) the purpose of the experiment.

It is acknowledged that the numbers used in the Nested experiment for estimating heriabilities and genetic correlations are only marginal for a properly designed quantitative

genetic experiment. However, they are considered adequate and certainly closer to optimality than most empirical investigations measuring these variables for life-history traits in natural populations. More importantly the design used allowed for the comparison of populations and the estimation of genotype-environment interactions.

3.1.3 The Experimental Procedures

(See Figure 3.1 for a diagrammatic presentation of the experimental procedure). For each population, sires and dams were randomly sampled from the virgin F_2 adults after the emergence of the last imago. Initially 36 sires were each mated with 5 dams in fresh medium (1 g) for seven days to allow females to reach peak egg lay (Bhat and Bhat 1974b) and establish fertility. Of these, only 27 sire groups with 4 dams per sire were ultimately used. Extra sires and dams were set up in case of infertility, and to keep the design as balanced as possible. After 7 days sires were removed, the dams isolated individually in fresh medium (1 g) and a 24 hour egg collection taken. The conditioned medium from each sire group was checked for the presence of larvae, and with fertility confirmed (no sires were infertile), 27 sires groups were chosen at random. After 24 hours the dams were transferred to fresh media (1 g) and returned to the incubator for 24 hours. This trial egg sample was counted and dams which had not produced any eggs discarded. For each sire group, only 4 of the remaining fecund dams (randomly chosen) were kept. This left 27 sire groups, with 4 dams inseminated by each sire, for each population.

Eight full-sib cultures per dam were collected by the following procedure. At the same time each day, over four consecutive days, the dams were transferred into fresh culture medium (1 g) and 24 hour egg samples taken (each sample constituted a "block" labelled 1, 2, 3 and 4). After the fourth collection each dam was individually remated with the same sire for a 12 hour period to re-establish fertility. They were then left undisturbed for a further 24 hours so that there was a three day gap between blocks 4 and 5. This remating was only precautionary, since *T. castaneum* females deprived of males take 3–4 weeks to show a significant drop in egg production (Bhat and Bhat 1974a; Espejo and Orozco 1966). The process of sequential egg collections was then repeated. At the same time each day, four consecutive egg lays were collected (representing blocks 5, 6, 7 and 8 respectively). Over the 11 day period a total of eight samples of eggs, each of fixed duration (24 hours) had been collected, giving 8 full-sib block cultures per dam. The offspring were collected in this manner to ensure both a sufficient number of full-sib progeny were available for each

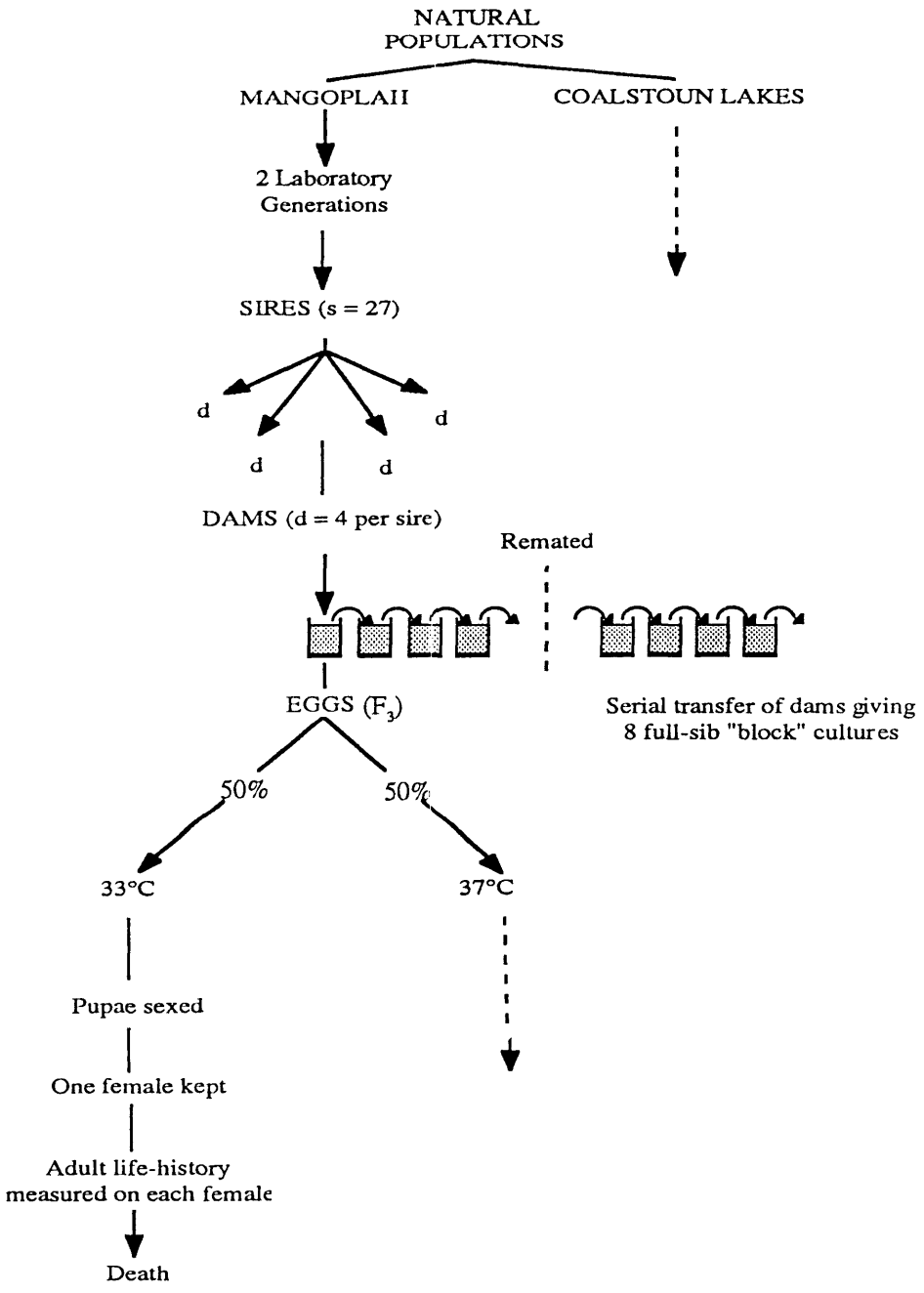

Figure 3.1: Schematic presentation of the Nested experiment

dam, and to stagger the workload at different stages of the experiment. At all times the periods spent outside the incubator were minimized.

For each block, after the dams had been removed and set up for the next egg collection, the eggs from each full-sib culture were separated from the medium, counted and half were placed in each of two separate vials containing fresh medium (2 g). One of these vials, from each dam full-sib culture, was returned to the same "optimal" environment (33°C), and the other placed in a different "stressful" environment (37°C). Obviously, all progeny had shared a common environment (33°C) for 24 hours. The common environment was needed to minimize sampling effects among progeny from the same genotypes, to guarantee sufficient progeny were available for assay under the more stressful temperature (37°C), and to initially establish constant density (eggs/g) among full-sib cultures across temperatures within blocks.

Within a block in each environment, there were 216 culture vials (108 dam full-sib cultures for each population) contained in two storage trays. To standardize incubator effects over the two populations, vials from each were intermingled and placed in alternate positions. The positioning of dam cultures within each population was completely randomized. For the same block, but in different incubators, the genotypes were placed in the same corresponding positions. However, the different blocks were separately randomized. After randomization the trays were placed in the appropriate incubator and left undisturbed until pupation.

At pupation (day 16 onwards), the pupae were removed and sexed. The males were discarded and the females placed in empty vials, since pupae do not consume food (Sokoloff 1977). One female pupa was randomly sampled from each full-sib culture and retained for assay, the remainder discarded. These female pupae were checked at the same time daily for adult emergence and the day of emergence recorded. Upon emergence each imago was weighed and placed in fresh medium (2 g) with an unrelated male from a black mutant stock for copulation. This procedure was repeated daily until all pupae had successfully completed adult emergence or had died. New males were provided for all females every four weeks.

The age-specific fecundity schedule for each female was assessed by sampling eggs at fortnightly intervals. This consisted of placing each female and its mate in fresh medium (1 g) for a fixed interval of 42 hours. After collection the pairs were removed, put into fresh medium (2 g) and left undisturbed for 12 days until the next census. This process was

repeated until all females had died (a maximum of 25 censuses). Death was measured as the lack of response to tactile stimuli under the microscope. In the event of a black male dying it was replaced with another of the same age. The first of these samples was taken for all females at day 35 (34–36), then day 49 (48–50), and so on. A 42 hour census meant that all blocks could be set up and females removed at the same time each day. In contrast, 24 or 48 hourly censuses would have meant that setting up and removal of females from some blocks would have coincided. After counting the second egg census (day 49), the eggs were retained and allowed to develop in fresh medium (2 g) for 21 days, at which time all surviving progeny were counted. This gave a measure of fertility. At all stages during the experiment, blocks were subjected to the same procedures but on different days.

3.1.4 The Life-History Traits Measured On Each Female

Non-Reproductive Traits:

a) Developmental Time (DT) = the time interval between egg collection and adult eclosion (days).

b) Adult Body Weight (BWT) = adult body weight on day of adult eclosion (μg).

c) Growth Rate (GR) = calculated as the ratio BWT/DT (μg/day).

d) Longevity

1) Age of Death (AOD) = time from egg collection to death (days).

2) Adult Lifespan (ALS) = the time interval between adult eclosion and death (days).

Reproductive Traits:

The reproductive curve of *T. castaneum* is generally platykurtic. After the onset of reproduction there is an early flat peak followed by a gradual and slowing loss of reproductive capabilities, conferring a long right tail to the curve — pronounced positive skewness (Mertz 1975).

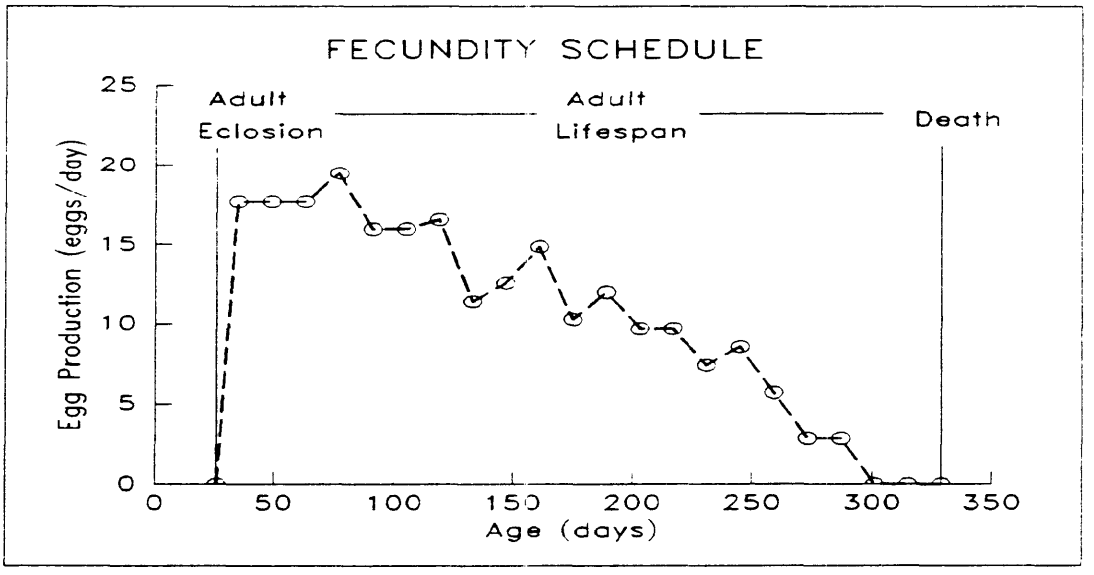

Figure 3.2: A sample fecundity schedule constructed by linear interpolation between successive egg censuses over the lifespan of the individual. Each point represents a census (42 hour samples standardized to a per day basis).

a) Individual Egg Lays.

day 35 (E1)
day 49 (E2) = at days 35, 49, 63, and so on
day 63 (E3) until day 371 (eggs/day).
⋮
day 371 (E25)

b) Total Fecundity for Given Periods of Adult Life. The number of eggs produced during a fixed period of the individual female's adult life determined by linear interpolation between egg censuses (see Figure 3.2). On the days of adult emergence and death females were assumed to have produced no eggs.

1. Total lifetime reproduction (TOTAL) = the total number of eggs produced in the lifetime of each female.
2. For each quarter of adult life:

   First quarter (Q1)

Second quarter (Q2)

Third quarter (Q3)

Last quarter (Q4)

3. For each half of adult life:

First half (H1) = Q1 + Q2

Second half (H2) = Q3 + Q4

c) Average Daily Fecundity for Given Periods of Adult Life.

1. For total adult lifespan (eggs/day):

Average daily fecundity for adult life (ADFTOT) = TOTAL/ALS

2. For each quarter of adult life (eggs/day):

First quarter (ADFQ1) = Q1/(ALS/4)

Second quarter (ADFQ2) = Q2/(ALS/4)

Third quarter (ADFQ3) = Q3/(ALS/4)

Last quarter (ADFQ4) = Q1/(ALS/4)

3. For each half of adult life (eggs/day):

First half (ADFH1) = (Q1 + Q2)/(ALS/2)

Second half (ADFH2) = (Q3 + Q4)/(ALS/2)

d) Age-specific Reproduction Relative to the Population Median.

1. The number of eggs laid by each female before and after the time at which 50% of eggs had been produced by all the females (the median) at that temperature (33° or 37°C) in that population (Mangoplah or Coalstoun Lakes):

(ALM) = the number of eggs produced before the population median

(ARM) = the number of eggs produced after the population median

2. Average daily fecundity relative to the population median. The average number of eggs laid per female per day, before and after the time at which 50% of eggs had been produced (the median) by all the females in that population at that temperature.

Average daily fecundity before the median (ADFALM)

= ALM/(Median - Age at eclosion)

Average daily fecundity after the median (ADFARM)

= ARM/(AOD - Median)

e) Maximum Egg Production.

(EMAX) = the maximum number of eggs produced in any one census (eggs/day)

f) Fertility.

(FERT) = the number of viable offspring from the day 49 egg census (standardized to a per day basis)

(HATCH) = the proportion of viable eggs from the day 49 egg census (those females which did not lay during this census were excluded)

Other traits were considered but results are not reported due to similarities with other traits. Reproductive lifespan (RLS) was estimated as the difference between the age at which reproduction ceased (the census after the last non-zero egg sample) and adult emergence (DT). Other indices based on RLS were in such close agreement with those based on ALS, that for all intents and purposes they were considered synonymous. The time of peak reproduction (TMAX) was not recorded since *Tribolium* generally maintain a plateau during peak reproduction (Mertz 1975).

3.1.5 Statistical Analyses

In the Nested experiment, for each population a number of sires ($s = 27$) were each mated to 4 dams, the sires and dams being randomly chosen and randomly mated. Where available, 8 full-sib female progeny from each dam in each of the two temperatures were used to provide the data. The individuals measured thus formed a population of half-sib and full-sib families.

3.1.5.1 Analysis of Variation

Estimation of Variance Components. Within each population and within each environment, an analysis of variance was made by which the phenotypic variance for individual traits is partitioned into observational components attributable to differences between the progeny of different sires (the between-sire component, σ_S^2), to differences between the progeny of dams mated to the same sire (between-dam, within-sires component, σ_D^2) and to differences between individual offspring of the same dam (within-progenies component, σ_W^2) (see Becker 1984; Falconer 1981). LSML76, a general purpose "mixed model least-squares" computer program (Harvey 1977, 1982) was used to estimate the variance components by equating computed mean squares to their expectations and solving the resulting equations.

As is commonly the case in many quantitative genetic studies, class sizes were unequal, due to differential fertility of dams, differential survival of offspring, and variation in the sex ratio. Data having unequal numbers of observations in the sub-classes are called unbalanced data (Searle 1979). The method of estimating variance components adopted by LSML76 is based on the fitting constants method, or Henderson's Method 3 (after Henderson 1953). The importance of the fitting constants method lies in its appropriateness for the mixed model, for which it yields variance component estimators that are unbiased by any fixed effects included in the model (Searle 1971). This method is considered appropriate for the Nested experiment where imbalance is not serious.

The Linear Statistical Model. Each population (Mangoplah and Coalstoun Lakes) was analyzed separately within each environment (33° and 37°C) since estimates of heritabilities and genetic correlations are specific to particular populations in particular environments (Falconer 1981). The following linear statistical model was assumed :

$$Y_{ijbl} = \mu + S_i + D_{ij} + B_b + e_{ijbl}$$

where Y_{ijbl} = the value of the dependent variable for the l^{th} individual beetle from the j^{th} dam mated to the i^{th} sire in the b^{th} block;

μ = value of the overall mean;

S_i = the random effect of the i^{th} sire;

D_{ij} = the random effect of the j^{th} dam nested within the i^{th} sire;

B_b = the fixed effect of the b^{th} block; and

ϵ_{ijbl} = random errors associated with each observation (assumed to be real and independent with expectations equal to zero).

Models that contain one set of cross-classified non-interacting random effects and one set of nested non-interacting random effects, where the nested random effects are nested within the cross-classified set of random classes are fitted by model IV of LSML76 to yield the appropriate variance components attributable to sires, dams nested within sires, and error (Harvey 1977).

Formulae for Calculating Heritabilities. Both paternal half-sib and full-sib heritability estimates were calculated for each life-history trait within each population and temperature.

a) paternal half-sib estimate:

$$h^2_S = \frac{4\hat{\sigma}^2_S}{\hat{\sigma}^2_S - \hat{\sigma}^2_D - \hat{\sigma}^2_W}$$

where h^2_S = the paternal half-sib heritability estimate;
$\hat{\sigma}^2_S$ = the estimated sire component of variance;
$\hat{\sigma}^2_D$ = the estimated dam component of variance; and
$\hat{\sigma}^2_W$ = the estimated within-progenies variance component.

The use of paternal half-sib families provides estimates of narrow-sense heritabilities, unconfounded with dominance or maternal effects. The sire variance component estimates the phenotypic covariance of half-sibs and thus one fourth of the additive genetic variance, because half-sibs have one fourth of their genes in common, on the average (Becker 1984; Falconer 1981). The disadvantage of these estimates based upon the covariance of paternal half-sibs is their relatively low precision.

b) full-sib estimate:

$$h^2_{S+D} = \frac{2(\hat{\sigma}^2_S + \hat{\sigma}^2_D)}{\hat{\sigma}^2_S + \hat{\sigma}^2_D + \hat{\sigma}^2_W}$$

where h^2_{S+D} = the combined heritability estimate from the sire and dam components; and the remaining terms are as above.

This calculation of heritability will overestimate the narrow sense heritability of a character since the numerator contains two times the variance due to maternal effects (V_M) and one half the dominance variance (V_D) (Becker 1984; Falconer 1981). The full-sib estimate is calculated for comparison with the paternal half-sib estimate. The full-sib estimate is also included for its statistical precision and because it represents a major portion of the total phenotypic variance which is genetically as opposed to environmentally determined.

Standard Errors of the Estimates. Standard errors of the heritability estimates computed by LSML76 are only approximations since they are computed in each case as if the analysis were simply a "between family" and "within family" analysis with unequal numbers, but with no adjustment for fixed effects (Harvey 1977). Therefore, the standard errors should be considered as minimum estimates of the true standard errors. A one-tailed t-test was used to test whether the estimated heritabilities are significantly different from zero. For any given heritability estimate, only individuals from dams with two or more full-sib progeny were included in the analysis.

3.1.5.2 Analysis of Covariation

Estimation of Covariance Components. The estimation of genetic correlations between different traits measured on the same individual relies on the resemblance between relatives in a manner analogous to the estimation of heritabilities (Falconer 1981). In addition to estimating the components of variance for each character separately, the components of covariance between the two characters are computed from an analysis of covariance. Just as the variance components are obtained by equating computed mean squares to their expectations, covariance component estimates are obtained by equating mean cross-products to their expectations and solving the resulting equations (Harvey 1982).

Formulae for Calculating Genetic Correlations. Genetic correlations are computed in each case by simply dividing the "family" covariance component estimate for the two traits (X and Y) by the geometric mean of the two "family" variance component estimates (Harvey 1977).

a) paternal half-sib estimate:

$$r_S = \frac{\widehat{cov}_{S(XY)}}{\sqrt{\hat{\sigma}^2_{S(X)} \cdot \hat{\sigma}^2_{S(Y)}}}$$

where r_S = the genetic (paternal half-sib) correlation estimate;
$\widehat{cov}_{S(XY)}$ = the estimated sire component of covariance between characters X and Y: and
$\hat{\sigma}^2_{S(X)}$ and $\hat{\sigma}^2_{S(Y)}$ are the estimated sire variance components for the respective traits.

b) full-sib estimate:

$$r_{S+D} = \frac{\widehat{cov}_{S(XY)} + \widehat{cov}_{D(XY)}}{\sqrt{\hat{\sigma}^2_{S(X)} + \hat{\sigma}^2_{D(X)}}\sqrt{\hat{\sigma}^2_{S(Y)} + \hat{\sigma}^2_{D(Y)}}}$$

where r_{S+D} = the genetic (full-sib) correlation:
$\widehat{cov}_{D(XY)}$ = the estimated dam component of covariance;
$\hat{\sigma}^2_{D(X)}$ and $\hat{\sigma}^2_{D(Y)}$ are the estimated dam variance components for the respective traits: and
$\widehat{cov}_{S(XY)}$, $\hat{\sigma}^2_{S(X)}$ and $\hat{\sigma}^2_{S(Y)}$ are as above.

This correlation estimate is confounded by dominance, maternal effects and epistasis (see Becker 1984 for genetic interpretation of components). Thus the full-sib genetic correlation estimate should be interpreted as genetic correlation in the "broad" sense.

The Phenotypic Correlation.

$$r_P = \frac{\widehat{cov}_{W(XY)} + \widehat{cov}_{S(XY)} + \widehat{cov}_{D(XY)}}{\sqrt{\hat{\sigma}^2_{W(X)} + \hat{\sigma}^2_{S(X)} + \hat{\sigma}^2_{D(X)}}\sqrt{\hat{\sigma}^2_{W(Y)} + \hat{\sigma}^2_{S(Y)} + \hat{\sigma}^2_{D(Y)}}}$$

where r_P = the phenotypic correlation between traits X and Y;
$\widehat{cov}_{W(XY)}$ = estimated within error covariance;
$\hat{\sigma}^2_{W(X)}$ and $\hat{\sigma}^2_{W(Y)}$ are the estimated error variance components for the respective traits: and the remaining terms are as above.

Standard Errors of the Estimates. The standard errors given for the genetic correlation by LSML76 are only approximate (Harvey 1977) and should be considered as minimum estimates of the true standard errors. A two-tailed t-test was used to test whether the estimated genetic correlations were significantly different from zero. For any given correlation estimate, only individuals from dams with two or more full-sib progeny were included in the analysis.

3.1.5.3 Analysis of Genotype-Environment Interaction

In the Nested experiment the full-sib cultures sampled from each dam (within a block) were divided into two random sub-groups and placed in different environments. In one environment (33°C) the parents (sires and dams) and the first group of randomly chosen progeny were reared continuously from birth, and the other environment (37°C) is the one in which the second group were reared for all except the first 24 hours of life. The measurement of life-history traits on the individual female progeny from each sub-group provides the necessary information to examine the extent to which temperature affects the expression of the genotype.

a) Analysis of Variance. In the analysis of variance, a mixed model was fitted to the data to test for the presence of genotype-environment interaction. The following linear statistical model was assumed:

$$Y_{ijbtm} = \mu + S_i + D_{ij} + B_b + T_t + (TxB)_{tb} + (SxT)_{it} + (DxT)_{ijt} + \epsilon_{ijbtm}$$

where Y_{ijbtm} = the phenotypic value of the m^{th} beetle;

μ = the value of the overall mean;

S_i = the random effect of the i^{th} sire;

D_{ij} = the random effect of the j^{th} dam mated to the i^{th} sire;

B_b = the fixed effect of the b^{th} block;

T_t = the fixed effect of the t^{th} temperature;

$(TxB)_{tb}$ = the interaction effect of the t^{th} temperature and the b^{th} block;

$(SxT)_{it}$ = the interaction effect of the i^{th} sire and t^{th} temperature;

$(DxT)_{ijt}$ = the interaction effect of the j^{th} dam mated to

the i^{th} sire and t^{th} temperature; and

ϵ_{ijbtm} = random errors associated with each observation (assumed to be real and independent with expectations equal to zero).

This model was fitted separately for each population using REG, a generalized linear models computer program (Gilmour 1985). Statistical significance associated with each effect is determined from F-ratios when: the TEMPS mean square is tested against the TxS interaction mean square; BLOCKS, TxB, DAMS(wS) and TxD(wS) mean squares are tested against the error term; SIRES mean square is tested against DAMS(wS) mean square; and the TxS interaction mean square is tested against the TxD(wS) interaction mean square.

b) Expression of Genotype-Environment Interaction as a Genetic Correlation. The biological importance of any genotype-environment interaction can be better appreciated by expressing the interaction as a genetic correlation between the expression of the same trait measured in the two environments. In this situation the two phenotypes are not measurable on one and the same individual, thus the environmental and phenotypic correlations have no reality (Falconer 1952). The usual statistical methods for calculating the genetic correlation between different traits measured on the same individual are not applicable, and alternative methods must be used.

The Method of "arbitrary pairing". In this method (see Via 1984b) observations made on individuals in different environments are arbitrarily paired within families. Covariance components are then estimated by equating mean cross-products to their expectations, as if the observations were made on the same individual. In the Nested experiment it was appropriate to pair full-sib progeny from one temperature with full-sibs from the other temperature, but only if they belonged to the same block. Even though up to 8 full-sibs were reared in each environment for each dam, only one combination of pairings was then possible. Any progeny which could not be paired with a full-sib complement from the other temperature were excluded from the analysis. Further, only dams which had two or more pairs of full-sibs were included in the analyses.

i) paternal half-sib estimate:

$$r_{S(arb)} = \frac{\widehat{cov}_{S(T1T2)}}{\sqrt{\hat{\sigma}^2_{S(T1)} \cdot \hat{\sigma}^2_{S(T2)}}}$$

where $r_{S(arb)}$ = the estimated genetic correlation between the performance of paternal half-sibs measured in different temperatures;
$\widehat{cov}_{S(T1T2)}$ = the estimated sire covariance component resulting from the arbitrary pairing; and
$\hat{\sigma}^2_{S(T1)}$ and $\hat{\sigma}^2_{S(T2)}$ are the genetic sire variance components for the character measured in temperatures 1 and 2 respectively.

ii) full-sib estimate:

$$r_{S+D(arb)} = \frac{\widehat{cov}_{S(T1T2)} + \widehat{cov}_{D(T1T2)}}{\sqrt{\hat{\sigma}^2_{S(T1)} + \hat{\sigma}^2_{D(T1)}}\sqrt{\hat{\sigma}^2_{S(T2)} + \hat{\sigma}^2_{D(T2)}}}$$

where $r_{S+D(arb)}$ = the genetic full-sib correlation estimate;
$\widehat{cov}_{D(T1T2)}$ = the estimated dam covariance component resulting from the arbitrary pairing;
$\hat{\sigma}^2_{D(T1)}$ and $\hat{\sigma}^2_{D(T2)}$ are the dam variance components for the character measured in temperatures 1 and 2 respectively;
and the other terms are as above.

For the "arbitrary pairing" method, the linear statistical model applicable to each environment is the same as for the estimation of heritability and genetic correlation when different traits were measured on the same individual. The variance and covariance components were obtained by model IV of LSML76 (Harvey 1977, 1982).

3.1.5.4 Genetic Differentiation Between Populations

Differentiation between the Mangoplah and Coalstoun Lakes populations in female life-history traits was investigated by analysis of variance techniques. The linear statistical model fitted within each temperature was as follows:

$$Y_{ijbpm} = \mu + P_p + S_{ip} + D_{ijp} + B_b + (BxP)_{bp} + e_{ijbpm}$$

where Y_{ijbpm} = the phenotypic value of the m^{th} individual beetle:
μ = value of the overall mean:
P_p = the fixed effect of the p^{th} population:
S_{ip} = the random effect of the i^{th} sire nested within the p^{th} population:
$D_{j:p}$ = the random effect of the j^{th} dam mated to the i^{th} sire and nested within the p^{th} population:
B_b = the fixed effect of the b^{th} block:
$(BxP)_{bp}$ = the interaction effect of the b^{th} block and p^{th} population: and
ϵ_{ijbpm} = random errors associated with each observation (assumed to be normally and independently distributed with zero mean and variance σ_ϵ^2).

This model was fitted for each trait using Model V of LSML76 (Harvey 1977, 1982). Population differentiation is indicated by significant F-ratios in the analysis of variance when the POPNS mean square is tested against the SIRES(wP) mean square. However, tests of significance are only approximate since the data are unbalanced.

3.1.5.5 Transformations

The valid application of tests of significance in the analysis of variance requires that experimental errors be independently and normally distributed with a common variance (Steel and Torrie 1981). However, the assumption of normality is not required for estimating components of variance (Searle 1971; Steel and Torrie 1981). Preliminary analyses were conducted to determine if the data violated the assumptions pertaining to analyses of variance. Measures of skewness and kurtosis were used to check for normality of distribution. Both Bartlett's test and the F_{max} test (see Sokal and Rohlf 1981; Steel and Torrie 1981) were used to check homogeneity of genetic variances between sire groups. The consequences of moderate heteroscedasticity are not too serious for overall tests of significance, but single degree of freedom comparisons may be far from accurate (Sokal and Rohlf 1981). The sire group variances were plotted against their respective means to check the assumption of independence of the mean and variance. When necessary, an appropriate transformation of the data was made to better satisfy these assumptions. Analyses based on transformed

data will be acknowledged along with the results.

3.2 Results

Paternal half-sib (h^2_S) and full-sib (h^2_{S-D}) heritability estimates for the various life-history traits (and derived indices) are presented in Tables 3.1 to 3.14.

The importance of distinguishing senescent from non-senescent deaths in *T. castaneum* has been emphasized by Mertz (1975) and Lavie (1981). Non-senescent deaths are considered to be a consequence of developmental injuries expressed during early adulthood (Lavie 1981). In both populations at 33°C the age-specific death rate prior to the first census (day 35) was higher than in the subsequent 4–8 weeks, over which time it declined, before increasing again. Thus, the distribution of the mortality patterns, at least under optimal (33°C) conditions, suggested that early deaths may have been of a non-senescent nature.

The estimates presented in Tables 3.1 to 3.4 are based on data from all individuals which had successfully eclosed as adults (i.e. includes early non-senescent deaths). In contrast, the estimates presented in Tables 3.5 to 3.8 are based on data from females which had survived for at least two egg censuses, that is beyond 49 days of age. Therefore, early deaths which are assumed to be predominantly of a non-senescent nature were excluded from these analyses.

Heritability estimates for individual egg censuses are presented in Tables 3.9 to 3.12. Only estimates which are based on a sufficient number of observations to give reasonable standard errors are reported. Females still alive at each census were considered in these analyses.

The calculation of some reproductive indices required the partitioning of the fecundity schedule into appropriate divisions. For measures of H1, H2, ADFH1, ADFH2, ALM, ARM, ADFALM and ADFARM to be meaningful, females must have survived beyond 49 days of age. When the fecundity schedule was partitioned into more divisions (quarters), only data from females surviving beyond 77 days of age were considered in the analyses. Only then were the fecundity schedules approximated from a sufficient number of censuses to give meaningful heritability estimates of Q1, Q2, Q3, Q4, ADFQ1, ADFQ2, ADFQ3 and ADFQ4, which are presented in Tables 3.13 and 3.14. These estimates are presented only for the optimal environment (33°C). At 37°C, the reproductive schedules generally were approximated from too few data points to be sufficiently sensitive for such divisions.

To compute HATCH it was necessary to exclude females which had not produced eggs at the second fecundity census (i.e. when E2 = 0). Therefore, heritability estimates for HATCH are based on fewer observations than those for the other traits presented in the same tables. In Tables 3.5 and 3.7, HATCH was based on sample sizes of n = 823 for the Mangoplah and n = 807 for the Coalstoun Lakes populations. In Tables 3.6 and 3.8, the sample sizes were 748 and 649 for the Mangoplah and Coalstoun Lakes populations respectively. All HATCH estimates are based on angular transformed data.

LSML76 sets all negative variance component estimates to zero. Heritability estimates are not computed for traits with negative estimates of genetic variance, and the genetic correlations with such a trait also are not computed (Harvey 1977).

Genetic correlation estimates from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) are presented in Tables 3.15 to 3.24. The estimates presented in Tables 3.15 to 3.18 are based on data from all females which had successfully eclosed as adults. In Tables 3.19 to 3.22 the estimates are based on data from females which had survived beyond 49 days of age. Only data from females surviving beyond 77 days of age were used to compute the correlation estimates in Tables 3.23 and 3.24.

Least-squares means are given in Tables 3.25 to 3.31 for each female life-history trait (or derived index). The values in Tables 3.25 and 3.26 are based on data from all females which had successfully eclosed as adults. The least-squares means in Tables 3.27 and 3.28 are based on data from females which had survived beyond 49 days. Those females alive at each fecundity census were used to compute the least-squares means in Tables 3.29 and 3.30. Only data from females surviving beyond 77 days of age were used to compute the values in Table 3.31.

Significant differences between the Mangoplah and Coalstoun Lakes populations, as indicated from significant F-ratios in an ANOVA also are given in Tables 3.25 to 3.31 for each life-history trait. The F-ratios are only approximate (Harvey 1977) since the data are unbalanced. The least-squares means for HATCH are based on angular transformed data. Sample sizes for HATCH in Tables 3.27 and 3.28 are the same as for the corresponding heritability estimates.

Tables 3.32 and 3.33 report results for the analyses of genotype-environment interaction. Results from the mixed model analyses of variance, which test for the statistical presence of genotype-environment interaction, are given in Table 3.32. In order to satisfy the assumptions underlying the analysis of variance, especially to stabilize the variances across

temperatures, it was necessary to transform the data. Therefore, analyses of DT, BWT, GR and ALS are based on $\log_{10}$ transformed data, while those for TOTAL and ADFTOT are based on square root transformed data. All females that had successfully eclosed were considered in the analyses, and included if they were from dams with two or more progeny.

In Table 3.33 genotype-environment interaction is expressed as a genetic correlation between performance of the same genotypes across the two laboratory temperatures. The estimates from paternal half-sibs ($r_{S(arb)}$) and full-sibs ($r_{S-D(arb)}$) are based on the method of arbitrary pairing (Via 1984b). All females which had successfully eclosed were considered in the analysis, and included if they were from dams with two or more full-sib pairs.

Table 3.1: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 33°C. All females which had successfully eclosed were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
DT	0.225 ± 0.097*	0.780 ± 0.213**
BWT	0.674 ± 0.193**	0.411 ± 0.142**
GR	0.823 ± 0.219**	0.172 ± 0.085*
AOD	0.173 ± 0.084*	0.191 ± 0.090*
ALS	0.174 ± 0.084*	0.197 ± 0.092*
RLS	0.168 ± 0.083*	0.181 ± 0.087*
TOTAL	0.121 ± 0.071	0.178 ± 0.087*
	n = 843	n = 823

* $P < 0.05$. ** $P < 0.01$

Table 3.2: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 37°C. All females which had successfully eclosed were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
DT	0.133 ± 0.076*	0.436 ± 0.156**
BWT	0.293 ± 0.115**	0.772 ± 0.220**
GR	0.428 ± 0.146**	0.493 ± 0.168**
AOD	0.357 ± 0.130**	0.092 ± 0.073
ALS	0.357 ± 0.130**	0.122 ± 0.081
RLS	0.364 ± 0.132**	0.124 ± 0.081
TOTAL	0.112 ± 0.070	0.012 ± 0.051
	n = 811	n = 684

* $P < 0.05$. ** $P < 0.01$

Table 3.3: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 33°C. All females which had successfully eclosed were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
DT	0.503 ± 0.089**	0.842 ± 0.105**
BWT	0.725 ± 0.101**	0.667 ± 0.100**
GR	0.877 ± 0.105**	0.686 ± 0.100**
AOD	0.219 ± 0.061**	0.267 ± 0.067**
ALS	0.221 ± 0.061**	0.274 ± 0.068**
RLS	0.213 ± 0.060**	0.261 ± 0.066**
TOTAL	0.195 ± 0.058**	0.268 ± 0.067**
	n = 843	n = 823

* $P < 0.05$, ** $P < 0.01$

Table 3.4: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 37°C. All females which had successfully eclosed were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
DT	0.686 ± 0.101**	0.694 ± 0.105**
BWT	0.613 ± 0.097**	0.740 ± 0.107**
GR	0.519 ± 0.091**	0.668 ± 0.104**
AOD	0.411 ± 0.083**	0.346 ± 0.081**
ALS	0.418 ± 0.083**	0.355 ± 0.082**
RLS	0.420 ± 0.083**	0.336 ± 0.080**
TOTAL	0.439 ± 0.085**	0.349 ± 0.082**
	n = 811	n = 684

* $P < 0.05$, ** $P < 0.01$

Table 3.5: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 33°C. Only females which had survived beyond 49 days were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
Non-Reproductive		
DT	0.226 ± 0.099*	0.805 ± 0.218**
BWT	0.688 ± 0.197**	0.400 ± 0.140**
GR	0.842 ± 0.223**	0.154 ± 0.081*
AOD	0.231 ± 0.100*	0.134 ± 0.076*
ALS	0.230 ± 0.100*	0.138 ± 0.077*
Reproductive		
E1	0.386 ± 0.136**	0.176 ± 0.087*
E2	0.426 ± 0.145**	0.416 ± 0.144**
EMAX	0.575 ± 0.176**	0.573 ± 0.176**
FERT	0.226 ± 0.098*	0.456 ± 0.152**
H1	0.125 ± 0.073*	0.181 ± 0.088*
H2	0.090 ± 0.064	0.091 ± 0.065
TOTAL	0.121 ± 0.072	0.152 ± 0.081*
ADFH1	0.435 ± 0.147**	0.402 ± 0.140**
ADFH2	0.113 ± 0.070	0.112 ± 0.070
ADFTOT	0.286 ± 0.113**	0.270 ± 0.110*
ALM	0.352 ± 0.128**	0.339 ± 0.127**
ARM	0.166 ± 0.083*	0.091 ± 0.066
ADFALM	0.370 ± 0.132**	0.452 ± 0.152**
ADFARM	0.107 ± 0.068	0.093 ± 0.066
HATCH	0.004 ± 0.040	0.399 ± 0.140**
	n = 827	n = 810

* $P < 0.05$. ** $P < 0.01$

Note: HATCH data were angular transformed

Table 3.6: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 37°C. Only females which had survived beyond 49 days were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
Non-Reproductive		
DT	0.206 ± 0.097*	0.446 ± 0.160**
BWT	0.318 ± 0.124**	0.794 ± 0.225**
GR	0.462 ± 0.156**	0.515 ± 0.174**
AOD	0.406 ± 0.144**	0.143 ± 0.089
ALS	0.406 ± 0.144**	0.177 ± 0.097*
Reproductive		
E1	0.221 ± 0.100*	0.178 ± 0.097*
E2	0.100 ± 0.069	0.104 ± 0.078
EMAX	0.299 ± 0.119**	0.413 ± 0.153**
FERT	0.056 ± 0.058	0.204 ± 0.104*
H1	0.085 ± 0.066	0.036 ± 0.060
H2	0.111 ± 0.072	0.083 ± 0.073
TOTAL	0.112 ± 0.072	0.069 ± 0.069
ADFH1	0.198 ± 0.095*	0.204 ± 0.104*
ADFH2	0.092 ± 0.067	0.030 ± 0.058
ADFTOT	0.189 ± 0.092*	0.158 ± 0.092*
ALM	0.221 ± 0.100*	0.171 ± 0.096*
ARM	0.191 ± 0.093*	0.060 ± 0.066
ADFALM	0.165 ± 0.086*	0.184 ± 0.099*
ADFARM	0.003 ± 0.043	0.103 ± 0.078
HATCH		0.202 ± 0.104*
	n = 770	n = 657

* $P < 0.05$, ** $P < 0.01$

Note: HATCH data were angular transformed

Table 3.7: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 33°C. Only females which had survived beyond 49 days were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
Non-Reproductive		
DT	0.520 ± 0.091**	0.870 ± 0.105**
BWT	0.740 ± 0.102**	0.661 ± 0.100**
GR	0.893 ± 0.105**	0.680 ± 0.100**
AOD	0.287 ± 0.069**	0.298 ± 0.071**
ALS	0.289 ± 0.070**	0.305 ± 0.072**
Reproductive		
E1	0.377 ± 0.079**	0.194 ± 0.059**
E2	0.334 ± 0.074**	0.262 ± 0.067**
EMAX	0.548 ± 0.093**	0.537 ± 0.093**
FERT	0.210 ± 0.060**	0.328 ± 0.074**
H1	0.231 ± 0.063**	0.306 ± 0.072**
H2	0.189 ± 0.056**	0.231 ± 0.063**
TOTAL	0.235 ± 0.063**	0.303 ± 0.072**
ADFH1	0.414 ± 0.082**	0.402 ± 0.082**
ADFH2	0.150 ± 0.052**	0.181 ± 0.057**
ADFTOT	0.300 ± 0.071**	0.331 ± 0.075**
ALM	0.366 ± 0.078**	0.338 ± 0.076**
ARM	0.261 ± 0.066**	0.287 ± 0.071**
ADFALM	0.380 ± 0.079**	0.413 ± 0.084**
ADFARM	0.146 ± 0.052**	0.247 ± 0.066**
HATCH	0.023 ± 0.033	0.434 ± 0.085**
	n = 827	n = 810

* $P < 0.05$. ** $P < 0.01$

Table 3.8: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 37°C. Only females which had survived beyond 49 days were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
Non-Reproductive		
DT	0.681 ± 0.102**	0.669 ± 0.105**
BWT	0.616 ± 0.098**	0.744 ± 0.108**
GR	0.546 ± 0.094**	0.670 ± 0.105**
AOD	0.394 ± 0.082**	0.396 ± 0.087**
ALS	0.400 ± 0.083**	0.409 ± 0.089**
Reproductive		
E1	0.439 ± 0.086**	0.170 ± 0.062**
E2	0.315 ± 0.074**	0.263 ± 0.074**
EMAX	0.511 ± 0.092**	0.431 ± 0.090**
FERT	0.309 ± 0.074**	0.218 ± 0.069**
H1	0.368 ± 0.080**	0.325 ± 0.081**
H2	0.460 ± 0.088**	0.341 ± 0.082**
TOTAL	0.454 ± 0.088**	0.378 ± 0.086**
ADFH1	0.476 ± 0.089**	0.373 ± 0.085**
ADFH2	0.319 ± 0.074**	0.307 ± 0.079**
ADFTOT	0.472 ± 0.089**	0.443 ± 0.092**
ALM	0.438 ± 0.086**	0.307 ± 0.079**
ARM	0.450 ± 0.087**	0.356 ± 0.084**
ADFALM	0.447 ± 0.087**	0.328 ± 0.081**
ADFARM	0.375 ± 0.080**	0.398 ± 0.088**
HATCH	0.159 ± 0.056**	0.246 ± 0.072**
	n = 770	n = 657

* $P < 0.05$. ** $P < 0.01$

Table 3.9: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 33°C. Females alive at each census were considered in the analyses.

	Mangoplah		Coalstoun Lakes	
Trait	h^2_S	(n)	h^2_S	(n)
E1	0.344 ± 0.126**	828	0.161 ± 0.082*	816
E2	0.426 ± 0.145**	827	0.416 ± 0.144**	810
E3	0.296 ± 0.116**	825	0.272 ± 0.111*	810
E4	0.144 ± 0.078*	816	0.213 ± 0.096*	807
E5	0.069 ± 0.059	809	0.204 ± 0.094*	800
E6	0.122 ± 0.074	795	0.058 ± 0.057	788
E7	0.051 ± 0.056	769	0.183 ± 0.094*	774
E8	0.018 ± 0.050	721	0.052 ± 0.059	736
E9		682	0.100 ± 0.074	698
E10	0.052 ± 0.065	651	0.064 ± 0.068	653
E11	0.023 ± 0.065	575	0.328 ± 0.141*	588
E12	0.086 ± 0.091	501	0.264 ± 0.132*	535
E13	0.247 ± 0.144*	433	0.416 ± 0.177*	465

* $P < 0.05$. ** $P < 0.01$

Table 3.10: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 37°C. Females alive at each census were considered in the analyses.

	Mangoplah		Coalstoun Lakes	
Trait	h^2_S	(n)	h^2_S	(n)
E1	0.225 ± 0.100*	794	0.179 ± 0.097*	671
E2	0.100 ± 0.069	770	0.104 ± 0.078	657
E3	0.048 ± 0.059	721		634
E4	0.092 ± 0.075	656	0.041 ± 0.067	599
E5	0.164 ± 0.100	590		559
E6	0.335 ± 0.151*	511	0.093 ± 0.094	493
E7	0.329 ± 0.170*	400	0.024 ± 0.092	407
E8	0.164 ± 0.171	282		274

* $P < 0.05$. ** $P < 0.01$

Table 3.11: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 33°C. Females alive at each census were considered in the analyses.

	Mangoplah		Coalstoun Lakes	
Trait	h^2_{S-D}	(n)	h^2_{S-D}	(n)
E1	0.353 ± 0.076**	828	0.204 ± 0.060**	816
E2	0.334 ± 0.074**	827	0.262 ± 0.067**	810
E3	0.267 ± 0.067**	825	0.314 ± 0.073**	810
E4	0.200 ± 0.059**	816	0.243 ± 0.065**	807
E5	0.136 ± 0.051**	809	0.272 ± 0.069**	800
E6	0.118 ± 0.049*	795	0.261 ± 0.068**	788
E7	0.083 ± 0.045*	769	0.109 ± 0.048*	774
E8	0.040 ± 0.041	721	0.179 ± 0.060**	736
E9	0.145 ± 0.058**	682	0.134 ± 0.055*	698
E10	0.163 ± 0.062**	651	0.096 ± 0.053*	653
E11	0.077 ± 0.055	575	0.164 ± 0.066**	588
E12	0.337 ± 0.093**	501	0.132 ± 0.067*	535
E13	0.250 ± 0.091**	433	0.215 ± 0.084**	465

* $P < 0.05$, ** $P < 0.01$

Table 3.12: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 37°C. Females alive at each census were considered in the analyses.

	Mangoplah		Coalstoun Lakes	
Trait	h^2_{S-D}	(n)	h^2_{S-D}	(n)
E1	0.383 ± 0.080**	794	0.171 ± 0.062**	671
E2	0.315 ± 0.074**	770	0.263 ± 0.074**	657
E3	0.214 ± 0.065**	721	0.277 ± 0.077**	634
E4	0.310 ± 0.079**	656	0.206 ± 0.070**	599
E5	0.389 ± 0.091**	590	0.169 ± 0.069*	559
E6	0.268 ± 0.085**	511	0.220 ± 0.081**	493
E7	0.169 ± 0.086*	400	0.322 ± 0.102**	407
E8	0.441 ± 0.136**	282	0.210 ± 0.114*	274

* $P < 0.05$, ** $P < 0.01$

Table 3.13: Heritability estimates (±SE) from paternal half-sibs (h^2_S) for two natural populations of *T. castaneum* measured at 33°C. Only females which had survived beyond 77 days were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
Q1	0.177 ± 0.0?7*	0.145 ± 0.079*
Q2	0.139 ± 0.077*	0.146 ± 0.079*
Q3	0.085 ± 0.053	0.049 ± 0.054
Q4	0.059 ± 0.056	0.096 ± 0.066
ADFQ1	0.448 ± 0.150**	0.426 ± 0.146**
ADFQ2	0.334 ± 0.125**	0.252 ± 0.106*
ADFQ3	0.136 ± 0.076*	0.123 ± 0.073
ADFQ4	0.045 ± 0.052	0.064 ± 0.058
	n = 816	n = 807

* $P < 0.05$, ** $P < 0.01$

Table 3.14: Heritability estimates (±SE) from full-sibs (h^2_{S-D}) for two natural populations of *T. castaneum* measured at 33°C. Only females which had survived beyond 77 days were considered in the analyses.

Trait	Mangoplah	Coalstoun Lakes
Q1	0.207 ± 0.060**	0.304 ± 0.072**
Q2	0.229 ± 0.063**	0.309 ± 0.072**
Q3	0.201 ± 0.059**	0.290 ± 0.070**
Q4	0.133 ± 0.050**	0.145 ± 0.052**
ADFQ1	0.379 ± 0.079**	0.440 ± 0.085**
ADFQ2	0.326 ± 0.07?**	0.313 ± 0.073**
ADFQ3	0.205 ± 0.060**	0.262 ± 0.067**
ADFQ4	0.079 ± 0.042*	0.080 ± 0.043*
	n = 816	n = 807

* $P < 0.05$, ** $P < 0.01$

Table 3.15: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Mangoplah population measured at 33°C. All females which had successfully eclosed were considered in the analyses (n = 843).

Traits	r_S	r_{S-D}	r_P
DT – BWT	−0.793 ± 0.158**	−0.476 ± 0.134**	−0.027
DT – ALS	−0.230 ± 0.313	−0.245 ± 0.186	−0.069
DT – TOTAL	−1.115 ± 0.234**	−0.371 ± 0.184	−0.076
BWT – ALS	0.044 ± 0.275	0.027 ± 0.184	0.089
BWT – TOTAL	0.057 ± 0.299	0.225 ± 0.180	0.228
GR – ALS	0.079 ± 0.270	0.078 ± 0.180	0.103
GR – TOTAL	0.234 ± 0.281	0.278 ± 0.173	0.237
ALS – TOTAL	0.626 ± 0.205**	0.748 ± 0.095**	0.811
ALS – RLS	0.999 ± 0.005**	0.999 ± 0.003**	0.980

* $P < 0.05$, ** $P < 0.01$

Table 3.16: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Mangoplah population measured at 37°C. All females which had successfully eclosed were considered in the analyses (n = 811).

Traits	r_S	r_{S-D}	r_P
DT – BWT	−0.727 ± 0.200**	0.377 ± 0.136*	0.382
DT – ALS	0.090 ± 0.317	−0.171 ± 0.163	0.021
DT – TOTAL	−0.849 ± 0.298**	0.007 ± 0.166	0.108
BWT – ALS	0.099 ± 0.268	0.155 ± 0.165	0.150
BWT – TOTAL	0.295 ± 0.313	0.455 ± 0.135**	0.341
GR – ALS	0.057 ± 0.256	0.242 ± 0.163	0.144
GR – TOTAL	0.423 ± 0.279	0.479 ± 0.136**	0.304
ALS – TOTAL	0.873 ± 0.100**	0.803 ± 0.061**	0.831
ALS – RLS	1.002 ± 0.002**	0.997 ± 0.002**	0.978

* $P < 0.05$, ** $P < 0.01$

Table 3.17: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Coalstoun Lakes population measured at 33°C. All females which had successfully eclosed were considered in the analyses (n = 823).

Traits	r_S	r_{S-D}	r_P
DT - BWT	0.832 ± 0.097**	0.310 ± 0.139*	0.327
DT - ALS	-0.295 ± 0.253	-0.265 ± 0.167	-0.015
DT - TOTAL	0.321 ± 0.253	0.047 ± 0.175	0.099
BWT - ALS	-0.422 ± 0.253	-0.031 ± 0.179	0.035
BWT - TOTAL	0.465 ± 0.237	0.379 ± 0.155*	0.318
GR - ALS	-0.378 ± 0.305	0.109 ± 0.177	0.045
GR - TOTAL	0.461 ± 0.279	0.376 ± 0.156*	0.290
ALS - TOTAL	0.596 ± 0.201**	0.679 ± 0.107**	0.738
ALS - RLS	1.001 ± 0.005**	1.007 ± 0.003**	0.973

* $P < 0.05$. ** $P < 0.01$

Table 3.18: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Coalstoun Lakes population measured at 37°C. All females which had successfully eclosed were considered in the analyses (n = 684).

Traits	r_S	r_{S-D}	r_P
DT - BWT	0.831 ± 0.107**	0.369 ± 0.141*	0.270
DT - ALS	-1.567 ± 0.307**	-0.216 ± 0.173	0.002
DT - TOTAL	-2.845 ± 5.077	0.054 ± 0.179	0.085
BWT - ALS	-0.621 ± 0.261*	-0.001 ± 0.178	0.065
BWT - TOTAL	0.842 ± 1.512	0.347 ± 0.159*	0.265
GR - ALS	-0.195 ± 0.327	0.083 ± 0.179	0.062
GR - TOTAL	2.200 ± 3.810	0.351 ± 0.161*	0.235
ALS - TOTAL	0.544 ± 0.709	0.735 ± 0.088**	0.795
ALS - RLS	0.997 ± 0.012**	0.996 ± 0.004**	0.971

* $P < 0.05$. ** $P < 0.01$

Table 3.19: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Mangoplah population measured at 33°C. Only females which had survived beyond 49 days were considered in the analyses (n = 827).

Traits	r_S	r_{S-D}	r_P
DT – BWT	−0.797 ± 0.157**	−0.450 ± 0.136**	−0.013
DT – ALS	−0.030 ± 0.303	−0.157 ± 0.179	−0.044
DT – EMAX	−0.821 ± 0.159**	−0.194 ± 0.161	−0.047
DT – FERT	−0.512 ± 0.263	−0.150 ± 0.191	−0.024
DT – H1	−0.707 ± 0.285*	−0.225 ± 0.185	−0.041
DT – H2	−1.313 ± 0.298**	−0.373 ± 0.188	−0.057
DT – TOTAL	−0.997 ± 0.246**	−0.297 ± 0.181	−0.053
DT – ADFH1	−0.716 ± 0.191**	−0.091 ± 0.171	−0.029
DT – ADFH2	−1.232 ± 0.235**	−0.240 ± 0.205	−0.021
DT – ADFTOT	−0.973 ± 0.158**	−0.158 ± 0.177	−0.013
BWT – ALS	−0.032 ± 0.261	−0.004 ± 0.175	0.056
BWT – EMAX	0.162 ± 0.223	0.474 ± 0.124**	0.461
BWT – FERT	−0.149 ± 0.256	0.193 ± 0.180	0.190
BWT – H1	−0.051 ± 0.298	0.119 ± 0.180	0.193
BWT – H2	−0.012 ± 0.329	0.288 ± 0.180	0.179
BWT – TOTAL	−0.038 ± 0.302	0.195 ± 0.175	0.208
BWT – ADFH1	0.011 ± 0.238	0.216 ± 0.157	0.316
BWT – ADFH2	−0.084 ± 0.305	0.345 ± 0.185	0.160
BWT – ADFTOT	−0.032 ± 0.252	0.275 ± 0.161	0.267
GR – ALS	−0.023 ± 0.257	0.032 ± 0.172	0.066
GR – EMAX	0.278 ± 0.208	0.456 ± 0.124**	0.449
GR – FERT	−0.038 ± 0.258	0.197 ± 0.177	0.185
GR – H1	0.072 ± 0.293	0.154 ± 0.176	0.195
GR – H2	0.195 ± 0.313	0.337 ± 0.173	0.187
GR – TOTAL	0.127 ± 0.292	0.237 ± 0.170	0.213
GR – ADFH1	0.130 ± 0.229	0.206 ± 0.155	0.306
GR – ADFH2	0.124 ± 0.298	0.355 ± 0.182	0.158
GR – ADFTOT	0.133 ± 0.243	0.291 ± 0.157	0.259
ALS – E1	−0.627 ± 0.217**	−0.304 ± 0.181	−0.065
ALS – E2	−0.677 ± 0.205**	−0.485 ± 0.172**	−0.087
ALS – EMAX	−0.480 ± 0.227*	−0.307 ± 0.171	−0.044
ALS – H1	0.692 ± 0.165**	0.786 ± 0.075**	0.860
ALS – H2	0.467 ± 0.297	0.628 ± 0.143**	0.486
ALS – TOTAL	0.640 ± 0.194**	0.758 ± 0.087**	0.779
ALS – ADFH1	−0.699 ± 0.194**	−0.376 ± 0.172*	−0.035
ALS – ADFH2	−0.552 ± 0.339	−0.332 ± 0.230	−0.191
ALS – ADFTOT	−0.663 ± 0.232**	−0.379 ± 0.187	−0.147
H1 – H2	0.775 ± 0.211**	0.815 ± 0.098**	0.607
ADFH1 – ADFH2	0.845 ± 0.166**	0.763 ± 0.123**	0.427
ALM – ARM	−0.300 ± 0.275	0.616 ± 0.148**	0.509
ADFALM – ADFARM	0.520 ± 0.252*	0.559 ± 0.159**	0.475

* $P < 0.05$, ** $P < 0.01$

Table 3.20: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Mangoplah population measured at 37°C. Only females which had survived beyond 49 days were considered in the analyses (n = 770).

Traits	r_S	r_{S-E}	r_P
DT – BWT	-0.503 ± 0.231*	0.346 ± 0.141*	0.382
DT – ALS	0.029 ± 0.289	-0.164 ± 0.166	0.024
DT – EMAX	-0.835 ± 0.170**	0.266 ± 0.153	0.192
DT – FERT	-0.813 ± 0.411	0.236 ± 0.169	0.116
DT – H1	-0.771 ± 0.316*	0.069 ± 0.171	0.136
DT – H2	-0.824 ± 0.267**	-0.037 ± 0.166	0.072
DT – TOTAL	-0.823 ± 0.271**	0.026 ± 0.166	0.120
DT – ADFH1	-0.758 ± 0.211**	0.301 ± 0.152	0.210
DT – ADFH2	-1.038 ± 0.294**	0.024 ± 0.177	0.051
DT – ADFTOT	-0.852 ± 0.202**	0.185 ± 0.160	0.150
BWT – ALS	0.052 ± 0.266	0.138 ± 0.168	0.140
BWT – EMAX	0.467 ± 0.215*	0.738 ± 0.082**	0.542
BWT – FERT	0.733 ± 0.343*	0.558 ± 0.133**	0.286
BWT – H1	0.351 ± 0.333	0.512 ± 0.133**	0.352
BWT – H2	0.062 ± 0.347	0.406 ± 0.143**	0.289
BWT – TOTAL	0.230 ± 0.326	0.473 ± 0.133**	0.351
BWT – ADFH1	0.352 ± 0.260	0.650 ± 0.102**	0.463
BWT – ADFH2	0.239 ± 0.348	0.447 ± 0.149**	0.241
BWT – ADFTOT	0.301 ± 0.274	0.578 ± 0.116**	0.400
GR – ALS	0.041 ± 0.253	0.219 ± 0.167	0.135
GR – EMAX	0.601 ± 0.175**	0.668 ± 0.099**	0.483
GR – FERT	0.861 ± 0.333*	0.496 ± 0.145**	0.256
GR – H1	0.511 ± 0.293	0.518 ± 0.135**	0.309
GR – H2	0.286 ± 0.306	0.434 ± 0.142**	0.268
GR – TOTAL	0.413 ± 0.283	0.488 ± 0.134**	0.315
GR – ADFH1	0.496 ± 0.222*	0.566 ± 0.120**	0.397
GR – ADFH2	0.456 ± 0.302	0.432 ± 0.153**	0.228
GR – ADFTOT	0.479 ± 0.232	0.522 ± 0.128**	0.356
ALS – E1	-0.722 ± 0.192**	-0.063 ± 0.178	0.075
ALS – E2	-0.976 ± 0.202**	0.035 ± 0.189	0.245
ALS – EMAX	-0.711 ± 0.173**	-0.089 ± 0.174	0.134
ALS – H1	0.908 ± 0.116**	0.768 ± 0.072**	0.842
ALS – H2	0.799 ± 0.167**	0.725 ± 0.091**	0.574
ALS – TOTAL	0.852 ± 0.117**	0.756 ± 0.075**	0.793
ALS – ADFH1	-0.812 ± 0.164**	0.019 ± 0.177	0.207
ALS – ADFH2	-0.562 ± 0.325	0.198 ± 0.186	-0.054
ALS – ADFTOT	-0.698 ± 0.208**	0.100 ± 0.176	0.089
H1 – H2	1.054 ± 0.109**	0.967 ± 0.028**	0.727
ADFH1 – ADFH2	1.087 ± 0.147**	0.882 ± 0.063**	0.554
ALM – ARM	-0.559 ± 0.232*	0.476 ± 0.137**	0.444
ADFALM – ADFARM	0.767 ± 3.755	0.796 ± 0.074**	0.626

* $P < 0.05$, ** $P < 0.01$

Table 3.21: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Coalstoun Lakes population measured at 33°C. Only females which had survived beyond 49 days were considered in the analyses (n = 810).

Traits	r_S	r_{S-D}	r_P
DT – BWT	0.847 ± 0.094**	0.315 ± 0.139*	0.329
DT – ALS	-0.242 ± 0.281	-0.234 ± 0.165	-0.014
DT – EMAX	0.631 ± 0.149**	0.387 ± 0.136**	0.223
DT – FERT	0.783 ± 0.116**	0.477 ± 0.138**	0.169
DT – H1	0.544 ± 0.216*	0.142 ± 0.168	0.125
DT – H2	0.294 ± 0.311	-0.035 ± 0.181	0.059
DT – TOTAL	0.506 ± 0.236*	0.085 ± 0.171	0.111
DT – ADFH1	0.814 ± 0.108**	0.507 ± 0.128**	0.230
DT – ADFH2	0.493 ± 0.267	0.235 ± 0.184	0.073
DT – ADFTOT	0.725 ± 0.151**	0.415 ± 0.145**	0.175
BWT – ALS	-0.496 ± 0.271	-0.041 ± 0.176	0.029
BWT – EMAX	0.824 ± 0.089**	0.718 ± 0.081**	0.626
BWT – FERT	0.654 ± 0.160**	0.444 ± 0.144**	0.284
BWT – H1	0.401 ± 0.250	0.393 ± 0.151*	0.323
BWT – H2	0.865 ± 0.221**	0.256 ± 0.174	0.261
BWT – TOTAL	0.589 ± 0.221*	0.369 ± 0.153*	0.335
BWT – ADFH1	0.858 ± 0.091**	0.607 ± 0.110**	0.496
BWT – ADFH2	1.066 ± 0.168**	0.291 ± 0.182	0.231
BWT – ADFTOT	0.966 ± 0.076**	0.533 ± 0.128**	0.427
GR – ALS	-0.572 ± 0.320	0.083 ± 0.175	0.038
GR – EMAX	0.731 ± 0.157**	0.550 ± 0.112**	0.559
GR – FERT	0.282 ± 0.277	0.215 ± 0.165	0.222
GR – H1	0.119 ± 0.341	0.338 ± 0.157*	0.285
GR – H2	1.167 ± 0.241**	0.286 ± 0.171	0.248
GR – TOTAL	0.479 ± 0.295	0.342 ± 0.156*	0.303
GR – ADFH1	0.593 ± 0.212**	0.368 ± 0.144*	0.419
GR – ADFH2	1.280 ± 0.227**	0.177 ± 0.190	0.208
GR – ADFTOT	0.877 ± 0.154**	0.317 ± 0.158	0.357
ALS – H1	0.321 ± 0.345	-0.066 ± 0.215	-0.006
ALS – H2	-0.411 ± 0.284	-0.263 ± 0.194	-0.012
ALS – EMAX	-0.144 ± 0.295	-0.024 ± 0.180	0.014
ALS – H1	0.529 ± 0.239*	0.720 ± 0.091**	0.784
ALS – H2	0.017 ± 0.433	0.440 ± 0.170*	0.360
ALS – TOTAL	0.392 ± 0.298	0.665 ± 0.108**	0.695
ALS – ADFH1	-0.20 ± 0.367	-0.161 ± 0.185	-0.076
ALS – ADFH2	-0.690 ± 0.420	-0.263 ± 0.225	-0.317
ALS – ADFTOT	-0.519 ± 0.315	-0.221 ± 0.199	-0.232
H1 – H2	0.647 ± 0.247*	0.721 ± 0.109**	0.569
ADFH1 – ADFH2	0.837 ± 0.161**	0.645 ± 0.132**	0.507
ALM – ARM	0.411 ± 0.307	0.435 ± 0.163*	0.361
ADFALM – ADFARM	1.058 ± 0.145**	0.710 ± 0.107**	0.543

* $P < 0.05$. ** $P < 0.01$

Table 3.22: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S+D}), and phenotypic correlations (r_P) for the Coalstoun Lakes population measured at 37°C. Only females which had survived beyond 49 days were considered in the analyses (n = 657).

Traits	r_S	r_{S+D}	r_P
DT – BWT	0.827 ± 0.109**	0.373 ± 0.142*	0.273
DT – ALS	-1.300 ± 0.180**	-0.275 ± 0.168	-0.013
DT – EMAX	0.473 ± 0.216*	0.347 ± 0.157*	0.202
DT – FERT	0.513 ± 0.251	0.271 ± 0.191	0.104
DT – H1	-1.380 ± 0.817	-0.018 ± 0.184	0.103
DT – H2	-0.820 ± 0.339*	0.086 ± 0.182	0.014
DT – TOTAL	-1.056 ± 0.372**	0.020 ± 0.179	0.079
DT – ADFH1	0.449 ± 0.257	0.330 ± 0.163	0.182
DT – ADFH2	-0.137 ± 0.584	0.221 ± 0.181	0.000
DT – ADFTOT	0.271 ± 0.304	0.280 ± 0.163	0.108
BWT – ALS	-0.481 ± 0.251	-0.033 ± 0.174	0.067
BWT – EMAX	0.819 ± 0.098**	0.702 ± 0.094**	0.540
BWT – FERT	0.853 ± 0.152**	0.471 ± 0.167**	0.200
BWT – H1	0.518 ± 0.486	0.345 ± 0.162*	0.293
BWT – H2	0.602 ± 0.306	0.326 ± 0.164	0.216
BWT – TOTAL	0.521 ± 0.337	0.346 ± 0.157*	0.291
BWT – ADFH1	0.985 ± 0.098**	0.565 ± 0.126**	0.421
BWT – ADFH2	1.454 ± 1.034	0.308 ± 0.170	0.164
BWT – ADFTOT	1.007 ± 0.131**	0.447 ± 0.140**	0.340
GR – ALS	0.132 ± 0.301	0.069 ± 0.176	0.072
GR – EMAX	0.869 ± 0.093**	0.621 ± 0.113**	0.473
GR – FERT	0.896 ± 0.158**	0.393 ± 0.179*	0.161
GR – H1	1.172 ± 0.667	0.376 ± 0.161*	0.257
GR – H2	1.086 ± 0.278**	0.319 ± 0.166	0.217
GR – TOTAL	1.062 ± 0.321**	0.364 ± 0.158*	0.266
GR – ADFH1	1.090 ± 0.098**	0.480 ± 0.142**	0.359
GR – ADFH2	1.921 ± 1.387	0.249 ± 0.176	0.170
GR – ADFTOT	1.187 ± 0.137**	0.374 ± 0.151*	0.306
ALS – E1	-0.606 ± 0.319	-0.177 ± 0.228	0.027
ALS – E2	-0.232 ± 0.408	0.258 ± 0.193	0.266
ALS – EMAX	-0.673 ± 0.234**	-0.036 ± 0.188	0.117
ALS – H1	0.694 ± 0.321*	0.806 ± 0.069**	0.814
ALS – H2	0.568 ± 0.344	0.447 ± 0.158**	0.427
ALS – TOTAL	0.597 ± 0.298	0.694 ± 0.098**	0.742
ALS – ADFH1	-0.737 ± 0.266*	-0.018 ± 0.193	0.120
ALS – ADFH2	-0.373 ± 0.795	-0.115 ± 0.202	-0.196
ALS – ADFTOT	-0.588 ± 0.345	-0.062 ± 0.187	-0.034
H1 – H2	1.279 ± 0.501*	0.897 ± 0.063**	0.636
ADFH1 – ADFH2	1.542 ± 0.936	1.019 ± 0.050**	0.549
ALM – ARM	0.368 ± 0.445	0.705 ± 0.117**	0.484
ADFALM – ADFARM	0.744 ± 0.220**	0.982 ± 0.044**	0.622

* $P < 0.05$, ** $P < 0.01$

Table 3.23: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S-D}), and phenotypic correlations (r_P) for the Mangoplah population measured at 33°C. Only females which had survived beyond 77 days were considered in the analyses (n = 816).

Traits	r_S	r_{S-D}	r_P
Q1 – Q2	0.968 ± 0.039**	0.982 ± 0.020**	0.887
Q1 – Q3	0.782 ± 0.198**	0.787 ± 0.104**	0.615
Q1 – Q4	0.701 ± 0.356	0.700 ± 0.180**	0.275
Q2 – Q3	0.907 ± 0.128**	0.864 ± 0.069**	0.737
Q2 – Q4	0.895 ± 0.319**	0.754 ± 0.157**	0.349
Q3 – Q4	1.199 ± 0.215**	0.885 ± 0.090**	0.659
ADFQ1 – ADFQ2	0.920 ± 0.062**	0.976 ± 0.037**	0.630
ADFQ1 – ADFQ3	0.723 ± 0.195**	0.720 ± 0.127**	0.315
ADFQ1 – ADFQ4	0.676 ± 0.410	0.507 ± 0.242*	0.123
ADFQ2 – ADFQ3	0.912 ± 0.110**	0.840 ± 0.083**	0.598
ADFQ2 – ADFQ4	0.905 ± 0.391*	0.623 ± 0.223**	0.244
ADFQ3 – ADFQ4	1.107 ± 0.258**	0.888 ± 0.126**	0.647
ALS – Q1	0.710 ± 0.143**	0.777 ± 0.080**	0.862
ALS – Q2	0.726 ± 0.152**	0.753 ± 0.088**	0.789
ALS – Q3	0.546 ± 0.272	0.593 ± 0.143**	0.541
ALS – Q4	0.492 ± 0.369	0.609 ± 0.182**	0.253
ALS – ADFQ1	−0.482 ± 0.224*	−0.371 ± 0.174*	0.049
ALS – ADFQ2	−0.621 ± 0.225*	−0.398 ± 0.184*	−0.160
ALS – ADFQ3	−0.610 ± 0.308	−0.390 ± 0.211	−0.236
ALS – ADFQ4	−0.158 ± 0.484	−0.004 ± 0.283	−0.156
ALS – ADFTOT	−0.543 ± 0.249*	−0.353 ± 0.191	−0.187

* P < 0.05, ** P < 0.01

Table 3.24: Genetic correlation estimates (±SE) from paternal half-sibs (r_S) and full-sibs (r_{S+D}), and phenotypic correlations (r_P) for the Coalstoun Lakes population measured at 33°C. Only females which had survived beyond 77 days were considered in the analyses (n = 807).

Traits	r_S	r_{S+D}	r_P
Q1 – Q2	0.971 ± 0.048**	0.966 ± 0.022**	0.852
Q1 – Q3	0.952 ± 0.260**	0.691 ± 0.110**	0.576
Q1 – Q4	0.137 ± 0.407	0.541 ± 0.189**	0.205
Q2 – Q3	0.985 ± 0.188**	0.860 ± 0.060**	0.733
Q2 – Q4	0.155 ± 0.402	0.720 ± 0.153**	0.310
Q3 – Q4	0.921 ± 0.250**	0.933 ± 0.076**	0.626
ADFQ1 – ADFQ2	0.940 ± 0.060**	0.908 ± 0.048**	0.662
ADFQ1 – ADFQ3	0.899 ± 0.153**	0.548 ± 0.140**	0.390
ADFQ1 – ADFQ4	0.586 ± 0.339	0.337 ± 0.247	0.141
ADFQ2 – ADFQ3	0.994 ± 0.083**	0.849 ± 0.068**	0.682
ADFQ2 – ADFQ4	0.466 ± 0.352	0.710 ± 0.201**	0.337
ADFQ3 – ADFQ4	0.913 ± 0.186**	0.937 ± 0.117**	0.657
ALS – Q1	0.385 ± 0.308	0.737 ± 0.086**	0.811
ALS – Q2	0.511 ± 0.270	0.699 ± 0.100**	0.681
ALS – Q3	0.064 ± 0.544	0.483 ± 0.153**	0.434
ALS – Q4	−0.372 ± 0.396	0.424 ± 0.205*	0.140
ALS – ADFQ1	−0.316 ± 0.299	−0.095 ± 0.182	0.044
ALS – ADFQ2	−0.338 ± 0.343	−0.179 ± 0.195	−0.214
ALS – ADFQ3	−0.780 ± 0.419	−0.243 ± 0.204	−0.338
ALS – ADFQ4	−0.720 ± 0.549	−0.076 ± 0.284	−0.272
ALS – ADFTOT	−0.514 ± 0.330	−0.178 ± 0.194	−0.260

* $P < 0.05$, ** $P < 0.01$

Table 3.25: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 33°C. Data from all females which had successfully eclosed were used to compute these values. Significant F-ratios from the ANOVAs are used to indicate population differentiation.

Trait	Mangoplah	Coalstoun Lakes	ANOVA
DT (d)	24.5445 ± 0.0678	26.3516 ± 0.1750	***
BWT (μg)	1528.9621 ± 17.9415	1600.9633 ± 20.0085	**
GR (μg/d)	62.4092 ± 0.8449	60.8171 ± 0.6195	NS
AOD (d)	204.4324 ± 3.5246	209.5136 ± 3.4188	NS
ALS (d)	179.8879 ± 3.5364	183.1621 ± 3.4529	NS
RLS (d)	173.1696 ± 3.4531	175.5237 ± 3.2813	NS
TOTAL (e)	1683.8225 ± 32.9263	1670.9385 ± 34.1663	NS
	n = 843	n = 823	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant
(Units: e = eggs, d = days)

Table 3.26: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 37°C. Data from all females which had successfully eclosed were used to compute these values. Significant F-ratios from the ANOVAs are used to indicate population differentiation.

Trait	Mangoplah	Coalstoun Lakes	ANOVA
DT (d)	22.8707 ± 0.0939	24.6773 ± 0.1441	***
BWT (μg)	1512.8680 ± 18.9552	1591.2015 ± 25.0647	*
GR (μg/d)	66.0234 ± 0.8164	64.5714 ± 0.8664	NS
AOD (d)	121.9742 ± 2.9951	129.8156 ± 2.3475	*
ALS (d)	99.1035 ± 2.9974	105.1383 ± 2.4246	NS
RLS (d)	94.1201 ± 2.9594	99.5457 ± 2.2947	NS
TOTAL (e)	704.0688 ± 22.4427	740.9234 ± 18.5270	NS
	n = 811	n = 684	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant
(Units: e = eggs, d = days)

Table 3.27: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 33°C. Data from females which had survived beyond 49 days were used to compute these values. Significant F-ratios from the ANOVAs are used to indicate population differentiation.

Trait	Mangoplah	Coalstoun Lakes	ANOVA
DT (d)	24.5348 ± 0.0682	26.3504 ± 0.1770	***
BWT (μg)	1531.5594 ± 18.1206	1601.7781 ± 19.8632	*
GR (μg/d)	62.5355 ± 0.3513	60.8487 ± 0.6109	NS
AOD (d)	207.7259 ± 3.6239	212.2467 ± 3.0744	NS
ALS (d)	183.1910 ± 3.6287	185.8963 ± 3.1012	NS
E1 (e/d)	14.1241 ± 0.2107	14.5980 ± 0.1853	NS
E2 (e/d)	12.6619 ± 0.2032	13.5430 ± 0.2261	**
EMAX (e/d)	15.2238 ± 0.1953	15.9553 ± 0.2280	*
FERT (prog)	11.6745 ± 0.2002	12.0668 ± 0.2851	NS
H1 (e)	1074.2988 ± 20.2267	1113.6086 ± 22.7239	NS
H2 (e)	642.3693 ± 13.9744	583.8488 ± 12.3124	**
TOTAL (e)	1716.6682 ± 31.7327	1697.4575 ± 31.9997	NS
ADFH1 (e/d)	11.7515 ± 0.1572	12.0367 ± 0.1811	NS
ADFH2 (e/d)	7.1819 ± 0.1436	6.5088 ± 0.1342	**
ADFTOT (e/d)	9.4668 ± 0.1371	9.2908 ± 0.1422	NS
ADFALM (e/d)	11.6584 ± 0.1574	12.2716 ± 0.1966	*
ADFARM (e/d)	7.3860 ± 0.1540	7.6528 ± 0.1365	NS
HATCH (rad)	1.3697 ± 0.0130	1.3321 ± 0.0294	NS
	n = 827	n = 810	

* P < 0.05, ** P < 0.01, *** P < 0.001, NS = non-significant
(Units: e = eggs, d = days, prog = progeny)
Note: HATCH data were angular transformed.

Table 3.28: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 37°C. Data from females which had survived beyond 49 days were used to compute these values. Significant F-ratios from the ANOVAs are used to indicate population differentiation.

Trait	Mangoplah	Coalstoun Lakes	ANOVA
DT (d)	22.8693 ± 0.0985	24.6878 ± 0.1439	***
BWT (μg)	1516.2082 ± 19.4958	1591.6331 ± 25.5947	*
GR (μg/d)	66.1739 ± 0.8437	64.5564 ± 0.8850	NS
AOD (d)	126.3415 ± 2.8115	133.5668 ± 2.2814	NS
ALS (d)	103.4722 ± 2.8144	108.8790 ± 2.3571	NS
E1 (e/d)	12.1643 ± 0.2124	12.6739 ± 0.1873	NS
E2 (e/d)	10.3323 ± 0.2084	10.6931 ± 0.2066	NS
EMAX (e/d)	12.9185 ± 0.2042	13.5101 ± 0.2210	NS
FERT (prog)	9.2393 ± 0.2071	9.0458 ± 0.2491	NS
H1 (e)	490.1741 ± 13.1832	530.2998 ± 12.2590	*
H2 (e)	248.7511 ± 8.7121	240.2996 ± 7.1024	NS
TOTAL (e)	738.9252 ± 21.3483	770.5994 ± 18.5168	NS
ADFH1 (e/d)	9.2960 ± 0.1640	9.6545 ± 0.1590	NS
ADFH2 (e/d)	4.8470 ± 0.1329	4.5502 ± 0.1181	NS
ADFTOT (e/d)	7.0717 ± 0.1414	7.1027 ± 0.1334	NS
ADFALM (e/d)	9.1183 ± 0.1592	9.6609 ± 0.1567	*
ADFARM (e/d)	5.6653 ± 0.1400	5.7740 ± 0.1467	NS
HATCH (rad)	1.3076 ± 0.0173	1.2453 ± 0.0297	**
	n = 770	n = 657	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant
(Units: e = eggs, d = days, prog = progeny)
Note: HATCH data were angular transformed.

Table 3.29: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 33°C. Data from females alive at each census were used to compute these values (units = eggs/day). Significant F-ratios from the ANOVAs are used to indicate population differentiation.

	Mangoplah		Coalstoun Lakes		
Trait	LSM	(n)	LSM	(n)	ANOVA
E1	14.1073 ± 0.2058	828	14.5220 ± 0.1908	816	NS
E2	12.6619 ± 0.2032	827	13.5430 ± 0.2261	810	**
E3	12.6553 ± 0.1943	825	13.1692 ± 0.2318	810	NS
E4	11.9126 ± 0.1738	816	12.0301 ± 0.2157	807	NS
E5	11.9066 ± 0.1653	809	11.8766 ± 0.2178	800	NS
E6	11.3260 ± 0.1916	795	11.3083 ± 0.1897	788	NS
E7	10.8869 ± 0.1820	769	10.7307 ± 0.2152	774	NS
E8	10.1874 ± 0.1598	721	9.7140 ± 0.1907	736	NS
E9	9.3217 ± 0.1795	682	8.6245 ± 0.2088	698	*
E10	8.1776 ± 0.2056	651	7.3433 ± 0.1841	653	**
E11	7.6210 ± 0.1827	575	7.0315 ± 0.2439	588	NS
E12	6.9414 ± 0.2448	501	5.9482 ± 0.2349	535	**
E13	6.3439 ± 0.2745	433	5.2291 ± 0.2677	465	**

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

Table 3.30: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 37°C. Data from females alive at each census were used to compute these values (units = eggs/day). Significant F-ratios from the ANOVAs are used to indicate population differentiation.

	Mangoplah		Coalstoun Lakes		
Trait	LSM	(n)	LSM	(n)	ANOVA
E1	12.0691 ± 0.2151	794	12.5237 ± 0.1998	671	NS
E2	10.3323 ± 0.2084	770	10.6932 ± 0.2067	657	NS
E3	9.8208 ± 0.2027	721	10.0180 ± 0.2085	634	NS
E4	8.1751 ± 0.2239	656	8.5135 ± 0.1869	599	NS
E5	7.2412 ± 0.2541	590	7.4355 ± 0.1793	559	NS
E6	5.9183 ± 0.2665	511	5.7870 ± 0.1985	493	NS
E7	4.6090 ± 0.2471	400	4.0838 ± 0.1900	407	NS
E8	4.1869 ± 0.2744	282	3.5761 ± 0.2050	274	NS

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

Table 3.31: Least-squares means (±SE) for two natural populations of *T. castaneum* measured at 33°C. Data from females which had survived beyond 77 days were used to compute these values. Significant F-ratios from the ANOVAs are used to indicate population differentiation.

Trait	Mangoplah	Coalstoun Lakes	ANOVA
Q1 (e)	533.2784 ± 10.5519	573.1354 ± 11.7712	*
Q2 (e)	552.4277 ± 10.2856	544.1259 ± 10.7898	NS
Q3 (e)	441.2873 ± 8.6667	411.7414 ± 7.8461	*
Q4 (e)	207.8300 ± 5.9895	174.0778 ± 5.2642	***
ADFQ1 (e/d)	11.5005 ± 0.1520	12.2716 ± 0.1859	**
ADFQ2 (e/d)	12.0619 ± 0.1690	11.8465 ± 0.1834	NS
ADFQ3 (e/d)	9.7711 ± 0.1786	9.1265 ± 0.1747	*
ADFQ4 (e/d)	4.6305 ± 0.1233	3.9188 ± 0.1109	***
	n = 816	n = 807	

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. NS = non-significant
(Units: e = eggs, d = days)

Table 3.32 Mixed model Analyses of Variance testing for the presence of genotype-environment interaction. Mean squares for each effect, with F-ratio significance, are given for six female life-history traits measured on two natural populations of *T. castaneum*. All females which had successfully eclosed were considered in the analyses.

SOURCE	df	DT	BWT	GR	TOTAL	ADFTOT	ALS
MANGOPLAH							
TEMPS	1	0.40330***	0.02165	0.23807***	88906.802***	83.07552***	29.87430***
BLOCKS	7	0.00511***	0.00379	0.01446***	78.634	0.20429	0.06631
T x B	7	0.00077*	0.00832**	0.00963***	45.778	0.16684	0.03273
SIRES	26	0.00298	0.03576***	0.04545***	241.298	1.20752**	0.19198**
DAMS(wS)	81	0.00187***	0.01327***	0.01304***	153.352***	0.51680**	0.09422**
T x S	26	0.00079	0.00904	0.00654	104.457	0.53818	0.06374
T x D(wS)	81	0.00067***	0.00548***	0.00407*	86.931	0.35913	0.06892
ERROR	1424	0.00031	0.00272	0.00249	67.965	0.29373	0.04940
COALSTOUN LAKES							
TEMPS	1	0.31523***	0.00350	0.25231***	71878.226***	67.99108***	24.17302***
BLOCKS	7	0.00311***	0.00287	0.00502	57.139	0.37728	0.03080
T x B	7	0.00048	0.00341	0.00301	16.376	0.09498	0.01100
SIRES	26	0.00983***	0.05208***	0.03659**	175.681	0.78782	0.12746*
DAMS(wS)	81	0.00298***	0.01341***	0.01590***	135.299***	0.51547***	0.06944*
T x S	26	0.00104*	0.00333	0.00330	103.334*	0.32486	0.08110
T x D(wS)	78	0.00060	0.00478*	0.00403*	61.733	0.29283	0.05705
ERROR	1280	0.00050	0.00294	0.00273	56.736	0.26132	0.04345

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Note: DT, BWT, GR and ALS were $\log_{10}$ transformed
TOTAL and ADFTOT were $\sqrt{}$ transformed.

Table 3.33: Genotype-environment interaction expressed as a genetic correlation between performance across temperatures 33° and 37°C. Estimates (±SE) from paternal half-sibs ($r_{S(a-b)}$) and full-sibs ($r_{S-D(a-b)}$) using the method of arbitrary pairing are given for six life-history traits measured in two natural populations of *T. castaneum*. All females which had successfully eclosed were considered in the analyses.

	$r_{S(a-b)}$	$r_{S-D(a-b)}$
Mangoplah		
DT	0.798 ± 0.188	0.754 ± 0.086
BWT	0.744 ± 0.142	0.639 ± 0.103
GR	0.881 ± 0.077	0.794 ± 0.071
TOTAL	0.417 ± 0.364	0.711 ± 0.149
ADFTOT	0.562 ± 0.260	0.720 ± 0.138
ALS	0.931 ± 0.182	0.780 ± 0.138
	Sample size = 797 pairs	
Coalstoun Lakes		
DT	0.803 ± 0.112	0.883 ± 0.056
BWT	1.165 ± 0.063	0.860 ± 0.062
GR	1.206 ± 0.115	0.880 ± 0.060
TOTAL		0.717 ± 0.150
ADFTOT	0.965 ± 0.335	0.780 ± 0.148
ALS	1.135 ± 0.345	0.617 ± 0.165
	Sample size = 665 pairs	

3.3 Discussion

In the Nested experiment, the methods of quantitative genetics have allowed the statistical description of phenotypic variation and covariation in several life-history traits measured on females from two natural populations of *T. castaneum*, under two constant laboratory temperatures.

Heritabilities were calculated for individual life-history traits for each population and temperature combination, using both the sire component of variance (paternal half-sib estimate) and the combined sire and dam components of variance (full-sib estimate). Comparisons of these two calculations of heritability (Tables 3.1 with 3.3, 3.2 with 3.4, 3.5 with 3.7, 3.6 with 3.8, 3.9 with 3.11, 3.10 with 3.12, and 3.13 with 3.14) emphasize the need for caution when drawing inferences based upon full-sib estimates. Generally, the full-sib estimates are higher than those from paternal half-sibs. The few exceptions to this trend show only minor differences between the two estimates. As expected the standard errors associated with the estimates from paternal half-sibs were generally larger than those from full-sibs.

The degree to which the heritability estimate from full-sibs exceeds that from paternal half-sibs indicates, in theory, the contribution of maternal common environment and non-additive genetic effects (Becker 1984; Falconer 1981). Maternal effects in *Tribolium* are due in part to the nutrients in the eggs that provide a developmental environment, and these nutrients may vary in both quality and quantity (Bondari, Willham and Freeman 1978). Density effects arising from the different number of eggs allowed to develop until pupation in the full-sib cultures also could have contributed to postnatal maternal influences. However, these density effects were not considered to be great, since the cultures were not crowded.

Under realistic conditions accidental and senescent causes interact to fix age-dependent mortality probabilities, with the senescent component predominating at more advanced ages (Mertz 1975a). Age at death (AOD) may give little information about the cause of death in a population where individuals deteriorate at varying rates. Mertz (1975) suggested using the reproductive pattern of individuals to characterize senescent decline and to give insights into the causes underlying individual mortalities. For individuals dying from senescent causes, it is assumed that after the onset of reproduction the age-specific reproductive schedule is characterized by an early flat peak, followed by a gradual and slowing loss of reproductive capabilities. On the other hand, females dying from non-senescent causes are

expected to display age-specific fecundity curves which are abruptly truncated. This may not always be the case, since senescence in the individual is due to the failure of many different physiological functions which affect an organisms viability, and in turn decreases its ability to cope with its environment (Comfort 1979).

In the Nested experiment, the exclusion of early deaths, which are considered to be predominantly a consequence of developmental injuries expressed during early adulthood (Lavie 1981), had little effect on the magnitude of heritability estimates for either population at either temperature. Differences between paternal half-sib heritability estimates for DT, BWT, GR, AOD, ALS and TOTAL based on data from females which had successfully eclosed (Tables 3.1 and 3.2) and estimates for the same traits, but based only on those females surviving beyond 49 days of age (Tables 3.5 and 3.6) are negligible. This is not unexpected since few adults had died before day 49. In the Mangoplah population only 1.9% and 5.1% of beetles died between the time of adult eclosion and day 49 at 33° and 37°C respectively. The proportions of beetles dying during this interval in the Coalstoun Lakes population were 1.6% at 33°C and 3.9% at 37°C. There is no guarantee that such early deaths were the result of non-senescent causes. However, their exclusion did marginally improve the normality of distribution for ALS within populations and allowed for more meaningful estimates of some reproductive indices. Later deaths due to non-senescent causes were assumed to be randomly distributed among the different genotypes. Further discussion about heritabilities will be restricted to estimates based on individuals which survived beyond 49 days.

There is evidence that abundant additive genetic variation exists for life-history traits in natural *T. castaneum* populations. Paternal half-sib heritability estimates significantly different from zero were found for both reproductive and non-reproductive traits in both populations at both laboratory temperatures (for examples, see Tables 3.5, 3.6, 3.9 and 3.10).

In agreement with the predictions of Fisher's (1930, 1958) Fundamental Theorem of Natural Selection, the reproductive traits generally display lower levels of heritable variation than the non-reproductive traits in both populations at both temperatures (see Tables 3.5 and 3.6). Furthermore, although age-specific components of reproduction (for example E1, E2, EMAX and FERT) sometimes display significant and high heritabilities, the estimates for lifetime reproduction (TOTAL) are low, and only significantly different from zero for the Coalstoun Lakes population at 33°C.

These results suggest that, although components of fitness may have significant heritability values, fitness itself need not be heritable. Such conclusions are based on the assumption that reproductive traits, and more specifically lifetime reproduction, are more highly correlated with fitness, than are non-reproductive traits. Gustafsson (1986) who reported an inverse relation between the heritability of a trait and its influence on fitness in a natural population of the collared flycatcher *F. albicollis*, also found the heritability of lifetime reproductive success did not differ significantly from zero.

Lewontin (1965) concluded that populations of colonizers should contain low amounts of additive genetic variance for developmental time. Modern practices associated with storage and seasonal movement of grain, where attempts are made to minimize damage due to *T. castaneum* and other grain infesting pests, provide conditions suitable for a sequence of colonizing episodes. This led Dawson (1977) to postulate that a lack of additive genetic variance for developmental rate in field populations of *T. castaneum* would result from selection for fast development in their role as colonizers. Contrary to this expectation, the natural populations from Mangoplah and Coalstoun Lakes display significant and sometimes high amounts of heritable variation for DT at both laboratory temperatures. This observation is more in keeping with studies on laboratory populations which often show significant amounts of additive genetic variance, evident from marked responses to artificial selection (Dawson 1965a; Englert and Bell 1970; Scheinberg, Bell and Anderson 1967). Previously it has been supposed that the laboratory environment, where synchronized cultures exist at high density in a habitat of fixed and limited resources, leads to stabilizing selection for intermediate developmental time which operates through cannibalism of early pupating individuals (Dawson 1975).

Obviously, selection pressures acting on populations in the laboratory and those in their natural habitat might be quite different. This, and the observation of significant differences between the biological characteristics of field and laboratory populations of *T. castaneum* (White 1984) serve as warnings against extrapolating heritability estimates from data based on long-established laboratory cultures to the field situation.

Body weight (BWT), which displays significant and high heritable variation in both populations under both temperatures, is an important life-history trait because many other characters showing genetic variability in natural populations are often correlated with body weight (Atchley 1983; David and Bocquet 1975a). Further, body size should be subjected to strong stabilizing selection, for there is obviously an optimal body size for each niche

(Masaki 1978).

In the Mangoplah population, the paternal half-sib heritability estimates (h^2_S) (see Tables 3.5 and 3.6) are generally higher at the optimum temperature (33°C) than under the more stressful environment (37°C) for both reproductive and non-reproductive traits. This trend is repeated in the Coalstoun Lakes population, but only for the reproductive traits. The non-reproductive traits are inconsistent: DT is higher, BWT, GR, and ALS are lower. These results are contrary to the expectation that additive genetic variance of a trait may increase when a colonizing species is stressed (Kohane 1987; Murphy et al. 1983; Parsons 1982, 1983). This is all the more interesting since the Mangoplah population, originating from a temperate climate, should be less well adapted to the stressful (37°C) temperature than the sub-tropical Coalstoun Lakes population which experiences higher temperatures more often in its natural habitat. The exception is longevity (ALS), which displays more significant additive genetic variation (higher h^2_S) under the stressful temperature.

Assuming the differences between the full-sib (Tables 3.7 and 3.8) and paternal half-sib (Tables 3.5 and 3.6) heritability estimates are largely due to non-additive dominance effects, rather than maternal effects, then dominance is important for longevity (ALS) in the Coalstoun Lakes population at both optimum and stressful temperatures, but not in the Mangoplah population at either temperature. This suggests that the expression of non-additive effects for longevity might be population specific. The significant heritability estimates (h^2_S) for ALS in both populations at both temperatures (see Tables 3.5 and 3.6) do indicate that longevity, with the appropriate selection pressures, is an adaptable trait and modifiable by natural selection.

This experiment has examined in detail the quantity of reproduction. However, fecundity may not always be an accurate guide to fertility (Hiraizumi 1985; Parker and Begon 1986; Wu 1981). The quality of an egg is also important in establishing the reproductive fitness of particular genotypes. For example, Wu (1981) found significant differences in egg size among strains of *T. castaneum*. In Tables 3.5 and 3.6 the heritability estimates for FERT and E2 are sufficiently different within populations and temperatures to warrant caution in predicting reproductive success from fecundity estimates alone. Furthermore, the populations differ in the amount of heritable variation in HATCH — the proportion of viable offspring (FERT) from the second egg census (E2). In contrast to the lack of heritable variation for HATCH in the Mangoplah population, the population from Coalstoun Lakes displayed moderate heritabilities significantly different from zero, at both temperatures.

Clearly, the most important point to be confirmed from the heritability estimates is that heritability is not a concept that can be applied to a life-history trait in general, but only to a given trait in a particular population, under a particular set of environmental conditions.

The response of a population to natural selection is, in theory, proportional to the heritable variation in fitness (Falconer 1981). Since life-history traits are largely synonymous with fitness components, the ability of these populations to respond to directional selection can be predicted, at least in these two environments, from the paternal half-sib (h^2_S) heritability estimates. Predictions based on these estimates can be useful for several generations (Hill 1974). However, for these predictions to be accurate, the genetic relationships between the different life-history traits and their relative contributions to overall fitness, must be known.

Genetic correlation estimates using variance and covariance components from full-sibs (r_{S-D}), like the heritability estimates, are confounded by non-additive genetic and maternal effects. A comparison of these estimates with those from paternal half-sibs (r_S) within Tables 3.15 to 3.24 confirm this. Further, genetic and environmental causes of correlation combine together to give the phenotypic correlation (r_P). The nature of the phenotypic correlation makes it clear that the magnitude and even the sign of the genetic correlation cannot be determined from the phenotypic correlation alone (Falconer 1981). This is evident from a comparison of the phenotypic correlation estimate (r_P) with the appropriate genetic correlation estimate from paternal half-sibs (r_S) for the different combinations of life-history traits in the two natural populations at both temperatures (see Tables 3.15 to 3.24). Therefore, the phenotypic correlation between different traits within populations may not accurately reflect the evolutionary constraints on major components of fitness imposed by genetic correlations.

In general, the exclusion of early deaths had little effect on the magnitude of the genetic correlation estimates. Paternal half-sib estimates (r_S) based on data from females which had successfully eclosed (Tables 3.15 to 3.18) and estimates between the same pairs of traits, but based only on those females surviving beyond 49 days of age (Tables 3.19 to 3.22) are in agreement.

In Tables 3.15 to 3.24, both positive and negative genetic correlations occur, some of which are significantly different from zero. Some of these negative correlations are of importance in establishing an empirical basis to the concepts of "trade-off" in general, and "reproductive cost" in particular. In life-history theory a "trade-off" means that an improvement in one fitness related character is associated with a decrement in some other

fitness related character (Reznick 1985). In particular, the reproductive cost hypothesis states that any increment in present reproduction is associated with a decrement in the expectation of future reproduction (Bell 1980, 1984a,b).

Evidence for the existence of reproductive costs may be of two kinds. Firstly, a negative genetic correlation between early reproduction and late reproduction may indicate the presence of a cost. In the Mangoplah population at 33°C (Table 3.19) the paternal half-sib genetic correlation between early-life average daily fecundity (ADFH1) and late-life average daily fecundity (ADFH2) was highly positive and significantly different from zero. At the stressful temperature (Table 3.20) a similar correlation was found. In the Coalstoun Lakes population (Tables 3.21 and 3.22) the correlations were also highly positive at both temperatures, but only at the optimum temperature was the correlation significantly different from zero. In Tables 3.23 and 3.24, ADFQ1 and ADFQ4 represent early and late-life fecundity respectively. Again the genetic correlations (r_g) between early and late reproduction in the optimal environment are highly positive in both populations. These consistently positive genetic correlations suggest that a cost associated with high early reproduction is not manifested through a reduction in later fecundity — those beetles which have a high rate of oviposition early in their life also oviposit at a relatively faster rate later in life.

Within a population, a change in ranking of individuals for early and late-life fecundity is necessary for a fecundity cost to be of evolutionary importance. For example, a greater rate of decline in age-specific fecundity in one group of individuals compared to another group with equivalent lifespan does not constitute a cost of evolutionary importance if one group consistently produces more eggs per day throughout their entire lifespan. Assuming fitter individuals are those which produce more eggs, then natural selection will always favour these individuals.

Negative genetic correlations between early reproduction and survival also would indicate the presence of reproductive costs. The genetic correlations (r_g) between early reproductive traits (E1, E2, EMAX and ADFH1) and longevity (ALS) in the Mangoplah population (Tables 3.19 and 3.20) are highly negative and significantly different from zero at both laboratory temperatures. For the Coalstoun Lakes population the same correlations (Tables 3.21 and 3.22) are mostly negative, but not always significantly different from zero. The exception is the correlation between ALS and E1 at 33°C, which is positive, but not significantly different from zero. In Tables 3.23 and 3.24 the genetic correlation between early reproduction (ADFQ1) and longevity (ALS) is negative for both populations

at 33°C, but only significantly different from zero in the Mangoplah population. These negative genetic correlations between early reproductive traits and survival suggest that a cost to reproduction is manifested through a reduction in future survival in these two natural populations of *T. castaneum*.

The quantity of offspring produced by an individual in a population is dependent upon two factors, the rate of oviposition and the duration of oviposition (reproductive lifespan). To better appreciate the net effect of reproductive costs incurred in these two natural populations it was necessary to examine the genetic relationships between early reproduction (ALM, ADFALM) and late reproduction (ARM, ADFARM) relative to the population median.

Positive genetic correlations (r_S) were found between ADFALM and ADFARM in both populations at both temperatures (Tables 3.19 to 3.22). With the exception of the Mangoplah population at 37°C, the correlations were significantly different from zero. These correlations show that those individuals which have higher early reproductive rates, also reproduce at a higher rate after the median number of eggs for the population had been produced.

The genetic correlations (r_S) between ALM and ARM (Tables 3.19 to 3.22) are negative in the Mangoplah population and positive in the Coalstoun Lakes population. Only for the Mangoplah population at the stressful temperature was the correlation significantly different from zero. These correlations suggest that in the Coalstoun Lakes population those individuals contributing more to early reproduction also contribute more at the later stages. However, in the Mangoplah population different individuals may contribute to early and late reproduction.

Generally, the detection of significant heritable variation in components of early reproduction and adult lifespan (see Tables 3.5 and 3.6), but not in lifetime fecundity, are consistent with the findings of reproductive cost. The genetic correlation (r_S) between lifetime fecundity (TOTAL) and longevity (ALS) is positive in both populations at both temperatures, but only significantly different from zero in the Mangoplah population (see Tables 3.19 to 3.22).

Negative genetic correlations between life-history traits not only address the problem of reproductive cost, but also the role of antagonistic pleiotropy in the maintenance of quantitative genetic variation. For the theory of maintenance of genetic variation by antagonistic

pleiotropy to hold, empirical studies are expected to disclose abundant additive genetic variability, associated with negative genetic correlations between genetically variable characters (Rose et al. 1987).

Evidence for abundant heritable variation in both non-reproductive and to a lesser extent reproductive traits, in both the Mangoplah and Coalstoun Lakes populations, has already been established. The negative genetic correlations between early reproductive traits and longevity (ALS), discussed in reference to reproductive costs, suggest that antagonistic pleiotropy is important in the maintenance of polygenic variation underlying life-history traits.

Other examples of negative genetic correlations between life-history traits are presented in Tables 3.19 to 3.22. In the Mangoplah population (Tables 3.19 and 3.20) at both temperatures, developmental time (DT) shows negative genetic correlations (r_S) with both reproductive and non-reproductive traits. Generally, in this population BWT and GR are not antagonistically correlated with other traits, and when the correlations are negative, they are close to zero. In the Coalstoun Lakes population (Tables 3.21 and 3.22) ALS is negatively correlated with DT, BWT and GR at both temperatures, but only significantly with DT at 37°C. At 37°C in the same population DT is negatively correlated with some reproductive indices (see Table 3.22), but these indices do not exhibit significant heritable variation (see Table 3.6). The above examples are further evidence that antagonistic pleiotropy could be responsible for the maintenance of some, or all, of the genetic variation in life-history traits observed in natural populations.

The adaptive response of the whole organism and its life-history may thus involve compromises or trade-offs between characters that are antagonistically correlated. Therefore, any attempt to elucidate the direction and rate of evolutionary change in the life-history of *T. castaneum*, or any other species, must consider the nature of these genetic constraints between individual fitness components. Further, it is obvious from these results that genetic correlations, like heritability estimates, refer to particular populations measured in particular environments.

The rust-red flour beetle, like most other species, occupies habitats in nature which differ spatially and or temporally in biotic and abiotic variables. Of particular interest to poikilothermic species, like *Tribolium*, are changes in temperature which can profoundly influence the expression of traits intimately associated with fitness. For example, Howe (1956, 1962) has documented evidence for phenotypic plasticity in rates of development,

mortality and oviposition in *T. castaneum* under a range of laboratory temperatures.

Comparisons between the least-squares means for each life-history trait measured at the different temperatures (Tables 3.25 with 3.26, 3.27 with 3.28, and 3.29 with 3.30) show that the Mangoplah and Coalstoun Lakes populations are phenotypically flexible for both reproductive and non-reproductive traits. The highly significant main effect of temperature in the mixed model analysis of variance (Table 3.32), confirms this for DT, GR, TOTAL, ADFTOT and ALS. These results indicate that the phenotypic expression of fitness traits in natural populations of *T. castaneum* is a function of environmental conditions. The only trait not displaying significant phenotypic flexibility across these temperatures in either population was adult body weight (BWT).

In the mixed model analyses of variance (Table 3.32) significant temperature-sire (TxS) and temperature-dam (TxD(wS)) interaction mean-squares indicate the presence of genetic differences in response to temperature by the different genotypes. The temperature-sire interaction effect was significant ($P < 0.05$) for DT and TOTAL in the Coalstoun Lakes population, but not for any of the six life-history traits in the Mangoplah population. A significant temperature-dam interaction was found for DT, BWT and GR in the Mangoplah population, and for BWT and GR in the Coalstoun Lakes population.

The prediction and understanding of evolution in heterogeneous environments requires not only a knowledge of the significance of genotype-environment interaction from an analysis of variance, but also the across-environment genetic correlation (Groeters and Dingle 1987; Mitchell-Olds and Rutledge 1986; Via 1984b; Via and Lande 1985, 1987). In Table 3.33, an additive genetic correlation ($r_{S(a)}$) between character states of unity implies that the same alleles or sets of alleles influence the character states in the same way in the two temperatures. In contrast, a cross-temperature correlation less than one indicates that the phenotypes in each environment are influenced either by some different alleles or differently by the same alleles (Via and Lande 1985, 1987), thus having the potential for somewhat independent evolution.

For both populations, the high and positive additive genetic correlations ($r_{S(a)}$) across-temperatures for DT, BWT, GR, ADFTOT and ALS suggests that the genetic control of each of these traits is largely the same in both environments. However, the genetic correlations were not all unity, showing that some temperature-specific responses had occurred. The large standard errors, which are minimal estimates of the true standard errors, emphasize the need for large sample sizes in order to statistically distinguish high from perfect

correlations among character states when the observations are made on different individuals.

If variation among sibships is non-significant, then the cross-temperature correlation is expected to be small and not significantly different from zero (Groeters and Dingle 1987). This is the case with lifetime fecundity (TOTAL), since the paternal half-sib heritabilities (h^2_S) within temperatures were low and generally not significantly different from zero (see Tables 3.5 and 3.6). In particular, for the Coalstoun Lakes population, no cross-temperature correlation estimate based on paternal half-sibs is given for TOTAL, since the estimated sire variance component ($\hat{\sigma}^2_S$) was negative in the stressful temperature (37°C).

Generally, the lack of significant sire-temperature interaction effects from the mixed model analysis of variance (Table 3.32) and the high and positive additive genetic correlations across temperatures (Table 3.33) are in qualitative agreement. At least the direction, if not the rate of evolution in these two natural populations, can be predicted in one of these temperatures from performance in the other. Of course, and rather naively, this assumes that natural selection acts on one trait independently of the others. Although the heritability and genetic correlation estimates for the two natural populations are, in general, compatible across the two laboratory temperatures, they are sufficiently different to suggest that the expression of the genetic architecture underlying the life-history of natural populations of this species may change under different environments.

The lack of significant genotype-environment interaction across these temperatures in both natural populations of *T. castaneum* suggests that fluctuating selection pressure in heterogeneous environments is not likely the sole factor responsible for maintenance of the considerable amounts of heritable variation for life-history traits observed in these and other natural populations. However, the widespread documentation of genotype-environment interaction in *T. castaneum* (Bell, Miles and Eich 1982; Benyi and Gall 1978; Bray, Bell and King 1962; Dawson 1965b; Dawson and Riddle 1983; Hardin and Bell 1967; Hardin, Rogler and Bell 1967; Kidwell, Freeman, Haverland and Rolfes 1964; Murthy and Taneja 1982; Navak and Friars 1969; Orozco 1976; Rich and Bell 1980; Riddle et al. 1986; Sokoloff, Shrode and Bywatters 1965; Taneja and Singh 1982; Yamada and Bell 1969, 1980) suggests further studies with larger sample sizes, and under more laboratory temperatures which adequately represent the biological range of *T. castaneum*, are needed to clarify this situation.

In this study, the two natural populations derived from geographically distant localities which presumably differed in ecologically important selective pressures, were expected to display genetic differentiation in response to their separate environments. The finding of

significant differences between them (Tables 3.25 to 3.31) within individual temperature treatments implies that there are genetically based life-history differences between these populations since the beetles were reared under uniform laboratory conditions.

The significantly faster rate of development from egg to adult eclosion (DT) and lower adult body weight (BWT) in the temperate Mangoplah population relative to the sub-tropical Coalstoun Lakes population, at both laboratory temperatures, may reflect adaptations to the different climatic regimes encountered by these populations in their natural habitats. However, the lack of significant population differentiation for growth rate (GR) at either temperature suggests that the relationship between DT and BWT may be physiologically constrained within the species. Significant differences between the populations in longevity (ALS) and lifetime reproduction (TOTAL) were not found at either laboratory temperature. This lack of differentiation in traits (ALS and TOTAL) which are often associated with fitness, could mean they are highly canalized in this species, or it may simply reflect their high coefficients of variation.

Contrasting trends in additive genetic variation and covariation underlying the life-history traits of these populations can be interpreted, not only as reflecting differences in the potential to respond to selection, but also the different selective regimes experienced by these populations in the past. Although the heritability (Tables 3.1 to 3.14) and genetic correlation (Tables 3.15 to 3.24) estimates from paternal half-sibs are associated with large standard errors, they do suggest the genetic architecture underlying the life-history of these populations may be different.

Of particular interest is the correlation between the rate of development (DT) and body weight (BWT). In the Mangoplah population at both temperatures, the paternal half-sib genetic correlation (r_S) is negative, large and significantly different from zero (see Tables 3.19 and 3.20). In contrast, the same correlations in the Coalstoun Lakes population are positive, large and significantly different from zero (see Tables 3.21 and 3.22). However, King and Dawson (1972) have indicated a great deal of caution must be exercised in the choice of statistics used to analyse developmental time, and in particular, the relationship between developmental time and body weight. The problem arises when the distribution of developmental time is distinctly non-normal, or even bimodal. Since the distributions of developmental time in the Nested experiment were approximately normal, it is postulated that bimodal distributions of developmental time may only be observed in cultures which are at high density as a consequence of increased cannibalism of pupae by larvae. Therefore,

the contrasting genetic relationship between DT and BWT in the Mangoplah and Coalstoun Lakes populations supports the notion that the populations are genetically differentiated. Clearly, heritability and genetic correlation estimates are particular to given populations and should not be extrapolated to the species as a whole. Presumably these laboratory derived estimates reflect those of their respective populations in their natural habitat.

Natural selection has probably played some role in moulding the observed life-histories in these two natural populations as they presumably evolved in grain habitats that differ with respect to biotic and abiotic conditions. However, attempts to attribute specific differences between the Mangoplah and Coalstoun Lakes populations to one, or a few, environmental variables may prove misleading. While climate, and especially ambient temperature, are expected to be important, confounding selection pressures are likely to contribute to the evolution of the life-history in natural populations of *T. castaneum*.