Chapter 4

The Clinal Experiment

The Clinal experiment examined genetic variation in certain early life-history traits (developmental time, adult body weight, growth rate and peak egg production) among 34 geographically distinct populations of *T. castaneum* that were sampled at approximately regular distances along a latitudinal gradient in Eastern Australia. The relationships among these life-history traits are described by intra-locality and inter-locality correlations. Individuals of both sexes were assayed (except for egg production) to allow estimation of genotype-sex interactions. The ecological relevance of temperature effects, expressed as genotype-environment interactions, was investigated by measuring progeny from each population under five constant laboratory temperatures (21°, 25°, 29°, 33° and 37°C) which are representative of those that the beetles experience in nature. Samples of the Mangoplah and Coalstoun Lakes populations also were included in this experiment to provide further evidence on the adaptive basis of any life-history differences between them.

4.1 Materials and Methods

4.1.1 The Experimental Populations

Thirty four natural populations of *T. castaneum* were sampled from the geographic locations shown in Table 4.1 and Figure 4.1. All population samples consisted of at least 200 wild caught adults taken from farm grain silos during late March and early April 1985. It was assumed the populations had infested their respective localities for many generations.

Table 4.1: Geographic location of the populations with corresponding climatic data.

Population	Popn abbrev.	Latitude (°S)	Longitude (°E)	TMEAN (°C)	RhMEAN (%)
Clermont	CLER	22° 42′	147° 39′	22.05	58.50
Emerald	EM	23° 28′	148° 10′	22.36	59.33
Rolleston	ROLL	24° 25′	148° 31′	21.49	56.84
Biloela	BIL	24° 26′	150° 32′	20.29	68.00
Monto	MONT	24° 55′	151° 09′	19.84	70.35
Mundubbera	MUND	25° 34′	151° 20′	20.54	66.92
Coalstoun Lakes	C.L.	25° 37′	151° 53′	20.54	66.92
Gayndah	GAY	25° 39′	151° 46′	20.54	66.92
Tansey	TANS	26° 02′	152° 03′	19.03	69.00
Goomeri	GOOM	26° 12′	152° 04′	17.52	71.08
Kingaroy	KING	26° 32′	151° 50′	17.52	71.08
Dalby	DALB	27° 06′	151° 18′	19.05	61.17
Cecil Plains	C.P.	27° 34′	151° 15′	18.44	63.13
Leyburn	LEYB	28° 06′	151° 34′	17.68	65.84
Goondiwindi	GOON	28° 29′	150° 22′	19.88	59.26
Warialda	WAR	29° 19′	150° 32′	17.12	65.01
Inverell	INV	29° 46′	151° 10′	15.53	64.00
Tamworth	TAM	31° 10′	150° 52′	17.91	68.91
Caroona	CAR	31° 24′	150° 26′	18.11	67.50
Coolah	COOL	31° 31′	149° 48′	14.81	67.57
Dunedoo	DUN	32° 05′	149° 14′	16.51	66.69
Wellington	WELL	32° 30′	148° 57′	17.09	60.33
Orange	OR	33° 10′	149° 00′	13.44	64.79
Cowra	COW	33° 47′	148° 42′	15.72	66.03
Barmedman	BARM	34° 10′	147° 24′	15.43	64.26
Temora	TEM	34° 38′	147° 31′	15.43	64.26
Mangoplah	MANG	35° 22′	147° 13′	16.44	63.42
Yerong Creek	Y.C.	35° 23′	147° 06′	16.44	63.42
Cookardinia	COOK	35° 29′	147° 21′	16.44	63.42
Holbrook	HOLB	35° 41′	147° 19′	16.36	61.96
Mullengandra	MULL	35° 55′	147° 09′	16.28	60.50
Benalla	BEN	36° 32′	146° 05′	14.85	68.80
Mansfield	MANS	37° 04′	146° 08′	13.80	70.86
Dromana	DROM	38° 20′	145° 03′	14.85	72.39

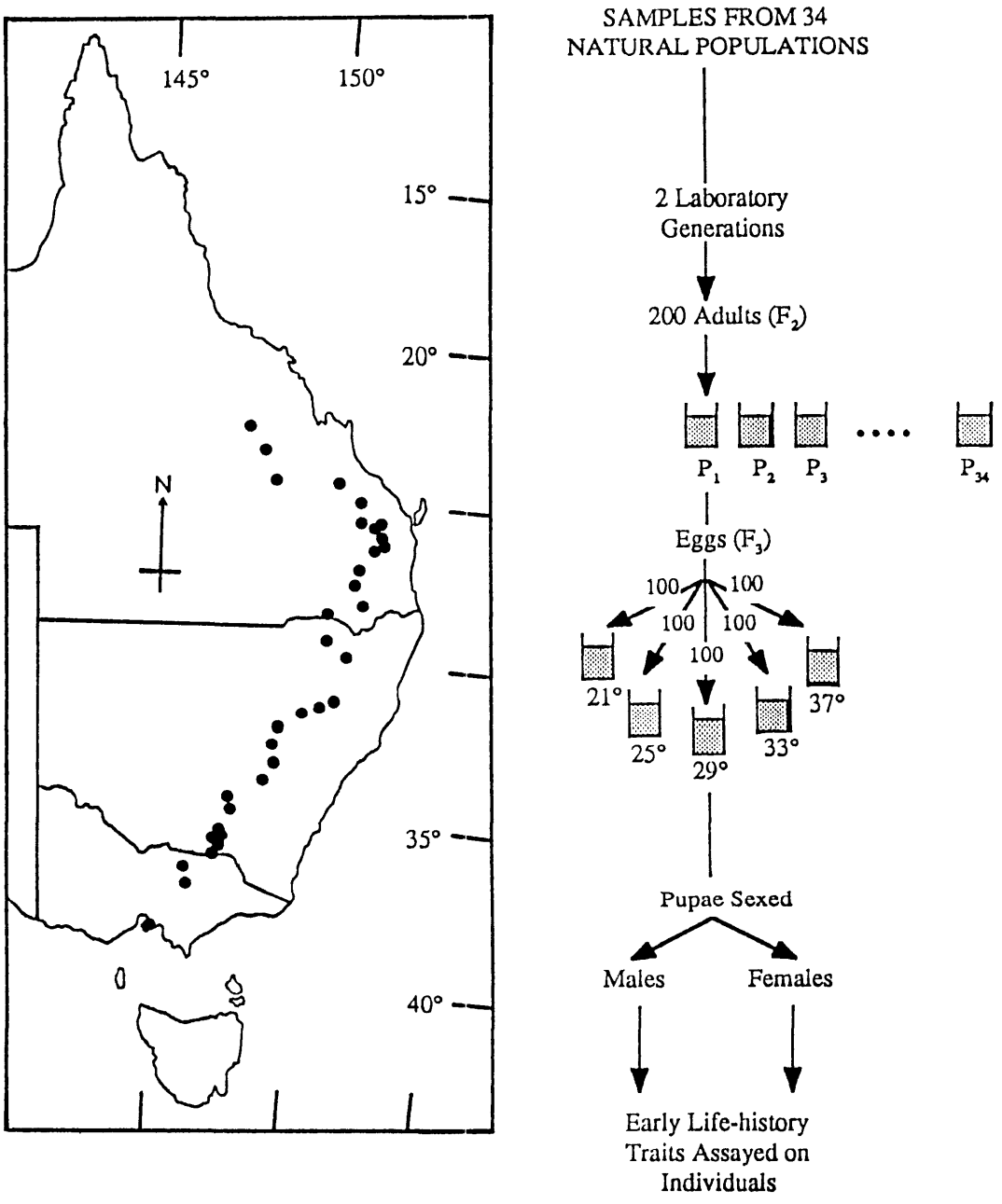

Figure 4.1: Schematic presentation of the Clinal experiment with a map of Eastern Australia showing the localities of populations sampled.

4.1.2 The Experimental Procedures

The experimental procedure (see Figure 4.1) described below was the same for each population. The population samples were individually classified according to species, placed in fresh culture medium (100 g), and incubated away from the experimental laboratory. After 5 days of acclimatization, the wild caught adults were allowed to deposit eggs *en masse* in fresh medium (100 g) for 48 hours. These eggs, constituting the F_1 generation, were counted and a random sample of 400 washed in a dilute solution of Benzalkonium Chloride (see General Materials and Methods). The washed eggs were placed in fresh medium (100 g), transferred to the experimental laboratory, incubated at 33°C and 70% relative humidity with position randomized, and left undisturbed until pupation. At this developmental stage, pupae were removed daily (to minimize cannibalism) and placed in fresh medium (100 g). After the completion of pupation and emergence of the last imago the culture medium was changed and the culture left for seven days to establish fertility and reach peak egg lay. Sperm precedence in the fertilization of eggs in *T. castaneum* (Schlager 1960) ensures against a preponderance of females being irreversibly inseminated by earlier developing and maturing males. A 24 hour egg sample was then taken in fresh medium (100 g) to establish the next generation (F_2). These eggs were counted, 400 randomly sampled, and placed in fresh medium (100 g). Once again the eggs were allowed to develop undisturbed until pupation. The pupae were removed daily and placed in fresh medium (100 g). The imagoes eclosing from these pupae constituted the parents of the individuals on which the early life-history traits were assayed.

The F_2 adults were placed in fresh medium (100 g) and left for seven days (after the last adult eclosed) to sexually mature and reach peak egg lay (after Bhat and Bhat 1974b). A 24 hour egg sample was then taken in fresh medium (100 g) from these adults. From these eggs, five sub-samples of 100 each were placed into separate bottles containing fresh medium (100 g). One sub-sample culture was randomly allocated to each of the five laboratory incubators preset at 21°, 25°, 29°, 33° and 37° C, and with relative humidity controlled by saturated salt solutions of sodium nitrate. Obviously, all cultures had shared a common environment (33°C) for the first 24 hours. The position of the population cultures was randomized within each temperature and the cultures then left undisturbed until pupation. Approximate developmental rates at the different temperatures are given by Howe (1956).

At pupation time, the pupae were removed from the cultures daily, sexed and placed into empty vials. The newly emerged imagoes were separated from the pupae at the same time

each day, and the day of adult emergence recorded. The males were individually weighed, and then discarded. The females were weighed and placed individually in fresh medium (1 g). After all female pupae had successfully eclosed at a given temperature, up to 30 individual females from each population were randomly sampled from those available and each put in fresh medium (1 g) with a black mutant male for copulation. These cultures were then fully randomized within each temperature and left for seven days to establish fertility and reach peak egg lay. From each of these females one 48 hour egg collection was then taken in fresh medium (1 g). These collections were set up on days 32, 41, 56, 71 and 134 for the 37°, 33°, 29°, 25° and 21°C laboratory temperatures respectively.

4.1.3 The Life-History Traits Measured

Developmental Time (DT)	=	the time interval between egg collection and adult eclosion (days).
Adult Body Weight (BWT)	=	adult body weight on day of eclosion (μg).
Growth Rate (GR)	=	calculated as the ratio BWT/DT (μg/day).
Peak Egg Production (EPEAK)	=	the number of eggs produced by individual females during the 48 hour sample (eggs/48 hrs).

4.1.4 Climatic and Location Variables

Quantitative climatological data for each locality were those from the nearest Australian Bureau of Meteorology station, the average distance (± standard error) between collecting locality and climatic station being 24.06 ± 3.01 km, with a maximum of 62 km. These data were estimated by Keig and McAlpine (1969) as weekly normals from the 30 year monthly normals published by the Bureau of Meteorology (1956). Values were averaged if collection sites were equidistant between meteorological stations.

In preliminary analyses six climatic variables were considered: the mean annual temperature (TMEAN); the mean maximum temperature for the hottest four consecutive weeks (TMAX); the mean minimum temperature for the coldest four consecutive weeks (TMIN); the mean annual (9 am) relative humidity (RhMEAN); the mean (9 am) relative humidity

for the most humid four consecutive weeks (RhMAX); and the mean (9 am) relative humidity for the least humid four consecutive weeks (RhMIN). The values of TMEAN and RhMEAN for each locality are given in Table 4.1.

Only results from regression analyses based on TMEAN and RhMEAN will be reported, since TMEAN is highly and significantly correlated with TMAX ($r = 0.69$) and TMIN ($r = 0.70$); and RhMEAN is highly and significantly correlated with RhMAX ($r = 0.65$) and RhMIN ($r = 0.65$) for the localities sampled. TMEAN and RhMEAN were not significantly correlated ($r = -0.33$). The results from the preliminary analyses reflected these correlations. Also the nature of the natural habitat, that is cereal grain in grain silos, is expected to act as a buffer against extremes of climate. Variation in heat load and moisture content of grain bulks would fluctuate less than that of the ambient climate. Furthermore, the occurrence of "hot-spots" (regions of relatively high temperature grain which have been largely created by the respiration of a large population of insects) can provide added protection against environmental extremes (Johnston 1981; Pimentel 1958).

In the preliminary analyses latitude (LAT), longitude (LONG) and distance inland from the eastern coast of Australia (DINLD) were used as coordinates to describe geographic location. Mulley et al. (1979) used distance inland in preference to longitude because climatological zones, in the area under study, tend to run parallel to the coast so that localities on the same longitude but at different latitudes could be in quite different climatological and ecological regions. From the results of these preliminary analyses, longitude and distance inland were considered to be unimportant in describing patterns of geographic variation among the populations under study. It is important to note that TMEAN is highly and significantly negatively correlated with LAT ($r = -0.86$), whereas RhMEAN is uncorrelated with LAT ($r = 0.13$).

4.1.5 Statistical Analyses

Unfortunately, in the Clinal experiment, there was no replication of population cultures at each laboratory temperature. Only one culture of each population was established in each temperature, with up to 100 individuals measured per culture and treated in the analyses as independent observations. Statistically, the individual observations within a population culture are not independent. Therefore, the following analyses of variance testing for genetic differentiation among geographic populations, genotype-environment interaction and genotype-sex interaction, as well as the intra-locality correlations, must be interpreted

with caution. The remaining analyses, which utilize population means, are unaffected. Future experiments would be better done with many single beetles or groups of beetles replicated in separate culture containers that are fully randomized, with respect to position, within each laboratory incubator.

4.1.5.1 Genetic Differentiation Among Geographic Populations

Before investigating relationships between genotypic (population) variation and location and/or climatic factors it is necessary to show statistically that such genotypic variation exists by performing oneway analyses of variance within temperatures and within sexes. The following linear statistical model was assumed:

$$Y_{pk} = \mu - P_p - \epsilon_{pk}$$

where Y_{pk} = the phenotypic value of the k^{th} individual beetle:
μ = value of the overall mean:
P_p = the fixed effect of the p^{th} population: and
ϵ_{pk} = random errors associated with each observation.

This model was fitted for DT, BWT, and GR in both sexes, and EPEAK in females using procedure ANOVA from SPSS^x (SPSS Inc. 1983). Population differentiation is indicated by a significant F-ratio in the analysis of variance. Further, any differences are expected to be genetically based since individuals are reared under uniform laboratory conditions.

4.1.5.2 Genotype-Environment Relationships

Regression analyses were used to describe the relationships between genetic variation among populations when measured under uniform laboratory conditions and variation in latitudinal and/or climatic factors associated with their geographic origin.

a) Simple Linear Regression. Simple linear regressions were used to describe the dependence of the population means for early life-history traits (DT, BWT, GR and EPEAK) on each of the independent variables, LAT, TMEAN and RhMEAN. The regression equations may be written in the form:

$$Y = a + bX$$

where Y = the expected value of the dependent variable;
a = the Y intercept;
b = the regression coefficient; and
X = the value of the independent variable.

The program P1R in the BMDP statistical package (Dixon 1983) was used to compute the regression equations, separately for each temperature and sex combination. Weighted means were used in the computations because the population sample sizes were unequal. Since the observations have a common variance, the weights are the numbers of observations (Steel and Torrie 1981).

b) Multiple Linear Regression. Studies testing the adaptive significance of geographic variation are often complicated by the fact that much of the genetic variation among populations in a particular character may be the product of natural selection by more than one environmental factor (Power 1969). The purpose of multiple regression is to establish a linear prediction equation that will enable a better prediction of a dependent variable than would be possible by any single independent variable.

The program P1R of the BMDP statistical package (Dixon 1983) estimates a least squares regression equation between a dependent (predicted) variable and one or more independent (predictor) variables. The multiple regression equation for a particular sample may be written in the form:

$$Y = a + b_1X_1 + b_2X_2 + \ldots\ldots\ldots + b_kX_k$$

where Y = the expected value of the dependent variable;
a = the intercept;
$X_1 \ldots\ldots X_k$ = the independent variables;
$b_1 \ldots\ldots b_k$ = the partial regression coefficients; and
k = the number of independent variables.

The partial regression coefficients may be used to estimate the degree of statistical relationship between the character and a single environmental variable, given that the effect of each was determined with all other independent variables held constant. However, partial regression coefficients can change, depending on which other independent variables are

included in the test. Further, the magnitudes of the partial regression coefficients are not independent of the scale in which the independent variables are measured. Therefore, standardized partial regression coefficients (b'), where the dependent and independent variables are transformed to standard deviates, were used to estimate the relative importance of each of the independent variables being considered in the equation. The coefficient of multiple determination (R^2) is an estimate of the proportion of the variation of the dependent variable jointly explained by independent variables X_1 through to X_k (Sokal and Rohlf 1981; Steel and Torrie 1981).

Each of the early life-history traits (DT, BWT, GR and EPEAK) were regressed simultaneously on TMEAN and RhMEAN, and then on TMEAN, RhMEAN and LAT. Latitude (LAT) was included as an independent variable in the latter regression equations to see if there were any geographical non-linearities in genetic patterns which were associated with corresponding non-linearities in environmental patterns, thus eliminating as far as possible associations due to gene flow (after Schaffer and Johnson 1974). Weighted means were used in the computations because the population sample sizes were unequal. The analyses were performed separately for each temperature and sex combination.

4.1.5.3 Estimation of Genotype-Environment Interaction

In the Clinal experiment the progeny sub-samples (100 eggs) from each population culture were randomly allocated to the five laboratory temperatures. The first group of randomly chosen progeny, like the parental generations, were reared continuously from birth at 33°C. The remaining sub-samples shared this common environment for the first 24 hours of life before being transferred and reared at another temperature (21°, 25°, 29° or 37°C). The measurement of the early life-history traits (DT, BWT, GR and EPEAK) on the individual beetles from each sub-sample provides the necessary information to examine the extent to which temperature affects the expression of the genotype. The following methods were used to investigate the importance of genotype-environment interactions on these early life-history traits.

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

$$Y_{ptk} = \mu + P_p + T_t + (PxT)_{pt} + e_{ptk}$$

where Y_{ptk} = the phenotypic value of the k^{th} individual:
μ = the value of the overall mean:
P_p = the fixed effect of the p^{th} population:
T_t = the fixed effect of the t^{th} temperature:
$(PxT)_{pt}$ = the interaction effect of the p^{th} population and the t^{th} temperature; and
ϵ_{ptk} = random errors associated with each observation.

The model was fitted separately for each sex and trait combination using procedure ANOVA from the SPSS^x statistical package (SPSS Inc. 1983). Genotype-environment interaction is indicated by a significant F-ratio when the PxT interaction mean square is tested against the error term.

b) Expression of Genotype-Environment Interaction as a Genetic Correlation. The biological importance of any genotype-environment interaction may be better appreciated by expressing the interaction in terms of a genetic correlation between the performance of the same genotype (population) measured in two or more environments. Here, any given individual is measured in one environment only.

i) Robertson's Method. Robertson (1959b) presented formulae for estimating the correlation between character states measured in two or more environments. These formulae are:

1. with two environments

$$r_g = \frac{A - B}{A + B - 2C} \qquad \text{and}$$

2. with more than two (t) environments

$$r_g = \frac{A - B}{A - C + (t - 1)(B - C)}$$

where r_g is the estimated genetic correlation: and A, B and C are the respective mean squares for genotypes (populations), genotype-environment interaction, and error obtained from the above two-way factorial analysis of variance.

ii) Yamada's Method. Yamada (1962) provided a method for estimating the genetic correlation of a character measured on different individuals in two or more environments. To

estimate the genetic covariance between characters measured in different environments, the character states are regarded as realizations of a single variable. The variance components derived from a two-way mixed model analysis of variance (with the effects of populations considered to be random) are used to estimate the genetic correlation. These formulae are:

1. with two environments

$$r_g = \frac{\hat{\sigma}_b^2 - \frac{1}{2}\hat{\sigma}_I^2}{\hat{\sigma}_b^2 - \frac{1}{2}\hat{\sigma}_I^2 - \frac{1}{2}(\hat{\sigma}_{b_1} - \hat{\sigma}_{b_2})^2} \quad \text{and}$$

2. with more than two (t) environments

$$r_g = \frac{\hat{\sigma}_b^2 - \frac{1}{t}\hat{\sigma}_I^2}{\hat{\sigma}_b^2 - \frac{t-1}{t}\hat{\sigma}_I^2 - \hat{\theta}(\sigma_{b_i})}$$

where $\hat{\sigma}_b^2$ and $\hat{\sigma}_I^2$ are the estimated between groups (populations) and interaction variance components from the two-way analysis of variance -- Model VI of LSML76 (Harvey 1977) was used to estimate these components. The terms $\hat{\sigma}_{b_1}$ and $\hat{\sigma}_{b_2}$ are the square roots of the between group variance components within temperatures 1 and 2 respectively, and $\hat{\theta}(\sigma_{b_i})$ is used to correct for inequality of genetic variance across environments.

With the mixed model formulae there is a possibility of getting negative estimates if the interaction component is very large compared to the genetic component, or if the genetic component is zero in the presence of interaction (Pani and Lasley 1972).

The genotype-environment interaction variance component can be partitioned into two parts, one associated with the genetic correlation and one associated with heterogeneity of genetic variances measured in each environment (Dickerson 1962; Robertson 1959b; Yamada 1962). Correcting for heterogeneity of variance provides unbiased estimates of the genetic correlation. Yamada's (1962) formulae include in the denominator, a factor to correct for bias introduced by heterogeneity of between group (genetic) variance. This correction factor equals zero if the between group variances or standard deviations are the same. Robertson (1959b) presented a method whereby this factor can be set to zero. This involves transforming the character states into standard measure by expressing them relative to their respective within temperature genetic standard deviations.

iii) The Standard Family Mean Correlation. A simple approximation to the genetic correlation between performance across two environments can be made via the

product-moment correlation of family (population) means (Via 1984b). The appropriate formula is:

$$r_m = \frac{cov_{m(XY)}}{\sqrt{(var_{m(X)})(var_{m(Y)})}}$$

where r_m = the standard family mean correlation;
$cov_{m(XY)}$ = the covariance of family means for trait X (environment 1) and Y (environment 2); and
$var_{m(X)}$ and $var_{m(Y)}$ are the respective within environment variances of family means.

This estimate is an approximation since the numerator may contain sampling error in addition to genetic covariance, and the denominator contains within-family error variance as a contaminant. For small numbers of observations the method may underestimate the absolute value of the genetic correlation, though it is expected to converge to the true value as the number of individuals measured per family increases (Via 1984b). Procedure PEARSON CORR in the SPSS^x statistical package (SPSS Inc. 1983) was used to estimate these correlation coefficients.

iv) **Spearman's Coefficient of Rank Correlation.** The consequences of a change in ranking of genotypes measured in different environments is more important from an evolutionary perspective than interaction arising from scaling effects. Spearman's correlation coefficient measures correspondence between ranks, and is not necessarily a measure of linear correlation (Steel and Torrie 1981). The appropriate formula is:

$$r_S = 1 - \frac{6\sum_i d_i^2}{n(n^2 - 1)}$$

where r_S = Spearman's rank correlation coefficient;
d_i = the difference in ranks for the i^{th} paired observations; and
n = the number of d's.

These correlation coefficients, based on the ranking of population means, were estimated by procedure NONPAR CORR in the SPSS^x statistical package (SPSS Inc. 1983)

4.1.5.4 The Relationships Between the Life-History Traits

Pearson's product-moment correlation coefficient measures the strength of association between two variables (Steel and Torrie 1981). In the Clinal experiment correlations between the early life-history traits can be assessed at different levels — that is, the intra-locality correlation and the inter-locality correlation (Thorpe 1976).

a) Intra-locality Correlation — is the correlation between characters, which are measured on the same individuals, in a sample taken from one locality (population). A high intra-locality correlation may reflect common epigenetic control of the characters but is not affected by geographic variation in the selection pressures which may influence the inter-locality correlation (Thorpe 1976). Since the identity of individual beetles was maintained in the Clinal experiment, estimates of DT, BWT and GR were available on each individual male and female, and EPEAK data also were available for a sample of females. The estimated product-moment correlations included DT with BWT in both sexes, and DT, BWT and GR with EPEAK in females. These correlations were estimated within each laboratory temperature using procedure PEARSON CORR from the SPSS^x statistical package (SPSS Inc. 1983). The part-whole correlation coefficients for DT with GR, and BWT with GR are not given because of the difficulties associated with their interpretation (see Sokal and Rohlf 1981). A t-test with (n-2) degrees of freedom is used to test whether the coefficients are significantly different from zero.

b) Inter-locality Correlation — the correlation between mean character states for each locality (population) measures the concordance of their patterns of geographic variation. A high inter-locality correlation between characters may result either from their common epigenetic control or else from independently controlled characters responding similarly to geographic variation in selection pressures. A similar response to geographic variation in selection pressures could mean the characters are responding to the same selection pressures, or else, the characters are responding to different selection pressures which have similar patterns of geographic variation (Thorpe 1976).

The inter-locality correlation coefficient is computed by using mean values, weighted for sample size. Correlations included DT with BWT in both sexes, and DT, BWT and GR with EPEAK in females. In the latter correlations involving EPEAK, only individuals which had been assayed for this trait were used to compute the means for DT, BWT and

GR. These correlations were also estimated within each laboratory temperature. A t-test with (n-2) degrees of freedom is used to test whether the coefficient is significantly different from zero.

4.1.5.5 Consistency of the Mangoplah and Coalstoun Lakes Populations with Clinal Patterns

Samples from the same collection site from which the Mangoplah and Coalstoun Lakes populations had been sampled for the Nested experiment were included in the Clinal experiment. Simple linear prediction equations with confidence intervals (see Steel and Torrie 1981) were again fitted to the data using weighted means from all populations, except those from these two localities. Compatibility of the Mangoplah and Coalstoun Lakes populations with these regressions, and therefore with any clinal trends, was determined by comparing their observed means with confidence intervals for the predicted values appropriate to each locality.

The simple linear regressions describing the dependence of the early life-history traits (DT, BWT, GR and EPEAK) on each of the independent variables LAT, TMEAN and RhMEAN were considered. Tests for compatibility were made only when the linear regressions were significant.

4.1.5.6 Genotype-Sex Interaction

To statistically detect the presence of population-sex interactions, two-way analyses of variance were carried out within temperatures for the life-history traits (DT, BWT and GR) which were measured on both sexes. The linear statistical model assumed was:

$$Y_{spk} = \mu + P_p + S_s + (P \times S)_{ps} + \epsilon_{spk}$$

where Y_{spk} = the phenotypic value of the k^{th} individual beetle;

μ = value of the overall mean;

P_p = the fixed effect of the p^{th} population;

S_s = the fixed effect of the s^{th} sex;

$(P \times S)_{ps}$ = the interaction effect of the p^{th} population and s^{th} sex; and

ϵ_{spk} = random errors associated with each observation.

Population-sex interaction is indicated by a significant F-ratio when the PxS interaction mean square is tested against the error term.

4.1.5.7 Transformations

Preliminary analyses were undertaken to determine if the data violated any of the major assumptions pertaining to the different statistical methods. In particular, 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 for checking homogeneity of genetic variances between population groups. The population group variances were plotted against their respective means to check for independence of means and variances. 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.

4.2 Results

Tables 4.2 and 4.3 present results from the oneway analyses of variance testing for population differentiation in early life-history traits (DT, BWT, GR and EPEAK) which were measured on males and/or females, respectively, at five laboratory temperatures.

Results from the simple linear regressions, investigating genotype-environment relationships, of the early life-history traits on geographical origin (LAT) and climatic factors (TMEAN and RhMEAN) are presented for males in Table 4.4, and females in Table 4.5, for each laboratory temperature.

The profile of phenotypes produced by the 34 natural populations of *T. castaneum* at each temperature are shown in Figures 4.2 to 4.8. For males, population means at each laboratory temperature with 95% confidence intervals are plotted against latitude of origin for DT (Fig. 4.2), BWT (Fig. 4.3) and GR (Fig. 4.4). Similar plots for females are shown for DT (Fig. 4.5), BWT (Fig. 4.6), GR (Fig. 4.7) and EPEAK (Fig. 4.8). For each plot the simple linear regression equation, based on means weighted for sample size, and level of significance are given.

Results from the multiple regression of early life-history traits on climatic factors (TMEAN and RhMEAN), then on geographical origin and climatic factors (LAT, TMEAN

and RhMEAN) are given for males (Table 4.6) and females (Table 4.7) within each laboratory temperature.

Results from the investigation of genotype-environment interaction in early life-history traits (DT, BWT, GR and EPEAK) are presented in Tables 4.8 to 4.11. Interactions across two laboratory temperatures at a time are given in Table 4.8 for males and Table 4.9 for females. Interactions across all five temperatures (21°, 25°, 29°, 33° and 37°C) and then across four temperatures (25°, 29°, 33° and 37°C) are given for both sexes in Tables 4.10 and 4.11, respectively. The analyses in Table 4.11 considered only the four higher temperatures since the proportion of progeny from a fixed number of eggs surviving to successfully eclose as adults (39.4%) at 21°C was approximately half that for other temperatures (81.8% at 25°C, 83.4% at 29°C, 81.8% at 33°C, and 78.5% at 37°C), and also because significant interactions across temperature pairs (Tables 4.8 and 4.9) frequently involved 21°C. For the ANOVAs in each of these tables DT, BWT and GR were based on $\log_{10}$ transformed data, and EPEAK on square root transformed data. For calculating the cross-temperature correlations using Robertson's (1959b) and Yamada's (1962) methods, the transformed data were also converted into standard measure.

The intra-locality product-moment correlation coefficients describing the relationship between DT and BWT in both sexes, and each of DT, BWT, and GR with EPEAK in females, are given in Tables 4.12 to 4.16 for each of the 34 natural populations at the five laboratory temperatures. Table 4.17 gives the inter-locality product-moment correlation coefficients which are based on population means weighted for sample size, for the same combinations of traits at each laboratory temperature.

The results from the two-way analyses of variance testing for the presence of genotype-sex interaction are given for DT, BWT and GR at each of the five laboratory temperatures in Table 4.18.

Samples of the Mangoplah and Coalstoun Lakes populations were assayed in the Clinal experiment to test the adaptive basis of life-history differences between them found in the Nested experiment. Because of the concordance of the simple linear regression equations, calculated with and without these two populations, specific results for the latter are not presented. Although the level of significance changed in a few circumstances with the exclusion of the Mangoplah and Coalstoun Lakes populations, they were very similar to those given in Tables 4.4 and 4.5. The regression of DT in males at 33°C on RhMEAN, which was non-significant when all 34 populations were included, became significant (P

< 0.05) after the exclusion of these two populations. When BWT in males at 21°C was regressed on RhMEAN, and when GR in males at 25°C was regressed on TMEAN, the regression equations changed from significant ($P < 0.05$) to non-significant with the reduced number of populations.

Generally, when there were significant clinal patterns in the early life-history traits associated with LAT, TMEAN and RhMEAN, the observed means for the Mangoplah and Coalstoun Lakes populations lay within the 95% confidence intervals corresponding to the appropriate predicted values from the regression equations. Of course, these simple linear regression equations were computed without these two populations. The only exceptions were in the Mangoplah population, when DT in males at 33°C was regressed on TMEAN and RhMEAN, and when DT was regressed on TMEAN at the same temperature in females.

Table 4.2: Population differentiation in early life-history traits (DT, BWT and GR) measured on males. The between populations (POPN) and within populations (ERROR) mean squares from oneway ANOVAs are given for each laboratory temperature. Significant F-ratios denote population differentiation.

Temp	Source	df	DT	BWT	GR
21°C	POPN	33	955.950***	305154.223***	29.933***
	ERROR	671	101.627	55198.035	6.495
25°C	POPN	33	76.955***	323811.164***	165.226***
	ERROR	1380	6.269	43743.428	16.977
29°C	POPN	33	18.593***	459607.436***	488.661***
	ERROR	1423	2.834	37035.284	36.479
33°C	POPN	33	12.611***	545177.341***	1153.163***
	ERROR	1395	1.571	36850.365	69.427
37°C	POPN	33	14.362***	466902.509***	1094.661***
	ERROR	1368	1.982	41795.517	78.066

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

Table 4.3: Population differentiation in early life-history traits (DT, BWT, GR and EPEAK) measured on females. The between populations (POPN) and within populations (ERROR) mean squares from oneway ANOVAs are given for each laboratory temperature. Significant F-ratios denote population differentiation.

Temp	Source	df	DT	BWT	GR	df	EPEAK
21°C	POPN	33	782.171***	275884.237***	37.247***	33	3.874*
	ERROR	669	95.512	54743.675	6.941	423	2.578
25°C	POPN	33	76.663***	382466.105***	203.015***	33	95.248***
	ERROR	1400	6.485	46560.367	18.388	978	15.698
29°C	POPN	33	21.741***	477745.580***	523.103***	33	153.046***
	ERROR	1411	3.171	39838.911	40.034	975	28.851
33°C	POPN	33	14.461***	525104.318***	1207.965***	33	140.036***
	ERROR	1387	1.595	39325.086	66.465	975	38.197
37°C	POPN	33	17.447***	345117.831***	870.875***	33	100.350***
	ERROR	1300	1.884	49491.784	83.731	966	37.477

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

Table 4.4: Simple linear regressions of the early life-history traits (DT, BWT and GR) on LAT, TMEAN and RhMEAN for males. Regression coefficients (b) and coefficients of determination (r^2) are given for each temperature with the level of significance for the regression equation.

	Temp	LAT (°S)		TMEAN (°C)		RhMEAN (%)	
Trait	(°C)	b	r^2	b	r^2	b	r^2
DT	21	-0.106	0.005	0.537	0.027	-0.166	0.008
	25	-0.239***	0.668	0.420***	0.551	-0.025	0.006
	29	-0.074**	0.264	0.156***	0.314	-0.016	0.010
	33	-0.082***	0.473	0.150***	0.439	-0.044	0.102
	37	-0.072**	0.235	0.139**	0.237	0.000	0.000
BWT	21	2.135	0.006	2.760	0.002	11.533*	0.123
	25	-2.374	0.016	0.278	0.000	10.424**	0.226
	29	-1.349	0.004	-0.808	0.000	13.021**	0.246
	33	1.735	0.005	-7.168	0.023	15.007**	0.280
	37	-2.834	0.015	0.046	0.000	12.521**	0.216
GR	21	0.037	0.017	-0.056	0.010	0.140*	0.184
	25	0.116	0.074	-0.283*	0.116	0.234**	0.223
	29	0.066	0.008	-0.246	0.030	0.418**	0.238
	33	0.287	0.063	-0.691	0.102	0.738***	0.320
	37	0.097	0.008	-0.429	0.040	0.581**	0.199

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

Table 4.5: Simple linear regressions of the early life-history traits (DT, BWT, GR and EPEAK) on LAT, TMEAN and RhMEAN for females. Regression coefficients (b) and coefficients of determination (r^2) are given for each temperature with the level of significance for the regression equation.

	Temp	LAT (°S)		TMEAN (°C)		RhMEAN (%)	
Trait	(°C)	b	r^2	b	r^2	b	r^2
DT	21	0.006	0.000	0.333	0.013	−0.342	0.042
	25	−0.233***	0.640	0.413***	0.536	−0.005	0.000
	29	−0.063*	0.163	0.124*	0.169	0.003	0.000
	33	−0.093***	0.536	0.189***	0.563	−0.034	0.051
	37	−0.082***	0.326	0.173***	0.386	−0.025	0.023
BWT	21	−1.443	0.003	6.529	0.014	10.380	0.109
	25	−1.784	0.008	−1.521	0.002	13.384***	0.316
	29	−1.204	0.003	−0.049	0.000	12.919**	0.237
	33	1.948	0.007	−10.396	0.047	15.959***	0.314
	37	−0.705	0.001	−0.368	0.000	11.439**	0.233
GR	21	−0.023	0.005	0.018	0.001	0.158*	0.187
	25	0.134	0.079	−0.333*	0.131	0.280**	0.262
	29	0.063	0.007	−0.197	0.018	0.398**	0.205
	33	0.337	0.084	−0.947*	0.170	0.747***	0.299
	37	0.238	0.055	−0.578	0.087	0.599**	0.253
EPEAK	21	−0.034	0.071	0.049	0.037	−0.002	0.000
	25	−0.246***	0.400	0.451***	0.360	0.081	0.033
	29	−0.216**	0.192	0.425**	0.199	0.155	0.074
	33	−0.281***	0.351	0.545***	0.354	0.069	0.016
	37	−0.095	0.056	0.203	0.069	0.132	0.082

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

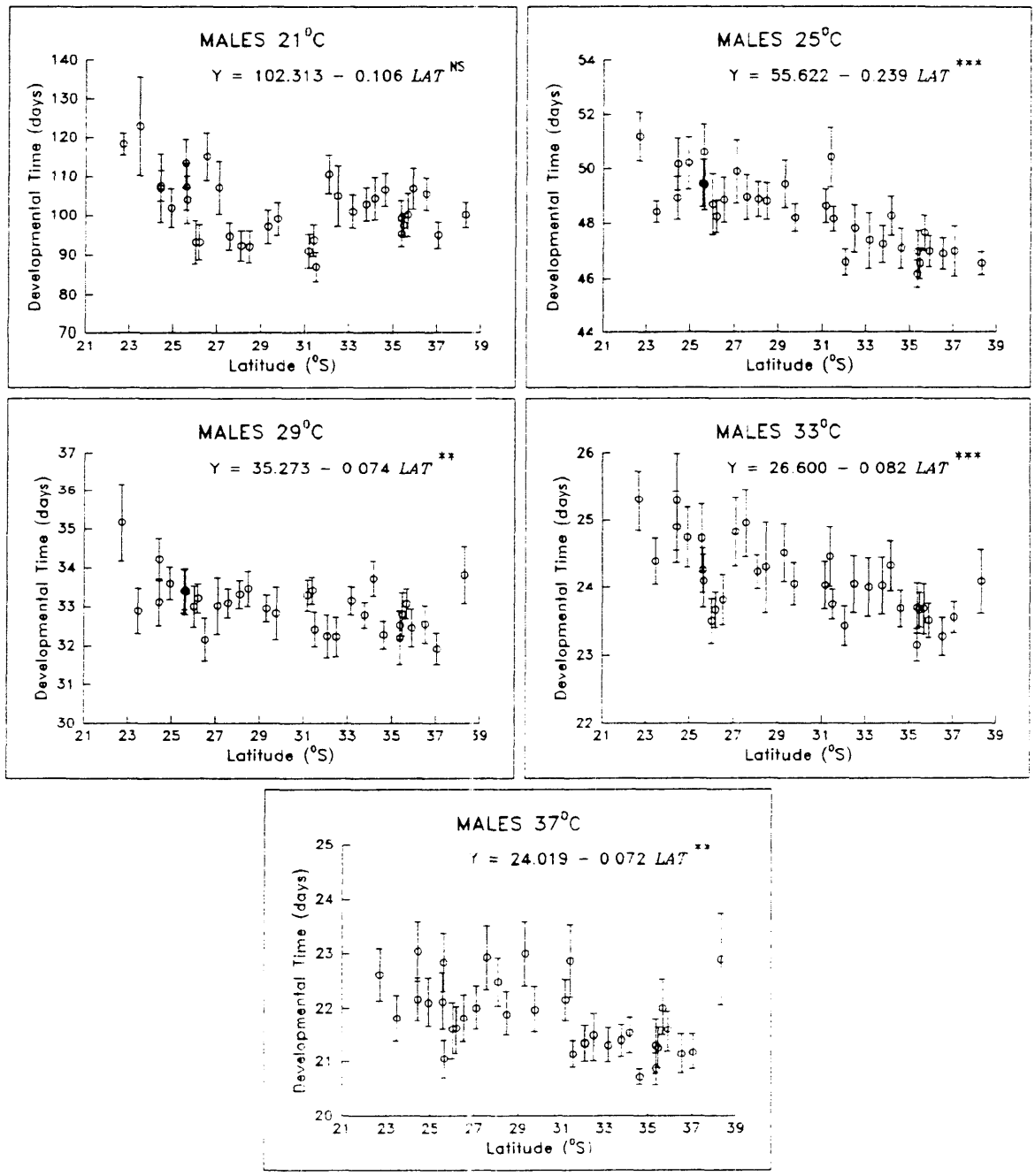

Figure 4.2: Phenotypic profiles for developmental period (DT) of males derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33° and 37°C), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

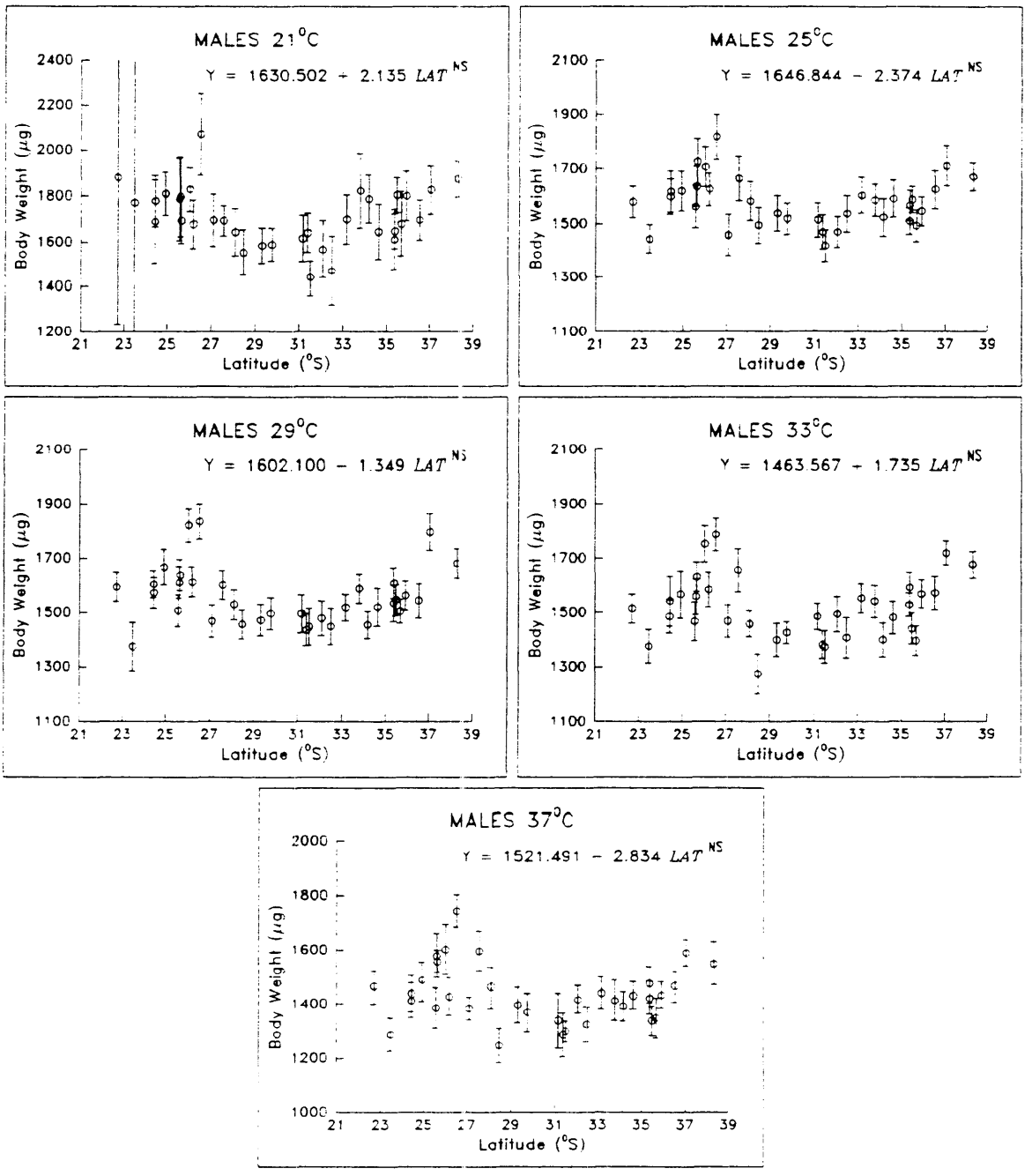

Figure 4.3: Phenotypic profiles for adult body weight (BWT) of males derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33° and 37 °), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

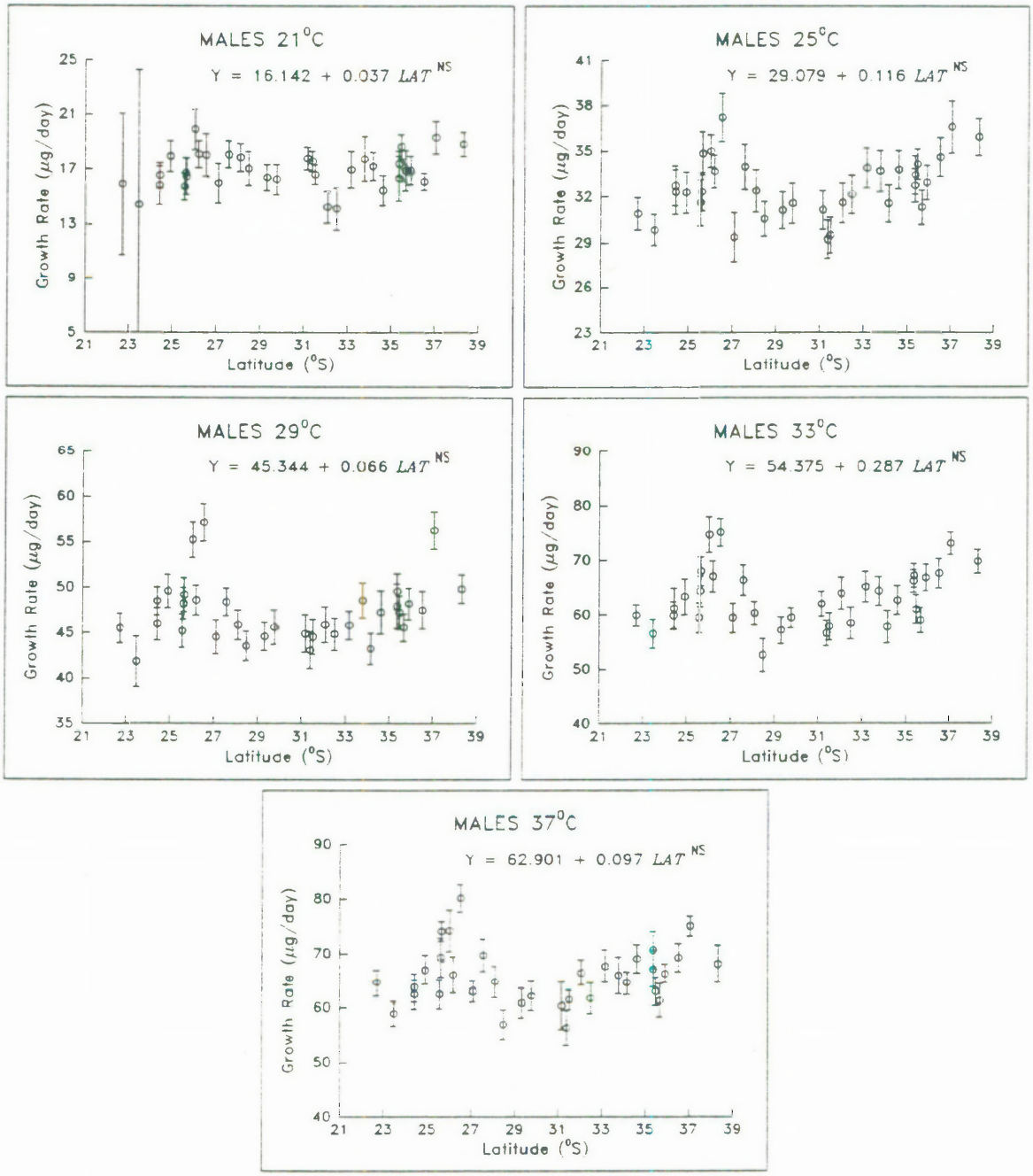

Figure 4.4: Phenotypic profiles for growth rate (GR) of males derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33° and 37°C), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

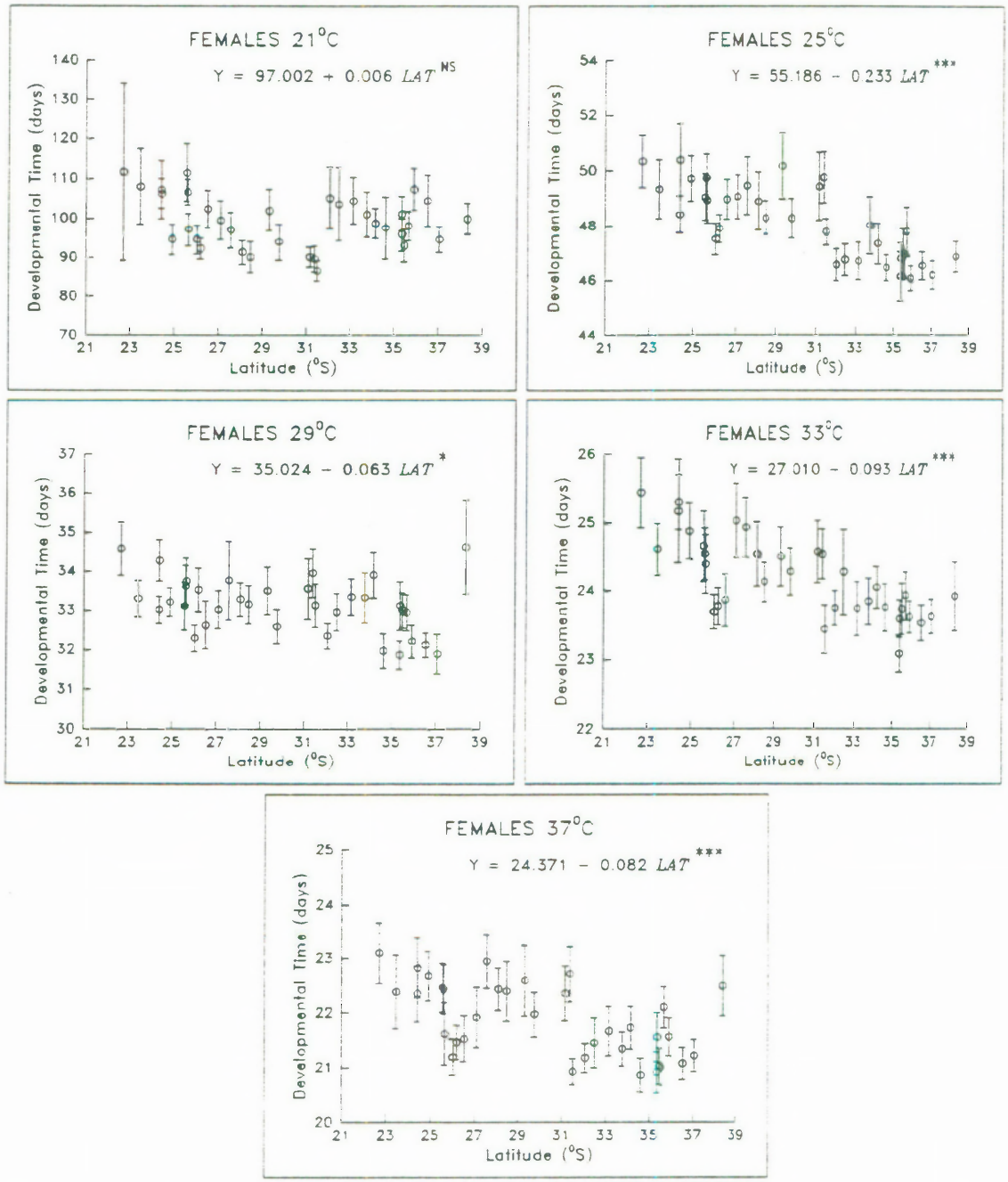

Figure 4.5: Phenotypic profiles for developmental period (DT) of females derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33° and 37°C), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

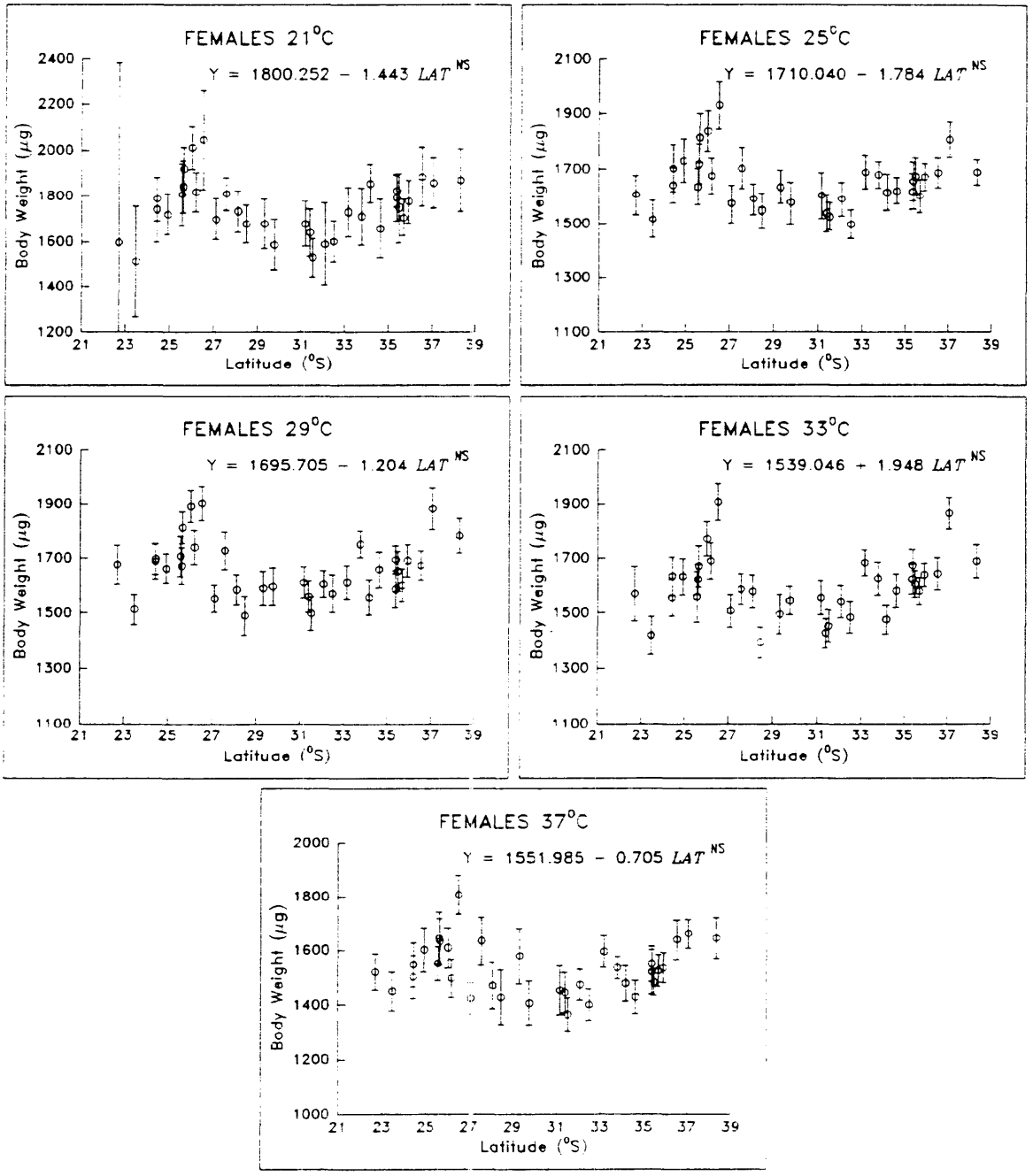

Figure 4.6: Phenotypic profiles for adult body weight (BWT) of females derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33° and 37°C), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

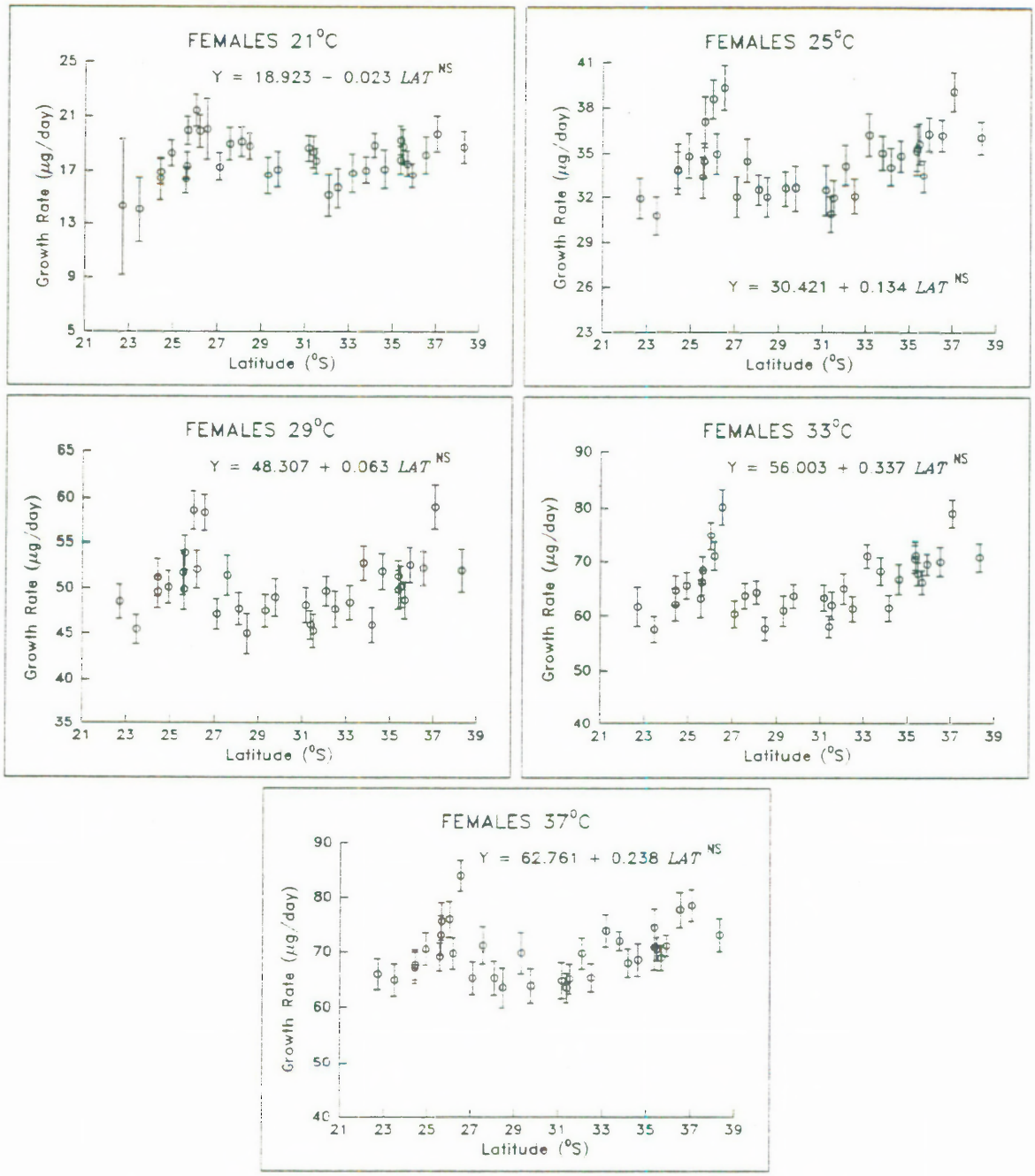

Figure 4.7: Phenotypic profiles for growth rate (GR) of females derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33° and 37°C), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

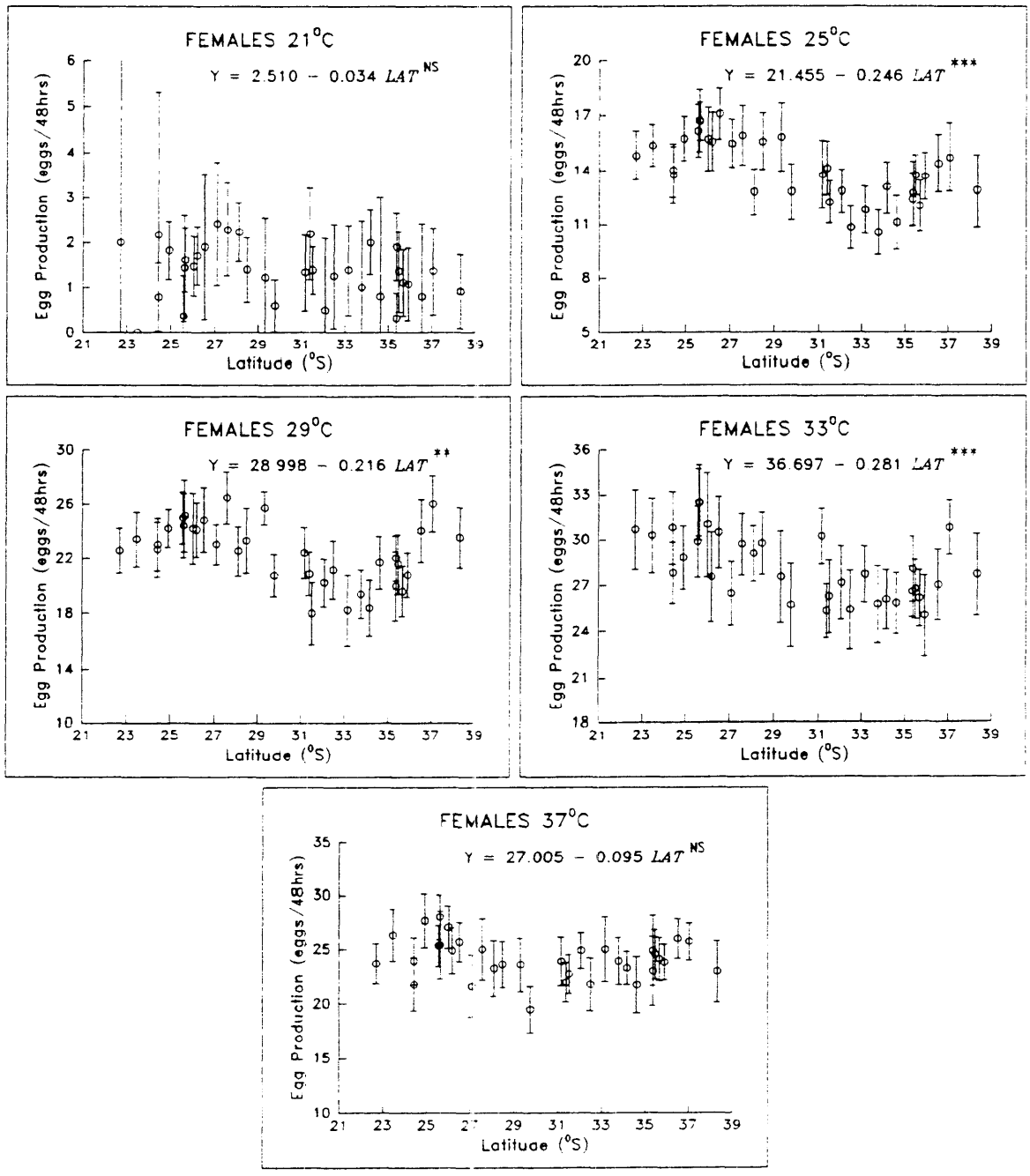

Figure 4.8: Phenotypic profiles for early fecundity (EPEAK) of females derived from 34 natural populations of *T. castaneum*. For each laboratory temperature (21°, 25°, 29°, 33 and 37 °), population means with 95% confidence intervals are plotted against latitude of origin. The simple linear prediction equation with significance level is given with each graph.

Table 4.6: Multiple regression for males. Standardized multiple regression coefficients (b') and multiple R^2 are given for each temperature when the early life-history traits (DT, BWT and GR) are regressed simultaneously on TMEAN and RhMEAN, then on LAT, TMEAN and RhMEAN. Significant coefficients and multiple regression equations are noted.

Trait	Temp	b'		Multiple	b'			Multiple
	(°C)	TMEAN	RhMEAN	R^2	LAT	TMEAN	RhMEAN	R^2
DT	21	0.153	-0.056	0.030	0.182	0.306	-0.030	0.041
	25	0.821***	0.220	0.593***	-0.636**	0.225	0.099	0.680***
	29	0.589***	0.091	0.321**	-0.081	0.516	0.078	0.323**
	33	0.634***	-0.075	0.444***	-0.528*	0.151	-0.157	0.513***
	37	0.555**	0.195	0.270**	-0.145	0.420	0.170	0.275*
BWT	21	0.132	0.380*	0.139	0.518	0.568	0.454*	0.226
	25	0.204	0.549**	0.263**	-0.130	0.082	0.524**	0.266*
	29	0.157	0.547**	0.268**	0.037	0.191	0.553**	0.269*
	33	0.060	0.552**	0.283**	0.052	0.107	0.560**	0.284*
	37	0.187	0.531**	0.247*	-0.187	0.013	0.498**	0.255*
GR	21	-0.002	0.428*	0.184*	0.346	0.290	0.478**	0.222
	25	-0.197	0.402*	0.257*	0.194	-0.016	0.439*	0.265*
	29	-0.018	0.482**	0.239*	0.070	0.046	0.495**	0.240*
	33	-0.119	0.519**	0.332**	0.189	0.054	0.549**	0.341**
	37	-0.050	0.428*	0.201*	-0.095	-0.138	0.412*	0.203

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$

Table 4.7: Multiple regression for females. Standardized multiple regression coefficients (b′) and multiple R^2 are given for each temperature when the early life-history traits (DT, BWT, GR and EPEAK) are regressed simultaneously on TMEAN and RhMEAN, then on LAT, TMEAN and RhMEAN. Significant coefficients and multiple regression equations are noted.

Trait	Temp	b′		Multiple	b′			Multiple
	(°C)	TMEAN	RhMEAN	R^2	LAT	TMEAN	RhMEAN	R^2
DT	21	0.083	-0.191	0.048	0.203	0.256	-0.148	0.060
	25	0.808***	0.241	0.588***	-0.576*	0.280	0.145	0.664***
	29	0.469*	0.173	0.196*	-0.086	0.389	0.157	0.197
	33	0.745***	-0.019	0.564***	-0.399	0.379	-0.097	0.598***
	37	0.639***	0.055	0.389***	-0.107	0.340	0.036	0.392**
BWT	21	0.177	0.359*	0.140	0.407	0.524	0.444*	0.187
	25	0.155	0.612***	0.338**	-0.122	0.044	0.591**	0.342**
	29	0.182	0.547**	0.266**	0.170	0.339	0.579**	0.272*
	33	-0.067	0.542**	0.318**	-0.045	-0.109	0.533**	0.318**
	37	0.163	0.535**	0.257*	0.191	0.339	0.569**	0.265*
GR	21	0.102	0.449**	0.197*	0.147	0.227	0.480*	0.203
	25	-0.222	0.441**	0.306**	0.154	-0.081	0.467**	0.312**
	29	0.020	0.459*	0.205*	0.197	0.202	0.496*	0.214
	33	-0.283	0.468**	0.373***	0.081	-0.208	0.484**	0.374**
	37	-0.148	0.455**	0.272**	0.239	0.072	0.498**	0.285*
EPEAK	21	0.194	0.006	0.037	-0.379	-0.121	-0.085	0.078
	25	0.746***	0.433**	0.527***	-0.207	0.555	0.397**	0.536***
	29	0.601***	0.471**	0.396***	0.093	0.687*	0.487**	0.398**
	33	0.711***	0.356*	0.468***	-0.102	0.617*	0.338*	0.470***
	37	0.402*	0.420*	0.226*	0.245	0.627	0.462*	0.239*

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