QUANTITATIVE GENETICS OF CLINAL VARIATION

THE QUANTITATIVE GENETICS OF CLINAL VARIATION IN *DROSOPHILA MELANOGASTER*

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Abstract

This work incorporates information from two sources in order to examine the nature of natural selection acting on phenotypic characters in *Drosophila melanogaster* along a North South cline. Isofemale lines were established from flies collected along a North South cline extending from Winnipeg, Manitoba to Tampa Bay, Florida. Offspring from different lines within each position were then cultured under standardized conditions and used to examine phenotypic variation in 10 morphological characters along the cline. In a separate set of experiments, flies from Vineland, Ontario were mated in a half-sib design in order to estimate the genetic covariance of the set of 10 characters. The results from the clinal and heritability experiments were then combined using Lande's (1979) equation, $\Delta\bar{\mathbf{z}} = \mathbf{G}\mathbf{P}^{-1}\mathbf{s}$, to estimate the net selective differentials and net selection gradients for each adjacent set of populations. The study concluded that:

1) Clinal variation is non-linear, with larger flies in the middle latitudes and smaller flies in the north and south.
2) Selection appears to act primarily on body characters in the north (wing width and femur length) and head characters in the south (eye and face width).
3) Scutellum width and wing length generally moderate the prevalent trends in directional selection on the other characters through antagonistic correlated responses.
4) Clinal patterns of variation may not be at equilibrium, but instead dominated by seasonal responses to selection pressures.

Acknowledgements

If all the world is a stage, then surely its performances require a cast of thousands. This work is no exception. In my years here, many of the people who have passed through the doors of Lab 505 have contributed in a meaningful way. Although they all cannot all be credited, thanks must be given to all the people at McMaster who have made my stay here enjoyable. Special thanks must be given to Ling-Wen Zeng, Dr. Larry Hale, Fayaz Alladina, Ozgur Huner, and Lee Geyer who have contributed many ideas though discussion that have benefitted this work. Additionally, both Walter Scott and Sue Laflair should be knighted for the patience they have shown in the last few months in dealing with their unshaven and often ethereal housemate.

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Dr. Richard Morton has been a colleague, friend, and foster supervisor during my stay at McMaster. Without the contributions he has made to this work it would be very different. But perhaps more importantly, without the influence he has had on my intellectual development I would be very different. Finally, I must thank my supervisor,

Dr. Rama Singh. Dr. Singh supported my work on a project unifying ecology and genetics, at a time when it seemed to me genetics had forgotten about the phenotype. Dr. Singh also introduced me to the exciting field of population genetics, he has served the true role of a supervisor in spawning an interest which will remain with me for many years. I have been fortunate in working with Dr. Singh and Dr. Morton as they have continually reminded me of the importance of stepping back from the tree that you think you know so much about, and examining it in the context of the entire forest.

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Introduction

1.1 Preamble: Temporal and Spatial Variation

An essential component of the synthetic theory of evolution is that the forces governing microevolution are the same as those governing macroevolution. It follows that inferences drawn from contemporary studies of populations can be extrapolated and used to predict the nature of change in these populations over the long term. More specifically an understanding of the mechanisms by which genetic change occurs within a population can be used to explain the nature of genetic variation between populations (or at any higher level of organization). Often times it is impossible to study the genetic nature of species change that has occurred over evolutionary time scales. If the only information regarding genetic variation is relative to shorter time intervals, the gradualist view of evolution can be a very powerful tool (via extrapolation) to understanding macroevolution. But what if even this information is not available? That is, it is likely that the time interval over which an empiricist can study any given population is too short to accurately observe the sort of genetic change which is likely to be important in macroevolution. It seems that a more clever paradigm is required than extrapolating from temporal observations.

Luckily a suitable substitution, spatial variation, presents itself. Two populations separated spatially are comparable to two populations separated temporally. Substituting spatial variation for temporal variation has been an important means of interpreting microevolution. In fact, Darwin's original theory of natural selection was largely a product

of his observations on geographical variation. This is evidenced by his devoting two chapters to the topic of geographic variation in his **The Origin of Species** (1859). Geographic, or spatial variation, is a useful tool for studying microevolution as two populations separated in space are in effect temporally separated from a common ancestor. Thus, through information gained from contemporary populations, it is possible to speculate about the causes of the differences between these groups. It is possible that the same events which cause populations to diverge geographically (eg; different selective optima) are responsible for temporal divergence. This fact makes the study of spatial variation both a blessing (evolutionary inferences can be drawn from contemporary populations) and a curse (it perpetuates the fallacy that one contemporary population is "descended" from another).

1.2 Why Study Clinal Variation?

The study of clinal variation takes the study of geographic variation one step further. If the difference between two populations is due to an underlying cause, then a series of populations which show a clinal trend can potentially provide strong circumstantial evidence of the cause of the divergence. A careful examination of such a clinal trend can possibly determine if variation is due to an environmental gradient paralleling the cline (a selectionist explanation), or the historical events which led to the species present day distribution along the cline (a neutralist explanation). As often times the true cause of a cline is a combination of the above explanations, the study of clinal variation can potentially determine the relative contribution of each. That is, clinal variation can uncover the total adaptive picture of a species' distribution. In addition, as studies of clinal variation can encompass both neutralist and selectionist elements; their results can provide useful grist for the theoretician's mills. In a similar vein, if clinal patterns are

consistent among a large number of species then persuasive theories can be developed which explain microevolution.

1.3 Clinal Variation in Quantitative Characters

This study will examine simultaneous clinal variation in a number of quantitative characters. This is a valuable approach to the study of clinal variation for a number of reasons. In order to understand the nature of adaptive evolution one must examine variation in characters of conceivable adaptive importance. The characters which are hypothesized to be of the greatest adaptive significance, such as morphological, physiological, behavioral and life history traits, are primarily polygenic in nature. That is, although models which deal with clinal variation in single gene traits are mathematically tractable, they may not be important with regards to traits which are of ecological significance (Haldane, 1948; May *et al.*, 1975; Barton and Hewitt, 1989). It would seem essential to understand clinal variation in quantitative characters if one wishes to understand the adaptive nature of geographic differentiation. Secondly, one cannot understand adaptive evolution by simply examining one quantitative character at a time. Quantitative characters in addition to being controlled by many genes are controlled by genes with potentially large pleiotropic effects. For example genes controlling arm length also control leg length, as presumably they both are manifesting themselves through general body size or limb length. In order to understand the means by which an organism adapts to its environment it is important to study a suite of characters simultaneously. This is the multivariate approach to geographic variation which we will presently deal with.

1.4 Phenotypic Geographic Variation

There is no shortage of studies which have dealt with multivariate variation in quantitative characters over a geographic range, perhaps there is even an excess. Thus this section will not exhaustively review such literature, but only examine some typical papers.

A complete review can be found in Reyment, Blackith, and Campbell (1984). Typical studies have examined clinal variation in mammals ranging from the wolf (Jolicoeur, 1959), to White Tailed Deer (Rees, 1970), to Long-Tailed Field-Mouse (Delany and Healy, 1964); birds such as the house sparrow (Lowther, 1977); snails (Galler and Gould, 1979); the Horseshoe Crab (Riska, 1981); and a wide variety of insects such as Aphids (Sokal *et al.*, 1980), Grasshoppers (Campbell and Dearns, 1980), and Dipterans (Atchley, 1971). The above studies, although differing in organism of study, have a number of common themes. Generally character measures were obtained from organisms raised in the wild at a number of geographic locations. The nature of geographic variation was then examined by multivariate statistical tests such as factor analysis, principal component scores, discriminant functions or MANOVA (for a review of these techniques see Pimental, 1979; Chatfield and Collins, 1980). Usually the populations differed, often in relation to latitude or an environmental variable. Little time will be spent reviewing this genre of work, as it often appears to be little more than an exercise in the new techniques of multivariate analysis made possible with the advent of cheap computer time. Futhermore it contributes very little to our understanding to the evolution of these characters in nature. This is because these studies lacked in two very important attributes. First they ignored the genetic basis of differences between the populations. As evolution can only proceed through genetic change in populations this is a major short coming. Although it is possible to assess what changes have occurred without studying the genetics of the situation, it is impossible to hypothesize as to how these changes occurred. Second the studies ignored, or off handedly dismissed, the environmental components of variation which contributed to the observed divergences. This is a more important concern because any variation in nature may be entirely (or chiefly) caused by environmentally - induced differences, and populations may not have actually diverged with respect to the characters of interest. The

extent of this effect is not trivial as cross fostering studies in birds have shown (James, 1983). Attempts to resolve these two problems will be discussed in the two sections that follow.

1.5.1 The Quantitative Genetics of Geographic Variation (Selection)

There is a large body of literature in the field of quantitative genetics which can be used to estimate the amount of selection that must be applied to a character or group of characters in order to achieve a desired level of change in a quantitative character (Falconer, 1981). Essentially if one knows the heritability of a trait (or genetic covariance matrix of a set of traits), then the population can be culled by not allowing those with a phenotype above or below a threshold (or some critical value of a discriminant value) to reproduce. Lande (1976, 1979) adapted the equations used in animal breeding to estimate the levels of natural selection necessary to cause observed levels of divergence among taxa or populations. Lande (1979) derives the equation for the change in mean phenotype, which is described in greater detail in section 2.8.1, the final result being;

$$\Delta\bar{z} = \mathbf{GP^{-1}S} = \mathbf{G\ V}\ \ln\ \bar{W} \qquad (1.5.1.1).$$

Although one can refer to section 2.8.1 for a complete description of this result it is sufficient to mention here that S will be referred to as the net selection differential and V ln $\bar{W}$ as the net selection gradient. Although equation 1.5.1.1 only describes the forces governing directional selection, Lande's model assumes selection is relatively weak, primarily stabilizing, and acts over long time periods. Such assumptions stand in contrast to the models used in animal breeding where selection is strong, directional, and over short time periods. As a result, Lande's result depends on a number of assumptions being met: 1) there is no genotype environment correlation, 2) there is a linear regression of the additive genetic values of characters i and j on the phenotypic values of j (ie; i and j are multivariate normal distributed, i and j being any two characters), 3) phenotypic variances

are nearly constant, that is the phenotypic variances are not a function of $\bar{z}_i$ and $\bar{z}_j$, and 4) that **G** remains constant over time (Lande, 1979). The first assumption can be met by controlling for G X E interactions by raising individuals under the same conditions in the lab, although it may be difficult to extrapolate results back to nature. Assumptions 2 and 3 are easily met if data are suitable transformed to a linear scale, although may not be met if such a transformation is not used, or the characters are of different 'type' (ie; they are not measured on the same scale, eg; morphological length characters versus area, number or colour measure). It follows that an experimenter that wishes to effectively use the equations describing multivariate evolution should carefully restrict their choice of characters. It also implies that equation 1.5.1.1 is not as generally applicable as the 'consumer' is led to believe, as it can only describe evolution in a character suite, not the phenotype as a whole. The forth assumption of Lande's model is the most tenuous and will be dealt with separately.

The most appealing aspect of Lande's model is that both the net selection differential (the genetic gain of each character including correlated responses of other characters) and the net selection gradient (the change in Malthusian mean fitness for a small change in each characters holding all others constant) can be estimated if $\Delta\bar{\mathbf{z}}$, **G**, and **P** are known or estimable. Thus, the selection differential can be used to represent the change in the multivariate phenotype over space weighted by the genetic covariance structure of the trait complex. The selection differential, used in this manner, can serve as a useful summary of the adaptive surface of a species over their range. The net selection gradient serves a different purpose. It can be used to interpret the nature of selection on a given character irrespective of selection on the others. Thus one can determine which traits selection primarily acted on and which ones evolved as a result of correlated responses to selection on other measured characters. A major problem with selection gradients is that

they only control for the characters measured. Thus apparent strong directional selection on a character may actually represent a correlated response to an unmeasured character that is being strongly selected for (Lande, 1979). As the response due to selection is, in part, a result of the off-diagonal structure of G, it is important to note that models based only on the phenotypic covariance structure of a set of traits may greatly err in predicting the response to selection. Lande (1979) points to this problem as a justification for the use of his model (1.5.1.1), even if assumptions are slightly violated. Support for this claim is provided by Falconer (1980: p. 284) who gives examples (from domestic animals) of genetic correlations which do not correspond to phenotypic correlations. Cheverud (1988) contrarily argues that genetic correlations and phenotypic correlations do correspond, but the statistical method used to justify their correspondence (correlating phenotypic and genetic correlations over a number of characters) only guarantees large phenotypic correlations correspond to large genetic correlations and not that they are necessarily proportional.

A second method by which one can examine the nature of selective divergence between two populations is by estimating the minimum selective mortality necessary to cause the observed divergence. This is accomplished by weighting each character by the strength of selection acting on it. This creates a univariate index similar to the discriminant functions used in animal breeding to maximize economic gain (Lande, 1979; Smith, 1936; Hazel, 1943; Lin, 1978; Hayes and Hill, 1981)(see section 2.8.2). If the time of divergence between two populations is known the minimum selective mortality per generation necessary to cause the observed divergence can be estimated. As truncated selection produces the greatest gain per unit of selection, the minimum selective mortality corresponds to the proportion of individuals that must be culled from the tail of the index's distribution in order to achieve the observed results. Of course, if selection is not

truncated, which is likely in nature, the selective mortality required will be greater Charlesworth, 1984). A third method similar to the index of selection involves estimating the selection differentials associated with principal components of the phenotypic data as opposed to the characters themselves. Principal components have the beneficial qualities of being orthogonal to one another (ie; independent), and usually the first few principal components account for most of the variation in a set of correlated characters. As a result, the analyses of selection on a large set of correlated characters can be reduced to selection on a few independent characters (Lande, 1979). This is convenient, but is of little use if the components are non-interpretable, as they are not true characters but linear combinations of the original character set. Much has been made of the interpretation of these scores (see Reyment *et al.*,.1984; Pimentel, 1979) but, beyond the first component which is generally accepted as 'size', this interpretation at times seems more of an art than science.

1.5.2 The Quantitative Genetics of Geographic Variation (Random Genetic Drift)

The above models assume that the observed phenotypic difference between two populations is both due to genetic causes (which is true if samples are lab reared) and directional selection. It is possible though, that the difference is not due to selection at all, but due to random genetic drift. Lande (1976) provides a statistical test with which to test whether the divergence in mean phenotype between two populations is due to random genetic drift:

$$N^* = \frac{(1.96)^2 h^2 t}{(z/\sigma)^2} = \frac{t (1.96)^2 G}{z^2} \qquad (1.5.2.1).$$

If N* is greater than N, the effective population size of each sub population, then it is possible that the divergence is due to random genetic drift. In 1.5.2.1, h^2 is the heritability of the trait, t is the time of divergence in generations, z is the mean phenotypic divergence

between the two populations with respect to the character under consideration, σ is the phenotypic variance of the trait, and G is the genetic variance of the trait. This test can be extended to the multivariate case and other tests are available which test the drift hypothesis (Lande, 1976; Lande,1979; Turelli *et al.*, 1988). These other tests will be ignored as any test of drift is sensitive to two parameters, which 1.5.2.1 will allow us to examine in a simple manner. First, 1.5.2.1 is sensitive to t, the divergence time. For a given degree of phenotypic divergence the larger the t the more likely one is to accept the hypothesis of random genetic drift. An obvious problem with this is that if the two population have diverged to an optimum and remained there, the more time that passes the greater the chance of an experimenter concluding the divergence was due to drift! One is tempted to carry this argument to a ludicrous extreme by comparing two widely diverged taxa for a homologous trait, but discretion is often better than valour. The second parameter such tests are sensitive to is the ratio of G to z^2. This is desirable in that a large degree of divergence will result in a rejection of the drift hypothesis, but is undesirable if the selective divergence is small with the same rational as above. I argue, albeit with little support, that tests of random genetic drift versus selection serve no purpose with regards to quantitative traits, as random genetic drift will be accepted if the difference between phenotypic optima is small, or the time of divergence has been great for a given two populations. Thus, although such tests can be used to reject the drift hypothesis they really **cannot** be used to accept it. It is really up to the researcher to make an *a priori* decision as to whether such a change could have occurred as a result of selection. This decision was easy in the current study as the phenotypes showed a consistent trend over latitude indicative of a response, at least in part, to an underlying environmental variable. Only molecular data can determine if, in fact, such an observed trend could be due to historical causes such as migration.

1.6 Constraints on Character Evolution

If the off diagonal structure of **G** is non-zero, that is the traits are genetically correlated, then one can immediately see the importance of multivariate formulations such as 1.5.1.1. Genetic correlations can result in evolutionary change, at least in the short term, which does **not** maximize the fitness of a given character. Thus, correlated characters in the face of antagonistic selection can be a barrier to adaptive evolution. That is, although the mean fitness of a set of characters will always increase according to Fisher's fundamental theorem, the mean fitness of any given character may decrease as a result of a correlated response to selection on another character. This effect can potentially create a thorny problem for the optimization school. Generally, optimalists create models which determine the morphological, behavioural, or physiological trait values which result in the maximum fitness (or feeding efficiency) of an individual (Maynard Smith, 1990). Often the solution to such models is coincident with the mean value in the study population, implying that natural populations are at or near a phenotypic optimum. Such models, almost by necessity, ignore possible reductions in fitness due to the effects of genetically correlated characters. That is, if the entire phenotype is being maximized at any given time it is possible that any given trait may not be at its optimum. That this outcome is rarely observed suggests a number of possible explanations:

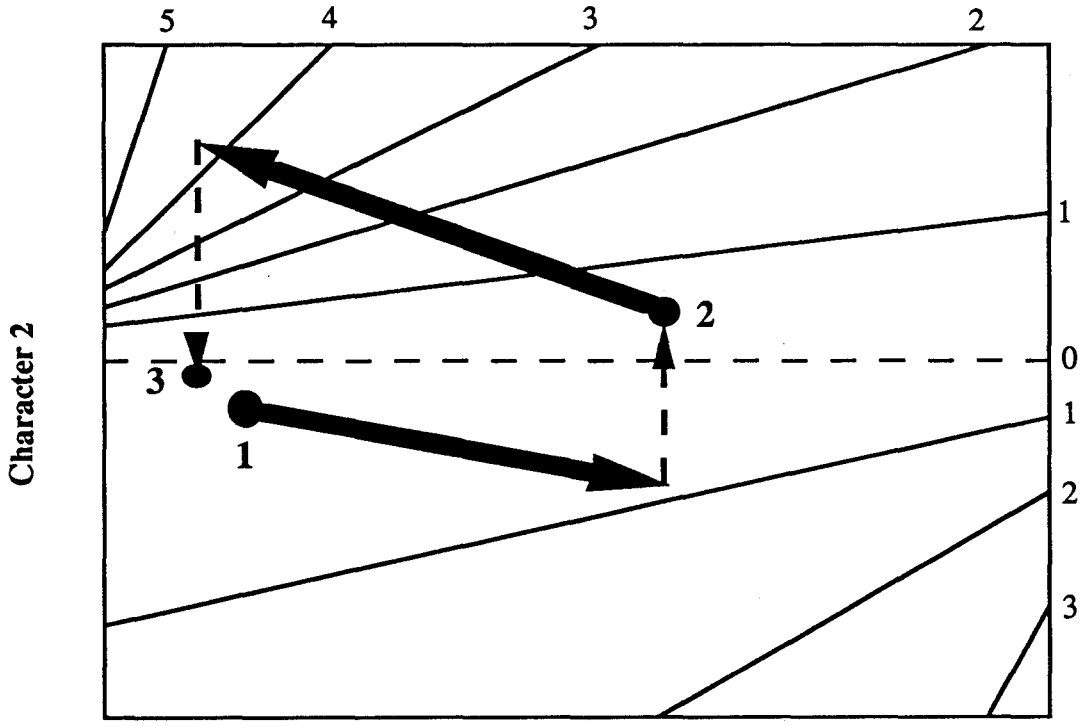
1. Practitioners generally choose characters which are highly correlated with fitness, and thus swamp out potential reductions in fitness due to correlated characters not as closely linked with fitness.
2. Work does not get published which concludes an optimum is other than that observed.
3. Models are tinkered with until they converge on the expected optimum.
4. Genetic correlations which impede the simultaneous maximization of fitness with respect to all characters tend to break down.

Postulate one is possible, but difficult to argue for or against. Possibilities two and three have been argued elsewhere by others and are only included for the sake of completeness. Four is quite probable and is addressed below.

Over the course of time it is probable that beneficial mutants affecting pleiotropic genes will become fixed and alter genetic correlations. In addition, if there is additive variation for all traits, regardless of the nature of **G**, eventually the phenotype will reach a fitness maximum with respect to all characters (Lande, 1979). But, as any given character will not necessarily proceed to that optimum via the most direct route it is possible that the optimum which the entire set of characters will proceed to may change. Consider figure 1.6, an adaptive topography in the style of Wright (1977), with the ordinate and abscissa representing two characters with a positive genetic correlation and labelled contour lines corresponding to some measure of fitness. It is clear from this figure that there are two fitness optima, one in the upper left corner (small character 1, big character 2), and one in the lower right (big character 1, small character 2). If selection were maximizing both characters independently, then starting at bold 1 we would expect a trajectory resembling the first bold arrow. But because of the genetic correlation of the characters the new position of the population would be somewhere near bold 2. Given the genetic correlation and fitness surface of figure 1.6, a population at 1 will end up at position 3 after 2 generations of selection as opposed to somewhere near the lower right of the figure. Of course, an exact determination of the position of the population after one generation of selection would depend on the degree of the genetic correlation and the level of selection acting on each character. Generally with weak selection the population will move a much shorter distance than that shown here. Nonetheless, it should be clear that even if the genetic correlation between the two characters does break down over time, the eventual fitness optimum that will be reached may not be a global maximum and will depend on the

initial genetic correlation between the characters and the n - dimensional starting position of the population (Lande, 1979). It would intuitively seem likely that evolution that changes mean phenotypes without changing the genetic covariance pattern is more common than the opposite. Although this statement, as with many others, critically depends on the constancy of genetic covariation patterns.

Figure 1.6
An Adaptive Topography for Two Genetically Correlated Characters



1.7 Problems with the Quantitative Genetic Methodology

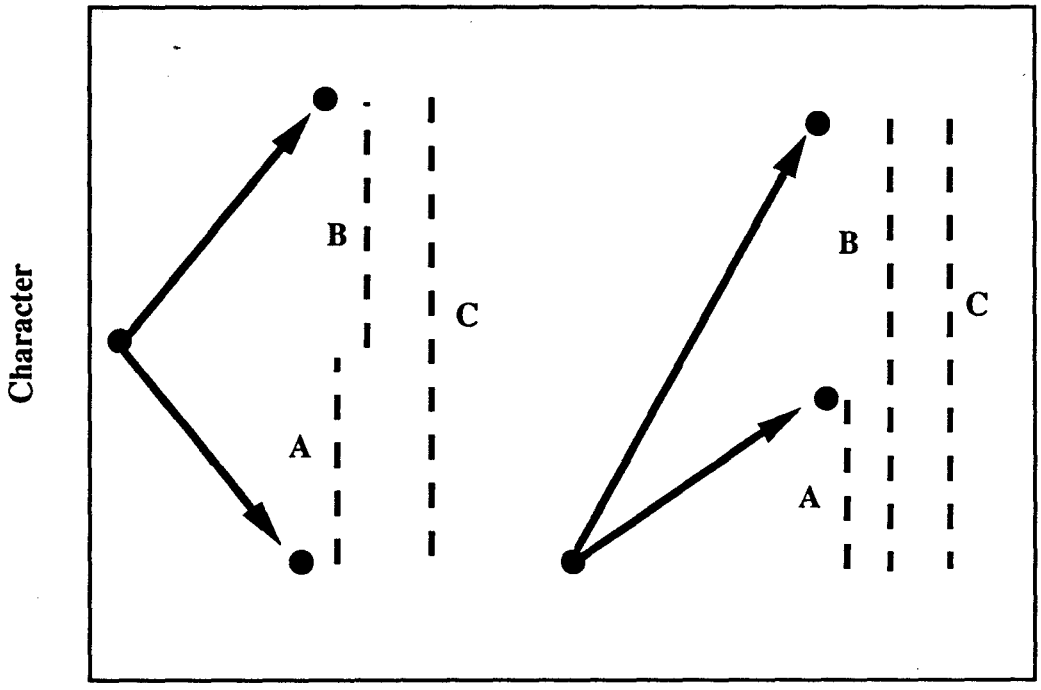
Even though the methods suggested by Lande for estimating the nature of selection on polygenic characters are an improvement over simply phenotypic models, a number of shortcomings still exist. First, and perhaps most importantly, one cannot ignore the effects on the phenotype of individuals raised in different environments. Nonetheless if the techniques proposed by Lande become popular in evolutionary ecology this will likely be a

common misapplication of the technique. As Gould (1972) points out ignoring this effect amounts to the now famous Jensen fallacy of equating the proportion of variance due to genetic causes within a population to the proportion of variance due to genetic causes between populations - there is absolutely no theoretical basis to this approach. It is important for future reviewers to accept the fact that no amount of 'fancy' statistical work can correct for a poor, or no, experimental design.

A second set of concerns with Lande's approach to phenotypic evolution relates to the concept of minimum selective mortality. This is indeed a minimum estimate and actual levels may be orders of magnitude higher. I will give three ways in which the minimum selective mortality may grossly underestimate the actual selective mortality. Firstly, the nature of selection in the wild is surely not truncated. As truncated selection is the most efficient means of changing the mean phenotype, any other form of selection (eg; one with a larger stochastic element) will require a greater number of selective 'deaths'. Secondly, Lande's method estimates the net selective mortality required to move the shortest distance between points in the n - space defined by the character set (ie; the generalized distance) (Lande, 1979). If the population takes a less direct route to its new multivariate mean, possibly as a result of a shifting fitness surface, the distance travelled will be greater. As the true selective mortality is a function of the total distance travelled any route other than the generalized distance requires greater levels of selection to achieve. Finally, as Lande's formulations will primarily be used with populations separated in time and space, the distance between two populations is really the sum of the two distances from a common ancestor. Consider figure 1.7. This figure depicts two examples of divergence of two populations from a common ancestor. In both examples C is the observed divergence between the two populations, and the actual divergence between the two populations is A + B. In the first example the phenotypic distance between the two populations accurately

reflects the nature of the change that has occurred to differentiate them. However in the second example, because the two populations have evolved in the same direction, the divergence between the two populations under-estimates the actual selective divergence which has occurred. In this case Lande's formulation will estimate more selection has occurred in the first case when really more has occurred in the second. This problem is especially acute when one considers that both the current populations exist in a similar set of environmental conditions (on a large scale) whereas the founding population may have existed in a different set of conditions (consider phyletic trends). It is truly impossible to determine phenotypic historical trends with no historical information.

Figure 1.7
An Example Showing an Under-Estimate of the Divergence Between Two Populations



The final potential weakness of Lande's model is the questionable assumption of the constancy of genetic covariance structure. Although it is obvious that G is not likely to

remain constant at higher taxonomic levels, it is difficult to determine if G is relatively constant at the level of sub-species or even populations within a species. It has been theoretically shown that G can change due to inbreeding in small populations (Lande, 1976; Avery and Hill, 1977, 1979) or vary among populations living in different environments as a result of a genotype X environment interaction (Via, 1984; see Via and Lande, 1987 for contradictory evidence). Additional empirical evidence suggests that G is not constant during ontogeny (Atchley and Rutledge, 1980; Cheverud et al., 1983) and can be changed by intense selection (Falconer, 1981; Sheridan and Barker, 1974). Although changes in G due to inbreeding or intense selection may not generally apply to wild populations the constancy of G must still concern us (as a corollary, comparison of G among domesticated animal may be of limited use). Turelli (1988) states that the degree to which G varies is largely an empirical question, albeit a difficult one, as a great deal of effort is required to estimate one G accurately, let alone a number. As small, but potentially important, within species differences in G may be swamped out by the errors associated with estimates of G, empirical studies have focused on differences in G between closely related or sub-species. Studies have generally concluded that the genetic correlation matrix has remained constant although the genetic covariance matrix has changed (Lofsvold, 1986; Kohn and Atchley, 1988). But these results must be interpreted with caution as covariance matrices are sensitive to characters measured on different scales (which may be very important if the characters are not normally distributed on the original measurement scale), and current statistical tests are designed for correlation not covariance matrices (Turelli, 1988). Even if we are able to determine the relationship between G's between species we still do not know their structure within species. As both Kohn and Atchley, and Lofsvold's studies indicate that differences in G are likely small between species, it is probably safe to assume that G's are fairly constant within species.

Nonetheless, a great deal of (mundane) work still needs to be done because of the importance of this assumption to evolutionary inference.

1.8 Studies Examining the Genetic Basis of Geographic Variation

Studies attempting to unravel the genetic basis of geographical variation fall into two broad categories. First those that measure phenotypes in a number of populations and then use genetic information via 1.5.1.1 to unravel the nature of selection separating the two populations. Examples of this sort of study include work done by Lofsvold (1988) on different species of Peromyscus (deer mouse) and a study by Arnold (1987) on coastal and inland populations of garter snake. Lofsvold found small selective mortalities would account for the observed phenotypic divergences between species. Otherwise we are not concerned with the work of Lofsvold as it deals with divergence at the species level, and measurements were made on wild caught individuals. Arnold (1987) examined divergence in tail and body vertebrae number in lab reared garter snakes and examined the selection gradients necessary to cause the observed divergence between coastal and inland populations. As the selection gradient was much greater for body vertebrae, Arnold concluded the change in tail vertebrae number could be largely a correlated response to selection for a change in body vertebrae number.

The second method which has been used to study geographic variation is to lab rear individuals from different localities and then examine trends in the distribution of phenotypes relative to the localities from which the parental samples were collected. Unfortunately few of these studies have used information from estimates of genetic correlations among traits, the above mentioned study by Arnold (1987) and a study by Coyne and Beecham (1987) being the only exceptions. Coyne and Beecham observed a North South cline in lab reared flies for both wing length and abdominal bristle number with the largest sizes/numbers being in the north. Coyne and Beecham concluded that the

change in bristle number over latitude could be accounted by the change in wing length over latitude and the genetic correlation of bristle number and wing length, but not vice versa. That is clinal variation in bristle number can be accounted for solely as a correlated response to selection on wing size (which probably was a correlated response to unmeasured selection on body size).

A large number of studies have examined quantitative geographic variation in lab reared Dipterans, possibly because of their wide distribution, ease of collection, and relative ease of lab rearing. Although the studies have not examined explicit genetic variation in the traits, and as a result it is difficult to determine the agent upon which selection is acting, some information can be gained from such studies. It appears that North South clinal variation is a general phenomena in the Dipterans studied. North South clinal variation of morphological traits have been observed in lab reared Face flies (Bryant and Turner, 1978), Houseflies (Bryant, 1977), *Drosophila melanogaster* (Hyytia et al., 1985), *D. simulans* (Hyytia et al., 1985), *D. pseudoobscura* (Sokoloff, 1965), *D. robusta* (Stalker and Carson, 1947), and *D. subobscura* (Misra, 1966). The strongest clines above were reported for characters such as wing length, thorax length, femur length or some linear function combining these and other characters (eg; discriminant scores or principal component scores). Interestingly, there is at least one report of such a cline being non linear in nature (Misra, 1966), although this was only discovered upon a reanalysis of the *D. robusta* data of Stalker and Carson (1947). It is not surprising few non-linear clines were observed as:

1. Few looked for them.
2. The clines were not sampled at regular intervals, so the analysis was confounded (all studies except Coyne and Beecham, 1987).
3. Some studies did not compare enough samples to observe a non linear trend (eg; Hyytia et al., 1985).
4. A non-linear trend may be the result of a confounding factor such as season of collection or altitude.

Observations of non linear trend are interesting as they suggest that local populations are not adapting simply to an environmental variable such as average temperature, and that a great deal of local differentiation is possible even for a species with relatively high dispersal rates such as *D. melanogaster* (Coyne and Milstead, 1987; Coyne *et al.*, 1987).

Additional observations support the thesis that quantitative characters respond very quickly to selection and are not the result of long term evolution. Stalker and Carson observed that lab reared flies from parents collected at different times of the year at the same locality showed differences in phenotype, with those flies collected in the hottest months showing the smallest size (1949). Bryant (1977) reported similar findings in the housefly, with seasonality accounting for a greater portion of the phenotypic variance observed than latitude. Stalker and Carson (1948) reported a difference in phenotype over a relatively short distance (a few kilometres) spanning an altitudinal gradient (small size at low altitudes). As gene flow would seem likely over this short of a distance, the selection causing such a divergence must be very powerful. In a similar vein, Bryant and Turner (1978) reported similar phenotypic patterns in geographic distribution of the Face fly and House fly which indicates both species may be near an optimum with respect to each location. This is particularly disturbing to those who believe weak selection caused such a distribution when one considers that the Face fly's first sighting in North America was in Nova Scotia in 1952. Thus it is probable that models which assume relatively weak selection has caused phenotypic evolution over long time periods may be incorrect. In Dipterans it appears that local populations genetically move to an optimum relatively quickly and then track short term deviations around that optimum. Of course, in mammals with longer generation times and smaller population sizes the rules may vary.

In the absence of genetic information which would allow one to interpret what characters selection has acted upon, causal explanations are often evoked in papers on

geographic variation. A common causal explanation for the trend seen in body size is selection in response to temperature (ie; Bergman's rule). As this rule is intended to imply that larger animals require less energy expenditure to thermo-regulate there is no obvious reason why this rule should apply to ecto-therms. In addition, although selection cage experiments using *D. pseudoobscura* held at different temperatures show phenotypic divergence in the direction expected, the response is far to slow to solely account for the rates of divergence seen in nature (Anderson, 1965, 1972). It seems more probable that the response is to desiccation threat at higher temperatures (Levins, 1969), or possibly even selection for a shorter larval period in hotter clines where there is a great risk of the larval food source desiccating. If this is indeed the case, it also serves as an effective demonstration of the pitfall of measuring a number of morphological characters and assuming one has a complete picture of the nature of selection acting between populations. A second interesting functional explanation for the common observation that the second principal component (or discriminant score contrasting wing length to thorax length) shows significant clinal variation is that high wing to thorax ratios are associated with a lower wing beat per unit of lift (Reed, Williams and Chadwick, 1942 in Stalker and Carson, 1947). In the north where the wing beat is slow, because of temperature constraints, a higher wing to thorax ratio would be needed to stay aloft.

1.9 The Choice of Organism

At this point in time the study of phenotypic evolution is approaching a turning point, no longer is it sufficient to merely describe phenotypic evolution, nor estimate allele frequencies because gel electrophoresis makes it possible to do so. In order to really understand evolution we must take one system, examine it at a number of levels, and perhaps more importantly interrelate what is happening at these levels. It would seem of little use to know all about molecular genetic variation in yeast, but only know about

phenotypic variation in Darwin's finch. Unfortunately though, this seems a likely scenario as Darwin's finch is not a convenient organism for molecular genetic work, nor does yeast seem particularly well suited to phenotypic or ecologically oriented work. It is clear that an appropriate organism for pioneering integrative work must be suited to both sorts of techniques. *D. melanogaster* is a particularly good organism for integrative work of this sort as it is suited to study at a molecular genetic level, yet is also phenotypically rich and can be found in a large number of ecological niches. Thus it seems appropriate to investigate some of the questions raised earlier using this species as a starting point for further research. Specifically, we can ask what is the nature of geographical variation in morphological characters in *D. melanogaster* ? How does the phenotypic variation relate to the underlying genetic variation controlling these characters? And finally, what is the nature of the selective forces necessary to cause the sort of phenotypic variation we observe?

Chapter 2
Materials and Methods

2.1 Collection of Isofemale Lines (Clinal)

2.1.1 Method of Collection

Traps constructed from one litre juice containers with sides partially cut out were used to capture the flies. The specifications of these traps are given in figure 2.1.1 a. Traps were baited with approximately two mashed bananas to which a tablespoon of live dehydrated yeast (Fleischman's Active Dry Yeast) had been added. Traps were suspended from trees or bushes so that they were 0.1 to 1 m from the ground. The practice of suspending the traps in most cases avoided their being infested with ants. The traps were set in the evening and generally collected in the morning 12 to 36 hours after being set. Flies were evacuated from the traps through the use of the mouth aspirator described in figure 2.1.1 b. After flies had been collected from a given trap the bait was discarded and the traps thoroughly cleaned to avoid the possibility of larvae pupating in the collection traps and erroneously being collected at a later site.

2.1.2 Establishment of Isofemale Lines

As soon as possible after the traps had been aspirated (15 min to 6 hours) the flies were anesthetized and sorted to remove unwanted species and isolate females. Flies were anesthetized using diethyl ether vapour, and then sorted with a artist's paint brush either by naked eye or with a small (10 X) magnifying lens. A number of females that appeared to

Figure 2.1.1 a
Specifications of Trap Used to Collect Flies

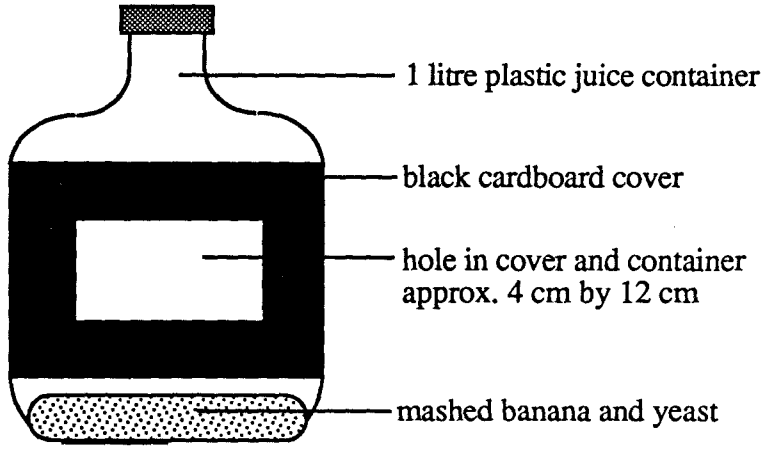
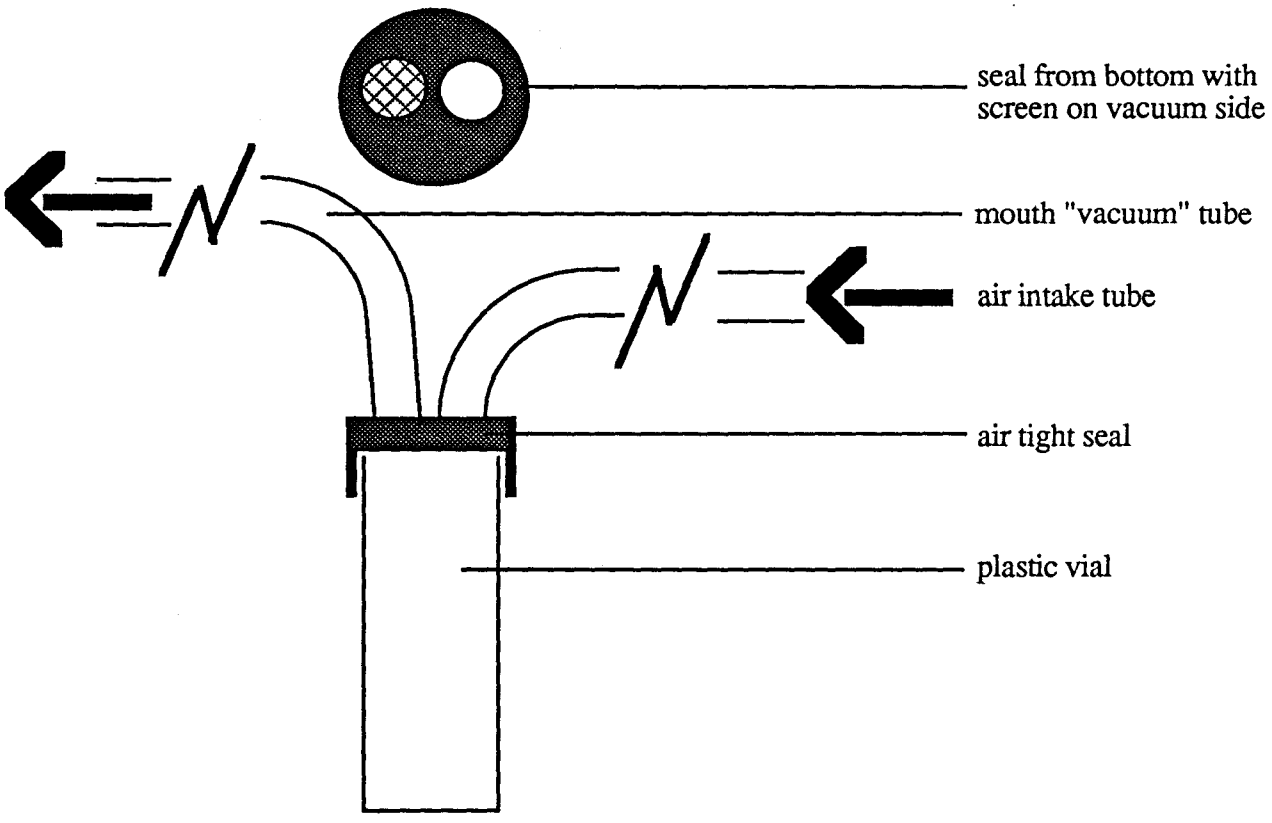


Figure 2.1.1 b
Mouth Aspirator



be *Drosophila melanogaster* (in the field one cannot differentiate between *D. melanogaster* and *D. simulans*) were placed in (15 ml) vials containing approximately 4 ml of instant Drosophila media and a pinch of live yeast. Initially only one female was placed in each vial, but viability and virility were low enough in wild flies that it became necessary to place a number of flies in each vial in order to increase the number of lines that could be effectively returned to the lab. Flies collected in this manner were mailed or brought back to the lab at which time each female was used to establish an isofemale line.

2.1.3 Preservation of Field Caught Flies

In addition to the females used to establish isofemale lines a number of wild caught males and females were preserved for later measurement. Flies that appeared to be *D. melanogaster* were anesthetized, placed in a filter paper "cup" and then placed in the preservation "highrise" show in figure 2.1.3. Approximately 0.5 ml of ethyl acetate was absorbed in a piece of foam in the bottom of the "highrise" to facilitate preservation of the flies. Flies preserved in this manner became dry and brittle with wings and appendages often extended. In addition, these flies can easily be kept a number of years with little or no deterioration in external features.

Figure 2.1.3
Preservation 'Highrise'

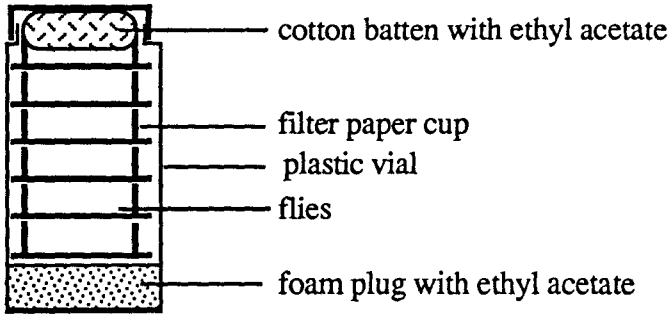


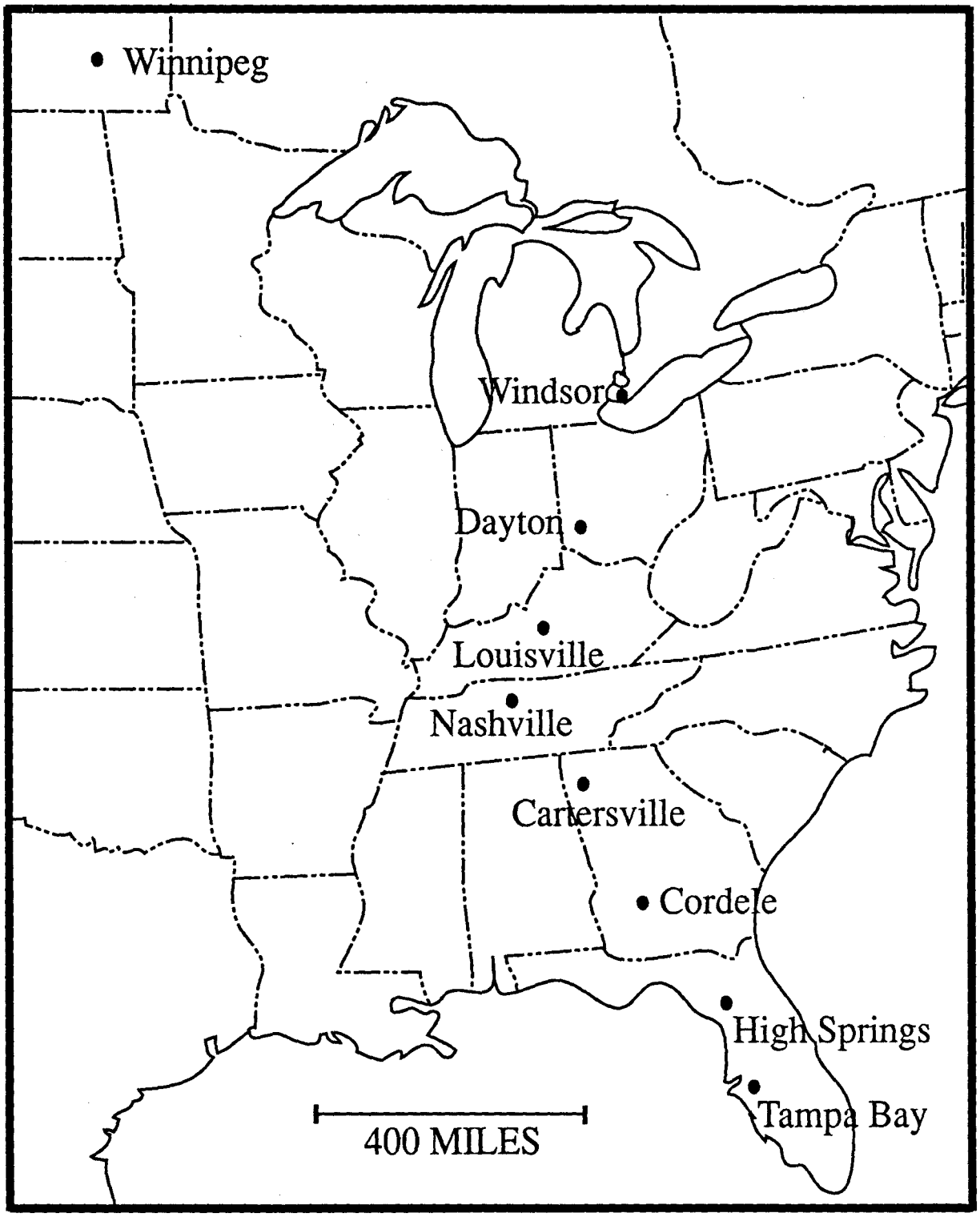
Table 2.1.4
Information Pertaining to the Collecting of Flies in Nature

Location	Site	lat (N)	long (W)	Date (1988)	Comments
Winnipeg, Man.	A	50.0	97.25	July 16	safeway food depot (onions)
	B				safeway (trap)
	C				Labatt's brewery on empties
Windsor, Ont.	A	42.0	83.0	Aug. 31	fruit stand
	B				pear trees
	C				KOA campsite
	D				barn at apple orchard
	E				near tomato field
Dayton, Ohio	A	40.25	84.25	Sept. 2	fruit stand
	B				commercial pear orchard
	C				sprayed commercial apple orchard
	D				KOA campsite
	E				private apple orchard
Louisville, Ken.	A	37.75	85.75	Sept. 4	apple tree
	B				KOA campsite
Nashville, Tenn.	A	36.00	86.75	Sept. 6	KOA campsite (hornets in trap)
	B				bushes
	C				bushes near shopping mall
Cartersville, Geo.	A	34.00	84.75	Sept. 8	apple tree
	B				apple tree in junkyard
	C				apple tree near campsite
	D				pear tree near site A
	E				grape vine in town
Cordele, Geo.	A	32.00	83.75	Sept. 10	peach orchard
	B				tree near church
	C	rainy weather			peach orchard (1 km from B)
	D				behind mall (not many *Dros.*)
	E				apple tree (not many *Dros.*)
High Springs, Fld.	A	29.75	82.5	Sept. 12	vines (very few)
	B	very hot and humid			blueberry patch
	C	with flooding			vines (not many *Dros.*)
	D				" "
Tampa Bay, Fld.	A	28.0	82.25	Sept. 14	orange grove (not many *Dros.*)
	B	very hot and humid			" "
	C	with flooding			" "
	D	recently sprayed?			" "

2.1.4 Location and Dates of Collection

A major objective of the field work entailed in this project was to establish a set of isofemale lines from regular intervals along a deliberately sampled North South cline. The

Figure 2.1.4
Map of Collection Sites



rational for this is discussed later. It follows that a number of lines were collected at approximately 300 km (200 mile or 2 degrees latitude) intervals along a transect from Windsor, Ontario to Tampa Bay, Florida that skirted to the West of the Appalachian mountains where possible. At each location traps were set two kilometres apart when possible to avoid sampling only one lineage in a given location. The collection took place over a relatively short time span to avoid seasonal effects. The sites, locations, and times of collection of the clinally collected flies are listed in table 2.1.4 and shown in figure 2.1.4.

2.2 Collection of Isofemale Lines (heritability)

2.2.1 Method of Collection

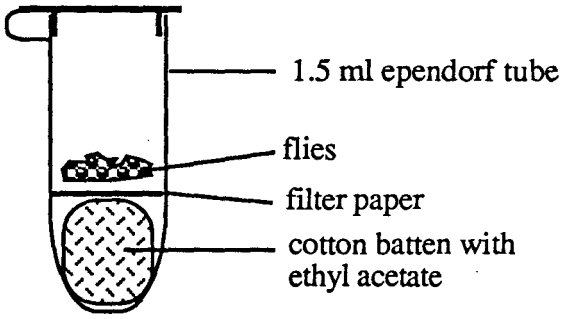
Flies were collected in a peach orchard on two occasions. On the first occasion they were collected as in 2.1.1, transferred *en masse* to bottles containing banana media, and soon after anesthetized and used to establish isofemale lines in the laboratory. In addition, a bottle containing approximately 200 individuals was maintained in a population cage. On the second occasion population levels were much higher and flies were collected by "sweeping" over fallen peaches, then transferring the flies to bottles and continuing as above. In both instances approximately 200 isofemales lines were established to use in the heritability study.

2.2.2 Preservation of Flies

The above collected flies were used in a breeding design with their offspring being preserved for later measurement. Flies collected from the mating design were placed in 1.5 ml epindorf tubes on top of filter paper which was directly on top of cotton batten containing approximately 3.0 µl of ethyl acetate (see figure 2.2.2). Generally between 2 and 20 same sex flies were placed in each vial prepared in this manner. In addition,

approximately 40 flies of mixed sex were placed in empty 15 ml epindorf tubes, frozen in liquid nitrogen, and stored at minus 70 C for later use.

Figure 2.2.2
Preservation Vials



2.2.3 Location and Date of Collections

The flies for this experiment were collected in a peach orchid at Vineland, Ontario. The first set of flies were collected on July 20/89 and the second set on Sept. 20/89.

2.3 Maintenance of Isofemale Lines

After their establishment isofemale lines were kept in 100 ml vials containing approximately 10 ml of banana media, being changed at 2-3 week intervals. The recipe for banana media can be found in table 2.3.1. The room in which the lines were maintained was generally kept between 18 and 26 C with approximately a 12 hour day/night cycle.

Table 2.3.1
Recipe for Banana Media

44.4	g	agar
4.0	l	water
3	tbsp	sugar
4	tbsp	corn syrup
3 - 4		small bananas
133.3	g	yeast

Boil agar and water. Mix remaining ingredients and add to boiling agar and water. Bring to a second boil and then allow to cool. When the media is 37 C add 80 ml tegosept (10 gm Methyl P-hydroxy Benzoate and 100 ml ethanol). Pour.

2.4 Measurement of Flies

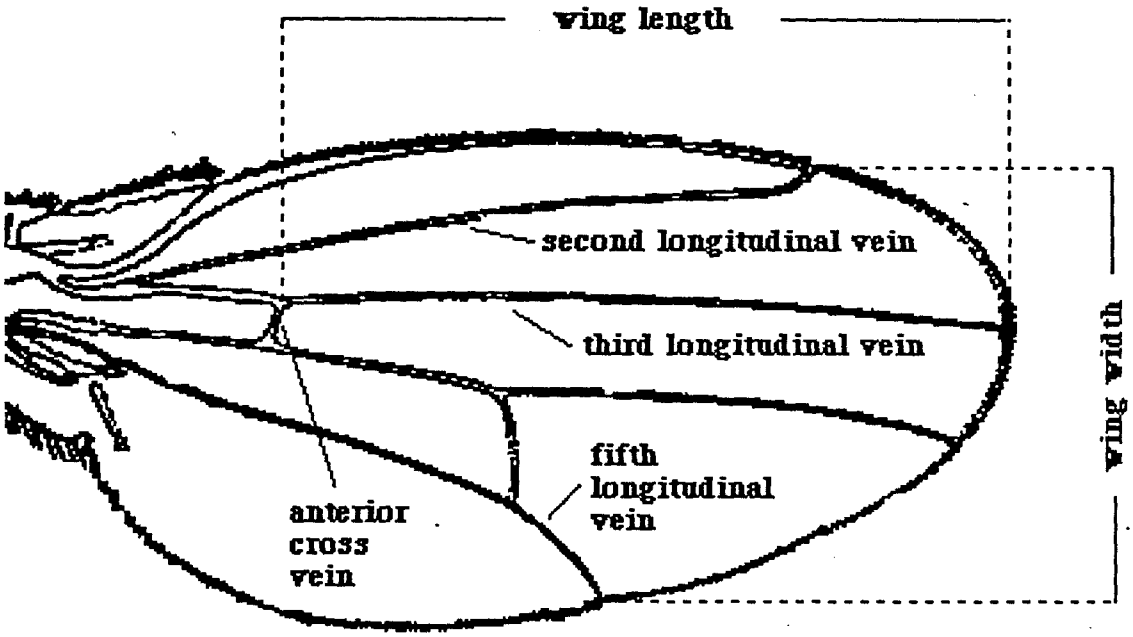
2.4.1 Characters Measured

On each fly 9 morphological characters were measured, with an additional sex limited trait being measured on male flies. If all the characters could not be measured on a given fly it was discarded from the study. The characters measured can be divided into four categories as described below. Taxonomic nomenclature is from Demerec (1965).

2.4.1.1 Wing Characters

Three characters in this study are derived from wing measures. The wing was dissected from the fly and placed beneath a cover slip on a slide such that the anterior (when extended or "in flight") part of the wing was upwards and the distal part of the wing was to the right (see figure 2.4.1.1). No preference was given to measuring the right or left wing as prior work has indicated differences between the right and left sides are small, nonheritable, and nonbiased (Long, unpublished). Given the positioning of the wing, and the bilateral symmetry of wings, it follows that when the right wing was measured the dorsal surface faced up, and when the left wing was measured the dorsal surface faced down. As the wings are semi-transparent, and the "veins" being measured dark, the above observation should be inconsequential with regards to the wing measurements. The first character measured was wing length, abbreviated throughout this work as WL or C1 (character 1). Wing length in this regard is defined as the distance from the intersection of the third longitudinal vein with the anterior cross-vein to the intersection of the third longitudinal vein with the edge of the wing (figure 2.4.1.1). Wing width, the second character measured, is similarly abbreviated WW or C2. Wing width is defined as the distance from the intersection of the fifth longitudinal vein with the wing edge to the intersection of the second longitudinal vein with the wing edge (figure 2.4.1.1). Wing

Figure 2.4.1.1
Details of Wing Characters

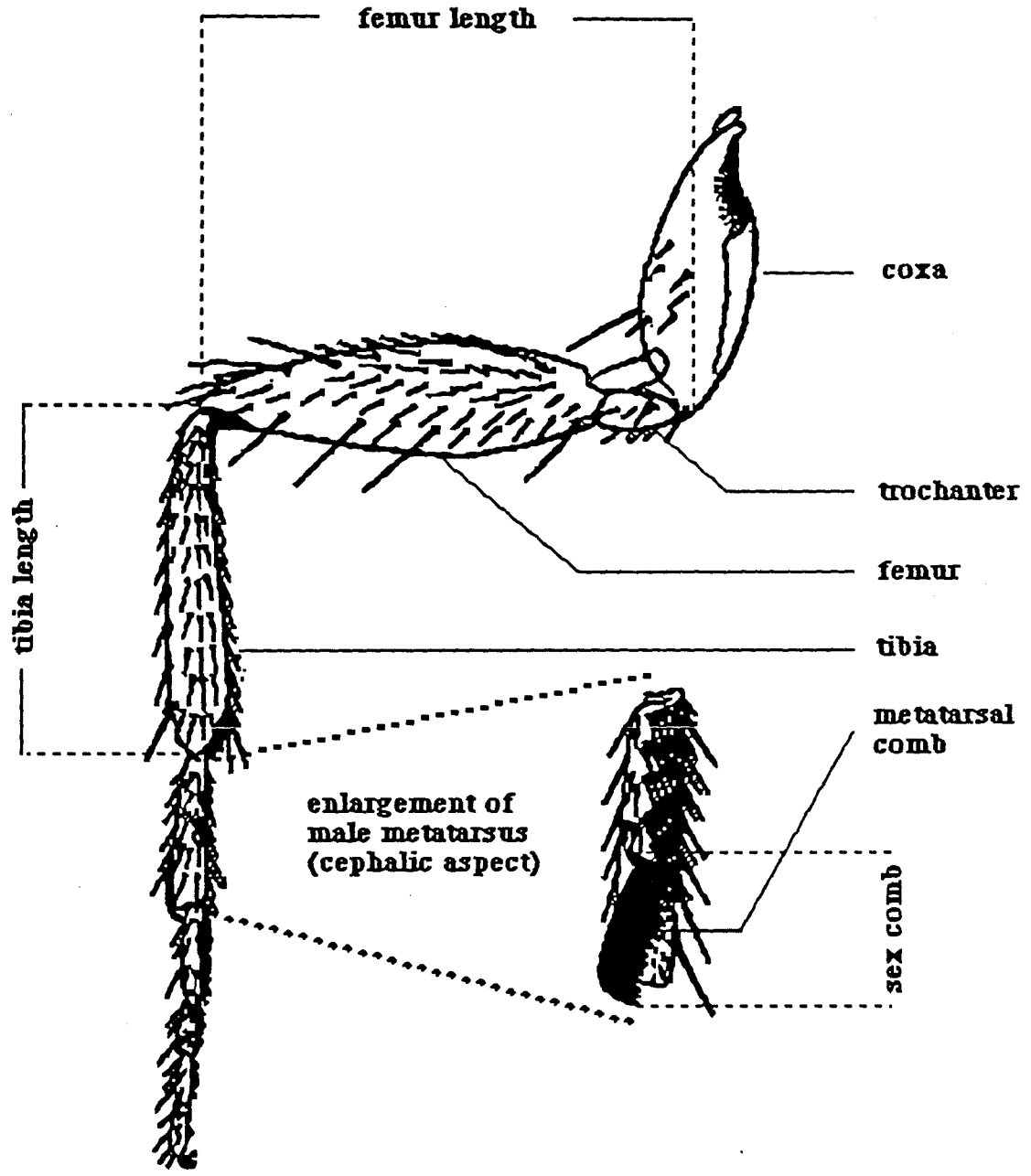


angle, WA or C10, is defined as the the upper right angle (in radians) between the lines prescribed by WL and WW (figure 2.4.1.1).

2.4.1.2 Foreleg Characters

The forelegs where dissected from the fly at or above the coxa. The leg was then positioned on the slide such that the caudal (outside) portion of the leg faced up. The coxa was further dissected from the fore leg above the trochanter. The femur, abbreviated Fem or C3, was then measured as the distance from the most proximal point on the trochanter to the most distal point on the femur. Similarly the tibia, Tib or C4, was measured as the distance from the last point marked above to the most distal part of the tibia (see figure 2.4.1.2). In males the fore leg was then flipped over so that the cephalic (inside) portion

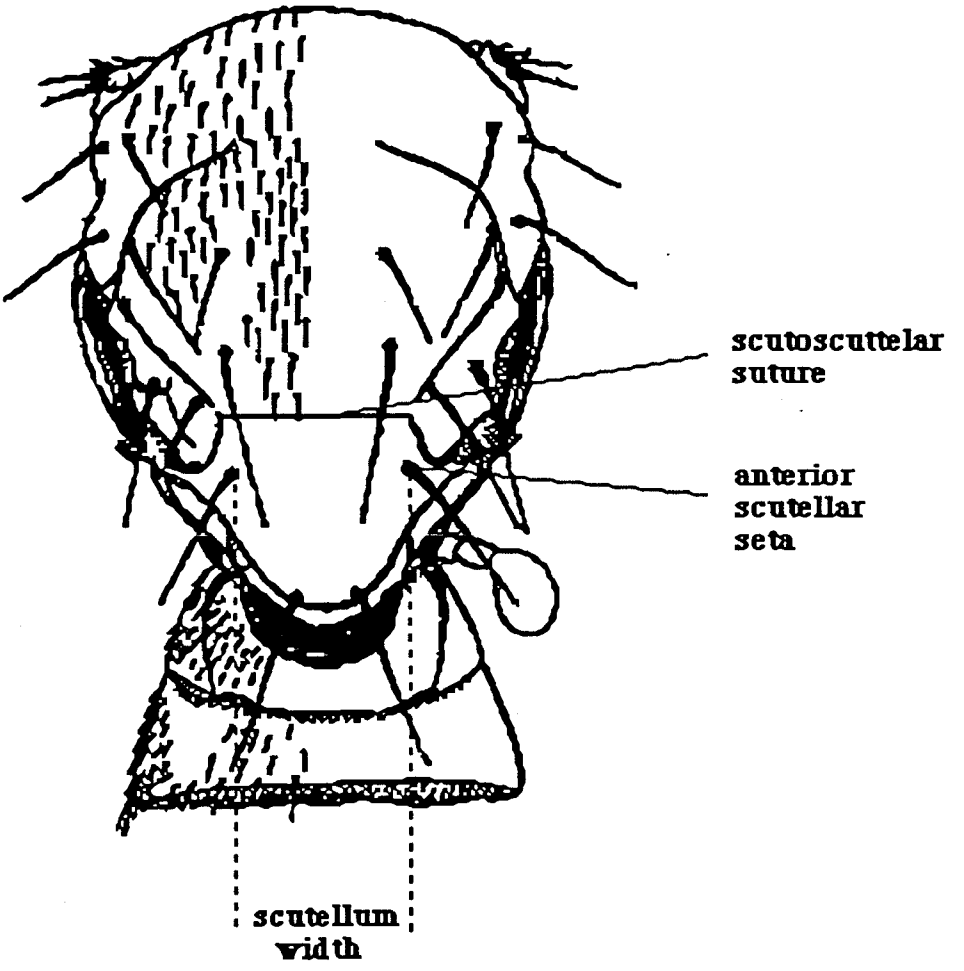
Figure 2.4.1.2
Details of Foreleg Characters
(left, caudal aspect)



was facing upwards and the lower portion of the foreleg was dissected off at the metatarsus/tibia intersection or on the lower part of the tibia. On males this allowed the

measurement of the metatarsus comb. The metatarsus comb, commonly referred to as the sex comb, is abbreviated sxcmb or C5 in the study. The sex comb was measured as the distance from the most proximal part of the comb to the most distal. As the comb "bristles" angle distally this always meant that the comb was measured diagonally from its proximal point, where it attaches to the metatarsus, to its distal, "free" end (see figure 2.4.1.2).

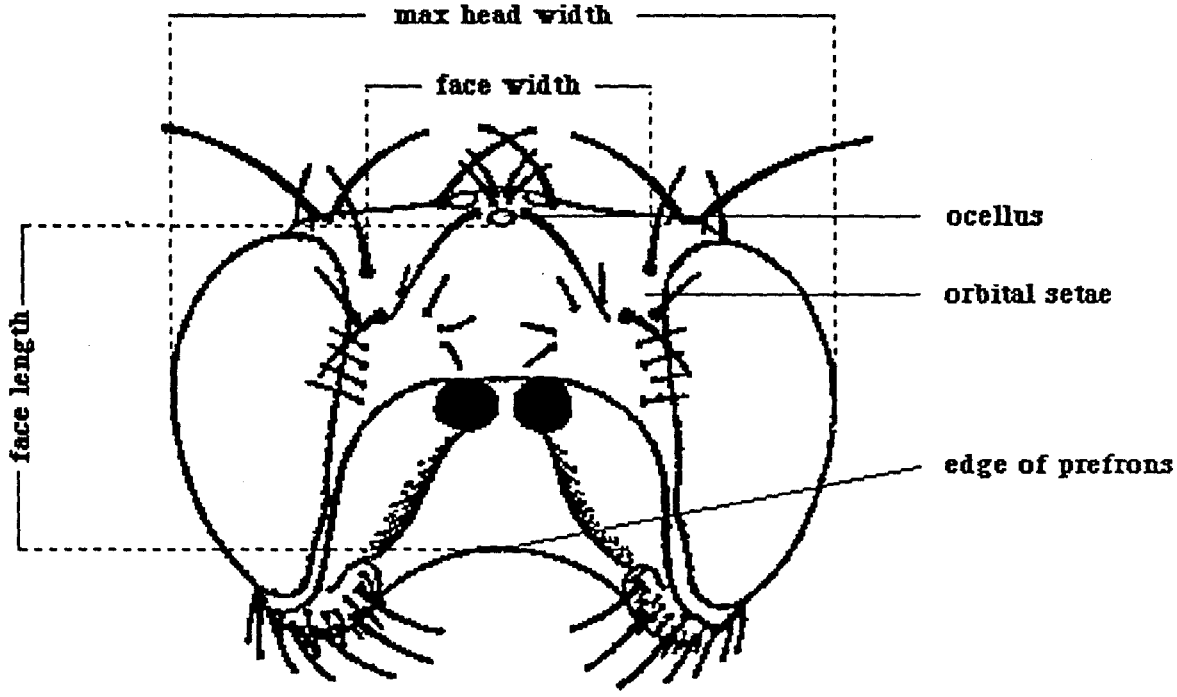
Figure 2.4.1.3
Details of Thoracic Characters



2.4.1.3 Thoracic Characters

Only one character was measured on the thorax, the width of the scutellum. The scutellum was dissected off along the scutoscutellar suture dorsally, and along the evagination where the scutellum and postscutellum or postnotum meet ventrally (figure 2.4.1.3). The scutellum, abbreviated Scut or C6, was then placed dorsal side up and measured as the distance between the two anterior scutellar setae.

Figure 2.4.1.4
Details of Head Characters



2.4.1.4 Head Characters

The head was dissected off the flies at the occipital foramen, being careful to obtain a clean cut so that the head would rest flat on its caudal surface. Face width, Fw or C7, was then measured as the distance between the paired dorsal most of the three principal orbital setae (see figure 2.4.1.4). The maximum width of the head was then measured along a line parallel to the face width measure. Face width was then subtracted

from the maximum head width to obtain eye width, abbreviated Eye or C8. Face length was finally measured as the distance between the ventral most point of the middle and ventral most of the three ocelli to the ventral edge of the face where then evagination begins that forms the mouth cavity (see figure 2.4.1.4). Face length, abbreviated Fl or C9, was measured along a line perpendicular to the face and eye width measures.

2.4.2 Measuring Equipment

Flies were dissected and measured on a glass side against a white background. All dissections and measurement were carried out under a Zeiss DRC stereomicroscope fitted with a 2X/4X/8X turret changer. The microscope was additionally fitted with a phototube DRC, and a Hitachi VK-C150 colour video camera. The camera was connected to a Commodore 1084 RGB/CVBS monitor in the CVBS mode via a VOCGA (Video Overlay/Colour Graphics Adapter) card housed in a long bus slot of a Laser 286X, IBM PC style, personal computer. The VOCGA card, available through K-systems of Texas, is a gen-lock card which allows an image generated by the computer to be overlaid on an image arriving from the microscope camera. It is important to note that the VOCGA card is not a frame grabber/digitizing card, but merely overlays a computer image on a camera image. Using version 2.0 of *VMP* (video measurement program, 1987) by D.R. McLaughlin of Texas, supplied by K-systems, points of interest were marked with a "mouse" controlled set of cross hairs and saved as a set Cartesian coordinates. As the *VMP* program only save points as Cartesian coordinates, two programs were written by T. Long of McMaster (1989) to convert these points to the desired measures. *CONVERT* changes points marked on clinal flies to appropriate sets of measures, with three additional coding columns intended for ANOVA software, and *HERMAT* performs similar operations on flies measured from a half-sib breeding design. The source code for *CONVERT* and *HERMAT* are provided on diskette.

2.4.3 Measurement Scale

The units used to measure characters 1 through 9 are arbitrary. The only constrain on the units of measure being that a distance measured in the x direction measure the same as the same distance in the y direction. This was not automatic in the *VMP* software, as it measures the position of a marked point in the pixel resolution of the monitor (eg; 100 pixels up, and 200 pixels to the right of 0,0 in the lower left corner), and pixels do not have the property described above. Thus any set of pixel coordinates were multiplied by an appropriate set of scaling constants (ie; (X,Y) transforms to (c_xX,c_yY)) to make the size of measured objects orientation independent. As the characters were to be log transformed to normalize the data and remove scaling effects, according to standard quantitative genetics practice, there was no need to have the raw measures on any true scale. To obtain measures of the mean and variance of the characters on a more familiar scale one can multiply the mean and variance on the arbitrary scale by c and c^2 respectively, where c is the ratio of distance measured on the desired scale to distance measured on the arbitrary scale.

2.5 Transformation of Raw Data

2.5.1 Transformation to a Linear Scale

Raw data were visually examined for outliers representing measurement errors for which the entire case was deleted. The measurements of wing length and wing width were initially transformed by multiplying by 2.011124 as these characters were measured at 40X and the other characters were measured at 80X. The constant 2.011124 was chosen by measuring 5 objects at both objective powers and calculating the mean value of the ratio of

Figure 2.5
Weighted Regression of St. Dev. on Mean
(size of circle is porportional to wieght)
(fill is different for each character)

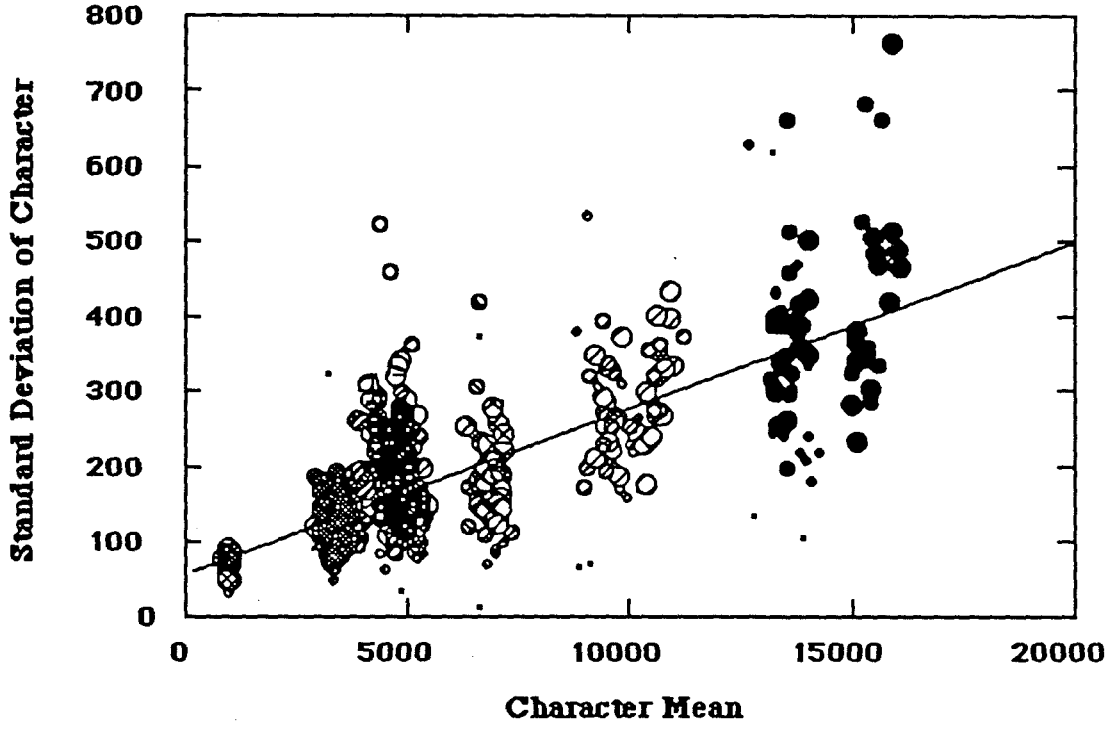


Table 2.5.1
ANOVA Table for Regression of St. Dev. on Mean Character

Variable	Coefficient	Std. Error	T	P
Constant	47.310	2.567	18.431	0.00
Mean	0.024	0.000	64.859	0.00

Source	SS	DF	MS	F	P
Regression	2.08 *E7	1	2.08 *E7	4206.653	0.00
Residual	1.24 *E7	2495	4958.53		

their sizes (s = 0.008092). A log transformation of the raw data was deemed necessary as the variance of the traits under consideration were a function of their mean (violating the homoscedasticity assumption for ANOVA) and the data were distributed in a log normal fashion. A suitable log transformation is X' = ln (X + a/b), where X is a value of a dependent variable in raw units, X' is the value of the same variable in transformed units, a and b are constants (Falconer, 1981). The natural logarithm will transform the variables to an additive scale and a suitable choice of constants (a and b) will decouple the relationship between the mean and variance of a given character. The constants were chosen from the weighted linear regression equation $\sqrt{n_{ijk}}\ \sigma_{ijk} = a + b\sqrt{n_{ijk}}\ \bar{x}_{ijk}$, where σ_{ij} is the standard deviation of the i^{th} trait in the j^{th} line of the k^{th} sex, $\bar{x}_{ij}$ is the mean of the i^{th} trait in the j^{th} line of the k^{th} sex, $\sqrt{n_{ijk}}$ is the square root of the sample size of the i^{th} trait in the j^{th} line of the k^{th} sex, a is the intercept, and b is the regression coefficient (Falconer,1981: pp.256-7). This regression is displayed in figure 2.5.1 and the ANOVA results are given in table 2.5.1. The values of a and b from this analysis were 47.310 and 0.024 respectively. This same values of a and b were used to transform the variables obtained from the heritability study.

2.5.2 Derivation of Four "new" Characters by PCA

The pearson product moment correlation matrix derived from the clinal data set is given in table 2.5.2 a for the 10 characters studied. As all the characters studied are highly correlated it was decided to create a new set of characters derived from the principle component scores of the ten characters studied excluding the sex comb and wing angle. Principle components are derived by solving $| S^2 - \lambda_i \mathbf{I} | = 0$ for λ_i, where **bold** type indicates a matrix, S^2 is a variance covariance matrix, **I** is the identity matrix, λ_i is the i^{th} characteristic root (or eigenvalue) of S^2, | x | denotes the determinant of x, and i = 1,2,...,n (where n is the rank of S^2, and **I** ,and designates the number of characteristic roots).

Table 2.5.2 a
Pearson Product Correlation Matrix for Clinal Flies
(all correlations based on 1486 observations except sxcmb (837))

	WL	WW	FEM	TIB	SCUT	FW	EYE	FL	SXCMB
WL	1.000								
WW	0.918	1.000							
FEM	0.694	0.689	1.000						
TIB	0.606	0.610	0.798	1.000					
SCUT	0.864	0.839	0.731	0.625	1.000				
FW	0.762	0.710	0.647	0.567	0.739	1.000			
EYE	0.669	0.693	0.603	0.533	0.698	0.566	1.000		
FL	0.655	0.648	0.490	0.431	0.618	0.553	0.524	1.000	
SXCMB	0.237	0.314	0.391	0.348	0.350	0.173	0.262	0.149	1.000

Table 2.5.2 b
Principal Component Analysis

EigenValues and Percentage of Variation

	1	2	3	4	5	6
λ	0.014567	0.001609	0.000912	0.000772	0.000595	0.000513
%	75.032580	8.285816	4.697510	3.976625		

Component Loadings

	1	2	3	4
WL	0.061941	0.006026	-0.011971	-0.007499
WW	0.052129	0.004706	-0.005723	-0.010239
FEM	0.024954	0.006369	0.005890	0.012083
TIB	0.020019	0.005486	0.006044	0.012319
SCUT	0.052465	0.008113	0.000729	0.002032
FW	0.034558	0.004524	-0.005459	0.015068
EYE	0.033749	0.004386	0.025129	-0.008664
FL	0.043633	-0.037068	0.001700	0.002639

Factor Score Coefficients

	1	2	3	4
WL	4.252028	3.745735	-13.126562	-9.713506
WW	3.578505	2.925616	-6.275705	-13.261708
FEM	1.713006	3.959402	6.458491	15.650906
TIB	1.374270	3.410186	6.627531	15.956088
SCUT	3.601593	5.043522	0.799495	2.632031
FW	2.372325	2.812512	-5.985574	19.517495
EYE	2.316785	2.726303	27.553884	-11.222257
FL	2.995261	-23.042553	1.864462	3.418509

Eigenvectors of S^2 are found by solving $(S^2 - \lambda_i \mathbf{I}) \mathbf{a}_i = 0$, where $\mathbf{a}_i$ is the eigenvector (rank n) corresponding to the i^{th} eigenvalue. A desirable property of eigenvectors is that they are orthogonal to one another. That is, component scores derived from the eigenvectors are independent of one another in the new space defined by these scores. This implies that analysis of component scores over untransformed scores is simplified as one can ignore the off diagonal structure of the component score SSCP matrices (as they are close to zero), and thus treat the component scores as a set of independent characters amenable to univariate analysis.

Usually eigenvalues and corresponding eigenvectors are arranged from largest to smallest so that λ_1 is the largest and λ_n the smallest. A second fortuitous quality of eigenvalues is that the λ_i^{th} eigenvalue equals the variance of the i^{th} component score. If characters are highly correlated usually the first few eigenvalues account for most of the variation in the original S^2 matrix. Analysing component scores can thus reduce the dimensionality of the problem under consideration.

Component scores can be derived by multiplying the raw data matrix (n rows corresponding to sample size by p columns corresponding to characters) by the first few eigenvectors (m column vectors each of order p). This will give m new characters columns with each value in the column equal to a linear combination of the raw values corresponding to the same case. M is chosen somewhat arbitrarily, eigenvectors corresponding to eigenvalues that account for very little of the variation in S^2 being dropped from the analysis. Thus $Y_i = \mathbf{X}\mathbf{a}_i$,where Y_i is a scalar corresponding to the i^{th} component score for a given case, $\mathbf{X}$ is the row vector of the original data (X1,X2,...,Xn), and $\mathbf{a}_i$ is the i^{th} eigenvector (column). For a more complete review of principle component analysis see R. Pimentel (1979) or C. Chatfield and A.J. Collins (1980).

The clinal data were subjected to PCA. Table 2.5.2 b gives the factor score coefficients, the variation accounted for by each factor, and the percentage of variation accounted for by each factor. A given column of factor score coefficients can be considered as the eigenvector associated with a given factor, although they include a term to transform the factor scores to unit variance. The variation accounted for by each factor is, of course, equal to its given eigenvalue. One can additionally see from the table that the first four factors accounted for 92 percent of the variation in the model, with the first factor accounting for 75 percent of the total variation.

2.6 Clinal Flies

2.6.1 Experimental Design

An experiment was carried out to measure clinal variation in lab reared flies. From each of a number of the isofemale lines collected along the cline 10 virgin females and 10 males were chosen and mass mated for approximately 12 hours in culture jars containing 40 ml of banana media. The parents were then removed from the jars and eggs were removed from each jar under a dissecting scope so that only approximately 80 eggs remained. It was decided to use virgin females from the isofemale lines to remove the known effect of maternal age on quantitative variation and egg density was controlled for similar reasons (Ashburner, 1989 : pp. 192-194). The jars were cultured with a 12 hour day/night cycle at a constant temperature of 24 C, as temperature can also influence adult body size (Ashburner, 1989: pp. 193). Offspring were collected from the jars on day 12 and preserved in the fly "highrise" described in 2.1.3. As it is probable that isofemale lines maintained in the lab are inadvertently selected for quantitative traits, the above experiment was carried out as soon as possible after the lines had been established in the lab. Nonetheless, this time was significant in some cases as some of the samples were misplaced by the postal carrier for close to a month. All flies (except Winnipeg) were

Table 2.6.1
Details of Lines Used in the Clinal Study

Location	N	Date: Collect	Cross	Sample	Line Name Study	Stock	Study	Stock
(1) **Winnipeg**	82	July 16	Aug. 8	Aug. 19	1	C 20	3	C 26
					2	C 23	4	C 29
(2) **Windsor**	50	Aug. 31	Sept. 21	Oct. 2	1	D 2	3	C 3
					2	A 1		
(3) **Dayton**	66	Sept. 2	Oct. 1	Oct. 12	1	E 5	6	B 1
					2	D 3	7	D 2
					3	B 2	8	E 4
					4	A 1	9	B 4
					5	E 1		
(4) **Louisville**	216	Sept. 4	Nov. 13	Nov. 28	1	C 2	6	B 3
					2	D 4	7	D 1
					3	C 4	8	E 1
					4	B 1	9	D 2
					5	C 1		
(5) **Nashville**	50	Sept. 6	Nov. 12	Nov. 28	1	B 6	4	B 4
					2	A 3	5	B 8
					3	B 5		
(6) **Cartersville**	145	Sept. 8	Nov. 12	Nov. 28	1	E 2	4	D 3
					2	C 2	5	B 3
					3	F 1	6	E 1
(7) **Cordele**	155	Sept. 10	Oct. 1	Oct. 13	1	B 2	5	D 1
					2	B 3	6	C 3
					3	E 4	7	C 1
					4	D 4	8	D 3
(8) **High Springs**	70	Sept. 10	Oct. 5	Oct. 17	1	A 5	4	A 7
					2	A 6	5	B 2
					3	A 3	6	A 1
(9) **Tampa Bay**	3	Sept. 14	Oct. 5	Oct. 17	1	D 1		

collected in the last week of August and first week of September of 1988 and the last set of crosses were carried out on November 13/89. This implies that some of the flies used in the experiment had been maintained in an isofemale line for several generations. Table 2.6.1 a lists the lines used in the study, the dates which flies were collected from nature, the date at which the crosses took place, and the date the flies were collected from these crosses (all dates are in 1988).

2.6.2 Statistical Model

Although initially flies of both sexes were measured it was eventually decided to concentrate on male flies only, as the heritability martrix was only going to be estimated for males and measurements were not obtained for female flies from all positions in the cline. Flies were analysed using the General Linear Model (GLM) procedure in SAS with Tampa Bay flies dropped from the analysis because of small sample size. It was decided to use the general linear model to analyse the data as the number of lines within each position and the number of flies measured within each line were unbalanced. The model used to analyse the flies was $Y_{ijk} = P_i + L_{j(i)} + \varepsilon_{ijk}$,where Y_{ijk} is a dependent measure, P_i is an effect due to the i^{th} position, $L_{j(i)}$ is an effect due to the j^{th} line nested in the i^{th} position, and ε_{ijk} is the error associated with the k^{th} fly in the j^{th} line in the i^{th} position. This model was tested for the first four principal components, all ten characters, and a multivariate composite of all of the above. The multivariate test used was that of an F- approximation based on Wilk's criterion. Wilk's criterion is based on a likelihood ratio of the determinate of the error matrix over the determinate of the error plus the hypothesis matrix, that is;

$$\Lambda = \frac{|\,E\,|}{|\,H + E\,|} = \prod_{i=1}^{p} \lambda_i$$

where p is the rank of the matrices (the number of variables) and the λ_i's are given by;

$$|\,R\,(H + R)^{-1} - \lambda\, I\,| = 0$$

(the eigenvalues of the first term). Given the null hypothesis an F statistic can be derived as

$$F = \frac{1-\Lambda}{\Lambda} * \frac{df_E + df_H - p}{p}$$

with p and $df_E + df_H - p$ degrees of freedom (df_x is the degrees of freedom of effect x). For a more complete discussion of multivariate tests of hypothesis see Chatfield and Collins (1980).

As the General Linear Model was used to analyse the data we can additionally construct contrasts to determine if specific sets of populations differ from one another. That is, although the parameter estimates from the general linear model are not unique, linear functions of the parameter estimates are unique (SAS, 1986; Searle, 1971). For example testing the difference between the means of position 1 and 2 is the same as testing if $-1*\mu_1 + 1*\mu_2 + 0*\mu_3 + ... + 0*\mu_n = 0$, similarly testing for a difference between the means of groups 1 plus 2 and groups 3 plus 4 is the same as testing if $-1*\mu_1 -1*\mu_2 +1*\mu_3 +1*\mu_4 +0*\mu_5 + ... +0*\mu_n = 0$. A sum of squares and mean square is calculated for a specific contrast and tested with an appropriate mean square error (line within position in this case). It is important to note that in order to maintain an alpha level equal to that of the entire design, contrasts should be tested against an alpha equal to the overall alpha level divided by the number of contrasts carried out. Multivariate contrasts can be constructed in an analogous manner and tested as above with Wilk's criterion.

2.7 Heritability Flies

2.7.1 Experimental Design

An experiment was carried out to estimate the heritabilities, and the genetic covariance matrix of the traits examined in the study. Virgin female flies were collected from isofemale lines which had been in the lab for less than a month derived from flies

caught in Vineland, Ontario. Females collected in this manner, each from a different isofemale line, were then individually mated to males collected from the large synthetic population maintained in the lab. The pair were allowed to mate and lay eggs in a 300 ml jar containing approximately 50 ml of media over a three day period. The parental flies were then anesthetized, the female discarded, and the male allowed to mate with a second female (collected in the same manner as the first) for an additional three days before both the male and second female were discarded. Eighteen days after the male and female were introduced to a given jar the offspring were collected, anesthetized, and preserved in small vials as described in section 2.2.2. All matings and culturing of flies were carried out at a controlled temperature and with a 12 hour day/night cycle. Four experimental replicates were carried out in this manner. The flies collected in July were used in experiments one and two, and the flies collected in September were used in experiments three and four. In addition, the flies from experiment two were measured by a technician different than those in the other three experiments.

2.7.2 Statistical Model

The experimental design described above is that of a classical half-sib design. In this design the covariance between half-sibs (flies with the same sire, but different dams) is equal to one quarter of the genetic covariance of the trait (Falconer, 1980). As with the clinal flies it was decided to use the General Linear Model to estimate variance components. This decision was made as the design was unbalanced due to general sampling errors (eg; death of flies, no offspring, etc.), in addition to being intentionally unbalanced at the level of the progeny in order to 'stack' the degrees of freedom up to the level of the sire. That is, within each paternal family three flies were measured, two from one dam and one from the other (Bainbridge, 1963; Cowley *et al.*, 1986; Cowley and Atchley, 1988). It follows that the design is balanced at the level of the sire but unbalanced at the level of the dam. The

variance is partitioned such that the deviation of a measure from the grand mean can be explained in terms components of the design:

$$y_{ijkl} = \mu + exp_i + sire_{ij} + dam_{ijk} + \varepsilon_{ijkl}$$

where y_{ijkl} is an observation, exp_i is the i^{th} experiment (i = 1 to 4), $sire_{ij}$ is the j^{th} sire nested in the i^{th} experiment, dam_{ijk} is the k^{th} dam nested in the j^{th} sire, and ε_{ijkl}, which can be considered the error term, is the l^{th} progeny nested in the k^{th} dam. The general linear model is $\mathbf{y} = \mathbf{Xb} + \varepsilon$, where $\mathbf{y}$ is a column vector of observations, $\mathbf{X}$ is a design matrix, $\mathbf{b}$ is the row vector of regression coefficients corresponding to the variables in the design matrix, and ε is the deviation of each y from its predicted value. With nested designs the number of parameters (or dummy variables) that b's must be estimated for can be very large. For example, in the above design a column must be specified in the design matrix for each dam, sire, and experiment (plus an addition column of 1's for the mean)(this is over 1,000 columns). As the solution to the general linear model is

$$\hat{\mathbf{b}} = (\mathbf{X}^T\mathbf{X})^{-1}\,\mathbf{X}^T\mathbf{y}$$

this design could not be analysed, as is, by the General Linear Model procedure of SAS, as the design matrix (X) is too large for $\mathbf{X}^T\mathbf{X}$ to be inverted. For this reason it was decided to analyse each experiment separately and then combine the estimated variance components. This is possible as the design is completely nested such that the experiments are independent of one another. Thus the reduced model

$$y_{ijkl} = \mu_i + sire_{ij} + dam_{ijk} + \varepsilon_{ijkl}$$

was fitted for each experiment, where μ_i is the grand mean of the i^{th} experiment.

Expected Mean Squares were calculated for each experiment with the **VARCOMP** procedure in SAS. These components have the form :

Source	SS	df	MS	EMS
sire	SS_s	#sires - 1	MSE_s	$\sigma^2_E + k'_1\sigma^2_D + k_2\sigma^2_S$
dam (sire)	SS_D	(#dams per sire -1)*#sires	MSE_D	$\sigma^2_E + k_1\sigma^2_D$
error	SS_E	$N - df_s - df_d$	MSE_E	σ^2_E

The terms in this chart should be self explanatory. Normally $k_1 = k'_1$ equals the harmonic mean of the number of offspring per dam and k_2 is equal to $k_1$times the number of dams per sire. But when the design is unbalanced the calculation of these components is more complex; exact solutions are provided by the **VARCOMP** procedure in SAS and formulae are given in Searle (1971; pp. 475-7). It is now simple to estimate the variance due to each component in the design by equating observed mean squares to expected mean squares:

$\hat{\sigma}^2_E = MSE_E$

$\hat{\sigma}^2_D = (MSE_D - MSE_E)/k_1$

$\hat{\sigma}^2_S = (MSE_S - MSE_E - k'_1 * \hat{\sigma}^2_D)/k_2$

As all the k's and df's are scalars and the experimental design is the same for all the characters these formulae apply equally well to estimating the variance/covariance matrix due to each source of variation. In this case one simply replaces the sum of squares for each level of the design with the Sum of Squares Cross Product matrix (SSCP) associated with the same level. The variance components from experiments 1,3, and 4 were averaged together (weighted by total number of observations) to obtain a better estimate of the variance due to a given design component. Experiment two's data was dropped as the

variance due to sires appeared inaccurate relative to the other measures. This was probably a result of the large number of missing cells within this experimental block.

2.7.3 Estimation of Genetic Components

The purpose of the above described design was to estimate the causal components (in Falconer's sense) which resulted in the observed variance components (design components). Table 2.7.3, taken from Falconer (1980), gives the covariance and causal components measured relative to the observed variance components.

Table 2.7.3
Observed and Causal Components of the Half-Sib Experimental Design

	COMPONENT:		
Observational		**Covariance**	**Causal**
Sires:	$\sigma_S^2 =$	cov_{HS}	$= \frac{1}{4} V_A$
Dams:	$\sigma_D^2 =$	$cov_{FS} - cov_{HS}$	$= \frac{1}{4} V_A + \frac{1}{4} V_D + V_{Ec}$
Progenies:	$\sigma_E^2 =$	$V_P - cov_{FS}$	$= \frac{1}{2} V_A + \frac{3}{4} V_D + V_{Ew}$
Total: $\sigma_T^2 = \sigma_s^2 + \sigma_D^2 + \sigma_E^2 =$		V_P	$= V_A + V_D + V_{Ec} + V_{Ew}$
Sires + Dams: $\sigma_s^2 + \sigma_D^2 =$		cov_{FS}	$= \frac{1}{2} V_A + \frac{1}{4} V_D + V_{Ec}$

In the above table the subscript HS refers to Half-sib, FS to Full-sib, A to additive genetic, D to dominant genetic, Ec to environmental effects common to full-sib family, and Ew to environmental deviations that are progeny specific within a full sib family. As full sibs in my design were raised in a common jar, it is clear from the above table that the Variance due to dominant genetic effects cannot be estimated as it will always be coupled with any Environmental variance common to full sib families. Thus it is logical to measure the additive genetic variance as simply $4 * \sigma_s^2$. In addition, the degree to which the variance

due to dams is larger than the variance due to sires will reflect primarily an effect due to common rearing bottle.

2.7.4 'Bending' of Genetic Covariance Matrices

A common problem in estimating genetic covariance matrices from 4 * σ_s^2 matrix is that often times the σ_s^2 matrix contains cells with impossible (usually negative or greater than $\pm$ 0.25 * σ_T^2) variance (or covariance) estimates. In order for the above matrix not to contain impossible parameter estimates all the eigenvalues of the matrix must be positive (this way all linear combinations of the parameters are positive). Thus the probability of a matrix not containing impossible parameter estimates is the probability of the same matrix being positive definite (all positive roots) or at least positive semi-definite (positive and zero roots). This problem is especially acute if a large number of characters are measured, the number of half-sib families is small, and/or trait heritabilities are low (Hill and Thompson, 1978). The reason that the σ_s^2 contains impossible roots is that, although the sum of the eigenvalues is unbiased, they are over dispersed. That is, large eigenvalues are biased upwards and small eigenvalues biased downwards (Hill and Thompson, 1978; Hayes and Hill, 1980; Hayes and Hill, 1981). As we are interested in constructing selection indices we wish to insure that we have accurate estimates of $\mathbf{P^{-1}G}$, that is we wish to insure that the ratio of genotypic to phenotypic variances is within its proper bounds. This is accomplished by solving the equation $| \mathbf{P^{-1}G} - \lambda \mathbf{I} | = 0$ for λ (ie; finding the eigenvalues), where **P** is the phenotypic covariance matrix and **G** is the genotypic covariance matrix. Hayes and Hill (1981) then suggest 'bending' $\mathbf{P^{-1}G}$ so that the smallest root is non-negative (ie; the whole thing is positive definite). That is, one can compute a modified $(\mathbf{P^{-1}G})^*$ such that

$$(\mathbf{P^{-1}G})^* = (1-\gamma)\mathbf{P^{-1}G} + \gamma \bar{\lambda} \mathbf{I}, \qquad 0 < \gamma < 1 \qquad (2.7.4.1)$$

where $\bar{\lambda}$ is the mean eigenvalue, and γ is the bending factor chosen so that the smallest root of $(\mathbf{P}^{-1}\mathbf{G})^*$ is non-negative. In the present study the smallest possible γ was chosen that resulted in $(\mathbf{P}^{-1}\mathbf{G})^*$ being positive definite. This was accomplished by evaluating 2.7.4.1 for a series of γ incremented by 0.05. As it is assumed **P** is estimated more accurately than **G**, only **G** was modified once γ was decided upon. The 'bent' genotypic covariance matrix is thus given as

$$\mathbf{G}^* = (1-\gamma)\mathbf{G} + \gamma\bar{\lambda}\,\mathbf{P}\,.$$

A new matrix corresponding to the genotypic covariance matrix as described in section 2.7.3 was then estimated with $\mathbf{G}^*$ replacing **G**. A complete discussion of bending can be found in Hayes and Hill (1981), and an example of bending used to correct additive genetic variance/covariance matrices can be found in Lofvold (1988).

2.8 The Estimation of Natural Selection in the Wild

2.8.1 Estimation of Selection Coefficients from the Change in Mean Phenotype

One of the fundamental relationships in quantitative genetics is that

$$\Delta\bar{z}_b = h_b^2 S_b = (V_{A(b)} / V_{T(b)})\, S_b \qquad (2.8.1.1)$$

where $\Delta\bar{z}_b$ is the change in the mean phenotype of character b after one generation of selection, h_b^2 is the narrow sense heritability of b (which is equal to the additive genetic variance over the total variance of the trait), and S_b is the selection differential (difference in means between selected and unselected adults) in the parental generation (Falconer, 1980; Lande, 1979). If we consider a second character, i, that is correlated with b we can predict the response of character i to selection on character b:

$$\Delta\bar{z}_i = (V_{A(ib)} / V_{T(b)})\, S_b \qquad (2.8.1.2)$$

where $V_{A(ib)}$ is the additive genetic covariance of characters i and b (Falconer, 1980). The above equation is true if: 1) there is no genotype environment correlation, 2) there is a linear regression of the additive genetic values of characters i and b on the phenotypic values of b (ie; i and b are multivariate normal distributed), and 3) phenotypic variances are nearly constant, that is the phenotypic variances are not a function of $\bar{z}_i$ and $\bar{z}_b$ (Lande, 1979). Lande (1979) extends the results of 2.8.1.2 to descibe the nature of evolution on any number of characters by providing a matrix formulation that describes the change in a vector of mean phenotypes. Lande's formulation for the change in a vector of mean phenotypes is

$$\Delta\bar{\mathbf{z}} = \mathbf{G}\mathbf{P}^{-1}\mathbf{S} = \mathbf{G}\,\mathbf{V}\ln\bar{W} \qquad (2.8.1.3)$$

where $\Delta\bar{\mathbf{z}}$ is a column vector (order equal to the number of characters) describing the change in the mean of the characters, **G** is the additive genetic covariance matrix, **P** is the phenotypic matrix, **S** is a column vector describing the selection differentials of the traits, and **V** is a column vector gradient operator. That is, **V** ln $\bar{W}$ describes the change in mean fitness with respect to each character, where the gradient operator is described as

$$\mathbf{V} = \left[\frac{\delta}{\delta\,\bar{z}_1}, \ldots, \frac{\delta}{\delta\,\bar{z}_n}\right]^T.$$

In the above equations any S_i includes genetic gains from both selection on character i and the correlated response of the other characters studied, whereas $\dfrac{\delta \ln \bar{W}}{\delta\,\bar{z}_i}$ is the change in Malthusian mean fitness of character i due to a small change in $\bar{z}_i$ holding fixed the change in the other characters (Lande, 1979). The implications to phenotypic evolution of the genetic contraints imposed by 2.8.1.3 have already been discussed.

2.8.2 Estimating the Net Selection Gradient and the Selection Index

If one can assume the genetic covariance matrix has remained relatively constant over time and that the divergence of any two populations is due to selection then the *net selection gradient* between two populations (i and j) can be estimated as;

$$\sum_{0}^{2t-1} \mathbf{V} \ln \bar{W} = \mathbf{G}^{-1} [\bar{z}_i - \bar{z}_j] \qquad (2.8.2.1)$$

where t is the number of generations since the two populations have diverged (Lande, 1979; with modification). Given the change in mean phenotype and phenotypic covariance matrices from the clinal flies, and using the genetic covariance matrix derived from the heritability study it was possible to measure the net selection gradient and the net selection differential for each adjacent pair of populations along the cline. Additionally, a selection index similar to those used in animal breeding can be constructed which weights the characters by the force of selection acting on them. This will, in effect, transform selection on a multivariate phenotype to selection on a univariate index. The index is given as $I = (\mathbf{V} \ln \bar{W})^T \Delta \bar{z}$ (Lande, 1979; Lin, 1978). The truncation point which gives the intensity of natural selection required to produce the observed change is given as;

$$b = \pm \sqrt{-2 \ln \left(\sqrt{2\pi} \frac{|I| \sqrt{P_{II}}}{G_{II}(2t-1)} \right)} \qquad (2.8.2.2)$$

where $P_{II} = (\mathbf{V} \ln \bar{W})^T \mathbf{P} (\mathbf{V} \ln \bar{W})$, and $G_{II} = (\mathbf{V} \ln \bar{W})^T \mathbf{G} (\mathbf{V} \ln \bar{W})$ (Lande, 1976; Lofvold, 1988). The proportion of the population that must be eliminated each generation (ie; truncated selection) in order to give the observed phenotypic divergence can be found in a standard normal table, or if b is much greater than 1 as

$$Q(b) = b^{-1} \phi(b) [1 - b^{-2} + 3b^{-4} \ldots]$$

where $\phi(b) = (1/\sqrt{2\pi}) \exp(-b^2/2)$ (Lofvold, 1988). Molecular data can be used to estimate times since divergence for adjacent pairs of populations and estimate the average selection gradient per generation and reconstruct the intensity of selection acting in nature.

2.8.3 Estimating the Direct and Correlated Response to Selection

Falconer (1981) gives equations for the response;

$$R_x = i\, h_x\, \sigma_{Ax} = i\, h_x^2\, \sigma_{Px} \qquad (2.8.3.1)$$

and correlated response to selection;

$$CR_x = i\, h_x\, h_y\, r_A\, \sigma_{Px} \qquad (2.8.3.2)$$

where i is the intensity of selection on character x in 2.8.3.1 and on y in 2.8.3.2, r_A is the genetic correlation between characters x and y, and σ_{Px} is the phenotypic standard deviation of character x. Furthermore;

$$r_A = \frac{cov_{Axy}}{\sigma_{Ax}\, \sigma_{Ay}}, \quad \text{therefore} \quad cov_{Axy} = r_A\, \sigma_{Ax}\, \sigma_{Ay} \qquad (2.8.3.3)$$

and

$$r_p = \frac{cov_{Pxy}}{\sigma_{Px}\, \sigma_{Py}}, \quad \text{therefore} \quad cov_{Pxy} = r_p\, \sigma_{Px}\, \sigma_{Py}. \qquad (2.8.3.4)$$

In addition, the 'coheritability' used later in this study is equal to;

$$h^2_{xy} = \frac{cov_{Axy}}{cov_{Pxy}} = \frac{r_A\, \sigma_{Ax}\, \sigma_{Ay}}{r_p\, \sigma_{Px}\, \sigma_{Py}} = \frac{r_A}{r_p} h_x\, h_y, \qquad (2.8.3.5)$$

therefore

$$r_A = \frac{h^2_{xy}}{h_x\, h_y} r_p. \qquad (2.8.3.6)$$

Finally by substituting 2.8.3.4 (r_p) into 2.8.3.6, and this result into 2.8.3.2 it is obvious that;

$$CR_x = i\, h^2_{xy}\, cov_{Pxy} / \sigma_{Py}. \qquad (2.8.3.7)$$

Using 2.8.3.1 and 2.8.3.7 the total response of trait x to selection is equal to;

$$TR_x = i\, h_x^2\, \sigma_{Px} + i\, h^2_{xy}\, cov_{Pxy} / \sigma_{Py} + i\, h^2_{xz}\, cov_{Pxz} / \sigma_{Pz} + \ldots \qquad (2.8.3.8)$$

Of interest in this study is the relative strengths of direct and correlated response to selection, thus we can substitute the net selection gradient on each character for the intensity

of selection, and if we additionally assume the phenotypic variances of the characters are equal (they should be close due to the scaling procedure used) we can remove and ignore a common factor of $1/\sigma_{Px}$. It follows that the total unit respose is proportional to;

$$TUR_x \ \alpha \ V_x \ln \bar{W} \, h_x^2 \sigma_{Px}^2 + V_y \ln \bar{W} \ h_{xy}^2 \ cov_{Pxy} + V_z \ln \bar{W} \ h_{xz}^2 \ cov_{Pxz} + \ldots \quad (2.8.3.9)$$

Later use will be made of 2.8.3.9 as it allows one to determine the relative importance of the direct and correlated response to selection on any given character.

Chapter 3
Results

3.1 Tests of Phenotypic Differences Along the Cline

Table 3.1 a gives F-statistics corresponding to a number of significance tests of phenotypic variation among male flies along the cline of this study as described in section 2.6.2. The row named total refers to the ANOVA on the total differentiation along the cline. This statistic was tested with 7 degrees of freedom in the numerator and 42 degrees of freedom in the denominator. A contrast between two positions is a test of the significance of the difference in means between adjacent populations. A contrast involving two positions against two other positions (eg; 12 vrs 34) is a test of the significance of the difference between the mean of the first two populations against the mean of the second two (see section 2.6.2). All such univariate contrasts were tested using the total error due to line within position and were thus tested with 1 and 42 degrees of freedom respectively in the numerator and denominator. The multivariate test of significance of the overall difference along the cline was tested with 98 and 192.33 degrees of freedom in the numerator and denominator and multivariate contrasts were tested with 14 and 29 degrees of freedom (see section 2.6.2). The results of the multivariate tests of significance are given in the column of table 3.1 a labelled M.

Table 3.1 b gives p-values corresponding to the F-values of table 3.1 a. As table 3.1 b presents close to 200 p-values it is important to adopt a low p-value to indicate significance in order to reduce the overall likelihood of obtaining a false positive. A

Table 3.1 a
F-Values for Various Contrasts

Contrast	F1	F2	F3	F4	C1	C2	C3	C4
total	10.22	7.22	5.47	3.94	5.83	6.70	16.08	11.90
1 vrs 2	8.62	3.25	8.91	1.72	1.63	4.94	17.75	20.58
2 vrs 3	1.43	1.62	0.56	1.18	0.22	0.31	6.91	1.92
3 vrs 4	0.24	0.92	3.35	0.03	1.90	0.00	2.04	2.94
4 vrs 5	8.19	0.54	4.91	0.15	2.39	9.81	11.80	17.61
5 vrs 6	14.63	0.40	5.84	0.65	3.29	24.29	11.66	14.23
6 vrs 7	3.91	15.22	0.23	12.99	5.27	0.16	7.92	3.24
7 vrs 8	0.56	0.18	13.38	3.10	2.66	0.68	1.98	0.25
12 vrs 34	20.62	16.96	5.37	7.23	6.99	7.04	44.36	21.52
23 vrs 45	10.89	2.99	0.09	0.75	9.08	5.90	5.88	2.44
34 vrs 56	2.36	0.01	0.00	0.98	3.19	0.66	1.68	3.59
45 vrs 67	27.98	3.42	6.54	0.23	10.01	30.36	21.51	16.18
56 vrs 78	17.59	10.19	0.13	3.36	22.62	13.60	18.37	17.24
	C5	C6	C7	C8	C9	C10	M	
total	9.25	18.33	6.52	5.09	1.42	1.64	3.05	
1 vrs 2	5.19	9.67	0.72	7.01	0.24	2.80	3.05	
2 vrs 3	7.93	7.03	0.04	0.20	0.02	4.96	2.02	
3 vrs 4	3.48	2.61	2.37	0.32	0.40	2.59	2.72	
4 vrs 5	10.94	0.33	0.07	4.58	5.32	3.63	3.61	
5 vrs 6	22.89	3.97	0.01	7.49	7.20	2.94	4.49	
6 vrs 7	4.45	2.89	27.32	2.67	3.27	0.40	4.54	
7 vrs 8	0.55	0.72	5.67	8.76	0.08	0.63	2.73	
12 vrs 34	27.26	61.57	3.97	5.71	0.09	1.99	8.82	
23 vrs 45	3.83	19.68	4.95	0.94	0.27	1.94	1.95	
34 vrs 56	0.03	0.23	1.90	0.35	0.67	6.32	0.83	
45 vrs 67	16.86	15.11	9.26	14.75	2.46	0.93	4.19	
56 vrs 78	0.56	6.46	13.49	2.31	0.01	1.29	2.17	

Table 3.1a : F1 to F4 are the first principal components. Other characters are Winglength (C1), Wingwidth (C2), Femur (C3), Tibia (C4), Sexcomb (C5), Scutellum (C6), Facewidth (C7), Eye (C8), Facelength (C9), and Wing angle (C10). M is a multivariate statistic derived from all the characters except C10.

Table 3.1 b
P - Values for Various Contrasts

Contrast	F1	F2	F3	F4	C1	C2	C3	C4
total	**0.0001**	**0.0001**	**0.0002**	0.0022	**0.0001**	**0.0001**	**0.0001**	**0.0001**
1 vrs 2	0.0054	0.0788	0.0047	0.1966	0.2093	0.0316	**0.0001**	**0.0001**
2 vrs 3	0.2392	0.2105	0.4597	0.2838	0.6437	0.5790	0.0119	0.1729
3 vrs 4	0.6290	0.3440	0.0743	0.8526	0.1756	0.9593	0.1603	0.0937
4 vrs 5	0.0065	0.4660	0.0322	0.6982	0.1293	0.0032	0.0013	**0.0001**
5 vrs 6	**0.0004**	0.5292	0.0201	0.4261	0.0768	**0.0001**	0.0014	**0.0005**
6 vrs 7	0.0546	**0.0003**	0.6326	**0.0001**	0.0268	0.6952	0.0075	0.0792
7 vrs 8	0.4569	0.6740	0.0007	0.0855	0.1106	0.4143	0.1671	0.6194
12 vrs 34	**0.0001**	**0.0002**	0.0250	0.0102	0.0110	0.0112	**0.0001**	**0.0001**
23 vrs 45	0.0020	0.0910	0.7630	0.3910	0.0044	0.0195	0.0197	0.1261
34 vrs 56	0.1318	0.9183	0.9580	0.3278	0.0810	0.4208	0.2015	0.0651
45 vrs 67	**0.0001**	0.0715	0.0140	0.6310	0.0020	**0.0001**	**0.0001**	**0.0002**
56 vrs 78	**0.0001**	0.0020	0.7180	0.0740	**0.0001**	**0.0001**	**0.0001**	**0.0002**

Contrast	C5	C6	C7	C8	C9	C10	M
total	**0.0001**	**0.0001**	**0.0001**	**0.0003**	0.2240	0.1500	**0.0001**
1 vrs 2	0.0278	0.0034	0.4013	0.0113	0.6277	0.1014	0.0054
2 vrs 3	0.0074	0.0113	0.8367	0.6557	0.8791	0.0313	0.0538
3 vrs 4	0.0690	0.1134	0.1315	0.5741	0.5312	0.1152	0.0110
4 vrs 5	0.0019	0.5661	0.7868	0.0382	0.0261	0.0635	0.0017
5 vrs 6	**0.0001**	0.0528	0.9284	0.0090	0.0104	0.0939	**0.0003**
6 vrs 7	0.0408	0.0963	**0.0001**	0.1098	0.0778	0.5296	**0.0003**
7 vrs 8	0.4606	0.4021	0.0219	0.0050	0.7815	0.4322	0.0108
12 vrs 34	**0.0001**	**0.0001**	0.0528	0.0215	0.7673	0.1661	**0.0001**
23 vrs 45	0.0570	**0.0001**	0.0316	0.3366	0.6067	0.1712	0.0630
34 vrs 56	0.8673	0.6316	0.1758	0.5557	0.4185	0.0158	0.6319
45 vrs 67	**0.0002**	**0.0004**	0.0040	**0.0004**	0.1240	0.3406	**0.0005**
56 vrs 78	0.4575	0.0148	0.0007	0.1361	0.9109	0.2622	0.0379

Table 3.1b : F1 to F4 are the first principal components. Other characters are Winglength (C1), Wingwidth (C2), Femur (C3), Tibia (C4), Sexcomb (C5), Scutellum (C6), Facewidth (C7), Eye (C8), Facelength (C9), and Wing angle (C10). M is a multivariate statistic derived from all the characters except C10. Values significant at $p < .0005$ are **bold**, those at $p < .00025$ are **bold and underline**.

suitable significance level given this criteria is 0.00025 (0.05/200) as it maintains the overall probability of a false positive at p = 0.05. Nevertheless, I have chosen a more liberal criteria of 0.0005 (albeit somewhat arbitrarily) in order to reduce the possibility of rejecting the alternate hypothesis of significance in favour of the null when the alternate hypothesis is indeed true. A liberal criteria is more suitable for observing trends in the data. The reader can make their own decision though, as the values in table 3.1 b that are significant at the 0.00025 level are in boldand underline, and those significant at the 0.0005 level are in bold. If one examines the significant values in table 3.1 b a number of trends are immediately apparent:

1. There is significant variation over the entire cline for all the characters except face length and wing angle.
2. Contrasts involving only adjacent pairs are less significant than those involving pooled populations. This indicates that the differences in mean phenotypes between adjacent populations are very small.
3. Contrasts involving positions 3 and 8 are generally less significant that others, possible due to small sample size (see table 2.6.1 a).
4. Multivariate comparisons are more likely to be significant that univariate comparisons because they increase the power of any given comparison.
5. Some characters exhibit stronger clinal variation than others. Specifically the first factor, wing length, wing width, femur length, tibia length, and scutellum width. These may additionally be the characters that have the lowest levels of measurement error.

There is a possibility that the p-values given in table 3.1 b are too conservative as the denominator for all the contrast F-statistics was derived from the total error of line within position, as opposed to only the error of line within positions for the specific contrasts being compared. Thus, later selection coefficents were estimated for each pair of populations regardless of the significance level for the pair given in table 3.1 b. It follows that inferences drawn from such non-significant differences are less robust than those drawn from significant differences.

Figure 3.2 a
Change in Wing Length Over Distance

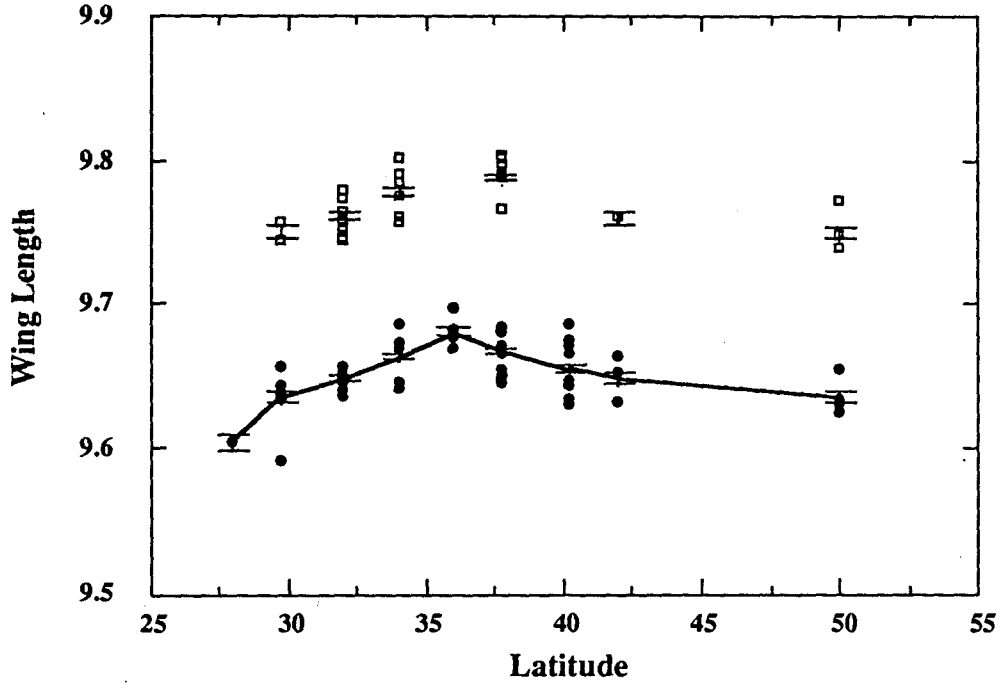


Figure 3.2 b
Change in Wing Width Over Distance

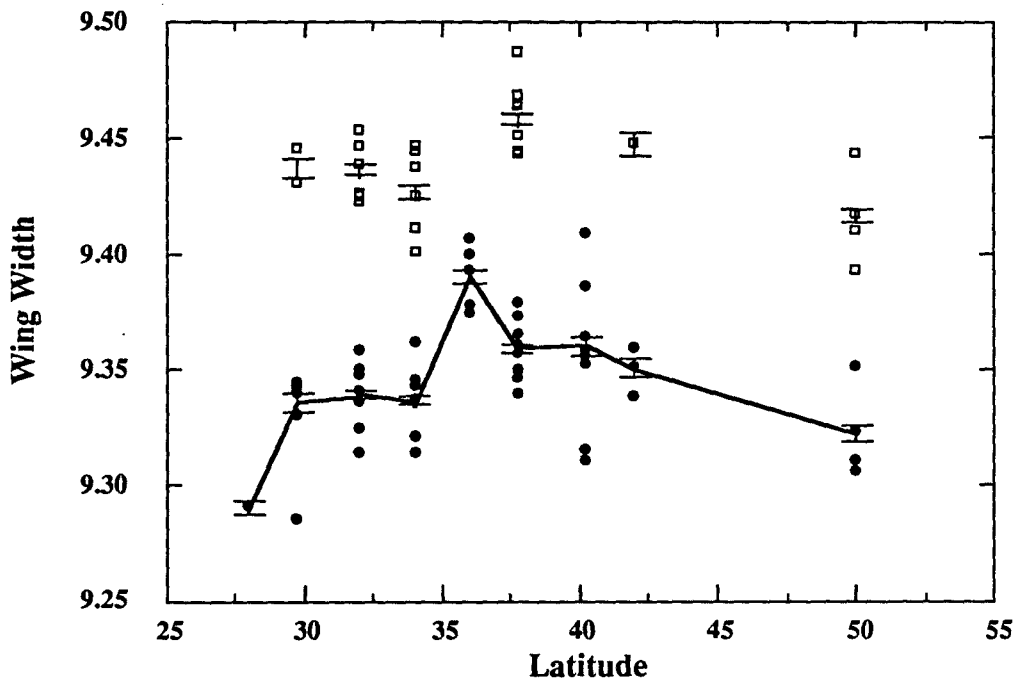


Figure 3.2 c
Change in Femur Length Over Distance

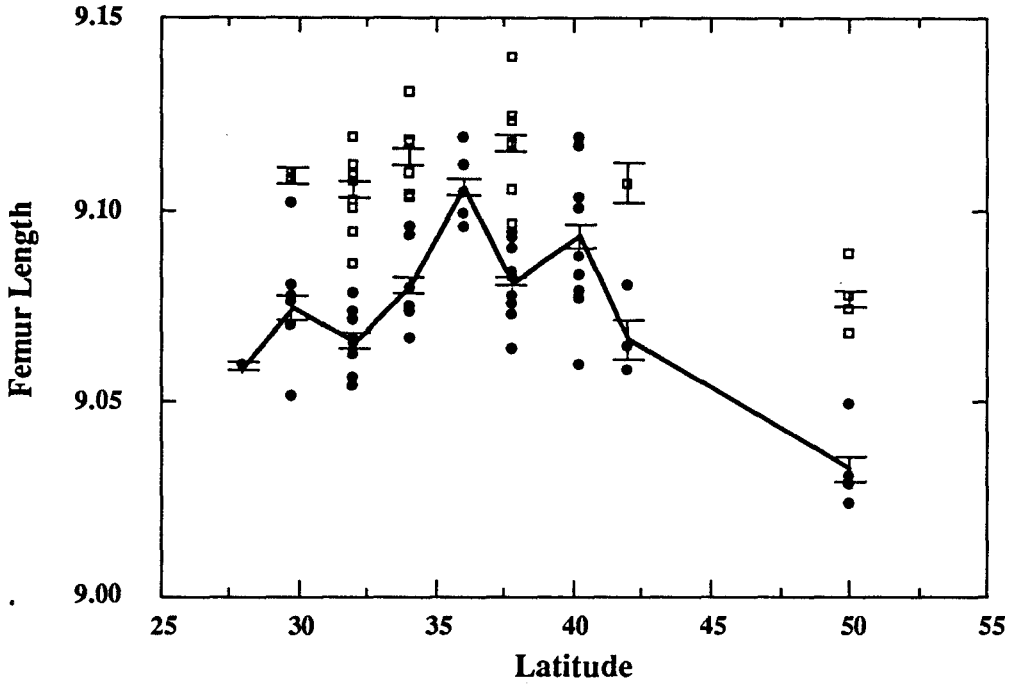


Figure 3.2 d
Change in Tibia Length Over Distance

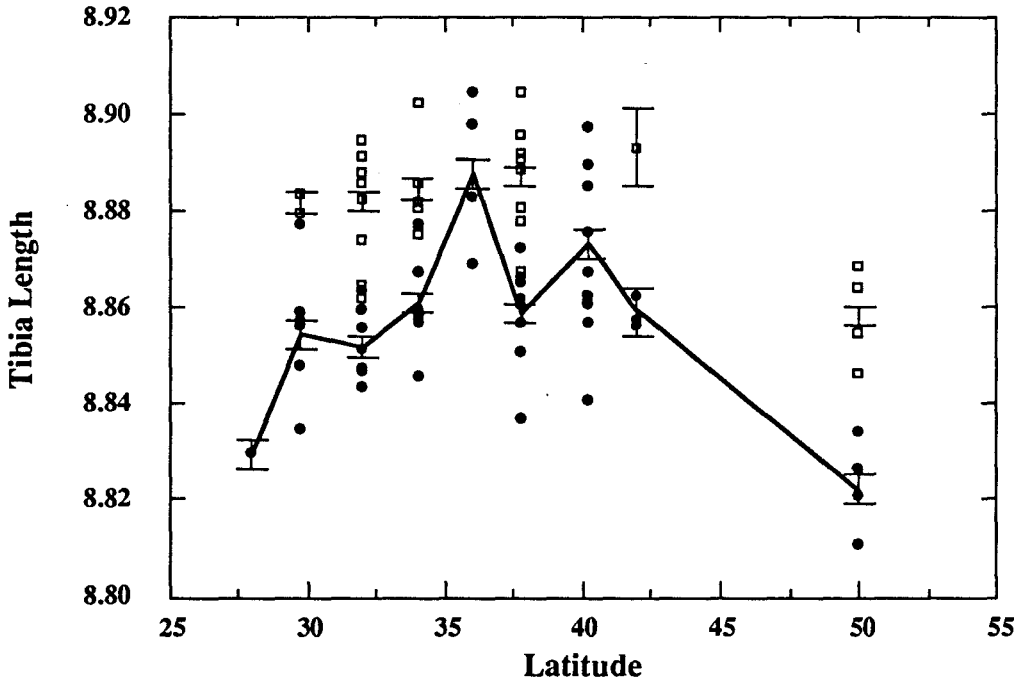


Figure 3.2 e
Change in Scutellum Width Over Distance

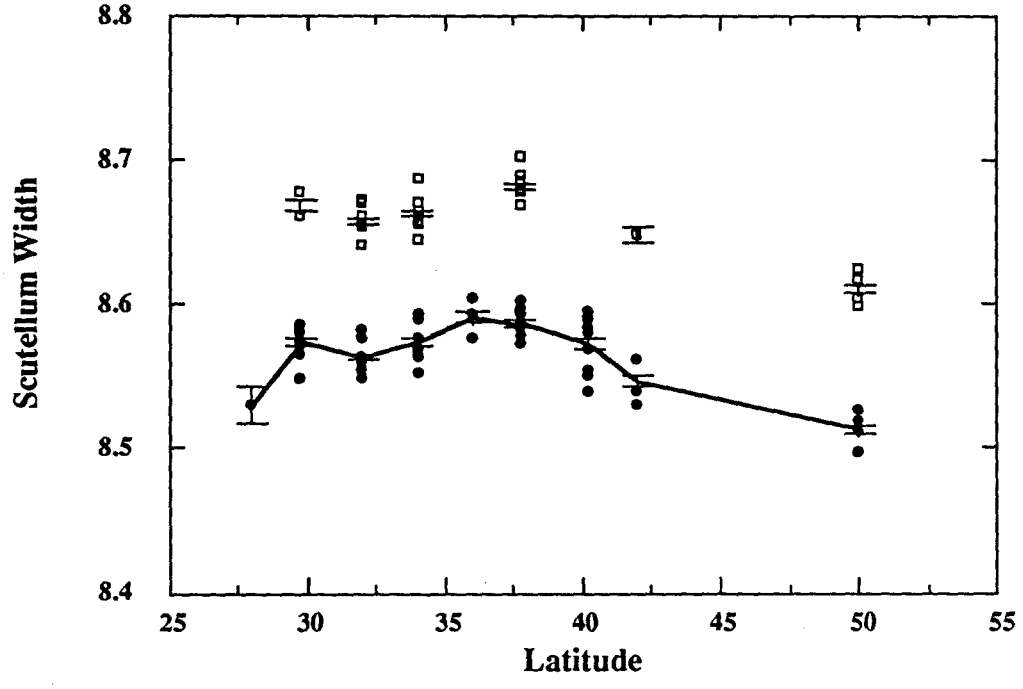


Figure 3.2 f
Change in Sex Comb Length Over Distance

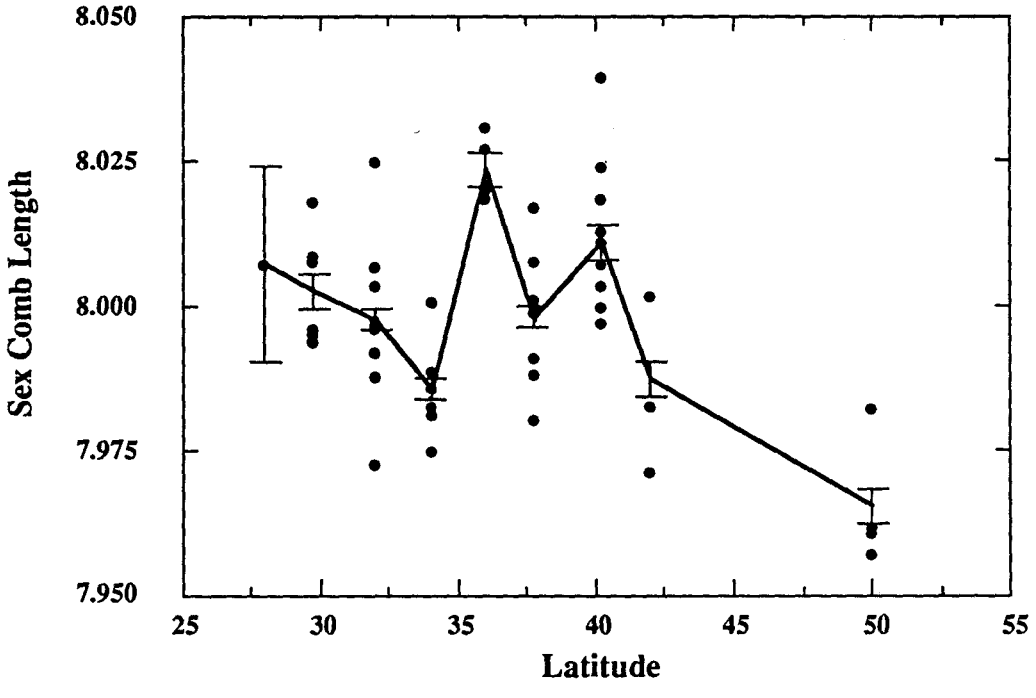


Figure 3.2 g
Change in Face Width Over Distance

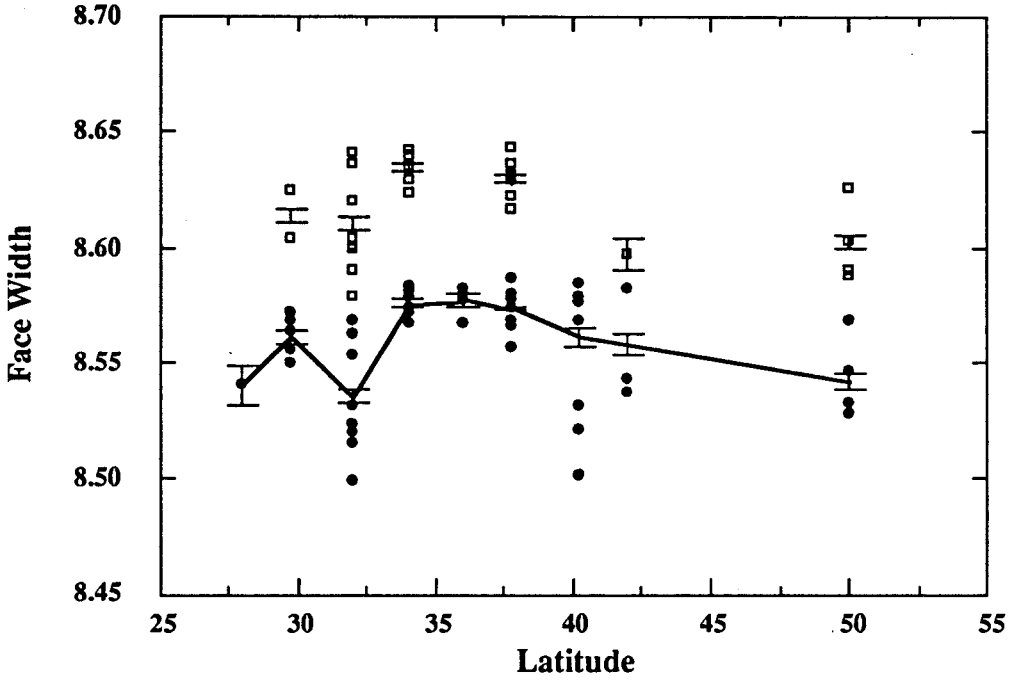


Figure 3.2 h
Change in Eye Width Over Distance

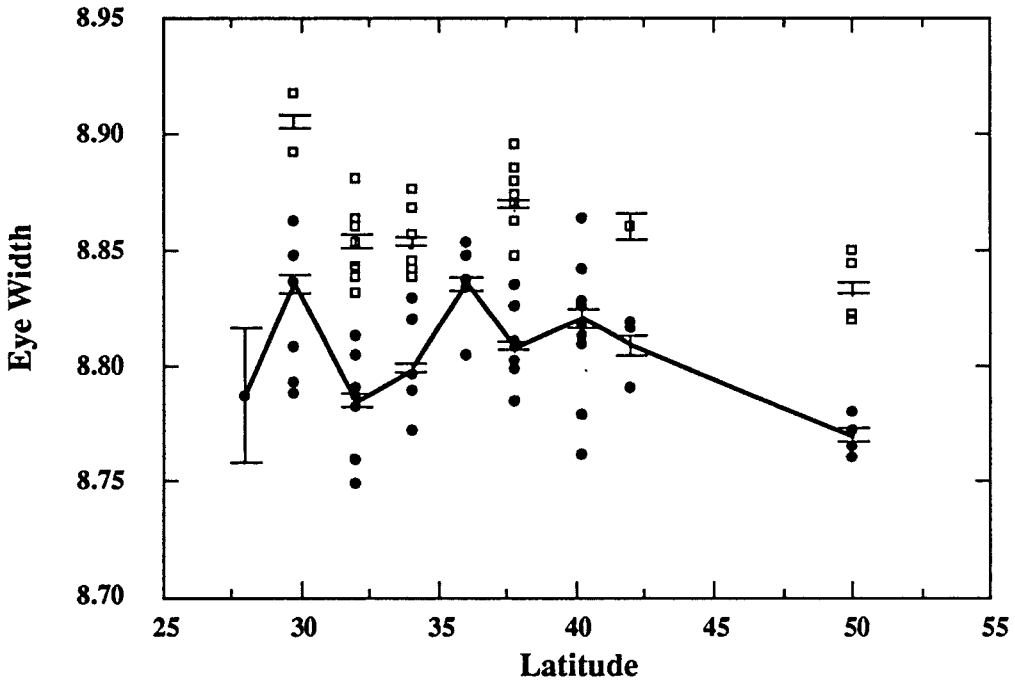


Figure 3.2 i
Change in Face Length Over Distance

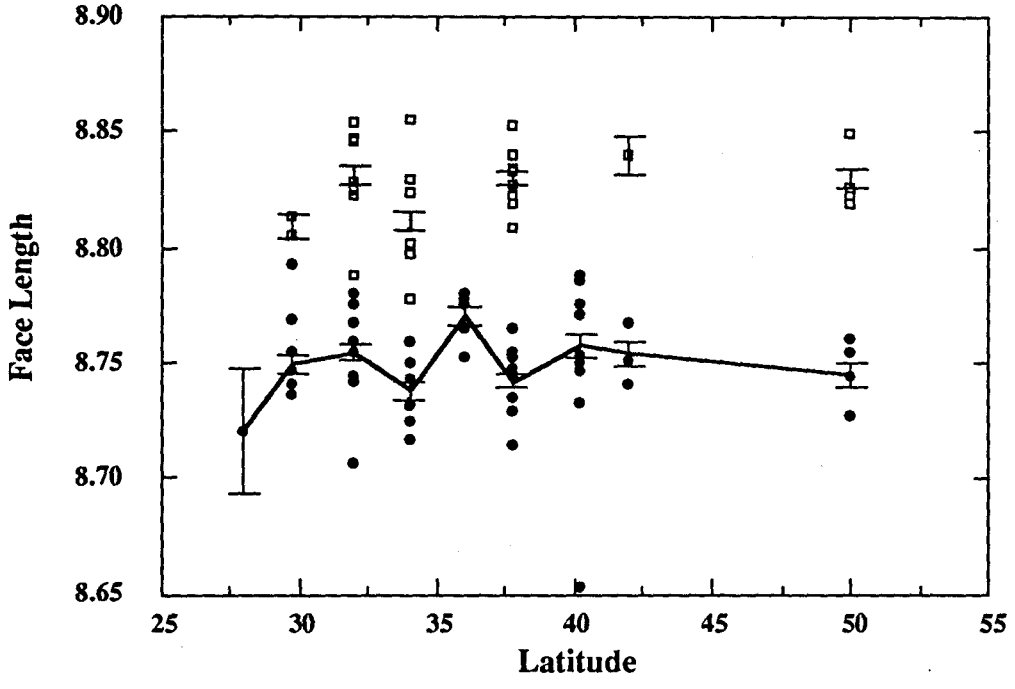


Figure 3.2 j
Change in First Principal Component Over Distance

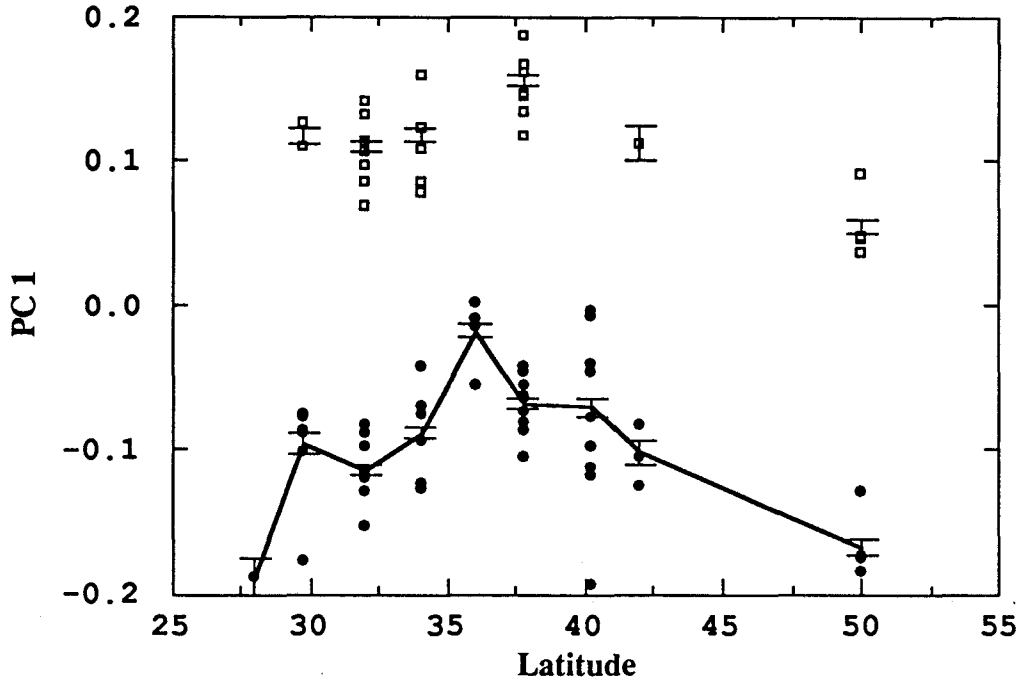


Figure 3.2 k
Change in Second Principal Component Over Distance

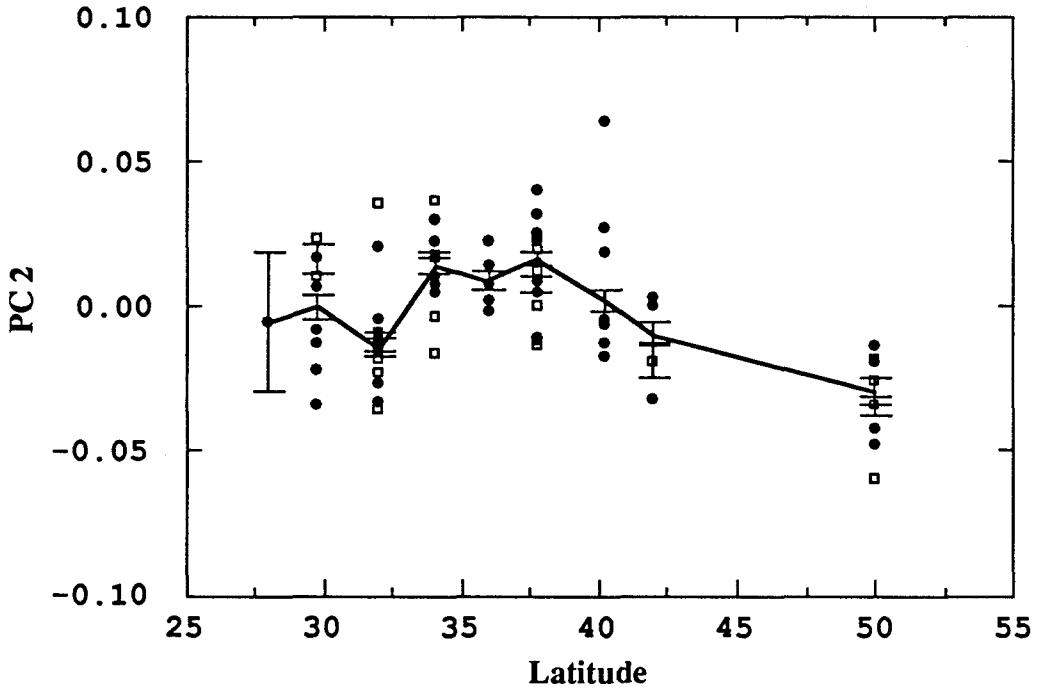


Figure 3.2 l
Change in Third Principal Component Over Distance

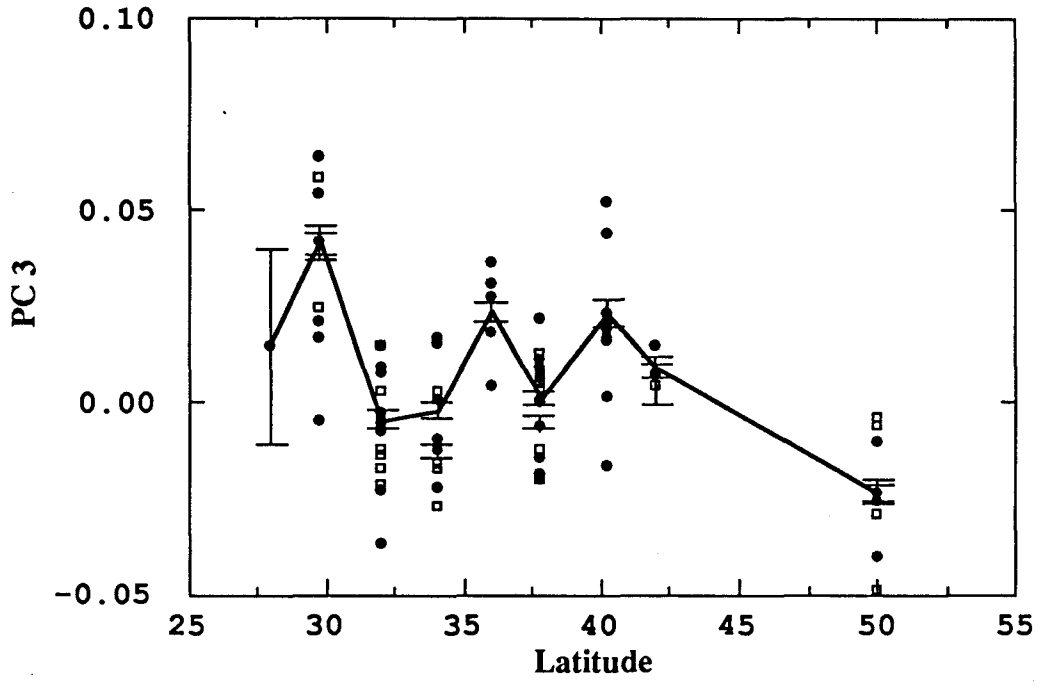
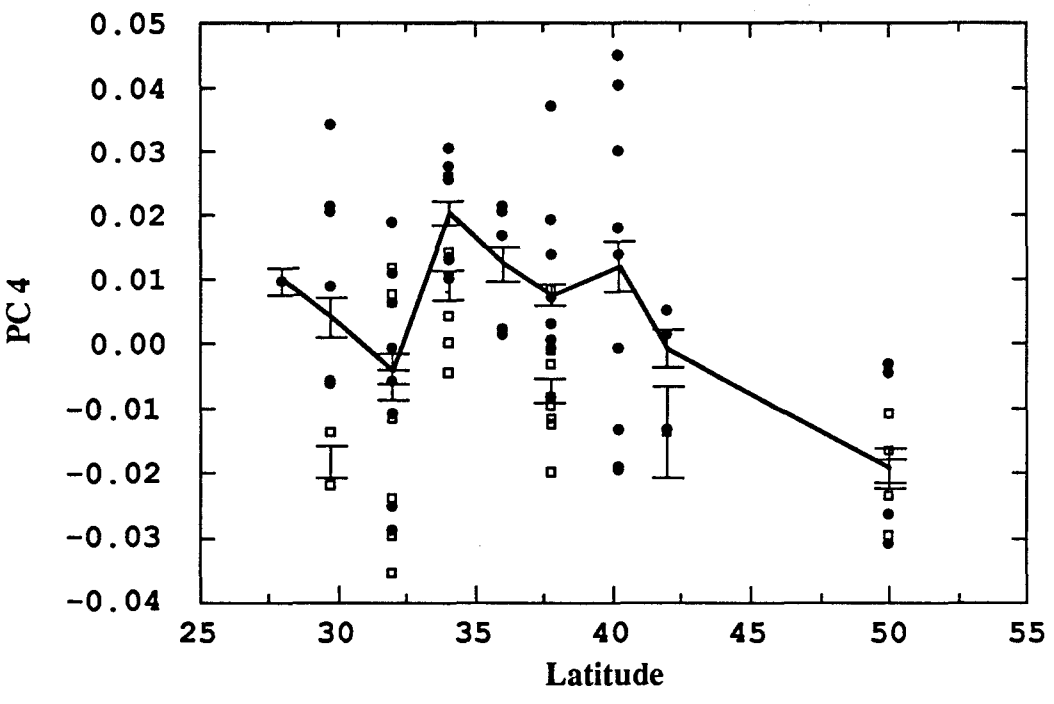


Figure 3.2 m
Change in Forth Principal Component Over Distance



3.2 Patterns of Variation Along the Cline

Figures 3.2 a through m show the patterns of phenotypic variation for male and female flies along the cline. Separate figures are provided for each character and the first four principal components in log scaled units. Filled circles represent the line means of male flies and hollow squares the line means of female flies. Error bars correspond to the standard error of the pooled mean of all the same sex flies from a given locality. Lines have been drawn connecting the location means for the male flies. The high correspondence between male and female flies may be an artifact of the data, as males and females from a given line were reared together. That is, males and females should not be considered as independent evidence for the strength of the observed cline as flies from a given line were reared together. The number of male flies measured from each position along the cline are given in table 2.6.1 a (column N). A number of patterns are apparent in the data:

1. The pattern of clinal variation is most apparent for principal components one and two, scutellum width, face width, and the wing characters. As with the comparisons of means these are the characters which would be expected to have the smallest measurement error.
2. There does not appear to be clinal variation for sex comb length nor the third and forth principal components.
3. The pattern of clinal variation is not linear as would have been expected but peaks at a latitude of 37 degrees and tapers off north and south of that point.

In the discussion the above points will be considered in more detail. It is important to note that a linear regression of the clinal variation involved in this study would be meaningless as the trend is obviously non-linear in nature.

3.3 Variance Components Estimated from the Heritability Flies

Appendix A, tables A1 to A12 show the mean square error matrices, by experiment, produced by the analyses described in section 2.7.2 for the 10 morphological characters, excluding sex comb length and wing angle. Sex comb length was excluded

from the study as in each experiment it gave large negative variance components due to sires, which suggests that either the heritability of the character is close to zero or that the character is measured with a very low level of repeatability. Wing angle was excluded for reasons similar to those for sex comb, in addition to its not matching the other characters in type. Tables A13 to A20 give estimates of the sire and dam variance components, by experiment, as described in section 2.7.2. The respective values of k_1, k'_1, and k_2 used to estimate the above variance components were; 1.34567, 1.68500, and 3.03129 in experiment one, 1.33655, 1.66715, and 2.98757 in experiment two, 1.33333, 1.66667, and 3.0 in experiment three, and 1.33025, 1.65117, and 2.98137 in experiment four. Note that the error variance matrices are equivalent to their respective Mean Square Error Matrices. Tables A21 to A25 give the total variance covariance matrices for sires and dams, and the genotypic, phenotypic, and heritability matrices described in section 2.7.3. Tables A26 and A27 give bent genotypic and heritability matrices as described in section 2.7.4. Finally, table A28 shows the mean square errors, variance components and heritabilities for each principal component by experiment and averaged over experiments one, three, and four. The off diagonal structure of the principal components are not examined as they are close to zero by definition.

3.4 Estimation of Selection Coefficients Along the Cline

Equation 2.8.1.3 gives a formula for the change in mean phenotype, if the genetic and phenotypic matrices, and the selection differential matrix are known. This equation can be rearranged to solve for the selection differential if the change in mean phenotype is known. The net selection differential is given as

$$\mathbf{S} = \mathbf{P}\mathbf{G}^{-1}\Delta\bar{\mathbf{z}} . \qquad (3.4.1)$$

Table 3.4 a gives the change in mean phenotype for adjacent pairs of populations. In this table the change is measured from north to south: that is, $\Delta\bar{z}_{1->2}$ is equal to $\bar{z}_2 - \bar{z}_1$. The

change in mean phenotype and selection differential from south to north would simply be negative that from north to south. Table 3.4 b gives the selection differentials between adjacent populations calculated from 3.4.1 using the phenotypic covariance matrix derived from the heritability flies (table 3.3 x). Table 3.4 c gives a similar set of selection differentials with the exception of the phenotypic covariance matrix that was calculated from the weighted average of the phenotypic covariance matrices of the two populations being compared. Figures 3.4 a to h give plots of the selection differentials over space for each of the eight morphological characters under consideration. The differentials are plotted against the mean latitude of the two populations being compared so that it is easy to pick out differences in selection differentials that are not simply a function of increasing geographic distance. In these figures the squares correspond to the selection differentials calculated from the heritability flies and the circles those from the clinal flies. The shadings are inconsequential, being intended to facilitate comparisons within character groupings if the plots are overlaid at a later date. It is apparent that the choice of which phenotypic matrix to

Table 3.4 a
Change in Mean Phenotype Between Adjacent Populations

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
WL	0.0130	0.0069	0.0122	0.0134	-0.0171	-0.0155	-0.0125
WW	0.0282	0.0088	-0.0007	0.0315	-0.0532	0.0021	-0.0033
FEM	0.0341	0.0271	-0.0121	0.0248	-0.0254	-0.0150	0.0088
TIB	0.0371	0.0139	-0.0142	0.0286	-0.0265	-0.0090	0.0025
SCUT	0.0345	0.0256	0.0142	0.0048	-0.0178	-0.0101	0.0099
FW	0.0163	0.0026	0.0128	0.0034	-0.0007	-0.0409	0.0256
EYE	0.0375	0.0118	-0.0114	0.0263	-0.0358	-0.0140	0.0502
FL	0.0093	0.0037	-0.0146	0.0277	-0.0327	0.0166	-0.0046

Table 3.4 b
Selection Differentials
(based on clinal phenotypic matrices)

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
AV Lat	46	41.25	39	36.625	35	33	30.875
WL	0.331	0.124	0.109	0.288	-0.503	-0.389	-0.14
WW	0.457	0.069	-0.184	0.716	-0.859	-0.161	-0.014
FEM	0.379	0.458	-0.266	0.524	-0.436	-0.304	0.043
TIB	0.289	-0.188	-0.214	0.496	-0.449	-0.28	0.114
SCUT	0.231	0.076	0.213	-0.095	-0.021	-0.022	-0.157
FW	0.383	-0.011	-0.039	0.288	-0.127	-0.981	1.957
EYE	0.354	0.037	-0.454	0.92	-0.788	-1.189	2.712
FL	-0.043	-0.038	-0.482	0.618	-0.428	0.553	0.079

Table 3.4 c
Selection Differentials
(based on heritability phenotypic matrix)

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
AV Lat	46	41.25	39	36.625	35	33	30.875
WL	0.257	0.101	-0.006	0.219	-0.239	-0.246	0.154
WW	0.475	0.109	-0.144	0.463	-0.662	-0.202	0.44
FEM	0.443	0.233	-0.176	0.372	-0.411	-0.298	0.372
TIB	0.402	0.11	-0.165	0.356	-0.368	-0.225	0.29
SCUT	0.274	0.146	0.054	0.103	-0.209	-0.105	0.181
FW	0.27	0.042	-0.046	0.229	-0.272	-0.273	0.327
EYE	0.741	0.164	-0.25	0.599	-0.769	-0.454	0.987
FL	0.17	-0.051	-0.174	0.322	-0.405	0.127	0.099

Table 3.4 d
Selection Gradients

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
AV Lat	46	41.25	39	36.625	35	33	30.875
WL	-4.749	-0.742	4.586	-4.738	7.784	-2.174	-6.856
WW	5.514	0.063	-2.942	6.747	-11.342	0.453	6.025
FEM	4.547	10.512	-4.903	4.131	-3.219	-4.819	2.441
TIB	1.735	-4.453	-1.601	2.28	-1.073	2.078	-1.129
SCUT	-3.051	0.455	4.591	-6.184	5.486	3.868	-5.53
FW	3.822	-1.198	-0.091	2.857	-3.439	-6.484	9.057
EYE	7.563	0.224	-2.366	5.921	-7.674	-6.011	13.149
FL	-2.051	-1.399	-1.01	0.315	-0.265	3.975	-3.227

Table 3.4 e
Selection Differentials for Principal Components

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
AV Lat	46	41.25	39	36.625	35	33	30.875
PC 1	1.259	0.611	0.049	0.974	-1.369	-0.493	0.353
PC 2	0.270	0.155	0.194	-0.105	0.083	-0.393	0.189
PC 4	0.057	0.041	-0.014	0.016	0.025	-0.077	0.026

use made little difference to the qualitative outcome of the analysis, although phenotypic matrices from the clinal flies generally gave more extreme selection differentials.

Equation 2.8.1.3 can also be rearranged to solve for the net selection gradient:

$$\mathbf{V} \ln \bar{W} = \mathbf{G}^{-1}\Delta\bar{\mathbf{z}} \ .$$

Table 3.4 d gives net selection gradients between adjacent pairs of populations. Figures 3.4 i to k give plots of the net selection gradients over space. The rational for the sign of the gradient and the choice of mean latitude as the spatial parameter is similar to that discussed above. Table 3.4 e gives the selection differentials for principal components 1, 2, and 4 using the phenotypic covariance matrix from the heritability study. Principal component 3 was omitted as it had a negative heritability estimate. As the off diagonal structure of the principal components are close to zero, the selection differentials are proportional to the selection gradients. Figure 3.4 l is a plot of the selection differentials of principal components 1, 2, and 4 over space.

3.5 Estimation of the Intensity of Selection

Equation 2.8.2.2 gives an expression for calculating the average truncation point corresponding to the intensity of natural selection required to produce the observed phenotypic change. Table 3.5 gives the values of $\mathbf{G}_{II}$, $\mathbf{P}_{II}$, and $|\mathbf{I}|$ that are necessary in order to calculate the truncation point (b). This table also gives the truncation point corresponding to various values of 2t - 1 , where t is the number of generations the two populations have diverged from a common ancestor. I have used values of 2t - 1 equal to

Figure 3.4 a
Wing Length Selection Differential Over Latitude

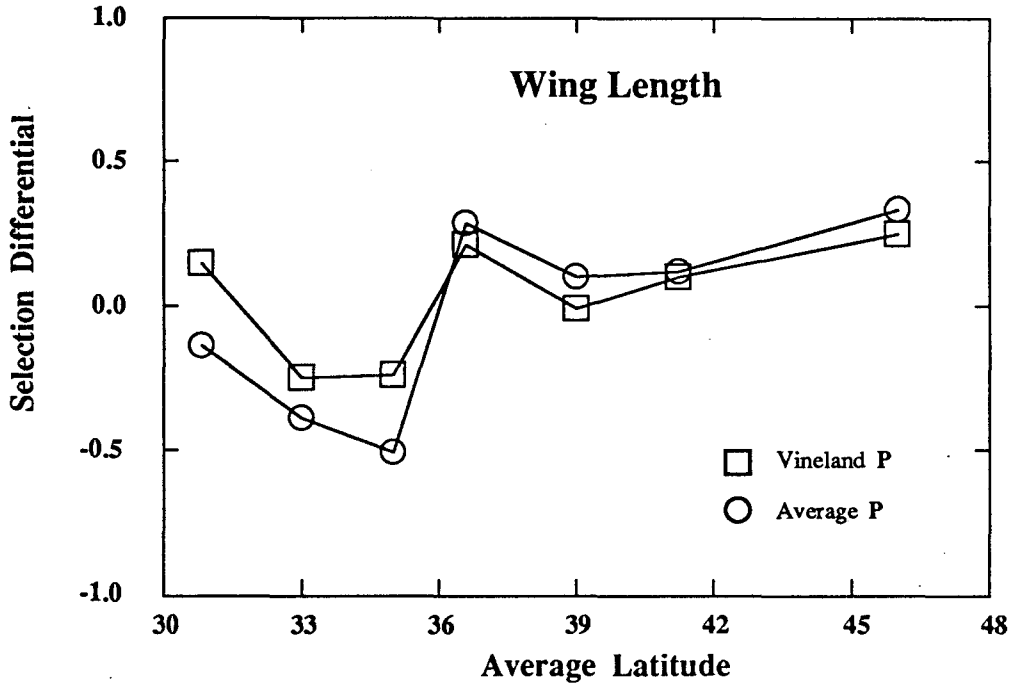


Figure 3.4 b
Wing Width Selection Differential Over Latitude

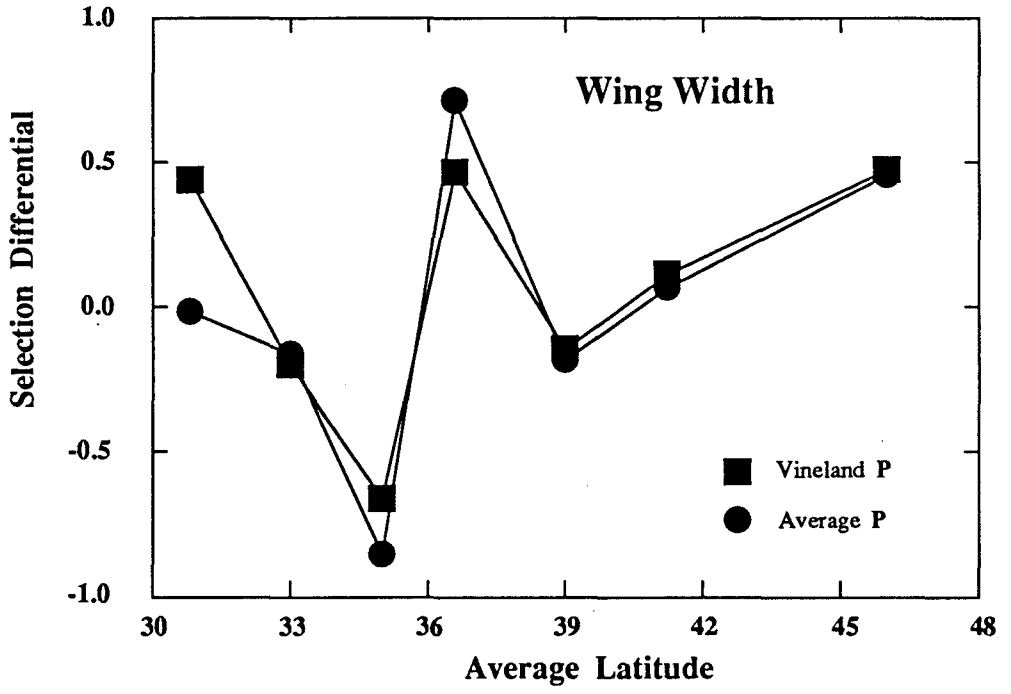


Figure 3.4 c
Femur Length Selection Differential Over Latitude

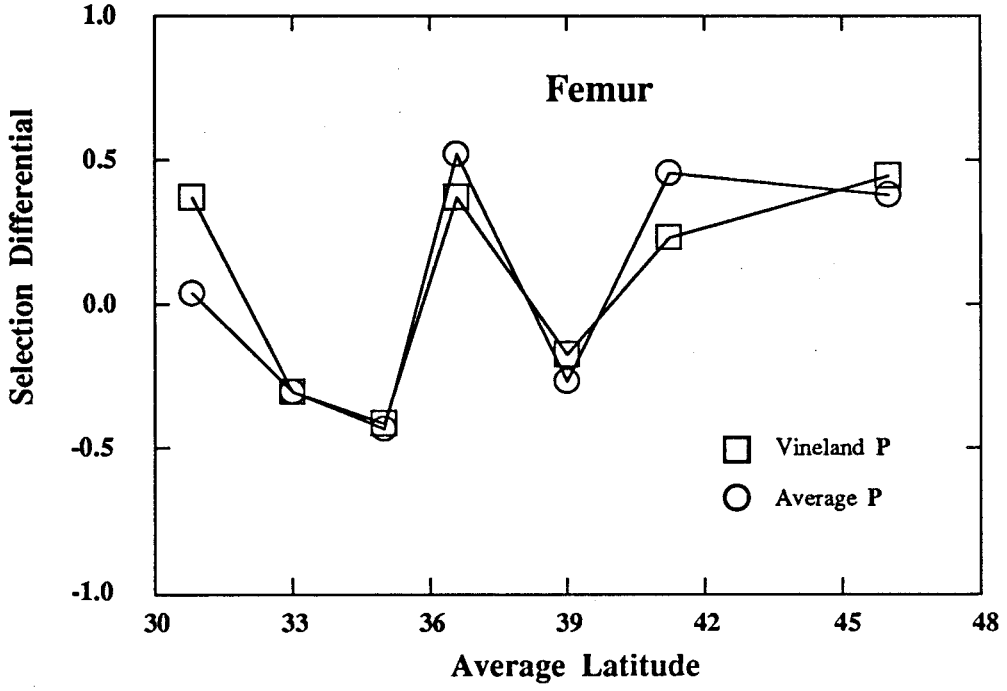


Figure 3.4 d
Tibia Length Selection Differential Over Latitude

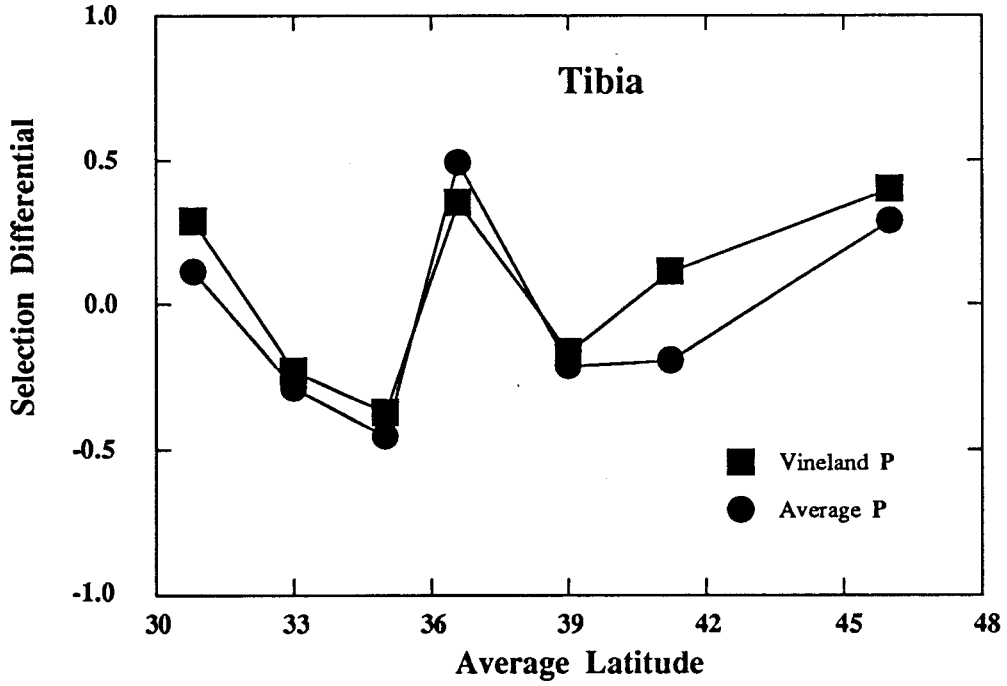


Figure 3.4 e
Scutellum Width Selection Differential Over Latitude

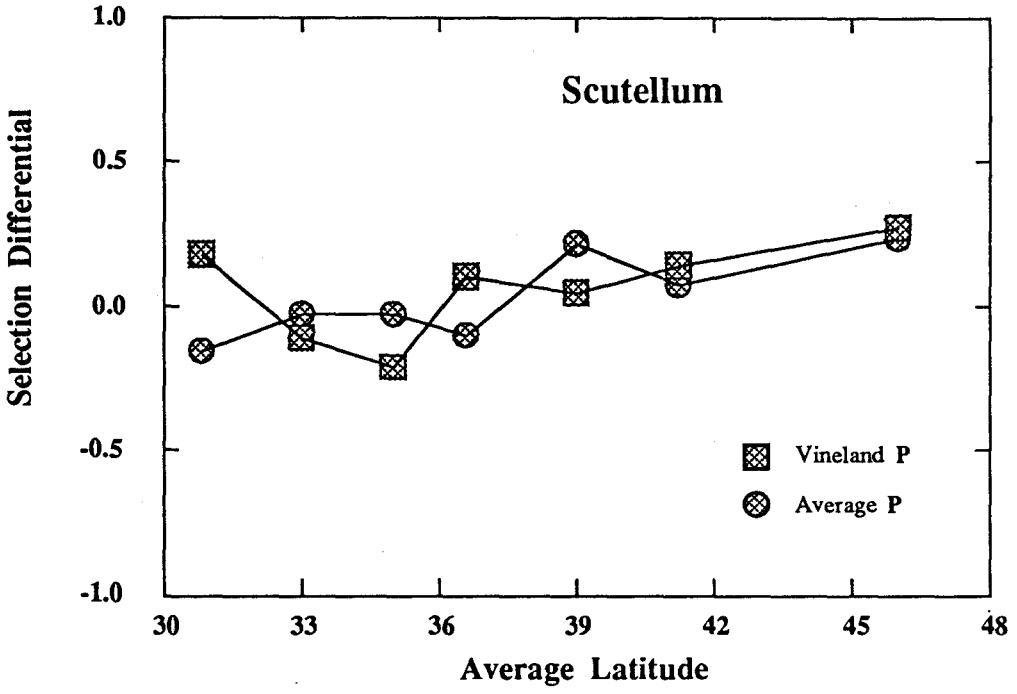


Figure 3.4 f
Face Width Selection Differential Over Latitude

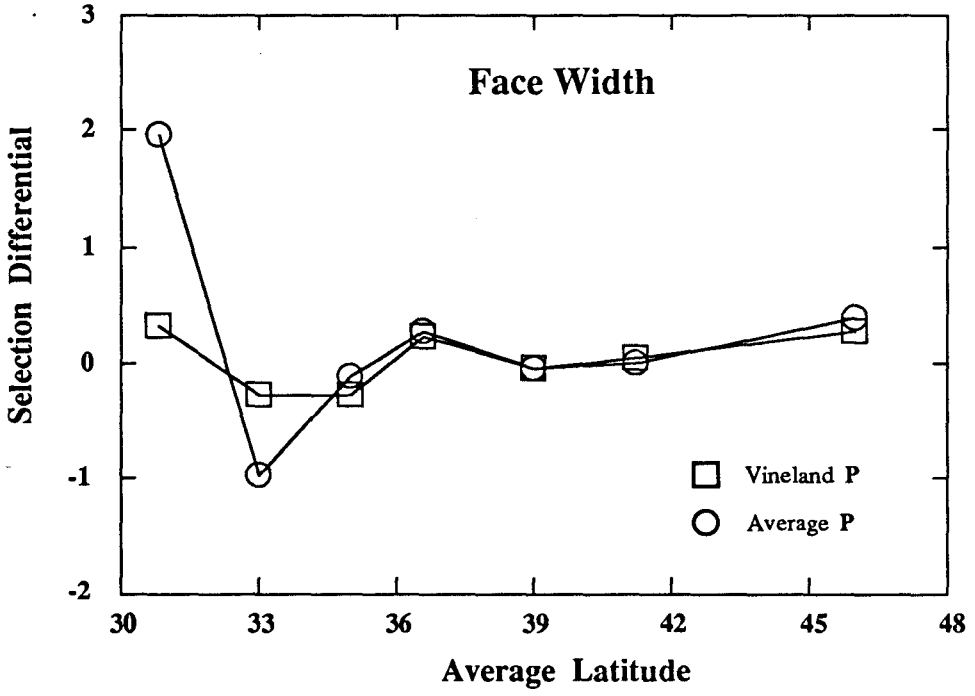


Figure 3.4 g
Eye Width Selection Differential Over Latitude

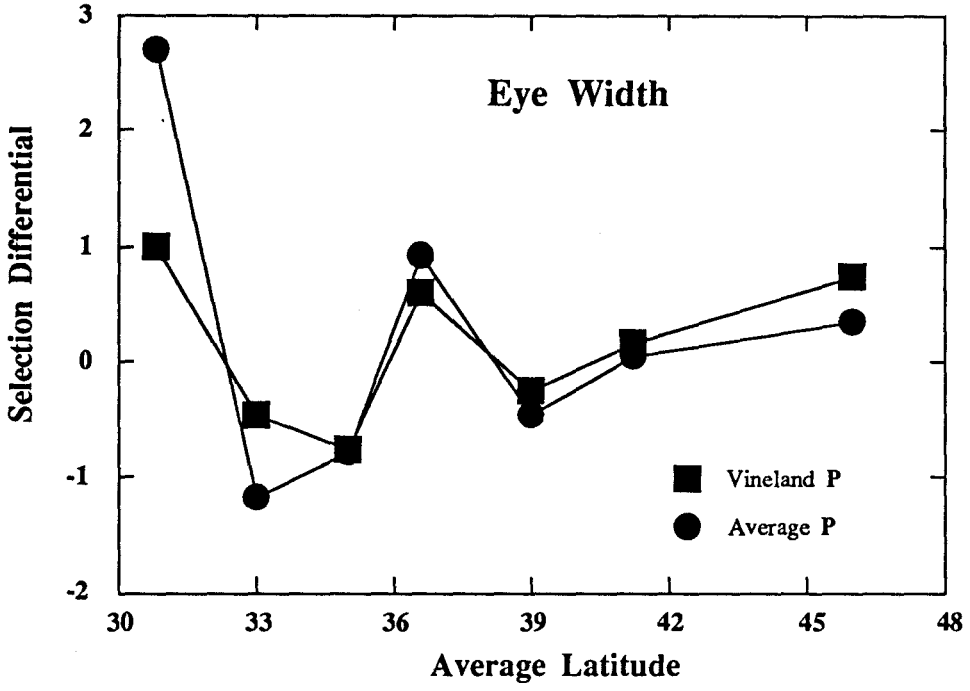


Figure 3.4 h
Face Length Selection Differential Over Latitude

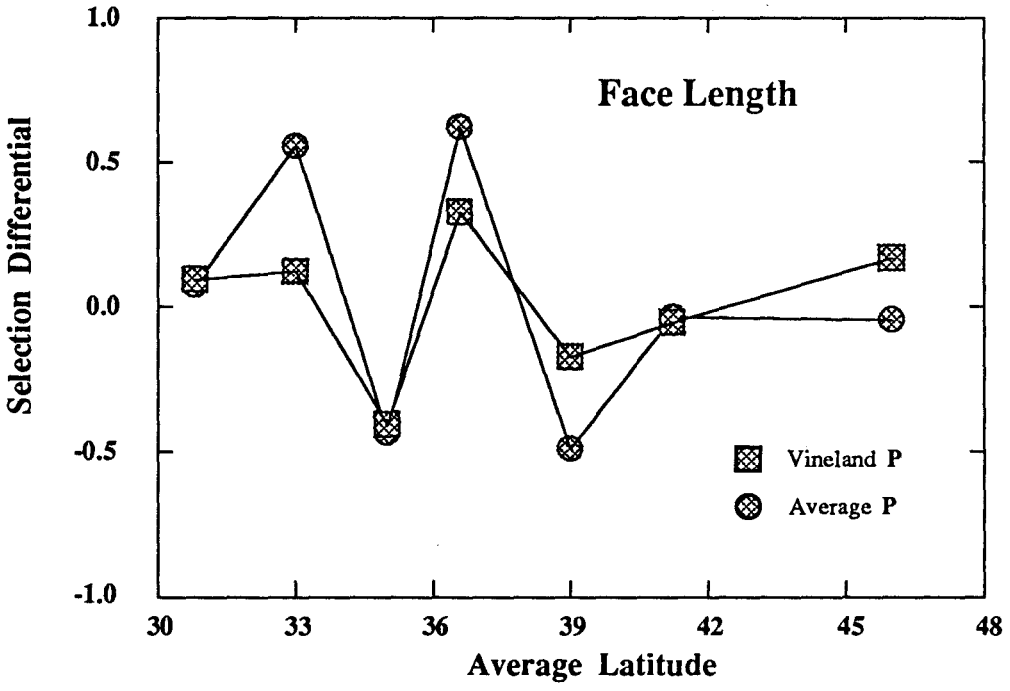


Figure 3.4 i
Wing Length and Width Selection Gradient Over Latitude

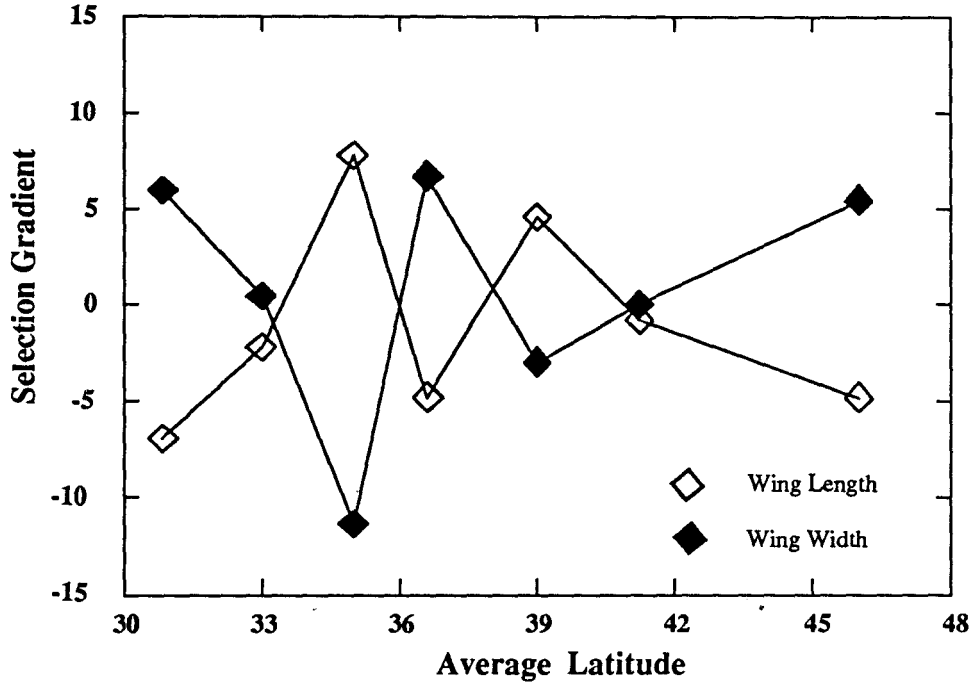


Figure 3.4 j
Femur Length, Tibia Length, and Scutellum Width Selection Gradient Over Latitude

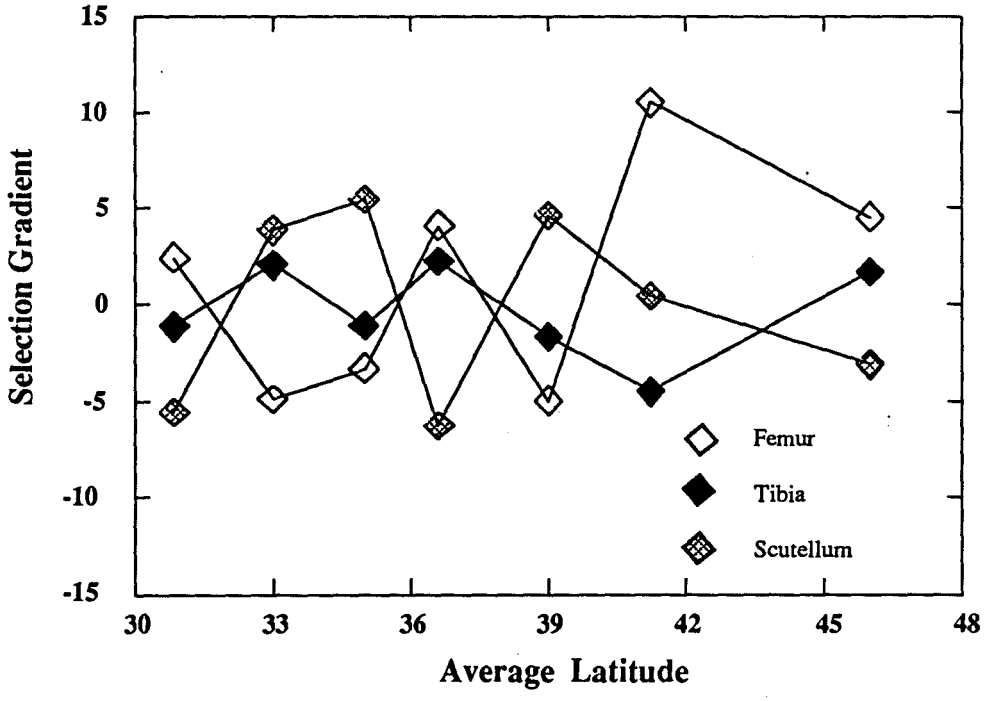


Figure 3.4 k
Face Width, Eye Width, and Face Length
Selection Gradient Over Latitude

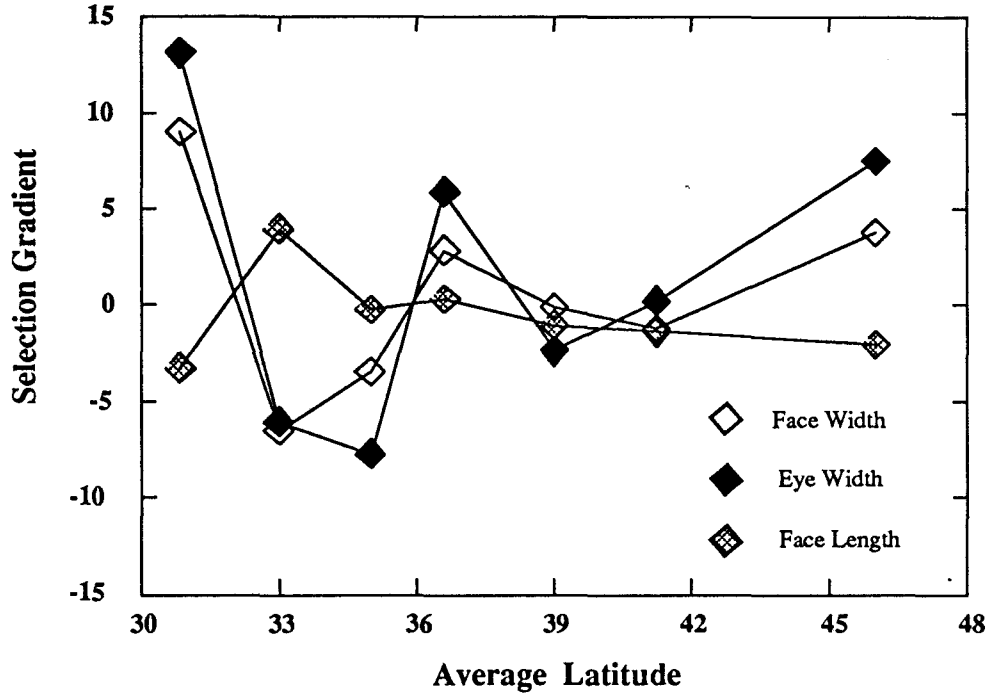
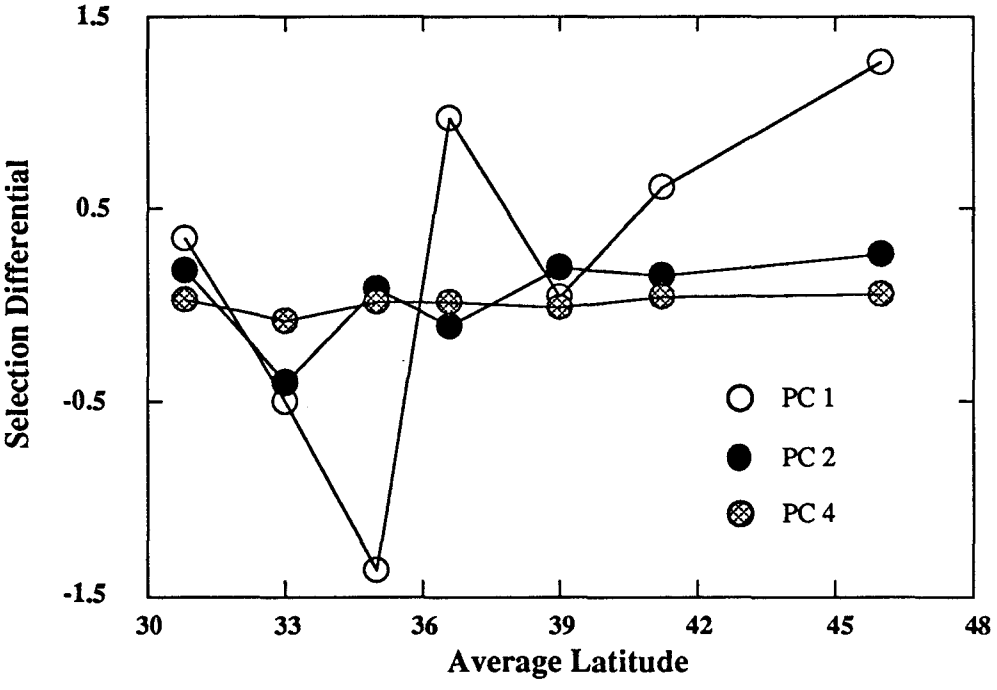


Figure 3.4 l
Principal Components 1, 2, and 4 Selection Differential Over Latitude



100, 1 000, 10 000, and 100 000 which correspond to b_1 through b_4 respectively. Values of the average selective mortality (SM) (the proportion of individuals culled) per generation which correspond to the various truncation points are also provided. Although there is migration between the populations, and thus they have not really diverged, it is useful to consider t as the effective time since divergence. Nei provides formulae for calculating this parameter if neutral molecular markers are available (1987). Although not included in this study, molecular data of this sort would provide more accurate estimates of the selective mortalities necessary to account for the phenotypic divergence of two populations. Regardless, the range of t provided should include such molecular estimates. It appears that very little selective mortality is needed to account for phenotypic evolution in natural populations.

Table 3.5
Selection Intensities and Truncation Points and Minimum Selective Mortality Required to Explain the Observed Phenotypic Divergence

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
G_{II}	0.5351	0.225	0.2451	0.4613	0.7693	0.4643	0.9366
P_{II}	6.6953	5.6733	5.2354	13.8103	14.1973	17.2785	54.851
$\|I\|$	0.5351	0.225	0.2451	0.4613	0.7693	0.4643	0.9366
b_1	2.339	2.374	2.391	2.179	2.172	2.127	1.835
b_2	3.174	3.200	3.213	3.058	3.054	3.021	2.824
b_3	3.833	3.853	3.864	3.736	3.732	3.706	3.547
b_4	4.392	4.410	4.420	4.308	4.305	4.282	4.145
SM_1	9.67×10^{-3}	8.79×10^{-3}	8.40×10^{-3}	1.47×10^{-2}	1.49×10^{-2}	1.67×10^{-2}	3.32×10^{-2}
SM_2	7.51×10^{-4}	6.86×10^{-4}	6.57×10^{-4}	1.11×10^{-3}	1.13×10^{-3}	1.26×10^{-3}	2.37×10^{-3}
SM_3	6.36×10^{-5}	5.83×10^{-5}	5.59×10^{-5}	9.35×10^{-5}	9.49×10^{-5}	1.05×10^{-4}	1.95×10^{-4}
SM_4	5.6×10^{-6}	5.2×10^{-6}	4.9×10^{-6}	8.2×10^{-6}	8.3×10^{-6}	9.2×10^{-6}	1.7×10^{-5}

3.6 Direct and Correlated Responses to Selection

Equation 2.8.3.9 was used to estimate the relative strengths of direct and correlated responses to directional selection for each character between each adjacent pair of populations. The results of these calculations are given in tables 3.6 a to g. Each table is for a different set of adjacent populations, with any given column representing the relative strengths of direct selection and correlated responses from selection on other characters. For example the third row gives the correlated response to selection in each character resulting from selection acting on femur, in the case of the column corresponding to femur this cell represents the the direct response to selection of femur. The bottom row is the relative net selection acting on each character. As the forces of selection were interpreted for each adjacent pair of populations we can additionally determine if the targets of selection vary along the cline. In the following sections the nature of selection will be examined for each adjacent pair of populations.

3.6.1 1 to 2

The change between populations 1 and 2 is positive for all characters, with face length showing a very small change and the other face characters an intermediate change. In referring to table 3.6 a it can be seen that direct selection on wing length accounts for a negative response equal to -320 % of the total response, which is countered by strong correlated selection on wing width (280 %) and femur (120 %), selection on the other characters creates both positive and negative correlated responses. Nonetheless, the net response for wing length is small relative to the other characters. The change in wing width is dominated by strong direct selection (190 %) and a negative correlated response from wing length (-110 %). The change in femur is dominated by direct selection (70 %) with correlated responses from the wing characters cancelling one another as well as a negative response from the scutellum (-50 %) being cancelled by the remaining characters.

Direct selection on the tibia accounted for 40 % of its observed response, which was less than the correlated response from selection acting on the femur (60 %). Correlated responses from selection on the wing characters were also larger than the direct response on tibia, but again they tended to cancel one another. As with face length, direct selection on the scutellum was large (-100 %) but negative, this trend was cancelled by positive correlated responses arising from femur length (70 %) and eye (80 %) with wing length and width again cancelling. Changes on face width and length were small with direct selection dominating the change in the former, and indirect selection on eye dominating the change in the latter (35 %). The trend in eye largely was due to direct selection acting on this character (165 %). Overall the change between 1 and 2 was dominated by positive selection on wing width and femur, which was partially countered by negative selection on wing length and scutellum. The exception to this trend was the face characters which responded in a more independent manner with eye and face width dominating.

4.4.2 2 to 3

Between populations 2 and 3 the total change in wing length, wing width, face width, and face length were small and all changes were positive. The change in femur was dominated by direct selection (210 %) with correlated selection on the tibia (-80 %) partially countering this trend. The change in tibia was similarly dominated by a correlated response to selection on the femur (390 %) with direct selection on the tibia countering this trend to a limited degree (-250 %). Again the change in scutellum was dominated by a correlated response to selection on the femur (210 %), with a correlated response to selection on tibia (- 80 %) and the direct response to selection on scutellum (-20 %) partially countering this trend. Direct selection on eye seemed inconsequential in determining its trend (15 %) as it was dominated by correlated responses on femur (150 %), face length (-60 %), and tibia (-

30 %). Overall the change from population 2 to 3 resulted from selection on femur length which was partially countered by negative correlated responses to selection on the tibia.

4.4.3 3 to 4

The change from population 3 to 4 was negative for all characters except wing length, scutellum and face width. As some of the changes were negative it is important to note that a new convention will be adopted whereby the sign of a percentage value is that of the numerator. That is, it does not matter whether the net change of the character is positive or negative (eg; net = -3, direct on A = -9 , therefore total = -300 %). The change in wing width and face width were small enough that they need not be considered. The change in

Table 3.6 a
Direct and Correlated Response to Selection on Each Character
Positions 1 and 2

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-0.042	-0.031	-0.016	-0.015	-0.022	-0.013	-0.007	-0.023
0.0362	0.0532	0.0166	0.0186	0.0257	0.0076	0.0013	0.0163
0.0151	0.0137	0.0248	0.0231	0.0238	0.0122	0.0075	0.0156
0.0056	0.0059	0.0088	0.0134	0.0084	0.0042	0.0014	0.0029
-0.014	-0.014	-0.016	-0.015	-0.035	-0.013	-0.01	-0.012
0.0108	0.0052	0.0102	0.0093	0.0158	0.0368	-0.008	0.0069
0.011	0.0018	0.0124	0.0061	0.026	-0.015	0.0617	0.0353
-0.01	-0.006	-0.007	-0.003	-0.008	-0.004	-0.01	-0.032
0.013	0.0282	0.0341	0.0371	0.0344	0.0159	0.0373	0.0092

Table 3.6 b
Direct and Correlated Response to Selection on Each Character
Positions 2 and 3

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-0.007	-0.005	-0.002	-0.002	-0.003	-0.002	-0.001	-0.004
0.0004	0.0006	0.0002	0.0002	0.0003	9E-05	1E-05	0.0002
0.035	0.0317	0.0573	0.0533	0.0549	0.0281	0.0173	0.036
-0.014	-0.015	-0.023	-0.034	-0.022	-0.011	-0.004	-0.008
0.0021	0.0021	0.0024	0.0022	0.0052	0.0019	0.0016	0.0018
-0.003	-0.002	-0.003	-0.003	-0.005	-0.012	0.0024	-0.002
0.0003	5E-05	0.0004	0.0002	0.0008	-4E-04	0.0018	0.001
-0.007	-0.004	-0.005	-0.002	-0.006	-0.003	-0.007	-0.022
0.0069	0.0088	0.0272	0.0138	0.0256	0.0027	0.0119	0.0037

Table 3.6 c
Direct and Correlated Response to Selection on Each Character
Positions 3 and 4

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0404	0.0301	0.0153	0.0147	0.0212	0.013	0.0067	0.0222
-0.019	-0.028	-0.009	-0.01	-0.014	-0.004	-7E-04	-0.009
-0.016	-0.015	-0.027	-0.025	-0.026	-0.013	-0.008	-0.017
-0.005	-0.005	-0.008	-0.012	-0.008	-0.004	-0.001	-0.003
0.0212	0.0214	0.024	0.0222	0.0526	0.019	0.0158	0.0187
-3E-04	-1E-04	-2E-04	-2E-04	-4E-04	-9E-04	0.0002	-2E-04
-0.003	-6E-04	-0.004	-0.002	-0.008	0.0047	-0.019	-0.011
-0.005	-0.003	-0.003	-0.002	-0.004	-0.002	-0.005	-0.016
0.0122	-7E-04	-0.012	-0.014	0.0141	0.013	-0.011	-0.014

wing length was dominated by direct selection (330 %) with correlated responses arising from wing width (-160 %), femur (-130 %), and scutellum (170 %) also being important. The change in femur was dominated by direct selection (-220 %), although the correlated responses from scutellum (200 %) and wing length (130 %) must be given consideration. Direct response to selection on tibia accounted for a small proportion of its total response (-90 %) relative to the correlated response of femur (-180 %), scutellum (160 %) and wing length (100 %). The positive change in scutellum was largely a result of direct selection (370 %), with correlated responses from femur (-180 %), wing length (100 %) and wing width (-100 %). The change in eye width was largely the result of direct selection (-170 %) with a correlated response from femur mediating (70 %). The change in face length was dominated by correlated responses arising from selection on wing length (150 %), scutellum (130 %) and femur (-120 %); direct selection accounting for -110 % of the response. Generally correlated responses from wing length and scutellum were large and opposite to the direction of the observed trend. Nonetheless the correlated response was not large enough to counter the direct selection on any given character plus the correlated response in the direction of the trend from femur and the sum of the small correlated responses on the remaining characters. With the exception of eye, the change from 3 to 4

was dominated by selection on wing length, scutellum, femur length, and to a lesser extent wing width.

4.4.4 4 to 5

The change from 4 to 5 was positive for all characters, although small for wing length, scutellum, face width and face length. The change in wing width resulted principally from direct selection (200 %) with negative correlated responses from wing length (-100 %) and scutellum (-90 %) being important. A correlated response from scutellum (-130 %) was the most important factor accounting for the change in femur, although it was out weighed by direct selection on femur (90 %), and a correlated response on wing width (80 %). The change in tibia (60 % direct) showed a similar trend to that of the femur with the addition of femur length as an important correlated response (70 %). The change in eye was largely accounted for by direct selection (180 %) mediated by correlated selection on scutellum (-80 %). In the non-face characters selection on wing width and femur length dominated, although strongly mediated by selection in the opposite direction on scutellum and to a lesser extent wing length.

Table 3.6 d
Direct and Correlated Response to Selection on Each Character Positions 4 and 5

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-0.042	-0.031	-0.016	-0.015	-0.022	-0.013	-0.007	-0.023
0.0444	0.065	0.0204	0.0228	0.0315	0.0092	0.0016	0.02
0.0138	0.0125	0.0225	0.021	0.0216	0.011	0.0068	0.0141
0.0073	0.0077	0.0116	0.0176	0.011	0.0056	0.0018	0.0039
-0.029	-0.029	-0.032	-0.03	-0.071	-0.026	-0.021	-0.025
0.0081	0.0039	0.0076	0.007	0.0118	0.0275	-0.006	0.0051
0.0086	0.0014	0.0097	0.0048	0.0203	-0.012	0.0483	0.0276
0.0015	0.0009	0.0011	0.0005	0.0013	0.0006	0.0015	0.005
0.0134	0.0314	0.0248	0.0286	0.0048	0.0031	0.0262	0.0276

Table 3.6 e
Direct and Correlated Response to Selection on Each Character
Positions 5 and 6

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0685	0.0512	0.0259	0.0249	0.0359	0.0221	0.0113	0.0377
-0.075	-0.109	-0.034	-0.038	-0.053	-0.016	-0.003	-0.034
-0.011	-0.01	-0.018	-0.016	-0.017	-0.009	-0.005	-0.011
-0.003	-0.004	-0.005	-0.008	-0.005	-0.003	-9E-04	-0.002
0.0253	0.0256	0.0287	0.0265	0.0629	0.0227	0.0188	0.0223
-0.01	-0.005	-0.009	-0.008	-0.014	-0.033	0.0068	-0.006
-0.011	-0.002	-0.013	-0.006	-0.026	0.0153	-0.063	-0.036
-0.001	-8E-04	-9E-04	-4E-04	-0.001	-5E-04	-0.001	-0.004
-0.017	-0.053	-0.025	-0.027	-0.018	-3E-04	-0.036	-0.033

4.4.5 5 to 6

The change from position 5 to 6 was negative for all characters, being small for both wing length and scutellum, and very small for face width. The change in wing width was primarily due to direct selection (-210 %) with wing length countering to a small extent (100 %). Wing width additionally dominated the change in femur (- 140 %) as the change due to direct selection was only -70 %: scutellum (110 %) and wing length (100 %) provided moderating influences. The change in tibia was similar to that of femur with femur providing less correlated response (-60 %) than wing width (-), scutellum (+) and wing length (+), but more than direct response on the tibia itself (-30 %). Strong direct selection on scutellum (350 %) and correlated selection on wing length (200 %) was countered by correlated responses in wing width (-300 %), femur (-90) and eye (-80) to give a small net negative response. As with earlier differences in clinal position the change in eye was primarily due to direct selection (-180 %). Overall the trend was dominated by correlated responses to selection on wing width and femur (though smaller), with wing length and scutellum acting antagonistically.

4.4.6 6 to 7

Between positions 6 and 7 all changes were negative with the exception of wing width and face length, with face width showing a large response and wing width showing

a small response. The change in wing length was a result of approximately equal levels of direct and correlated response on wing length (-120 %), scutellum (120 %), face width (-120 %), face length (120 %) and femur (-100). This pattern of direct and correlated response was different than those from earlier transitions, as wing width had a very small effect on the change in wing length and a large correlated response resulted from selection on the face characters (although they cancelled). Although the change in femur was primarily due to direct selection (-180 %), a correlated response to selection on face width

Table 3.6 f
Direct and Correlated Response to Selection on Each Character Positions 6 and 7

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-0.019	-0.014	-0.007	-0.007	-0.01	-0.006	-0.003	-0.011
0.003	0.0044	0.0014	0.0015	0.0021	0.0006	0.0001	0.0013
-0.016	-0.015	-0.026	-0.024	-0.025	-0.013	-0.008	-0.016
0.0066	0.007	0.0105	0.0161	0.0101	0.0051	0.0017	0.0035
0.0179	0.0181	0.0202	0.0187	0.0443	0.016	0.0133	0.0157
-0.018	-0.009	-0.017	-0.016	-0.027	-0.062	0.0129	-0.012
-0.009	-0.001	-0.01	-0.005	-0.021	0.0119	-0.049	-0.028
0.0193	0.0118	0.0136	0.0067	0.0162	0.0072	0.0185	0.0628
-0.016	0.0021	-0.015	-0.009	-0.01	-0.041	-0.014	0.0167

(-120 %) again made an important contribution to the trend which was partially cancelled by scutellum (140 %). Positive direct selection on tibia (180 %) and a correlated response from scutellum (210 %) were negated by correlated responses to selection on femur (-270 %) and face width (-180 %) to give a net negative response. In a similar manner strong positive direct selection on scutellum (440 %) was largely negated by correlated responses from selection on face width (-270 %), femur (-250 %) and eye (-210 %). The negative responses in face width (-150 %) and eye (-360 %) were largely a result of selection acting directly on these characters which was moderated by a correlated response to selection on face length and scutellum (and face width in the case of eye). Similarly, the positive change in face length was largely due to direct selection (380 %) being moderated by

correlated responses to eye (-170 %) and wing length (-120 %). With the exception of scutellum, which still had a large antagonistic relationship with the general trend, the pattern of selection operating between 6 and 7 is markedly different from that seen earlier. Wing width seems relatively unimportant, whereas wing length is important and in the same direction as the observed character trend. In addition, a face character, face width, appeared very important in determining both the observed trends in face shape as well as those seen in the other characters, although eye still had a strong component due to direct selection.

4.4.7 7 to 8

The change from 7 to 8 was dominated by large positive trends in face width and eye, with additional intermediate positive trends in scutellum and femur, and negative trend in wing length. The change in wing length was dominated by direct selection (-480 %) and a correlated response to selection on scutellum (-200) which was mediated by correlated responses to selection on wing width (320 %) and face width (200 %). The positive change in femur was dominated by correlated responses to selection on face width (270 %), eye (250 %) and wing width (210 %) which were mediated by negative correlated responses to selection on scutellum (-330 %) and wing length (-260 %). The positive trend seen in scutellum was largely the result of a correlated response to selection on eye (460 %), face width (380 %) and wing width (280 %), which was partially cancelled by direct selection on scutellum (-640 %) and negative correlated response to selection on wing width (-320 %). The large trend seen in face width mostly resulted from direct selection (350 %) which was mediated by negative correlated responses to selection on eye (-110 %) and scutellum (-90 %). Similarly the large trend seen in eye was largely due to direct selection (220 %). As in all other cases scutellum had a large antagonistic effect on all characters. Like the transition from 6 to 7, face width and

eye were important in the transition from 7 to 8: direct selection dominated the change in face width and eye which in turn effected the trends seen in the other characters. Wing width and femur were important, but only with regards to the body characters. Finally wing length may be under intense selective pressures as large direct selection over-rode negative correlated responses from other sources and correlated responses on other characters were generally antagonistic to the net direction of selection on the other characters.

Table 3.6 g
Direct and Correlated Response to Selection on Each Character
Positions 7 and 8

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-0.06	-0.045	-0.023	-0.022	-0.032	-0.019	-0.01	-0.033
0.0396	0.0581	0.0182	0.0203	0.0281	0.0083	0.0014	0.0179
0.0081	0.0074	0.0133	0.0124	0.0128	0.0065	0.004	0.0084
-0.004	-0.004	-0.006	-0.009	-0.005	-0.003	-9E-04	-0.002
-0.026	-0.026	-0.029	-0.027	-0.063	-0.023	-0.019	-0.022
0.0257	0.0124	0.0242	0.0221	0.0375	0.0871	-0.018	0.0163
0.0192	0.0031	0.0216	0.0106	0.0452	-0.026	0.1073	0.0613
-0.016	-0.01	-0.011	-0.005	-0.013	-0.006	-0.015	-0.051
-0.013	-0.003	0.0088	0.0025	0.0099	0.0249	0.0498	-0.005

Chapter 4
Discussion

4.1 Interpretation of Principal Components

In section 2.5.2 it is suggested that principal components represent a convenient way of summarizing a set of correlated characters. The reasons given for this are that usually the first few principal components account for most of the total variation in a set of correlated characters (thus reducing the number of characters under study), and that principal components are independent of one another. A problem with principal components though is that they have no meaning in their own right, as they are merely a linear combination of the original characters. Usually each principal component is interpreted to have some meaning based on the relative contribution of each of the original characters to the score. It follows that any interpretation of such scores must be accepted with some scepticism and caution as they reflect the interpreter's intuition. It is useful for the reader to refer to the component loadings of table 2.5.2 b in order to understand the interpretation that follows. All the characters load positively on the first principal component suggesting it is a general size factor. It is generally agreed that the first principal components derived from a positive definite covariance matrix (eg; morphological characters) represents a general size factor (Chatfield and Collins, 1980; Pimentel *et al.*, 1979; Reyment, 1984). The second component is dominated by a contrast between face length and the other characters. Other studies of morphological variation in Drosophila have found the second component to be dominated by a wing 'size' to thorax length

contrast (eg; Stalker and Carson, 1947, 1948, 1949; Sokoloff, 1965). As the present study did not measure thorax length, it should not come as a surprise that it did not note such a contrast. It is interesting that face length, the only character measured on the same axis as thorax length, showed the contrast that would be expected of thorax length, had it been measured. An alternative explanation, suggested by R. A. Morton (pers. comm.), for the face length contrast is a functional constraint imposed on the ptilinum. The ptilinum is a specialized structure in the fore face of Drosophila that is everted to aid the adult fly in emergence from its pupa, but is otherwise inverted. It is possible that this character is under strong selection and may even show clinal variation. The final two components both show contrasts between the wing and body/head characters. It is possible that these principal component represent a wing to thorax contrast as suggested above. These two components also account for very little of the total variation (< 10 %) and as a result may be unique to the sample they are drawn from, that is they may have no general significance. As these components additionally show no trends over the cline they are difficult to interpret. Inclusion of thorax length in the study may have been instructive, but was difficult technically to accomplish because of the method used to preserve the flies.

4.2 The Nature of Phenotypic Trends Over the Cline

The following discussion relates to figures 3.2 a to m. A number of characters show very strong non linear clinal trends. As it is known from this study that the characters are genetically correlated this discussion will revolve around general trends as opposed to a character by character treatment. The clinal trends are most readily apparent for the wing characters, the leg characters, scutellum width, face width, and the first two principal components. Although a measurement repeatability study was not conducted on these characters prior to the entire study it is probable that these are the same characters that are measured with the least error. As measurement error of the other characters was often

related to dissection problems (eg; the head resting at different angles due to an unclean cut of the foramen magnum), or preservation problems (eg; sometimes the eyes would slightly collapse), even a repeatability study would not completely resolve which characters were measured most accurately. Similarly measures of the length of the sex comb were plagued with the problem of it being small on the measurement screen (ie; inaccuracies in measurement due to the pixel resolution of the monitor) and being dependent on the angle the meta-tarsus was positioned, a high power microscope would alleviate the first problem but not the second. Given the measurement problems with some of the characters the trends observed in the others are probably reflective of true trends over the cline. Thus we will reduce further discussion to those traits showing the 'true' trends.

The characters show non linear trends, generally being small in the north and south and peaking near the middle of the cline at 36 degrees N latitude (Nashville). Interestingly Nashville was the western most collection site (excluding Winnipeg), and one of the higher sites. Ignoring Tampa Bay flies, because of small sample size, it is apparent that the difference between the northern sites and southern sites, if any, is a negative one. Thus, the findings of this study differ markedly relative to previous studies observing north south clines. Its seems unlikely that long term differences in temperature can explain the patterns observed, as even though at a higher altitude the middle clinal positions are still much warmer than the northern locations such as Dayton, Windsor, and Winnipeg. If one assumes that the adaptation in body size occurs over shorter intervals (ie; seasonally), as would seem plausible by the studies of Stalker and Carson (1949) and Bryant (1977), a more suitable explanation for the trend seems apparent. In the higher altitude, middle latitudes, the temperatures at, and in the few months prior to, collection times are more moderate and less likely to select for desiccative resistance (ie; through a shorter larval period). If the phenotypic trends over the cline were established in the summer months

prior to collection the observed trend would be expected given the very hot temperatures associated with July and August in the north. This effect could have acted in combination with selection acting on short generation time in the north where the food sources for Drosophila are suddenly abundant and then disappear (eg; in fruit orchards where many of the northern collections were made: see table 2.1.4 a). In the middle latitudes, temperatures in July and August might have been slightly more moderate, and food sources may have been relatively constant, as flies were collected from more natural sites. In the south, where very hot temperatures are the norm, we would expect smaller flies. The data of this study supports the hypothesis that morphological adaptation in Drosophila is not slow and responding to long term trends, but is occurring relatively quickly as the result of intense selection on a seasonally varying optimum. It follows that models for phenotypic evolution that contain phenotypic optima that are a function of simply a spatial parameter may give an unrealistic view of adaptive evolution in Dipterans (eg; Slatkin, 1978).

An important consideration that must be given to the observed trends is that although the grand phenotypic mean shows a strong clinal trend, there is a great deal of variation within any given clinal position. That is, different lines collected from a given clinal position show a great deal of variation in mean phenotype when reared in the lab. In fact, within any given clinal position it is probable that one could find lines with a mean phenotype equal to the grand mean of any other clinal position. This suggests a possible mechanism for the speed of evolution in local populations of Drosophila. If the selective regime changed in a given locality there would already be sub-populations pre-adapted to the new selective regime. Thus selection may operate within a given locality by booms and busts of preadapted lines (these could occur over one generation) as opposed to relatively slow selection on the population as a whole. This explanation may be lacking though, as it is difficult to imagine how populations of fruit flies separated by a few kilometres can

maintain genetic variation for the genes controlling the quantitative genetic characters under consideration if selection is indeed strong.

4.3 Heritability Estimates

The variance due to sires was estimated in each of four breeding experiments and then combined to give estimates of the genotypic variances/covariances and heritabilities of each trait and trait pair. The ANOVA method of genetic parameter estimation used in this study is known to give unbiased but over dispersed parameter estimates (Hayes and Hill, 1981). It was decided that the genotypic matrix was indeed over dispersed as it gave parameter estimates outside their proper bounds. In an attempt to alleviate this undesirable property the genotypic matrix was 'bent' (Hayes and Hill, 1981). 'Bending' contracts genotypic parameter estimates towards the grand mean of the eigenvalues of the matrix, and as a result tends to 'wash out' both high and low parameter estimates. Although little is known of the sampling properties of 'bent' matrices, it was decided to use the 'bent' matrix in this study as simulation studies have shown that 'bent' genetic covariance matrices generally give better predictions of the expected response to selection (Hayes and Hill, 1981). Unfortunately, it is difficult to determine if the bending factor used was too large or small, if too small parameter estimates would continue to be over dispersed and if too large all parameter estimates will tend toward the same value. As the bending factor of this study reduced the range of trait heritabilities from -0.186 - 0.252 to 0.09 - 0.1778 it is possible the genotypic matrix was over 'bent', even though the bending factor removed two impossible parameter estimates (femur length and eye width variance estimates). It is possible that bending may not be appropriate if one character tends to dominate the dynamics of the genotypic matrix. This may have been the case in the present study as genetic correlation between eye width and face width was large and negative relative to their phenotypic variances. This may have resulted from erroneous parameter estimates if the

heritability of eye width was actually close to zero or had a negative genetic correlation with the other characters. It follows that estimates of genetic parameters might have been better if eye width had been dropped from the genotypic matrix before bending, although this would be a questionable practise with no *a priori* reason for doing so.

A second solution to the problem of estimating genetic parameters may lie in an entirely different approach. It is possible better parameter estimates could have been obtained using an iterative maximum likelihood method as opposed to a least squares approach. Shaw (1987) reviews the potential of this approach to replace least squares approaches given newer more efficient computer algorithms and faster more powerful computers than were previously available. This approach was not used in the present study because of an incomplete understanding of the underlying theory on the author's part, the computer programs necessary to carry out this task not becoming available until late in the work, and these programs requiring additional modification to correctly analyse the data set at hand.

4.4 The Nature of Selection Over the Cline

Section 2.8.3 describes a method for calculating the relative intensities of direct and correlated response to selection given selection gradients, heritabilities and phenotypic covariances (2.8.3.9). Tables 3.6 a to g present the results of applying equation 2.8.3.9 to the data of this study. Given these tables we can determine if selection was strong on a given character or if its trend was likely due to a correlated response to selection acting on other characters. As was mentioned earlier results such as these are compromised as they only represent the direct and correlated responses to selection relative to the characters measured. It is conceivable that a strong 'direct' response observed here may really be a correlated response to an unmeasured variable. As the forces of selection were interpreted

for each adjacent pair of populations we can additionally determine if the targets of selection vary along the cline.

4.4.1 Summary of Direct and Correlated Responses to Selection

Table 4.4.1 summarizes the results presented in sections 3.6.1 to 3.6.7. The fraction in each cell of the table is made up of two parts. The numerator summarizes the net trend for a given character: a plus '+' implies the trend was positive, a minus '-' negative, with either being modified by a 's' if the trend was small or 'm' if intermediate. In the

Table 4.4.1
A Summary of Direct and Correlated Responses to Selection

	1 -> 2	2 -> 3	3 -> 4	4 -> 5	5 -> 6	6 -> 7	7 -> 8
WL	s+ / -	m+ / 0	+ / -	m+ / s-	m- / -	m- / +	m- / -
WW	+ / +	m+ / 0	s- / s+	+ / +	- / +	s+ / 0	s- / s+
FEM	+ / +	+ / +	- / +	+ / +	- / s+	m- / s+	m+ / s+
TIB	+ / 0	+ / s-	- / 0	+ / 0	- / 0	s- / 0	s+ / 0
SCUT	+ / -	+ / 0	+ / -	s+ / -	m- / -	m- / -	m+ / -
FW	s+ / d+	s+ / 0	+ / 0	s+ / 0	s- / 0	- / +	+ / +
EYE	s+ / d+	+ / 0	- / d+	+ / d+	- / d+	m- / 0	+ / +
FL	s+ / 0	s+ / 0	- / 0	+ / 0	- / 0	m+ / 0	s- / 0

denominator a plus signs (+) refers to a strong response in the same direction as the observed trend as a result of selection on this character in both the marked and other characters. Similarly a negative sign (-) in the denominator refers to a strong antagonistic response relative to the observed trend. An added 's' implies the effect was small, and an added 'd' implies the effect was strong by mostly just effecting the marked character. The signs do not indicate that a given character effected all the others in the indicated manner. That is, a plus character may have created a negative correlated response in some other

characters (relative to the trend in those characters). For example a cell containing $\frac{s+}{-}$ would tell the reader that a small positive trend was noted for this character and between these two positions this character had a significant negative effect on the other characters relative to their trends. From table 4.4.1 it can be seen that:

1. Correlated responses from scutellum and wing length generally acted antagonistically relative to the individual character trends.
2. Wing width and femur generally produced strong correlated responses in the same direction as observed trends, especially in the northern and central parts of the cline.
3. Changes in tibia could generally be accounted for as a correlated response to selection on femur.
4. Face width and eye generally responded to selection independently of the other characters in the northern and central portions of the cline.
5. In the southern part of the cline selection on face width and eye came to dominate changes in the other characters, with femur and wing width becoming less important.

Thus it appears that although phenotypically the flies in the north and south resemble one another more than those in the central regions, the nature of the selection maintaining this similarity differs. In the north the phenotypic distribution is maintained principally by selection on body characters, whereas in the south the distribution is maintained by selection on the head characters. Of course such conclusions should be treated cautiously as any trends may simply be due to sampling errors.

4.5 The Selective Mortalities Required to Produce the Observed Cline

Table 3.5 gives the selective mortalities necessary to create the observed differences in means. These mortalities appear small even given relatively short times of divergence. Of course if the trends observed occur rapidly and are dynamic over time much high selective mortalities would be required. Additionally the present methodology cannot differentiate between intense selection causing the divergence between populations over a relatively short time span and then the difference remaining constant (ie; at an optimum),

relatively weak constant selection creating the observed phenotypic divergence, or a wandering dynamic population mean for which any estimated selection inferences are mathematical mirages. It follows that studies which use these relatively small selective mortalities as support for a gradualist view of evolution are unfounded. In fact, Lande (1976) estimated selective mortalities of the order of 10^{-6} for horse populations which had diverged 10^6 generations; Lofsvold (1988) observed selective mortalities of the order of 10^{-6} for deer mice populations which had diverged 10^6 generations, and this study estimated selective mortalities of the same order if it is assumed populations have diverged 10^5 generations. This implies that selective mortalities are more a function of assumed divergence times than actual phenotypic divergence which is relatively constant. Manly (1985, p.358) points out that Lande's methodology can only detect small proportions culled per generation as higher proportions culled per generation give unrealistic expectations for phenotypic divergence. In citing Lande's (1976) example of the change in tooth height in the fossil horse he shows that a proportion culled per generation equal to a z score of -3.0 as opposed to -4.6 gives an expected change in tooth height of e^{2043} mm. Thus it is impossible for the sort of theory used in this work to reject a dynamic or punctuated equilibria model.

4.6 Other Considerations

4.6.1 Constancy of the Genetic Covariance Matrix

As was mentioned in the introduction the relative constancy of genetic covariance matrices both within and between species is an empirical question of great importance. If the infinite alleles or other gaussian models are not true and quantitative traits are governed by a few genes, possibly with important non-additive effects, then models of the sort used in this work may not be appropriate for describing long term evolution. Work needs to be completed which will determine the number, nature, and dynamics over time (space) of loci

governing quantitative traits. Only after such work is completed will it be known whether gaussian models are an appropriate avenue for the exploration of phenotypic evolution.

4.6.2 Migration

Although this work has largely assumed that the observed cline is due to selection for an optimum at each position it is possible that the actual cline is entirely the result of migration between populations with different phenotypes or, more likely, that the cline is smoothed by migration. Previous studies have concluded that North American *D. melanogaster* populations are largely panmictic (Singh and Rhomberg, 1987; Hale and Singh, 1987). Nonetheless this data is of little use as it estimates migration levels on the continent as a whole, usually under the assumption of an island model. That is, island models assume that migration rates are the same between each pair of populations. In addition, estimates may not come from truly neutral markers in the case of allozymes. In order to assess the relative importance of migration patterns in determining the observed phenotypic clines migration data is needed for each pair of populations. Once such information is available the rates of migration can be compared to the rates of change (or selection intensities) between adjacent populations, migration would appear to be important if these rates are inversely proportional (although this will most likely be on a non-linear scale). The proportion of variation not explained by such a model (ie; the residuals from the line of best fit) will represent the minimum proportion of the effect due to selection. An even better estimate of the proportion of the effect due to selection could be obtained if one had a theoretical curve relating migration rates to phenotypic differences under the assumption of neutral characters which the observed trend could be compared to, as information would not be lost in fitting the line.

4.7 How is the Observed Cline Created and Maintained

A possible explanation for the peak in the intermediate part of the cline is seasonal weather patterns. If the flies are responding to environmental changes occurring over the short term (eg; monthly climatic shifts), then it is possible that intermediate positions on the cline are indeed the coolest. Flies were collected in August and September which are generally preceded by very warm temperatures in both the North (great lake effect) and the South. In the intermediate clinal positions July and August may actually be cooler as a result of being further inland and moderated by altitude. A more careful scrutiny of meteorological data from the clinal positions in July and August may support or refute this hypothesis.

If indeed populations are responding this quickly to environmental pressures, one wonders how this is accomplished. It seems unlikely that local populations are responding in a 'Fisherian' manner to intense selective pressure. Although this mode of selection may be important in the species as a whole it would not seem capable of responding quickly enough to the changing environment. As was noted earlier there is a great deal of variation among lines within clinal position. It appears that lines can be easily found at any given location that have a mean phenotype which would be ideal for any other clinal position. Thus it seem possible that local populations may adapt by the expansion and contraction of small demes pre-adapted to the new phenotypic optimum at any given time. This would provide a relatively rapid means of adaptation to a new optimum for the species. Nonetheless if this is the predominant mode of adaptation one wonders why all genetic variation is not quickly depleted when expanded demes genetically intermingle with other contracted demes.

Dr. Rama Singh (per comm.) has suggested high migration rates from surrounding populations may explain the high levels of variability seen within a given clinal position. If

migration levels are quite high then the level of selection necessary to maintain the cline must be high as well. Nonetheless, migration levels of this magnitude may completely eliminate any differences between populations even with strong selection. Further empirical and theoretical studies may determine if a balance between high migration and strong selection will produce the same qualitative outcome as one between weak migration and weak selection.

Another explanation may be that selection does little, in the short term, to alter gene frequencies but only selects offspring from any given mating with beneficial recombinant genotypes. That is, although there is a great deal of additive variation present, short term selection acts on beneficial linked gene combinations or epistatic and dominant gene combinations. It is possible for a great deal of genetic variation to be maintained by a linkage disequilibria even in the face of strong selection (Bulmer, 1980; Lande, 1979; computer simulations by the author). This scenario implies that unless selection is maintained or linkage is tight, such beneficial gene combinations will quickly disappear.

Chapter 5
Conclusions

5.1 The Value of Estimates of Net Selection

5.1.1 Pitfalls

A topic which has been touched upon a number of times is the value of estimates of the net selection causing the divergence between populations. As has been discussed, such estimates may grossly under estimate the actual selective mortalities which have occurred, and provide no information as to the path taken to an optimum (or even if the optimum is stable). This is a serious short coming. An important question in evolutionary theory is how quantitative variation is maintained in the face of selection. If selection acting in the wild is greatly under estimated then models attempting to explain the maintenance of variation may be entirely invalid, as the parameters used in the model (eg; mutation rates) may have to be increased by orders of magnitude to account for higher intensities of selection. A second problem centres on evolutionary ecologist who wish to explain how organisms adapt (eg; the optimization school). Knowing the adaptive path travelled between populations is important for this group. If populations respond very quickly to selection, with respect to a few characters, and then stay near the new optima, selection will then be free to act on a new set of characters. This scenario reinforces the assumption of the optimization school that characters can be treated as largely independent. Contrarily, if the adaptive path is direct and slow then only a few characters can be optimized at one time, some characters may never reach a global maximum with respect to fitness, and organisms

are truly 'constrained' by their own genetic make up. Questions such as these are difficult to answer within the framework provided by the models utilized in this work. Instead they must be addressed with temporal studies of single populations or studies which address the underlying genetic basis of phenotypic traits.

5.1.2 Benefits

Nonetheless, it does appear that some useful information can be gleaned from such studies. Regardless of the path taken it is possible to infer the characters which were the most important in adaptive evolution between two populations. In this study for example. it appears that selection acted primarily on head characters in the south (through face width), but acted more on body size in the north (through femur and wing width). It also appears that character compromises are the norm. That is selection to increase one character is often offset by a correlated response from selection to decrease another. In this study selection, opposite the direction of the prevailing trend, on wing length and scutellum often impeded changes in other characters. Negative pleiotropy of this sort should be common if populations are close to their fitness optimum, and character compromises reflect functional constraints. In fact, if two characters are positively and strongly correlated with fitness then at equilibrium the two characters must show negative pleiotropy (otherwise the mean would change). Nonetheless studies of this sort can give a misleading picture, as unmeasured characters of potential importance are not accounted for. Ideally to obtain a complete picture one wishes to know the genetic correlation of each character with fitness, although this is probably impossible to accomplish in practise.

5.2 Using Knowledge of Historical Forces to Benefit the Study

The picture assembled in this work of phenotypic evolution along a cline is far from complete. A great deal of information would be gained if the populations along the cline were molecularly characterized in a such manner that inferences could be drawn about

migration rates between populations. Information of this sort would alow one to determine if the steepness of the cline at different positions is due to increased selection differentials or decreased gene flow with relatively constant selection differentials. As there is no way to infer this from phenotypic data alone, one cannot even speculate as to the importance of historical forces in population differentiation. Although it was intended to combine molecular data on migration rates with the phenotypic data, the molecular work was not completed at the time of this writing. When such data does become available this study will greatly profit.

5.3 How Little Do We Know?

This title is borrowed from a review paper by Barton and Turelli which summarizes the problems faced by geneticists studying phenotypic evolution (1989). Unfortunately we know close to nothing about the underlying nature of the genetic system controlling quantitative characters. In fact we do not even know if the nature of the system is fundamentally the same as that controlling traditional Mendelian traits. We do not know the number of genes, the distribution of effects, nor the effects of pleiotropy, dominance, and epistasis in regards to quantitative genetic variation. We additionally do not know the rate or nature of mutations in polygenic characters nor how the evolution of such characters effects the genetic correlation matrix. Although we know a little of how selection operates on phenotypes in the short term we do not know if such results can be extrapolated beyond the barnyard. Finally, we are ignorant of the forces of natural selection and its targets. Thus, the methodology used in this work may only serve as a first step to uncovering the forces governing adaptive evolution. Empirical work must be completed which will uncover the mechanisms governing phenotypic evolution and only then can theoretical models be created which will allow a fuller understanding of natural selection.

Appendix A

Table A1
Experiment 1: SSCP Sire

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0659	0.0496	0.0343	0.0334	0.0468	0.0277	0.0280	0.0261
0.0496	0.0888	0.0386	0.0361	0.0547	0.0313	0.0287	0.0233
0.0343	0.0386	0.0518	0.0448	0.0513	0.0353	0.0341	0.0205
0.0334	0.0361	0.0448	0.0588	0.0460	0.0327	0.0255	0.0190
0.0468	0.0547	0.0513	0.0460	0.1161	0.0481	0.0441	0.0333
0.0277	0.0313	0.0353	0.0327	0.0481	0.0765	0.0010	0.0219
0.0280	0.0287	0.0341	0.0255	0.0441	0.0010	0.1048	0.0278
0.0261	0.0233	0.0205	0.0190	0.0333	0.0219	0.0278	0.0990

Table A2
Experiment 1: SSCP Dams

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0680	0.0481	0.0371	0.0371	0.0397	0.0235	0.0308	0.0253
0.0481	0.0856	0.0332	0.0297	0.0532	0.0340	0.0323	0.0386
0.0371	0.0332	0.0394	0.0350	0.0366	0.0254	0.0232	0.0217
0.0371	0.0297	0.0350	0.0466	0.0331	0.0265	0.0173	0.0247
0.0397	0.0532	0.0366	0.0331	0.0896	0.0396	0.0382	0.0401
0.0235	0.0340	0.0254	0.0265	0.0396	0.0582	-0.0070	0.0272
0.0308	0.0323	0.0232	0.0173	0.0382	-0.0070	0.0940	0.0231
0.0253	0.0386	0.0217	0.0247	0.0401	0.0272	0.0231	0.1036

Table A3
Experiment 1: SSCP Error

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0384	0.0274	0.0183	0.0197	0.0222	0.0185	0.0123	0.0205
0.0274	0.0540	0.0180	0.0170	0.0178	0.0193	0.0079	0.0191
0.0183	0.0180	0.0269	0.0232	0.0182	0.0141	0.0153	0.0124
0.0197	0.0170	0.0232	0.0321	0.0174	0.0148	0.0146	0.0108
0.0222	0.0178	0.0182	0.0174	0.0466	0.0159	0.0155	0.0189
0.0185	0.0193	0.0141	0.0148	0.0159	0.0429	-0.0051	0.0191
0.0123	0.0079	0.0153	0.0146	0.0155	-0.0051	0.0531	0.0109
0.0205	0.0191	0.0124	0.0108	0.0189	0.0191	0.0109	0.0690

Table A4
Experiment 2: SSCP Sires

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0842	0.0622	0.0465	0.0352	0.0532	0.0496	0.0282	0.0368
0.0622	0.0835	0.0464	0.0371	0.0369	0.0527	0.0314	0.0367
0.0465	0.0464	0.1053	0.0429	0.0402	0.0537	0.0451	0.0412
0.0352	0.0371	0.0429	0.0556	0.0397	0.0388	0.0258	0.0406
0.0532	0.0369	0.0402	0.0397	0.1517	0.0542	0.0282	0.0584
0.0496	0.0527	0.0537	0.0388	0.0542	0.1299	-0.0046	0.0573
0.0282	0.0314	0.0451	0.0258	0.0282	-0.0046	0.1203	0.0288
0.0368	0.0367	0.0412	0.0406	0.0584	0.0573	0.0288	0.1585

Table A5
Experiment 2: SSCP Dams

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0929	0.0566	0.0002	0.0386	0.0348	0.0847	0.0209	0.0166
0.0566	0.0772	0.0122	0.0266	0.0276	0.0518	0.0269	0.0124
0.0002	0.0122	0.1523	0.0371	0.0266	-0.0263	0.0454	0.0402
0.0386	0.0266	0.0371	0.0569	0.0325	0.0420	0.0251	0.0176
0.0348	0.0276	0.0266	0.0325	0.0973	0.0406	0.0221	0.0305
0.0847	0.0518	-0.0263	0.0420	0.0406	0.2023	-0.0215	0.0437
0.0209	0.0269	0.0454	0.0251	0.0221	-0.0215	0.1056	0.0267
0.0166	0.0124	0.0402	0.0176	0.0305	0.0437	0.0267	0.1423

Table A6
Experiment 2: SSCP Error

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0255	0.0162	0.0051	0.0095	0.0134	0.0094	0.0102	0.0059
0.0162	0.0396	0.0079	0.0066	0.0173	0.0085	0.0088	0.0069
0.0051	0.0079	0.0761	0.0277	0.0133	0.0109	0.0098	0.0000
0.0095	0.0066	0.0277	0.0320	0.0093	0.0074	0.0158	0.0033
0.0134	0.0173	0.0133	0.0093	0.0942	0.0117	0.0092	0.0154
0.0094	0.0085	0.0109	0.0074	0.0117	0.0374	0.0014	0.0218
0.0102	0.0088	0.0098	0.0158	0.0092	0.0014	0.0506	0.0171
0.0059	0.0069	0.0000	0.0033	0.0154	0.0218	0.0171	0.1421

Table A7
Experiment 3: SSCP Sires

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0672	0.0495	0.0347	0.0334	0.0342	0.0355	0.0203	0.0302
0.0495	0.0738	0.0284	0.0308	0.0342	0.0341	0.0218	0.0262
0.0347	0.0284	0.0488	0.0436	0.0326	0.0266	0.0324	0.0314
0.0334	0.0308	0.0436	0.0581	0.0271	0.0280	0.0263	0.0314
0.0342	0.0342	0.0326	0.0271	0.0709	0.0294	0.0384	0.0405
0.0355	0.0341	0.0266	0.0280	0.0294	0.0641	0.0075	0.0389
0.0203	0.0218	0.0324	0.0263	0.0384	0.0075	0.1083	0.0419
0.0302	0.0262	0.0314	0.0314	0.0405	0.0389	0.0419	0.1269

Table A8
Experiment 3: SSCP Dams

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0524	0.0361	0.0298	0.0294	0.0249	0.0235	0.0284	0.0260
0.0361	0.0579	0.0314	0.0240	0.0287	0.0292	0.0343	0.0322
0.0298	0.0314	0.0428	0.0346	0.0268	0.0273	0.0355	0.0301
0.0294	0.0240	0.0346	0.0423	0.0220	0.0240	0.0261	0.0350
0.0249	0.0287	0.0268	0.0220	0.0560	0.0195	0.0344	0.0229
0.0235	0.0292	0.0273	0.0240	0.0195	0.0515	0.0189	0.0305
0.0284	0.0343	0.0355	0.0261	0.0344	0.0189	0.1021	0.0444
0.0260	0.0322	0.0301	0.0350	0.0229	0.0305	0.0444	0.1131

Table A9
Experiment 3: SSCP Error

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0361	0.0252	0.0179	0.0159	0.0178	0.0131	0.0138	0.0095
0.0252	0.0410	0.0169	0.0164	0.0157	0.0117	0.0148	0.0148
0.0179	0.0169	0.0282	0.0219	0.0185	0.0151	0.0174	0.0113
0.0159	0.0164	0.0219	0.0294	0.0165	0.0144	0.0135	0.0144
0.0178	0.0157	0.0185	0.0165	0.0311	0.0161	0.0101	0.0119
0.0131	0.0117	0.0151	0.0144	0.0161	0.0328	-0.0024	0.0143
0.0138	0.0148	0.0174	0.0135	0.0101	-0.0024	0.0470	0.0066
0.0095	0.0148	0.0113	0.0144	0.0119	0.0143	0.0066	0.0597

Table A10
Experiment 4: SSCP Sires

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0781	0.0521	0.0422	0.0438	0.0320	0.0113	0.0337	0.0507
0.0521	0.0741	0.0382	0.0332	0.0383	0.0158	0.0346	0.0552
0.0422	0.0382	0.0582	0.0531	0.0369	0.0246	0.0272	0.0344
0.0438	0.0332	0.0531	0.0708	0.0349	0.0245	0.0222	0.0308
0.0320	0.0383	0.0369	0.0349	0.0755	0.0192	0.0280	0.0256
0.0113	0.0158	0.0246	0.0245	0.0192	0.0663	-0.0069	0.0222
0.0337	0.0346	0.0272	0.0222	0.0280	-0.0069	0.1147	0.0475
0.0507	0.0552	0.0344	0.0308	0.0256	0.0222	0.0475	0.1640

Table A11
Experiment 4: SSCP Dams

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0506	0.0350	0.0362	0.0368	0.0282	0.0208	0.0286	0.0276
0.0350	0.0586	0.0362	0.0377	0.0223	0.0236	0.0372	0.0284
0.0362	0.0362	0.0654	0.0564	0.0317	0.0288	0.0466	0.0206
0.0368	0.0377	0.0564	0.0703	0.0290	0.0310	0.0483	0.0247
0.0282	0.0223	0.0317	0.0290	0.0601	0.0154	0.0283	0.0206
0.0208	0.0236	0.0288	0.0310	0.0154	0.0545	0.0090	0.0286
0.0286	0.0372	0.0466	0.0483	0.0283	0.0090	0.1321	0.0249
0.0276	0.0284	0.0206	0.0247	0.0206	0.0286	0.0249	0.1364

Table A12
Experiment 4: SSCP Error

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.0313	0.0209	0.0171	0.0166	0.0128	0.0134	0.0163	0.0143
0.0209	0.0401	0.0172	0.0172	0.0147	0.0062	0.0194	0.0239
0.0171	0.0172	0.0348	0.0275	0.0150	0.0127	0.0212	0.0228
0.0166	0.0172	0.0275	0.0426	0.0149	0.0106	0.0218	0.0196
0.0128	0.0147	0.0150	0.0149	0.0373	0.0048	0.0239	0.0275
0.0134	0.0062	0.0127	0.0106	0.0048	0.0380	-0.0004	0.0113
0.0163	0.0194	0.0212	0.0218	0.0239	-0.0004	0.0652	0.0293
0.0143	0.0239	0.0228	0.0196	0.0275	0.0113	0.0293	0.1582

Table A13
Experiment 1: Variance Due to Sires (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-0.2427	-0.0940	-0.1935	-0.2078	0.0751	0.0797	-0.1893	-0.0113
-0.0940	-0.1165	0.0439	0.0879	-0.1829	-0.1635	-0.2468	-0.5235
-0.1935	0.0439	0.2440	0.1802	0.2677	0.1876	0.2352	-0.0895
-0.2078	0.0879	0.1802	0.2280	0.2384	0.0881	0.1978	-0.2379
-0.1201	-0.0332	0.1663	-0.0109	-0.1116	-0.1199	0.2469	-0.0386
0.0751	-0.1829	0.2677	0.2384	0.4222	0.0725	0.0113	-0.3141
0.0797	-0.1635	0.1876	0.0881	0.0725	0.3814	0.2230	-0.1898
-0.1893	-0.2468	0.2352	0.1978	0.0113	0.2230	0.0230	0.0453
-0.0113	-0.5235	-0.0895	-0.2379	-0.3141	-0.1898	0.0453	-0.3398

Table A14
Experiment 1: Variance Due to Dams (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
1.8022	1.2638	1.1350	1.0514	1.0602	0.3235	1.1032	0.3198
1.2638	1.9462	0.9207	0.7728	2.0995	0.8972	1.4400	1.1729
1.1350	0.9207	0.7809	0.7339	1.1133	0.6842	0.4903	0.5652
1.0514	0.7728	0.7339	0.9065	0.9484	0.7105	0.1838	0.8363
0.1953	0.2203	-0.2099	0.1886	0.3657	0.4530	-0.6821	0.1007
1.0602	2.0995	1.1133	0.9484	2.5980	1.4172	1.3563	1.2765
0.3235	0.8972	0.6842	0.7105	1.4172	0.9721	-0.1256	0.5082
1.1032	1.4400	0.4903	0.1838	1.3563	-0.1256	2.4920	0.7326
0.3198	1.1729	0.5652	0.8363	1.2765	0.5082	0.7326	2.1469

Table A15
Experiment 2: Variance Due to Sires (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
-1.0260	-0.1760	1.9148	-0.4293	0.5273	-2.1677	0.1885	0.7071
-0.1760	-0.1240	1.3309	0.2205	0.2709	-0.3945	-0.0002	0.9233
1.9148	1.3309	-2.6649	0.1358	0.4101	3.5966	-0.3666	-0.3572
-0.4293	0.2205	0.1358	-0.3014	0.0595	-0.4780	-0.0639	0.7874
4.0511	2.7138	-6.2800	0.1362	0.4840	6.8942	-1.9313	-1.8522
0.5273	0.2709	0.4101	0.0595	2.1499	0.2594	0.1154	0.9716
-2.1677	-0.3945	3.5966	-0.4780	0.2594	-4.5727	0.9108	0.3276
0.1885	-0.0002	-0.3666	-0.0639	0.1154	0.9108	0.0347	-0.0094
0.7071	0.9233	-0.3572	0.7874	0.9716	0.3276	-0.0094	0.6346

Table A16
Experiment 2: Variance Due to Dams (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
6.1015	3.6525	-0.4362	2.6315	1.9439	6.7954	0.9765	0.9682
3.6525	3.4330	0.4030	1.8106	0.9422	3.9127	1.6372	0.5038
-0.4362	0.4030	6.9499	0.8809	1.2203	-3.3401	3.2201	3.6174
2.6315	1.8106	0.8809	2.2753	2.0983	3.1325	0.8500	1.2876
-7.8065	-4.9144	13.4049	0.0624	-0.9590	-11.1445	4.4242	3.2558
1.9439	0.9422	1.2203	2.0983	0.3794	2.6191	1.1758	1.3853
6.7954	3.9127	-3.3401	3.1325	2.6191	14.9054	-2.0643	1.9922
0.9765	1.6372	3.2201	0.8500	1.1758	-2.0643	5.0179	0.8792
0.9682	0.5038	3.6174	1.2876	1.3853	1.9922	0.8792	0.1670

Table A17
Experiment 3: Variance Due to Sires (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.3379	0.3338	0.0692	0.0253	0.2343	0.2926	-0.3446	0.0075
0.3338	0.3707	-0.1914	0.1564	0.0751	0.0254	-0.5165	-0.3033
0.0692	-0.1914	0.0828	0.1858	0.1237	-0.1070	-0.2199	-0.0932
0.0253	0.1564	0.1858	0.3938	0.1180	0.0553	-0.0796	-0.2556
0.0966	-0.0588	-0.0903	-0.2018	0.1699	-0.1375	0.5461	-0.2124
0.2343	0.0751	0.1237	0.1180	0.2823	0.2798	-0.0505	0.4565
0.2926	0.0254	-0.1070	0.0553	0.2798	0.2555	-0.5026	0.1420
-0.3446	-0.5165	-0.2199	-0.0796	-0.0505	-0.5026	-0.1993	-0.3463
0.0075	-0.3033	-0.0932	-0.2556	0.4565	0.1420	-0.3463	0.0459

Table A18
Experiment 3: Variance Due to Dams (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
1.1025	0.7366	0.7998	0.9141	0.4827	0.7052	0.9825	1.1177
0.7366	1.1465	0.9789	0.5143	0.8819	1.1837	1.3207	1.1765
0.7998	0.9789	0.9871	0.8608	0.5549	0.8271	1.2186	1.2737
0.9141	0.5143	0.8608	0.8747	0.3766	0.6524	0.8479	1.3907
-0.3293	-0.4052	0.2737	0.3935	0.3789	0.1255	-0.9673	0.4201
0.4827	0.8819	0.5549	0.3766	1.6786	0.2302	1.6437	0.7416
0.7052	1.1837	0.8271	0.6524	0.2302	1.2686	1.4405	1.0921
0.9825	1.3207	1.2186	0.8479	1.6437	1.4405	3.7254	2.5530
1.1177	1.1765	1.2737	1.3907	0.7416	1.0921	2.5530	3.6092

Table A19
Experiment 4: Variance Due to Sires (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
0.7372	0.4462	0.0585	0.0812	0.0152	-0.3458	0.0805	0.6330
0.4462	0.3687	-0.0680	-0.2814	0.4517	-0.3661	-0.2022	0.8195
0.0585	-0.0680	-0.4312	-0.3007	0.0493	-0.2413	-0.7812	0.4583
0.0812	-0.2814	-0.3007	-0.1646	0.0918	-0.3453	-0.9977	0.1628
-0.0329	-0.8225	-1.2846	-1.0906	-0.5668	-1.2572	-1.6560	-0.8895
0.0152	0.4517	0.0493	0.0918	0.3347	0.0452	-0.0329	0.2179
-0.3458	-0.3661	-0.2413	-0.3453	0.0452	0.2661	-0.5659	-0.3198
0.0805	-0.2022	-0.7812	-0.9977	-0.0329	-0.5659	-0.9979	0.7510
0.6330	0.8195	0.4583	0.1628	0.2179	-0.3198	0.7510	1.0894

Table A20
Experiment 4: Variance Due to Dams (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	EYE	FL
1.3014	0.9498	1.3036	1.3797	1.0492	0.4987	0.8347	0.9089
0.9498	1.2352	1.3027	1.4070	0.5143	1.2039	1.2152	0.2787
1.3036	1.3027	2.0841	1.9750	1.1414	1.1014	1.7349	-0.1833
1.3797	1.4070	1.9750	1.8687	0.9588	1.4075	1.8128	0.3263
0.0483	1.6697	2.6635	2.9824	0.8874	2.0712	3.6055	0.7797
1.0492	0.5143	1.1414	0.9588	1.5389	0.7333	0.2777	-0.5175
0.4987	1.2039	1.1014	1.4075	0.7333	1.1014	0.6526	1.1886
0.8347	1.2152	1.7349	1.8128	0.2777	0.6526	4.5693	-0.3426
0.9089	0.2787	-0.1833	0.3263	-0.5175	1.1886	-0.3426	-1.7209

Table A23
Total Genotypic Variance (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	FL
0.9864	0.8409	-0.1254	-0.1747	0.4301	0.0646	0.7767
0.8409	0.7588	-0.2645	-0.0181	0.3847	-0.6630	-0.1487
-0.1254	-0.2645	-0.0667	0.1324	0.6160	-0.1581	0.3158
-0.1747	-0.0181	0.1324	0.6381	0.6178	-0.2263	-0.4781
0.4301	0.3847	0.6160	0.6178	1.4013	0.5217	0.3894
0.0646	-0.6630	-0.1581	-0.2263	0.5217	1.2216	-0.4953
0.7767	-0.1487	0.3158	-0.4781	0.3894	-0.4953	0.9047

Table A24
Total Phenotypic Variance (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	FL
4.7094	3.3201	2.5839	2.5684	2.4953	1.8066	2.2231
3.3201	5.5380	2.4907	2.3358	2.6999	1.9781	2.5259
2.5839	2.4907	3.8258	3.2822	2.5832	2.0238	1.9810
2.5684	2.3358	3.2822	4.3530	2.3257	1.9927	2.0306
2.4953	2.6999	2.5832	2.3257	5.6298	2.0077	2.3296
1.8066	1.9781	2.0238	1.9927	2.0077	4.6799	2.0629
2.2231	2.5259	1.9810	2.0306	2.3296	2.0629	9.9366

Table A25
Heritability

WL	WW	FEM	TIB	SCUT	FW	FL
0.2095	0.2533	-0.0485	-0.0680	0.1724	0.0357	0.3494
0.2533	0.1370	-0.1062	-0.0078	0.1425	-0.3352	-0.0589
-0.0485	-0.1062	-0.0174	0.0403	0.2384	-0.0781	0.1594
-0.0680	-0.0078	0.0403	0.1466	0.2656	-0.1136	-0.2355
0.1724	0.1425	0.2384	0.2656	0.2489	0.2598	0.1672
0.0357	-0.3352	-0.0781	-0.1136	0.2598	0.2610	-0.2401
0.3494	-0.0589	0.1594	-0.2355	0.1672	-0.2401	0.0911

Table A26
Bent Genotypic Matrix (X 10^4)

WL	WW	FEM	TIB	SCUT	FW	FL
1.7300	1.3469	0.3663	0.3203	0.8358	0.3892	1.0887
1.3469	1.6837	0.2275	0.4144	0.8337	-0.2153	0.3353
0.3663	0.2275	0.6464	0.7204	1.0144	0.2346	0.6410
0.3203	0.4144	0.7204	1.3598	0.9686	0.1692	-0.0440
0.8358	0.8337	1.0144	0.9686	2.2623	0.8260	0.7696
0.3892	-0.2153	0.2346	0.1692	0.8260	1.9302	-0.0531
1.0887	0.3353	0.6410	-0.0440	0.7696	-0.0531	2.6215

Table A27
Bent Heritability

WL	WW	FEM	TIB	SCUT	FW	FL
0.3674	0.4057	0.1418	0.1247	0.3349	0.2155	0.4897
0.4057	0.3040	0.0913	0.1774	0.3088	-0.1089	0.1327
0.1418	0.0913	0.1690	0.2195	0.3927	0.1159	0.3236
0.1247	0.1774	0.2195	0.3124	0.4165	0.0849	-0.0217
0.3349	0.3088	0.3927	0.4165	0.4018	0.4114	0.3304
0.2155	-0.1089	0.1159	0.0849	0.4114	0.4124	-0.0257
0.4897	0.1327	0.3236	-0.0217	0.3304	-0.0257	0.2638

Table A28
Genetic Parameter Estimates for the First Four Principal Components

Comp	exp	MSE_s	MSE_d	MSE	V_s	V_d	V_t	h^2
1	1	0.166	0.1552	0.0808	-0.003	0.0553	0.1335	-0.079
	2	0.3113	0.2456	0.1055	0.0103	0.105	0.2208	0.1859
	3	0.1753	0.1505	0.08	0.0024	0.0529	0.1353	0.071
	4	0.191	0.1579	0.1019	0.0065	0.0421	0.1506	0.1735
	avg				0.0018	0.0504	0.1393	0.052
2	1	0.411	0.3572	0.2349	0.0076	0.0909	0.3334	0.0911
	2	0.7902	0.8376	0.9025	-0.01	-0.049	0.8434	-0.05
	3	0.4595	0.4033	0.2659	0.0073	0.103	0.3762	0.0777
	4	0.6369	0.6197	0.6721	0.01	-0.039	0.6427	0.0623
	avg				0.0082	0.0544	0.4429	0.0745
3	1	0.715	0.5766	0.4078	0.0316	0.1254	0.5649	0.224
	2	1.3946	1.7414	0.6221	-0.21	0.8393	1.2516	-0.67
	3	0.9422	0.7127	0.3844	0.0491	0.2462	0.6798	0.2891
	4	0.843	1.0333	0.5693	-0.101	0.3488	0.8169	-0.495
	avg				-0.004	0.2333	0.6797	-0.023
4	1	0.7231	0.5855	0.34	0.025	0.1824	0.5474	0.1824
	2	1.5164	1.5934	0.7944	-0.093	0.5991	1.3008	-0.285
	3	0.6451	0.4876	0.418	0.0467	0.0522	0.5169	0.3615
	4	0.9339	0.6791	0.4695	0.0684	0.1576	0.6954	0.3932
	avg				0.0454	0.133	0.5835	0.311

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