INVESTIGATIONS OF HOMEOSTASIS AND OTHER GENETICAL PROBLEMS USING VARIANCE COMPONENTS WITH APPLICATION TO HUMAN FAMILY DATA

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CONTENTS

ABSTRACT

This thesis is concerned primarily with the issue of heterozygote advantage. One of the ways in which it is believed that heterozygote advantage manifests itself is by the reduced effect that environmental changes have on heterozygous individuals, thus rendering their traits less variable than those of homozygotes. In the analyses described in this thesis, no consistent support for or refutation of this hypothesis has been obtained. The reasons for these indeterminate findings are discussed in detail as each methodology is evaluated.

Three data sets have been analysed in this thesis. The first consisted of 414 mother-newborn baby pairs for which nine quantitative traits and the genotypes of several polymorphic loci were available. This set was used in chapter 3. The second consisted of 43 same-sex twin pairs for which height, IQ and six genotypes had been ascertained. The third data set consisted of 99 pairs of dizygous twins and the variable of interest was lung function. These two sets were used in chapter 4.

The first data set was used to investigate more appropriate statistical methods for analysing variances of several related traits. In particular, interest lay in finding statistical methods which were more appropriate than those used in earlier work on this problem. Methods such as multivariate parametric and nonparametric tests for equality of dispersion matrices were found to be superior to multivariate coefficients of variation and principal components analysis. The appropriateness of the data to the problem of examining heterozygote advantage has also been discussed.

These data were also used to consider an adaptive distances model, an alternative approach suggested in the literature. The problems of fitting this model in practice have been discussed fully in chapter 3.

In the final part of chapter 3 a new way of estimating heritability using multivariate analysis of variance (MANOVA) has been suggested. The third data set was also used to estimate heritability by the MANOVA method but was less successful and these results appear in the final part of chapter 4.

Most of chapter 4 is concerned with modifying a pedigree model to include components of variance for heterozygotes and homozygotes. The pedigrees used were the twin pairs of the second data set.

Previous research reported in the literature has found that if the alleles at a locus act additively on a particular trait and if there are several such loci all acting additively then the variance of the trait will decrease with increasing heterozygosity, even though there may be no heterozygote advantage *per se*. Other research has shown that this effect depends on the relative frequencies of different alleles, when there are more than two alleles at each locus. In chapter 2 of this thesis, algebraic calculations and computer simulations have been used to show that these effects change if the assumptions of additivity are relaxed.

SIGNED STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the text. I consent to this thesis being made available for photocopying and loan if applicable, if it is accepted for the award of the degree.

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I have already submitted some of this work for publication. Most of section 3.5 on heritability estimates has appeared in Bishop, Mayo and Beckman (1987). Much of the rest of chapter 3 will appear in Bishop, Mayo and Beckman (1988). Beckman provided the data for these two publications and made some helpful comments after reading the manuscripts.

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1. LITERATURE REVIEW

1.1 Introduction

In his book, Lerner (1954) developed a thesis to explain the processes underlying genetic homeostasis to establish a connection between genetic and developmental homeostasis and to suggest that the most likely mechanism for both types of homeostasis was the superiority in fitness of the heterozygous over the homozygous genotypes.

Lerner defined homeostasis as the property of the organism to adjust itself to variable conditions or, alternatively, as the self-regulatory mechanisms of the organism which permit it to stabilize itself in fluctuating inner and outer environments. He stated that developmental homeostasis, or ontogenetic self-regulation, was based on "the greater ability of the heterozygote to stay within the norms of canalized development", while genetic homeostasis, or self-regulation of populations, was based on "natural selection favouring intermediate rather than extreme phenotypes".

Lerner suggested that an ideal test of whether heterozygotes were better canalized than homozygotes would consist of examining the environmental variability in fitness of the two types. Since useful fitness data were not usually available, individual phenotypic characters would have to be used instead to investigate the buffering capacity of homozygotes and heterozygotes. He discussed several different ways in which this buffering capacity could express itself. First, under normal environmental conditions the variability of some traits would be less among heterozygotes than among homozygotes. Second, when there has been a change in the environment or a mutant has appeared, the extent of phenotypic difference from the original form would be less for the heterozygotes than for the homozygotes. Third, the variation in expression of bilateral or serially arranged characters would be less in heterozygotes than in homozygotes. Finally, the amplitude of expression of repeatable reactions to some stimulus would be less for heterozygotes than for homozygotes.

Experimental evidence obtained by Lerner related to marker genes which had major morphological effects and which were therefore likely to influence fitness directly. Since that time genes determining widespread biochemical polymorphisms have been discovered (Harris

1966, Lewontin and Hubby, 1966) and have been used extensively to test for heterozygote advantage.

Mitton and Grant (1984) summarize a number of studies which looked at protein heterozygosity and developmental stability - morphological variability in marine fish, sparrows and monarch butterfly, fluctuating asymmetry in rainbow trout, the number of fin rays in the guppy and the plaice and fluctuating asymmetry among populations of side-blotched lizards. Then they turn their attention to protein heterozygosity and growth rate where studies have been conducted on marine invertebrates, the tiger salamander, deer, pigs, sheep, quaking aspen, conifers and humans. These studies have all been conducted within populations and in almost all cases the more heterozygous individuals have exhibited some advantage in growth characteristics.

However, there have been some problems associated with these studies, not the least of which is statistical methodology. Mitton and Grant do not address this issue. Many researchers collect several measurements of morphological characters or meristic traits for each individual and wish to analyse them as a group. The search for methods of analysis has sometimes resulted in the misuse of some statistical techniques and even in the spurious invention of others. Suitable statistical methods will be discussed in Section 1.4 of this chapter.

Once results have been obtained there may be some difficulty in interpreting them and fitting them into the spectrum of other results. Studies conducted by other researchers will be discussed and appraised in two sections - Section 1.2 consists of studies performed on various organisms to give a general picture, while Section 1.3 consists only of studies performed on humans.

1.2 Investigation of various organisms

As Zink,Smith and Patton (1985) point out, Lerner's hypothesized inverse relationship between individual heterozygosity and variance (developmental homeostasis) does not necessarily carry over to the same relationship between the population heterozygosity averaged over all individuals and the variance among populations (genetic homeostasis). What is true on

an individual basis may not be true when comparing several populations because environmental variance may be negligible or may change from one population to the next.

Zink *et al.* (*ibid.*) also summarize three assumptions which are not mutually exclusive and which occur in the literature relating to investigations of homeostasis. They are:

(i) the degree of heterozygosity of the loci examined reflects the heterozygosity of the whole genome;

(ii) the loci examined are in linkage disequilibrium with the loci controlling the traits whose variances are being measured;

(iii) the loci examined are directly involved in the expression of the traits being measured.

They conclude that the first assumption is an unlikely one for positive results given the small numbers of loci which are usually examined. Other researchers agree with this. For instance, Mitton and Pierce (1980) and Chakraborty (1981) have shown that the correlation between heterozygosity of a few observed loci and that of the whole genome is small. Smouse (1986) argues that nothing can be gained from invoking sections of the genome which are segregating independently of the loci under observation. His view is that loci closely linked to the loci under observation may be responsible for the fitness-homozygosity relationships found by several authors and so what are of interest are "observed segments of chromosome" rather than "observed loci". Zink *et al.* (*ibid.*) suggest that Lerner's hypothesis could be tested using individual heterozygosity if either of the second two assumptions holds.

It is somewhat surprising that they then investigate the relationship between average heterozygosity and morphometric variation across several populations of fox sparrows and pocket gophers when they have already pointed out that it is impossible to predict what this relationship should be.

Because of the uncertainty of the predictions under the hypothesis of genetic homeostasis, it is worth looking at some of the studies of this hypothesis which have been reported in the literature. Leary, Allendorf and Knudsen (1985a) bred 14 families from a single population of rainbow trout which had been raised in isolation for several generations. The 14 families were raised in very similar environments in a hatchery. The experimental conditions for this study do not tend to favour fluctuations in the environment which is nearly constant for

all of the 14 families. Leary, *et al.* (*ibid.*) measured eight meristic characters and point out that much of the variation of these characters is controlled by additive genetic variation. They plot mean family asymmetry for the meristic traits against mean family heterozygosity for 13 isozyme loci and find a significant negative relationship. However, with minimal environmental fluctuations, it is a matter for speculation just what buffering effect heterozygosity would need to afford. In fact Leary *et al.* (*ibid.*) continued their investigation of these fish by examining individuals with extreme vertebral counts in more detail and found a significantly higher average number of heterozygous loci among them than among those individuals with normal counts. So perhaps these results demonstrate the problem of examining individuals versus populations.

Soulé (1979) reports a significant negative correlation between fluctuating asymmetry of four morphological characters and heterozygosity of 18 loci for 15 populations of side-blotched lizards. He argues that this sort of relationship is hard to detect at an individual level because an individual may have different directions of and degrees of asymmetry for different characters. He also argues that the loci which control different traits within an individual may not be equally heterozygous, but this does not preclude a difference in average level of asymmetry among populations. Reference is made to a study by Soulé, Yang, Weiler and Gorman (1973) in which they examined eight species of *Auolis* lizards and 13 populations of *Uta* lizards and found a positive correlation between variance of morphological traits and mean percentage heterozygosity for respectively 19 and 22 loci controlling enzymes and other proteins. They state that the morphological data provide information on variation environmental heterogeneity whereas the electrophoretic data provide information on a much smaller fraction of the genome but their variation is almost exclusively genetic. They conclude that the positive correlation supports the hypothesis that both types of data are estimating overall genetic variation and, in consequence, that the sample of enzymes and structural proteins measured is representative of all gene products. Thus they support the assumption that heterozygosity of the sample reflects the heterozygosity of all loci.

Possible manifestations of homeostasis considered so far have been variance of morphological traits and fluctuating asymmetry of bilateral characters. Another one examined

by Hawkins, Bayne and Day (1986) is growth rate. They found a positive correlation between mean heterozygosity for five enzyme loci and shell length of mussels of similar age. However, they also found the same relationship for one of the single loci which implies that this locus is directly involved in the growth mechanism, i.e. the third assumption of Zink *et al.* applies here.

Leary, Allendorf and Knudsen (1985b) compare a hatchery population of rainbow trout with four wild populations for fluctuating asymmetry and find that the wild populations have similar values of mean asymmetry per individual. However, the hatchery fish are significantly more asymmetric than the wild population from the same stream. They conjecture that because of their previous finding of a negative correlation between heterozygosity and asymmetry (see later), the salmonids' asymmetry is very sensitive to inbreeding. Thus they conclude that the loci measured reflect the heterozygosity of the whole genome, in accordance with assumption (i) of Zink *et al.*

Danzmann, Ferguson, Allendorf and Knudsen (1986) have also studied the rainbow trout. They find that developmental stability is associated with developmental rate, viz. fish which develop faster have less time for accidents during critical periods of development and are therefore more stable and so they use hatching time as a measure of developmental stability. They use families of fish from the same population and bred in very similar environments to find a negative correlation between average number of heterozygous loci and mean hatching time. The mean hatching time for heterozygotes at both of two loci is significantly lower than for homozygotes over all families. They conclude that similar research using other strains of rainbow trout will reveal whether the enzyme products of a particular locus directly influence the developmental rate or whether the locus marks a chromosome segment containing other loci which control development rate. They further add that if heterozygotes are more buffered against environmental insult then under some conditions, e.g. high temperatures, they may develop more slowly.

Many researchers have tackled the issue of homeostasis on an individual level. A significant relationship between heterozygosity and developmental stability seems more likely when the organism under investigation is a poikilotherm rather than a homeotherm. For

instance, Handford (1980) found no difference in variation of 11 metrical characters between heterozygotes and homozygotes at each of four enzyme loci for sparrows. He also found no difference in variation across classes of individuals heterozygous for different numbers of loci.

Fleischer, Johnston and Klitz (1983) also examined sparrows; they measured 14 skeletal variables and two or four enzyme polymorphisms for different groups of birds. They compared pooled variances of the skeletal variables for individuals with 0, 1 and 2 or more heterozygous loci and obtained significant results for the three of the eight groups.

The problem with both of these papers on sparrows is that their results rely on the use of a modification of Levene's test, which will be discussed later (see Section 1.4). Even if the results of Fleischer, Johnston and Klitz (1983) were significant, the trend could be due to the effect of pooling over loci as described by Chakraborty and Ryman (1983). (This will be discussed in detail in Chapter 2.) They mistakenly discount this effect because rank orderings of homozygote and heterozygote means for the individual loci do not show a predominance of sandwiching of the heterozygote means. It will be shown in Chapter 2 that the rank ordering of the means at a locus is irrelevant since, whether the alleles at a given locus act additively or with dominance, the genotypic variance will decrease as heterozygosity increases if the loci considered have some effect on the quantitative trait. If there is dominance the heterozygote mean will theoretically equal one of the homozygote means. There will also be some phenotypic variation and so, regardless of the theoretical order which the means should take, in practice they may take any ordering.

The research which has been done on trout indicates that heterozygosity at several different enzyme loci reflects that of the whole genome. The usual measures of developmental stability are fluctuating asymmetry and growth rate.

Leary, Allendorf and Knudsen (1983) examined five bilateral meristic characters and the genotypes of 40 loci in a sample from a population of rainbow trout; of the 40 loci only eight had at least five heterozygotes in the sample. A significant negative correlation was found between the proportion of heterozygous loci and the proportion of asymmetric characters and there was also a significant negative correlation between the number of heterozygous loci and the magnitude of the asymmetry. Heterozygotes at seven of the eight loci considered separately

had a smaller mean proportion of asymmetric traits, but these means were significant for only two loci. Leary *et al.* (1983) suggest that there may be a significant mechanism for asymmetry controlled by these two loci.

Danzmann *et al.* (1986) also find a mechanism controlled by a specific locus. A very significant proportion of trout which hatch early are heterozygous (*a*/*b*) at the Pgm1-t locus, late-hatchers being predominantly homozygotes (*a*/*a*). This implies that the presence of the *b* gene speeds up the hatching time. Other findings by this group have been discussed earlier.

Leary *et al.* (1985a) in their study of rainbow trout, reported earlier, find that there is a much stronger negative correlation between proportion of individuals asymmetric and the heritability of bilateral characters than there is between the coefficient of variation (cv) and heritability of meristic characters and this "suggests that asymmetry is the better estimator of the amount of developmental noise and environmental variability affecting a trait." They make the very important point that cv has an additive genetic component whereas fluctuating asymmetry does not and so the latter may be a better measure of developmental stability.

Research on poikilothermic and a few homeothermic animals suggests that fluctuating asymmetry and development time are better measures of environmental variability than the variance of meristic characters. When considering artificially bred populations one may reasonably assume that the degree of heterozygosity at some enzyme loci reflects that of the whole genome but often one or two of the loci examined seem to be directly involved in the expression of the trait and it may be coincidental that heterozygosity at such loci results in lower variance or less asymmetry of the trait. Most studies of natural populations consider genetic homeostasis and it is more difficult to draw conclusions.

1.3 Research relating to humans

Let us now turn to human populations to see what has been discovered. Some researchers have deliberately set out to look for the presence or absence of developmental homeostasis while others have commented on the effects of heterozygosity in passing. On the whole, there is little evidence to support an inverse relationship between variance of a quantitative trait and degree of heterozygosity.

Propping, Friedl, Huschka, Schlor, Reiner, Lee-Vaupel, Conzelmann and Sandhoff (1986) find an association between reduced arylsulphatase A (ASA) activities and neuropsychiatric disorders; the reduced ASA activity is indicative of a person being heterozygous for an ASA deficiency allele. The deficiency allele is deleterious in the homozygous state. Chronic disease states such as severe mental retardation and dementia do not seem to be affected by these alleles. Heterozygotes would appear to be at a selective disadvantage to the homozygotes for the normal allele. Thus we have a situation involving enzyme loci which are directly involved in the expression of a trait but which exhibit the opposite of developmental homeostasis.

In the same vein, Beckman, Beckman and Perris (1980) find a disproportionate number of Gc 2-1 heterozygotes among a sample of schizophrenics. They conclude that there is no evidence for an association between the Gc 2 allele and schizophrenia, as was previously thought but that some particular type of the syndrome may be associated with Gc 2-1 group. This is another example of heterozygote disadvantage.

In an effort to find a general relationship between heterozygosity and developmental stability, several researchers have studied a variety of loci and a number of quantitative traits. For instance, Chakraborty, Ferrell, Barton and Schull (1986) investigated the relationship between several fertility parameters (number of pregnancies, number of live births, surviving offspring at one year and surviving children at the time of the survey) and heterozygosity at 17 loci among the Aymara of Chile and Bolivia. The polymorphisms were pooled to give a single value of heterozygosity for each individual (HET). Ethnicity and altitude significantly affect some of the fertility parameters, but there are no significant effects for HET and no significant correlations between HET and the fertility parameters. Eleven of the most polymorphic loci were examined individually to see how much of the variance of each fertility parameter was explained by heterozygosity at a given locus. No general pattern emerged but the Rh polymorphisms have significant effects on a few variables. Chakraborty *et al.* suggested a specific mechanism for these effects, but were unable to test it with their data.

Ward, Sarfarazi, Azini-Garakani and Beardmore (1985) consider the variances of human birth weight for homozygotes and heterozygotes at each of five loci and find no

Fourth line from bottom, insert after "variance."

Their conclusion implies that there is an inverse relationship between the size of the modal class, as defined, and variance.

significant differences between the heterozygotes and homozygotes. They also find that the low birth weight infants do not exhibit a higher degree of homozygosity than those of normal birth weight. They conclude that, although it is subject to stabilizing selection (Ulizzi, Gravina and Terrenato, 1981), human birth weight may not be a good trait to use in such investigations because foetal genotype plays such a small part in its determination. Maternal genotype is a more important contributor to human birth weight (Robson, 1955; Morton, 1955) and is, in fact, used later in this thesis in a similar investigation. Maternal age and parity also strongly affect birth weight (Millis and Seng, 1954).

Another study of newborns was conducted by Bottini, Gloria-Bottini, Lucarelli, Polzonetti, Santoro and Varveri (1979) in which the genotypes for several loci (ABO, Rh(D), PGM_1, ACP_1, ADA) in a group of light for dates (LFD) and preterm (PT) babies were compared with the genotypes of the adult population. LFD babies showed a higher proportion of homozygotes than the adult population at all loci; LFD babies also showed a higher proportion of homozygotes than PT babies at all loci except ADA where both were high. Bottini *et al.* conjecture that different homozygotes may show a diverse susceptibility towards growth retardation. For instance, among LFD babies there is an excess of PGM_1^{22} homozygotes and also an excess of ACP_1^{BB} homozygotes, but no excess of PGM_1^{11} or ACP_1^{AA}.

Livshits and Kobyliansky (1984) purported to show an inverse relationship between variance of morphological traits and degree of heterozygosity for seven loci. The latter was determined by counting the number of loci for which an individual was heterozygous. They divided the range of values of a morphological trait into three classes -

$$M = (\overline{X} - .67s, \overline{X} + .67s),\ B = (-\infty, \overline{X} - .67s) \text{ and } T = (\overline{X} + .67s, \infty)$$

and then they ascertained what proportion of each heterozygosity class lay in the modal category. When they found a relationship between degree of heterozygosity and size of modal class M, they mistakenly attributed this to an inverse relationship between heterozygosity and variance. A simple example will show that this is a non sequitur (see Table 1-1). Samples of size 10 are drawn from a population of 100 (see Snedecor and Cochran, 1967, Table 3.2.1) which can be divided into three classes as follows: B = (0,23), M = (23.3, 36.7), T = (37, 60) with 25, 50 and 25 elements respectively. One can see from Table 1-1 that there is no direct

relationship between size of modal class, M, and variance. For instance, there are no significant differences among the variances of samples 1, 2, 4 and 5 which have modal class sizes of 1, 5 or 8. However, there is a significant difference between the variances of samples 3 and 6 and also 5 and 6 even although these three samples all have modal classes of the same size.

So one cannot agree with Livshits and Kobyliansky (*ibid*) that the relationship which they found between degree of heterozygosity and modal class size implies an inverse relationship between variance and degree of heterozygosity.

Ashton (1986) administered a battery of cognitive ability tests to a large number of individuals from two different racial backgrounds and also ascertained phenotypes for eighteen polymorphisms. He found that scores for verbal and spatial tests were higher with increasing homozygosity, but that speed and memory tests were not affected by zygosity. His conclusion was that there was a distinct advantage in being homozygous, but that this was offset by the very small probability of being so. This must be tempered by the fact that he did not analyse the variances of these test scores, but the means and so little can be said about the ability of the homozygote to maintain superior test scores under environmental insult.

Several researchers have considered enzymatic acitivity associated with the common phenotypes of electrophoretically determined genetic polymorphisms. Usually the common phenotypes were due to codominant alleles at an autosomal locus and were examples of isozyme loci being directly involved in the expression of a particular trait.

Scacchi, Corbo, Calzolari, Laconi, Palmarino and Lucarelli (1985) presented data showing the glucose dehydrogenase (GDH) phenotypes of human placentae and the corresponding GDH enzyme activities. A summary of their results is presented in table 1-2. It can be seen that there is no trend in standard deviations or coefficients of variation across phenotypes. Since raw data have not been presented it is not possible to pool phenotypes into homozygotes and heterozygotes.

Golden and Sensabaugh (1986) have investigated the red cell acid phosphatase polymorphism (ACP_1) and the associated phenotypic expression of phosphotransferase activity ratios. Their results together with coefficients of variation are shown in table 1-3. Since many researchers pool all heterozygous genotypes and all homozygous genotypes, the mean standard deviation and coefficient of variation were calculated for each of these two pooled classes.

Here is a situation which at face value displays the reverse of developmental homeostasis with heterozygotes more variable than homozygotes.

Another study examining the specific effects of a polymorphic locus was conducted by Daiger, Miller and Chakraborty (1984). They measured the concentration of human group specific component (Gc) which is the plasma protein used to transport vitamin D. They also determined electrophoretically the alleles at the Gc locus for pairs of monozygous twins, dizygous twins and unrelated controls. Their findings were that environmental variance was much higher among females than males and that there were significant differences in group specific component concentration among the different genotypes at the Gc locus, with concentration decreasing from Gc^{11} to Gc^{12} to Gc^{22}. Here is another situation in which a specific locus affects a particular quantitative trait.

Szathmary (1987) extended these results to show that fasting insulin level, after adjusting for body mass index, was significantly affected by Gc genotype, the mean insulin level being lower among Gc^{11} individuals than among Gc^{12}.

The metabolically active form of vitamin D is involved in the regulation of insulin level. The serum group specific component (Gc) binds vitamin D, but the relationship between Gc and vitamin D is not clear cut. Szathmary (*ibid.*) suggested that the mechanism controlling the Gc polymorphism would best be examined by studying Gc concentration, vitamin D and insulin.

Two papers examine the relationship between levels of α_1-antitrypsin (AAT), the major serum protease inhibitor, and alleles of the protease inhibitor locus (Pi). It is thought that differences in levels of AAT affect its elastase inhibitory capacity (EIC) and elastase, in turn, plays a role in tissue destruction in such conditions as emphysema and chronic obstructive lung disease (Oakeshott, Muir, Clark, Martin, Wilson and Whitfield 1985). They point out that the S and Z alleles of the *Pi* locus are associated with reduced levels of AAT and also with greater susceptibility to respiratory conditions.

Beckman and Beckman (1980) tabulated means and standard deviations of AAT levels for M_1, M_2 and M_3 homozygotes and heterozygotes of the *Pi* locus. Applying Bartlett's test to the variances revealed no significant difference among homozygotes ($\chi^2_2 = 1.69$, $P > .05$), a significant difference among heterozygotes ($\chi^2_2 = 6.91$, $P < .05$) and a significant difference

among all *Pi* M subtypes ($\chi^2_5 = 37.05$, $P < .001$). The shortcomings of Bartlett's test should be noted (see Section 1.4) but it was not possible to perform a more robust test since the raw data were not available.

Oakeshott *et al.* (1985) presented means and standard errors for AAT and EIC for the same M subtypes of the Pi locus as above. They did not carry out any tests for homogeneity of variances but claimed that M subtype homozygotes in their sample "show lower means and higher variances for EIC, AAT... than the M subtype heterozygotes." However, Bartlett's test revealed no difference among variances of EIC for any subtypes ($\chi^2_5 = 6.81$, $P > .05$) and differences among the AAT variances appeared to be due to one high homozygote variance ($\chi^2_5 = 19.93$, $P < .01$). Once again, in the absence of the raw data, it is not possible to apply a more suitable test for homogeneity of variance. Perhaps this is a case of different homozygotes showing a diverse susceptibility to environmental insult as conjectured by Boltini *et al.* It seems that heterozygosity at the *Pi* locus does not offer any buffering effect through lower variance of EIC nor through lower levels of EIC; the mean for heterozygotes is 15.68 and for homozygotes, 15.75.

Boerwinkle, Chakraborty and Sing (1986) suggested that the best way to study the genetics of a quantitative phenotype in humans was to use the "measured genotype" approach. This involves ascertaining individuals' genotypes at loci which are known or hypothesized *a priori* to be involved in the aetiology of the phenotype of interest and then relating variability at these loci to variability in that phenotype. This method is becoming more accurate because of increased understanding of the aetiology of quantitative phenotypes and better techniques for ascertaining genotypes at relevant loci.

Boerwinkle *et al.* point out that loci with large effects on the individual do not contribute greatly to population phenotypic variance because some genotypes are rare and that the contribution of common alleles at marker loci is small because of miniscule effects on individual differences. Thus they hypothesize that loci having alleles at polymorphic frequencies and having moderate phenotypic effects contribute the majority of the genetic

variance for many quantitative phenotypes. They predict that the unmeasured polygenic random component will be reduced as more alleles are identified at more loci. Consequently, fundamental questions about quantitative phenotypic variability will be addressed.

Their comments taken in conjunction with all of the above evidence seem to support the idea that there is little point in examining several loci picked apparently at random to investigate phenotypic variability. One should be considering those loci which are involved in the aetiology of the trait in question. Chakraborty (1987) also supports this approach. It should be noted that choice of loci is non-random in two separate ways: first, only those which can be assayed are examined; secondly, only those shown to be polymorphic in a given sample can be considered. Both of these factors are likely to cause bias, of unpredictable direction and magnitude.

In summary, many studies have found no relationship between heterozygosity and variance of quantitative traits. When such a relationship does exist, it can often be attributed to a particular allele at some locus. The statistical methodology used is often inadequate for the task.

1.4 Statistical techniques

Testing for differences in developmental homeostasis between homozygotes and heterozygotes will often involve testing for difference among variances of quantitative traits of genotypic classes of polymorphisms. Consequently, it is necessary to examine statistical techniques for testing equality of variances for the univariate and multivariate cases and also for situations when the traits are distributed normally or have unknown distributions. Let us begin with the univariate case.

A widely used test for determining whether variances from two or more samples are equal is Levene's test (Levene, 1960). This test supposes that we have c independent samples with observations: $X_{k1},..., X_{kn_k}$ $\quad k = 1, ..., c,$

where the $X_{k.}$ have an unknown distribution with mean μ_k and variance σ_k^2.

The null hypothesis is H_0: $\sigma_1^2 = ... = \sigma_c^2$

and the alternative: H_1: $\sigma_s^2 \neq \sigma_t^2$ for some $s \neq t$

We form a new variable $Z_{ka} = | X_{ka} - X_{k.} |$ $\quad a = 1, \ldots n_k, k = 1, \ldots c$

$$\text{Then } W = \frac{(N-c) \sum_{k=1}^{c} n_k (Z_{k.} - Z_{..})^2}{(k-1) \sum_{k} \sum_{a} (Z_{ka} - Z_{k.})^2}$$

where $N = \sum_{k=1}^{c} n_k$

and W is distributed as F with (c-1) and (N-c) degrees of freedom.

In other words, Levene's test involves performing a one-way analysis of variance of the Z_{ka} values. This test is robust for symmetric distributions with sample sizes of at least ten (Kotz, Johnson and Read, 1983).

Two modifications have been suggested for Levene's test. Instead of W, W' is obtained using sample medians instead of means. Conover, Johnson and Johnson (1981) have recommended this modification since it is also robust for asymmetric distributions. Alternatively W" may be obtained using 10% trimmed means in place of sample means. Both of these modifications are robust when the distributions have very heavy tails (*ibid.*). On the occasions when all three test statistics are robust (e.g. symmetric distributions with large sample sizes) then W, calculated from the sample means, typically has greatest power.

Handford (1980) proposes a multivariate extension to Levene's test. This extension involves lumping all of the traits together to form a single measurement for each individual.

Let $X_1^{(k)}, \ldots, X_{n_k}^{(k)}$ be the n_k observations from sample k

$$\text{where } X_a^{(k)'} = \left(X_{a1}^{(k)}, \ldots, X_{ap}^{(k)} \right) \quad a = 1, \ldots, n_k$$

$$\text{Then form a new variable } Y_a^{(k)} = \left[\sum_{i=1}^{p} \left(X_{ai}^{(k)} - \overline{X}_{.i}^{(k)} \right)^2 \right]^{1/2}$$

where $\overline{X}_{.i}^{(k)}$ is the mean of trait i in sample k, and $Y_a^{(k)}$ is the single value for case a of sample k. Since there were two samples, Handford performed a *t* test on the $Y_a^{(k)}$'s and Fleischer, Johnston and Klitz (1983) also used this test.

However, neither Handford (1980) nor Fleischer *et al.* (1983) examine the distributional properties of the $Y_a^{(k)}$'s. One obvious problem is that if some of the p traits have heavy-tailed distributions then more weight will be given to them than to other traits. It might have been preferable to use $Y_a^{(k)} = \sum_{i=1}^{p} |X_{ai}^{(k)} - \overline{X}_{.i}^{(k)}|$ or $Y_a^{(k)} = \sum_{i=1}^{p} |X_{ai}^{(k)} - \tilde{X}_{.i}^{(k)}|$ where $\overline{X}_{.i}^{(k)}$ is the mean and $\tilde{X}_{.i}^{(k)}$ is the median of trait i in sample k.

Handford proposes his test as an alternative to principal components. Other researchers have reduced a large number of variables to one by using the first principal component. See, for instance Fleischer *et al.* (1983), Zink *et al.* (1985). Handford's main objection is that there is no objective method available for deciding how many of the principal components should be used. Anderson (1984) gives three tests using characteristic roots which would be appropriate for deciding which principal components to include. However, the underlying distribution of the multivariate random variables must be multivariate normal to conduct these tests validly. In the event that the underlying distribution precludes the use of these tests, Morrison (1967) gives a formula for determining the maximum amount of variance explained by the first principal component. However, this formula is imprecise when the covariance matrix has widely differing diagonal elements. The sample estimate of the proportion of the variance accounted for in the first principal component is given by l_1/tr(S) where S is the sample covariance matrix, l_1 is the first characteristic root of S and tr(S), the trace of S, is the sum of the diagonal elements of S. If this proportion is large enough, say at least 70%, then there seems little point in resorting to the abovementioned tests if all that we want is some overall measure of variance.

Problems arise when the p traits are measured on widely differing scales. Linear compounds of the original quantities have little meaning and so an alternative is to use standardised measurements. Now the ith principal component explains the largest portion of the variance not already explained by the previous (i–1) principal components and maximizing the variance of standardised measurements has an artificial quality. Furthermore the sampling theory of principal components obtained from a correlation matrix is much more complex than that obtained using a covariance matrix (Morrison, 1967). Gower (1987) recommends taking logarithms of uncentred data since normalisers are much influenced by sampling vagaries.

Either method diminishes the role of outliers. Sometimes taking logarithms gives more meaning to the first principal component, e.g. if it were

$$Y = a_1 \log X_1 + a_2 \log X_2 + a_3 \log X_3$$

where X_1 is length, X_2 is breadth and X_3 is depth, then Y represents some measure of volume, i.e. $\log \left(X_1^{a_1} X_2^{a_2} X_3^{a_3} \right)$ but such an interpretation is not always possible.

If the sample variance-covariance matrix is S, then |S|, the determinant of S, is called the generalized sample variance and this is another way of assigning a single numerical value to the variation expressed by S (Johnson and Wichern, 1982).

$$\mathbf{S} = \begin{bmatrix} s_{11} & s_{12} & \cdots & s_{1p} \\ s_{12} & s_{22} & \cdots & \cdot \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ s_{1p} & \cdot & & s_{pp} \end{bmatrix}$$

where p is the number of variables, n is the number of observations and

$$s_{ij} = \frac{1}{n-1} \sum_{a=1}^{n} (X_{ia} - \overline{X_i})(X_{ja} - \overline{X_j})$$

If X (pxn) is the matrix of observations, then we can form the matrix of deviation vectors

$$\begin{bmatrix} e'_1 \\ \cdot \\ \cdot \\ e'_p \end{bmatrix} = \begin{bmatrix} X_{11} - \overline{X}_1 & X_{12} - \overline{X}_1 & \cdots & X_{1n} - \overline{X} \\ \cdot & \cdot & & \cdot \\ \cdot & \cdot & & \cdot \\ X_{p1} - \overline{X}_p & X_{p2} - \overline{X}_p & \cdots & X_{pn} - \overline{X}_p \end{bmatrix}$$

and $|S| = (n-1)^p \, (\text{volume})^2$

where volume is the volume generated in n space by the p deviation vectors $e_1, \ldots, e_p$. Clearly the length of $e_i = \sum_{a=1}^{n} (X_{ia} - \overline{X_i})^2 = \sqrt{(n-1)s_{ii}}$.

Thus |S| or volume increases as the length of any e_i, i.e. $\sqrt{s_{ii}}$, increases and also as vectors of the same length are moved until they are at right angles to one another. |S| will be small if only one of the s_{ii} is small and/or if one of the deviation vectors lies nearly in the

hyperplane formed by the others. In fact the generalized variance is zero if and only if at least one deviation vector lies in the hyperplane formed by a linear combination of all the others.

To overcome the problem caused by an unduly large or small s_{ii}, the sample correlation matrix, **R**, may be used to calculate the generalized sample variance of the standardized variables, |**R**|. The standardized deviation vectors will all have the same length and so the generalized sample variance of the standardized variables will be large when the deviation vectors are nearly perpendicular. If ϑ_{ij} is the angle between $\frac{1}{\sqrt{s_{ii}}} e_i$ and $\frac{1}{\sqrt{s_{jj}}} e_j$, then $\cos(\vartheta_{ij}) = r_{ij}$, the sample correlation coefficient. Thus |**R**| is large when all the r_{ij} are near zero and small when one or more of the r_{ij} is near +1 or –1.

In summary, a very small generalized sample variance will occur if one of the p variables has a comparatively small variance or if two of the variables are highly correlated.

Van Valen (1974) introduces a multivariate coefficient of variation $CV_p = \frac{100s}{|\overline{X}|}$

where $s^2 = s_{11} + s_{22} + \ldots + s_{pp} = \text{tr}(S)$

and $|\overline{X}| = \sqrt{\sum_{i=1}^{p} \overline{X}_i^2}$.

This has the advantage of alllowing very different organisms to be compared but does not address the problem of differing scales for the p variates. He indicates that Reyment (1960) proposed an analogous statistic using |**S**| instead of s. A combined coefficient of variation using |**S**| will have the same shortcomings as the generalized inverse itself.

Rather than reduce the variance-covariance matrix to a single numerical value, it is possible to test for equality of dispersion matrices. Anderson (1984) discusses criteria for testing equality of covariance matrices from several populations which are normally distributed. The test statistic is a multivariate extension of Bartlett's test for the univariate case (Bartlett, 1937). An exact distribution for the test statistic can be derived when there are only two bivariate populations. But for other cases an asymptotic expansion must be used to find probabilities.

Suppose there are c p-variate populations. Let $X_a^{(k)}$, $a = 1,..., n_k$, $k = 1,..., c$ be an observation from the kth population $N(\mu^{(k)}, \Sigma_k)$. The hypothesis to be tested is

$$H_0 : \Sigma_1 = \Sigma_2 = ... = \Sigma_c = \Sigma$$

Let $\sum_{k=1}^{c} n_k = N$

and $A_k = \sum_{a=1}^{n_k} \left(X_a^{(k)} - \overline{X}^{(k)}\right)\left(X_a^{(k)} - \overline{X}^{(k)}\right)'$ $k = 1,..., c$

and $A = \sum_{k=1}^{c} A_k$.

Then the test statistic is $V_1 = \dfrac{\prod_{k=1}^{c} |A_k|^{\frac{1}{2}(n_k-1)}}{|A|^{\frac{1}{2}(N-c)}}$.

The asymptotic distribution obtained for

$$\lambda_1^* = V_1 \prod_{k=1}^{c}\left(\frac{N-c}{n_k-1}\right)^{\frac{1}{2}p(n_k-1)}$$

is $\Pr\{-2\rho\log\lambda_1^* \le Z\} = \Pr\{\chi_f^2 \le Z\} + \omega_2\left[\Pr\{\chi_{f+4}^2 \le Z\} - \Pr\{\chi_f^2 \le Z\}\right] + O(n^{-3})$

where $\rho = 1 - \left(\sum_{k=1}^{c} \frac{1}{n_k-1} - \frac{1}{N-c}\right)\frac{2p^2+3p-1}{6(p+1)(c-1)}$,

ω_2 depends on p, c, n_k, N, ρ and is often very small in practice, and

$$f = \tfrac{1}{2}(c-1)p(p+1).$$

For some values of n_k, c and p, $-2\log\lambda_1^*$ has been tabulated, but otherwise χ_f^2 should be used as the limiting distribution.

Unfortunately, the populations from which the samples are drawn are often not normally distributed. Transformations may help but it is probably more satisfactory to consider a non parametric test.

Puri and Sen (1971) discuss rank tests for homogeneity of dispersion matrices. They consider three hypotheses. Under $H_0^{(1)}$ the dispersion matrices are tested for equality, with the assumption that the location vectors for the different populations are equal. The alternative hypothesis is that not all dispersion matrices are equal. Under $H_0^{(2)}$ the dispersion matrices are once again tested for equality but without assuming that the location vectors are equal. The alternative hypothesis is as before. Under $H_0^{(3)}$ the equality of dispersion matrices and the equality of location vectors are tested simultaneously against the alternative that not all of the dispersion matrices are equal or that not all of the location vectors are equal or both.

The particular dispersion matrices used in these tests are invariant under certain transformations of the variables and not very sensitive to outlying observations.

It may be enlightening to consider a simple example to illustrate the formulation of the dispersion matrices and the test statistic for $H_0^{(1)}$. The data in this example are a subset of data from an example given by Morrison (1978), page 167.

Two populations have been sampled, four observations from the first and six from the second. Three variates have been measured for each observation. The data are as follows:

	Sample 1				Sample 2					
variate 1	1.21	0.92	0.80	0.85	1.40	1.17	1.23	1.19	1.38	1.17
variate 2	0.61	0.43	0.35	0.48	0.50	0.39	0.44	0.37	0.42	0.45
variate 3	0.74	0.71	0.75	0.68	0.73	0.69	0.70	0.72	0.71	0.70

So $n_1 = 4$, $n_2 = 6$, $N = 10$ and $X_{ia}^{(k)}$ is observation a of variate i from population k. The observations for each variate are then ranked across samples to form a rank matrix.

$$\mathbf{R}_{10} = \left[\begin{array}{cccc:cccccc} 7 & 3 & 1 & 2 & : & 10 & 4.5 & 8 & 6 & 9 & 4.5 \\ 10 & 5 & 1 & 8 & : & 9 & 3 & 6 & 2 & 4 & 7 \\ 9 & 5.5 & 10 & 1 & : & 8 & 2 & 3.5 & 7 & 5.5 & 3.5 \end{array}\right]$$

If $F_{(i)}^{(k)}(x)$ is the marginal cumulative distribution function (c.d.f.) for variate i in population k, i = 1,...,p, k = 1,...,c, then $H_{(i)}(x) = \sum_{k=1}^{c} \lambda_N^{(k)} F_{(i)}^{(k)}(x)$ is the combined population c.d.f. for variate i, where $\lambda_N^{(k)} = n_k/N$. ($\Sigma n_k = N$ as before). If the elements of $\mathbf{R}_N$ are divided by (N+1), the values in a row represent observations from $H_{(i)}(x)$; we can call them $H_{N(i)}(x)$ since they are functions of sample size.

The next step is to find a function J such that $J_{(i)}(u)$, i = 1,...,p, is an absolutely continuous function of u defined on (0,1). $J_{(i)}(u)$ is normalized as follows

$$\int_0^1 J_{(i)}(u)du = 0 \qquad \int_0^1 J_{(i)}^2(u)du = 1; \qquad i = 1,...,p. \tag{1.1}$$

Grade functionals are formed by taking J functions of H functions of the original observations, i.e. $J_{(i)}[H_{(i)}(X_{ia}^{(k)})]$. These grade functionals are then used to construct the dispersion matrices. J is initially defined at the points $\left(\frac{\alpha}{N+1}\right)$, $\alpha = 1,...,N$ and held constant for $\frac{\alpha}{N+1} \le u < \frac{\alpha+1}{N+1}$. Thus in practice there is a sequence of functions $J_{N(i)}(u)$ which converges to $J_{(i)}(u)$ as $N \to \infty$ for all $0 < u < 1$ and i = 1,...,p.

The purpose of the J function is to transform the matrix of ranks, **R**, so that the new matrix has rows with zero means and approximately unit variances.This new matrix is a score matrix, **E**. Puri and Sen (1971) give several examples of J functions to suit different purposes. If J is monotone then the resultant dispersion matrix will be invariant under monotone transformations of the coordinate variables. For our example, let us use $J_{(i)}(u) = \sqrt{12}\,(u - \frac{1}{2})$, which satisfies the conditions in (1.1). The score matrix is formed by dividing each element of $\mathbf{R}_N$ by N+1 and substituting this value for u in $J_{(i)}(u)$.

$$\text{Then } \mathbf{E}_{10} = \left[\begin{array}{cccc:cccccc} .472 & -.787 & -1.417 & -1.102 & : & 1.417 & -.315 & .787 & .157 & 1.102 & -.315 \\ 1.417 & -.157 & -1.417 & .787 & : & 1.102 & -.787 & .157 & -1.102 & -.471 & .472 \\ 1.102 & 0 & 1.417 & -1.417 & : & .787 & -1.102 & -.630 & .472 & 0 & -.630 \end{array}\right]$$

The test is based on some functions obtainable from the score matrix, $\mathbf{E}_N$. In practice the simplest functions to use are:

$$S^{(k)}_{N,ij} = \frac{1}{n_k - 1}\left\{ \sum_{a=1}^{n_k} E^{(i)}_{N,R^{(k)}_{ia}} E^{(j)}_{N,R^{(k)}_{ja}} - \frac{1}{n_k}\left[\sum_{a=1}^{n_k} E^{(i)}_{N,R^{(k)}_{ia}} \right]\left[\sum_{a=1}^{n_k} E^{(j)}_{N,R^{(k)}_{ja}} \right] \right\}$$

Insert after the expressions and before the numerical example:

$S^{(k)}_{N,ij}$ represents the corrected sum of products of the scores for the variables i and j for one sample k while $S^{*}_{N,ij}$ is the corrected sum of products of the scores for variables i and j over all samples. These values can be arranged in matrix form. Our interest is in whether there are differences among the matrices $\left(S^{(k)}_{N,ij}\right)$, k=1,...c. If there are no differences, these matrices will all be similar to the pooled matrix, $\left(S^{*}_{N,ij}\right)$.

$$S^{*}_{N,ij} = \frac{1}{N-1}\left\{\sum_{k=1}^{c}\sum_{a=1}^{n_k} E^{(i)}_{N,R^{(k)}_{ia}} E^{(j)}_{N,R^{(k)}_{ja}} - N\,\overline{E}^{(i)}_{N}\,\overline{E}^{(j)}_{N}\right\}$$

$$\text{where } \overline{E}^{(i)}_{N} = \frac{1}{N}\sum_{k=1}^{c}\sum_{a=1}^{n_k} E^{(i)}_{N,R^{(k)}_{ia}}$$

Using the numerical example,

$$\left(S^{(1)}_{10,ij}\right) = \begin{bmatrix} .686 & .793 & .285 \\ & 1.521 & -.579 \\ & & 1.643 \end{bmatrix};\quad \left(S^{(2)}_{10,ij}\right) = \begin{bmatrix} .545 & .278 & .352 \\ & .692 & .140 \\ & & .530 \end{bmatrix} \text{ and } \left(S^{*}_{10,ij}\right) = \begin{bmatrix} .904 & .336 & .146 \\ & .909 & .083 \\ & & .898 \end{bmatrix} \begin{matrix} .14(\\ .08: \\ .89(\end{matrix}$$

One can consider the N! equally likely permutations of the columns of $\mathbf{R}_N$ and obtain an exact test for $H^{(1)}_0$. However, the expressions involved are complicated and so, if N is not small, it is more convenient to use an asymptotic permutation test. To show how this is done, the relevant statistic will be calculated for the example even though N is small.

In order to decide whether there are differences among these dispersion matrices, the variance matrix for the dispersions must be calculated as follows:

$$v_{ij,i'j'}(\mathbf{R}_N) = \frac{1}{N}\sum_{k=1}^{c}\sum_{a=1}^{n_k} E^{(i)}_{N,R^{(k)}_{ia}} E^{(j)}_{N,R^{(k)}_{ja}} E^{(i')}_{N,R^{(k)}_{i'a}} E^{(j')}_{N,R^{(k)}_{j'a}} - S^{*}_{N,ij}\,S^{*}_{Ni'j'}$$

for all i,i',j,j' = 1,....p

The notation is a result of the fact that one element of a dispersion matrix is formed from variables i and j while another element is formed from variables i' and j', not necessarily all distinct. Thus the covariance of two such elements is formed from variables i,j,i' and j'.

The dispersion matrices may be expressed as vectors by setting

r=[(i-1)(2p-i)/2] + j for $i \le j = 1,....,p$.

and rewriting

$$\left\{ S^{(k)}_{N,ij},\ i \le j = 1,\ldots,p \right\}$$

as $$S^{(k)}_{N} = \left\{ S^{(k)}_{N,r},\ r = 1,\ldots,p(p+1)/2 \right\}, \qquad k = 1,\ldots,c$$

and $\left\{ S^{*}_{N,ij},\ i \le j = 1,\ldots,p \right\}$ as $S^{*}_{N} = \left\{ S^{*}_{N,r},\ r = 1,\ldots,p(p+1)/2 \right\}$

Thus $V_N(R_N) = \left((v_{rs}(R_N))\right)_{r,s=1,\ldots,p(p+1)/2}$

where s is defined as for r, substituting i' for i and j' for j.

Then the test statistic is

$$L_N = \sum_{k=1}^{c} N_k \left[S^{(k)}_N - S^{*}_N \right] V_N^{-1}(R_N) \left[S^{(k)}_N - S^{*}_N \right]'$$

and $H_0^{(1)}$ is rejected if $L_N > L_{N,\varepsilon}$.

Puri and Sen (*ibid*) show that for large samples $L_{N,\varepsilon} \to \chi^2_{\varepsilon,(c-1)p(p+1)/2}$.

In the example S^{*}_{10} = (.904 .336 .146 .909 –.083 .898) and

$$V_{10}(R_{10}) = \begin{bmatrix} .369 & 275 & -.135 & -.012 & -.027 & .029 \\ & .696 & -.381 & .348 & -.004 & .131 \\ & & .819 & -.165 & -.444 & -.056 \\ & & & .362 & .005 & .143 \\ & & & & .952 & -.232 \\ & & & & & .370 \end{bmatrix}$$

Once this matrix has been inverted, it is easy to obtain the test statistic L_{10}.

Since we are primarily concerned with the changes in variances of quantitative traits among genotypes and since there is no good reason to assume that the location vectors for different genotypes will be identical, our main interest is in the second hypothesis.

Under $H_0^{(2)}$ the basic permutation argument is no longer tenable and so Puri and Sen (*ibid*) derive a rank order test by centring the observations at the respective estimates of location parameters and working with the centred observations. The assumption that the test based on

the centred observations is asymptotically equivalent to the test based on the observations centred at the true locations means more stringent conditions must be applied to the cumulative distribution functions and their asssociated density functions and also the grade functionals. This means that $J_{(\omega)}(u) = \mathcal{E}[\Phi^{-1}(u)]$ is a more suitable function, where $\mathcal{E}$ is the expected value operator.

The only assumption regarding the estimates of the location parameters is that they must be consistent and so the mean or the median of each variate in each population will suffice.

1.5 Conclusions

There are several directions one could take to further examine the issue of heterozygosity affording some selective advantage in humans.

First, it may be useful to consider theoretical arguments and this will be done in chapter 2.

More data can be analysed to increase the pool of loci and quantitative traits which have been examined. More appropriate statistical techniques may be used in order to obtain as much information as possible from the data. These two approaches will be adopted simultaneously in chapter 3 and 4.

Table 1-1. Six samples of size 10 with associated variances and numbers in three classes - B,M,T (see text).

Item Number	Sample number 1	2	3	4	5	6
1	33	32	39	17	11	21
2	53	31	34	22	24	34
3	34	11	33	20	26	33
4	29	30	33	19	28	33
5	39	19	33	3	30	33
6	57	24	39	21	31	39
7	12	53	36	3	33	36
8	24	44	32	25	34	32
9	39	19	32	40	36	32
10	36	30	30	21	45	30
s^2	169.8	151.6	9.0	112.3	78.0	21.8
B, M, T	1, 5, 4	3, 5, 2	0, 8, 2	8, 1, 1	1, 8, 1	1, 8, 1

Table 1-2 Glucose dehydrogenase activity in six common phenotypes (µmol of NADH/min/g wet weight) (Scacchi *et al.* (1985)).

Phenotype	N	Glucose dehydrogenase activity mean	s.d	c.v.
1-1	249	26.77	12.78	0.477
2-2	26	24.73	12.34	0.499
3-3	9	20.44	10.14	0.496
2-1	182	23.97	12.68	0.529
3-1	103	25.46	12.69	0.498
3-2	34	24.62	11.66	0.474

Table 1-3 Phenotype variation in phosphotransferase activity ratios. (Golden and Sensabaugh (1986)

Genotype	N	Phosphotransferase activity ratio mean	s.d	c.v.
B	23	3.74	0.25	0.067
BA	22	3.49	0.46	0.132
A	25	3.38	0.22	0.065
CB	8	2.94	0.33	0.112
CA	10	2.74	0.34	0.124
C	1	2.43		
homozygotes	49	3.53	0.36	0.102
heterozygotes	40	3.19	0.52	0.163

2. PHENOTYPIC VARIABILITY OF POLYGENIC TRAITS

2.1 Introduction

This chapter is concerned with the phenotypic variability of quantitative traits which are controlled by many loci. However, the problems of the origin of variation (e.g. Frankham 1980) and its possible maintenance by stabilizing selection acting directly on the phenotype for the trait of interest (e.g. Turelli 1984) are peripheral to the relationship between heterozygosity and variability, and will not be considered. In what follows, it will be assumed that the loci which are examined are directly involved in the expression of those traits being measured.

Chakraborty and Ryman (1983) have shown that the fact that, "within a population, the least phenotypic variation exists among the most heterozygous individuals can be explained by simple additivity of genic effects that control the quantitative character in question". In order to show this they make a number of assumptions: allelic effects are additive and the same for each locus, gene frequencies are the same at each locus, independent segregation at all loci and they confine their discussion to just two segregating alleles. They also discuss what happens when the gene frequencies at each locus vary and when phenotypic variance includes an environmental component.

Mani (1988) extends their argument by increasing the number of segregating alleles at each locus. His evidence is presented as the results of simulations. He makes the same assumptions as Chakraborty and Ryman. So it is not surprising to find that the results of his simulations for the two-allele case confirm the results which Chakraborty and Ryman found by mathematical methods, i.e. the mean converges to a fixed point and the genotypic variance decreases linearly to zero with increasing heterozygosity.

When the model is extended to three or more alleles, the mean does not converge to a fixed point any more and the variance decreases with increasing heterozygosity only if the alleles are present at similar frequencies to one another. Such a situation occurs in a selective model. If one allele is present at much higher frequencies than other alleles, as in a neutral model, the variance increases with increasing heterozygosity.

Mani (*ibid*) concludes that increasing or decreasing variance with increasing heterozygosity could be used to determine whether selection was at work. However, his model assumes additivity and small environmental effects.

The rest of this chapter deals with models which I have developed by extending those of Chakraborty and Ryman, and Mani.

2.2 Extending the two-allele model

It is possible to relax some of Chakraborty's and Ryman's assumptions and still obtain the same effect for 2 alleles. I will use their notation (i.e. two segregating alleles A_i & B_i with gene frequencies p and q at each of n loci).

Suppose first that the allelic effects are not additive. For instance let the genotypes, $A_i A_i$ and $A_i B_i$, both take the value 1 and $B_i B_i$ have the genotypic value 0 (i.e. complete dominance); a homozygous individual can be $A_i A_i$ or $B_i B_i$ at each locus, i=1,....n. Suppose an individual is homozygous for the A allele at m loci and for the B allele at n-m loci. So if the locus effects are additive the genotypic value for the individual is m and

$$\mu_0 = \frac{\sum_{m=0}^{n} m \binom{n}{m} (p^2)^m (q^2)^{n-m}}{(p^2+q^2)^n}$$

$$= \frac{np^2}{(p^2+q^2)}$$

$$\sigma_0^2 = \frac{\sum_{m=0}^{n} \left(m - \frac{np^2}{p^2+q^2}\right)^2 \binom{n}{m} (p^2)^m (q^2)^{n-m}}{(p^2+q^2)^n}$$

$$= \frac{np^2q^2}{(p^2+q^2)^2}$$

An individual heterozygous at k loci, with genotypic value k, will be homozygous at n-k loci. Suppose m of these are $A_i A_i$, with genotypic value m, and n-k-m are $B_i B_i$ with genotypic value 0.

$$\mu_k = \frac{\sum_{m=0}^{n-k} (m+k) \frac{n!}{k!\, m!\, (n-k-m)!} (p^2)^m (2pq)^k (q^2)^{n-k-m}}{\binom{n}{k} (2pq)^k (p^2+q^2)^{n-k}}$$

$$= \frac{(np^2+kq^2)}{(p^2+q^2)}$$

and this equals n when k = n.

$$\sigma_k^2 = \frac{\sum_{m=0}^{n-k} \left\{(m+k)-k-\frac{(n-k)p^2}{p^2+q^2}\right\}^2 \binom{n}{k} \binom{n-k}{m} (p^2)^m (2pq)^k (q^2)^{n-k-m}}{\binom{n}{k} (2pq)^k (p^2+q^2)^{n-k}}$$

$$= \frac{(n-k)\, p^2q^2}{(p^2+q^2)^2}$$

and this equals 0 when k=n.

Thus the variance decreases from $\frac{np^2q^2}{(p^2+q^2)^2}$ to $\frac{(n-k)p^2q^2}{(p^2+q^2)^2}$ to 0 as the number of heterozygous loci, k, increases from 0 to n.

The genotypic mean increases from $\frac{np^2}{p^2+q^2}$ to $\frac{np^2+kq^2}{p^2+q^2}$ to n as k increases from 0 to n, regardless of whether p > q or not.

A second way of relaxing the assumption of additivity is to introduce partial dominance. Suppose that the allelic effects of A and B are essentially additive but with partial dominance, so that for locus i the genic effect of $A_i A_i$ is 2, $A_i B_i$ is 1+s and $B_i B_i$ is 0, s > 0.
This is heterosis if s>1.

Then the mean and variance for completely homozygous individuals are

$\frac{2np^2}{(p^2+q^2)}$ and $\frac{4np^2q^2}{(p^2+q^2)2}$ respectively. Suppose an individual is heterozygous at k loci with genic value k (1+s) and homozygous for A at m of the remaining (n-k) loci with genic value 2m.

$$\mu_k = \frac{\sum_{m=0}^{n-k} (2m+k+ks) \binom{n}{k} \binom{n-k}{m} (p^2)^m (2pq)^k (q^2)^{n-k-m}}{\binom{n}{k} (2pq)^k (p^2+q^2)^{n-k}}$$

$$= k + ks + 2 \sum_{m=0}^{n-k} m \binom{n-k}{m} (p^2)^m (q^2)^{n-k-m}$$

$$= k (1+s) + \frac{2(n-k)\, p^2}{(p^2+q^2)}$$

$$\sigma_k^2 = \frac{\sum_{m=0}^{n-k} \left\{2m+k(1+s)-k(1+s)-\frac{2(n-k)p^2}{p^2+q^2}\right\}^2 \binom{n}{k} \binom{n-k}{m} (p^2)^m (2pq)^k (q^2)^{n-k-m}}{\binom{n}{k} (2pq)^k (p^2+q^2)^{n-k}}$$

$$= \frac{4 \sum_{m=0}^{n-k} \left\{m - \frac{(n-k)p^2}{p^2+q^2}\right\}^2 \binom{n-k}{m} (p^2)^m (q^2)^{n-k-m}}{(p^2+q^2)^{n-k}}$$

$$= \frac{4\,(n-k)\, p^2q^2}{(p^2+q^2)^2}$$

Thus as k, the number of heterozygous loci, increases the genotypic variance decreases from $4np^2q^2/(p^2+q^2)^2$ to $4(n-k)p^2q^2/(p^2+q^2)^2$ to zero while the mean changes linearly from

$2np^2/(p^2+q^2)$ to $k(1+s) + 2(n-k)p^2/(p^2+q^2)$ to $n(1+s)$ and this has a negative slope if $p>(1+\varepsilon)q$ where $\varepsilon>0$ depends on s. It should be noted that the change in genotypic variance is independent of s, the size of the selection coefficient.

I have shown that, if there are only two possible alleles at each locus, genotypic variance among individuals in a population decreases with increasing heterozygosity. This is true whether the allelic effects exhibit complete dominance, partial dominance, heterosis or ibit additivity, provided that the loci have a direct effect on the trait under investigation.

2.3 Developing a more complex model

A locus effect is defined to be the net effect of the alleles at a particular locus whether they be acting additively, with dominance or with some heterosis. In all of the preceding work of this chapter, it has been assumed that the locus effects were additive. Mayo (1980) discusses the doubtful validity of this assumption and illustrates his discussion with examples of additive, multiplicative and asymptotic locus effects. He also points out that if appropriate data were examined the last would be readily detectable but it would be hard to distinguish between multiplicative and additive locus effects.

One extension that I will make to the original model of section 2.2 will be the inclusion of multiplicative locus effects.

If more than two alleles are segregating at each locus, the property of additive allelic effects is easily dealt with. However, if the alleles are not additive how do the different alleles interact?

A classic example is the ABO blood group locus. Alleles I^A and I^B are codominant to each other (i.e. both are expressed in an I^AI^B genotype) and dominant to allele *i*. Similarly Santachiara-Benerecetti (1970), when investigating the Babinga pygmies, found a three-allele polymorphism at the peptidase C locus. Two alleles $PEPC^1$ and $PEPC^2$ were codominant to each other while the third, $PEPC^0$, was recessive.

The galactose 1-phosphate uridyltransferase locus (GALT) has three main alleles, $GALT^A$, $GALT^G$ and $GALT^D$ (Tedesco, 1972). If the homozygote for $GALT^A$ has an enzyme activity of 1.0, then the activities for the possible genotypes are as follows :

AA	AD	DD	AG	GG
1.0	0.75	0.5	0.5	0

The G allele is deleterious in that homozygous individuals exhibit galactosaemia. So this is an example of three alleles all acting additively.

These are just three examples to indicate what feasible assumptions I have made when constructing the model. When more than two alleles are present in the model, additive allelic effects will mean that all alleles act additively as at the GALT locus. Dominant allelic effects, will mean that the first allele is recessive and each of the others exhibits dominance to the first but these others show overdominance among themselves.

To determine actual values to be used in the model, adult female height was taken as an example of a quantitative trait. Using the twin data described in chapter 4 of this thesis the estimates of mean and standard deviation for female height were $\bar{x} = 163.3$ and $s = 7.32$. Assuming that height is distributed normally with a range $\mu \pm 3\sigma$, the range of female heights estimated from this sample would be (141, 185). These values were used as the extremes when determining values for allelic effects.

If allelic effects and locus effects are both additive the method for determining the phenotypic value of an individual in the absence of environmental effects will now be described.

Suppose there are n loci and k possible alleles for each locus. Suppose that each locus is homozygous for the allele which has the smallest effect on the quantitative trait. Then an individual with such a genotype will have the lower extreme value for that trait. By the same token, an individual who is homozygous at each locus for the allele having the largest effect will have the upper extreme value for the trait. Let us extend the model of Mani for k alleles by assuming that the effect of allele j is $\frac{j-1}{k-1}s$, so that the allele with least effect is allele 1, contributing zero, and allele k will have the greatest effect, contributing s, where s is a scale parameter. If locus effects and allelic effects are both additive then the genotypic value will be

$$x + \sum_{\text{loci}} \sum_{\text{alleles}} \frac{j-1}{k-1} s,$$

where x is some base value.

Using the example of height with 10 loci and the range of heights given above we get

$$x = 141$$

$$x + 10\,(2s) = 185$$

$$\text{i.e.} \quad s = (185 - 141)/20$$

If there were three alleles per locus then a woman who was homozygous for allele 2 at each locus would have a genotypic value for height of

$$x + 10.2.\tfrac{1}{2}\, s$$

$$= \quad 141 + 10.\frac{(185-141)}{20} \quad = \quad 163.$$

For additive allelic and locus effects, $s = (L_U - L_L)/2n$, $x = L_L$ and the genotypic value is $x + s \sum_{\text{loci}} \sum_{\text{alleles}} \frac{j-1}{k-1}$, where L_L and L_U are the lower and upper extremes of the trait respectively.

If locus effects are still additive but alleles exhibit dominance, as previously defined, individuals at the lower extreme will again be homozygous for the allele with smallest effect. However individuals at the upper extreme will be heterozygous for the two dominant alleles with the biggest effects since these are additive. If the effect of allele j is $\frac{j-1}{k-1}s$ then the genotypic value for an individual at the upper extreme is

$$x + \sum_{\text{loci}} \left(1 + \frac{k-2}{k-1}\right) s$$

$$= \quad x + n\left(\frac{2\,k-3}{k-1}\right) s$$

$$\text{Again } x = L_L \text{ and so } s = \left(\frac{L_U - L_L}{n}\right)\left(\frac{k-1}{2k-3}\right)$$

Individuals who are heterozygous at all loci for one of the dominant alleles and the recessive allele will have a genotypic value of $x + \sum_{\text{loci}} \left(\frac{j-1}{k-1}\right) s$, $1 < j \leq k$ while individuals homozygous for the same dominant allele will have the same genotypic value. Thus if there are three alleles,

a locus effect can have the values $\{0, \frac{1}{2}s, s, \frac{3}{2}s\}$ whereas if there are only two alleles, the locus effect can have values 0 or s only.

Now suppose that locus effects are multiplicative. Then if alleles are additive, the smallest genotypic value is x^n while the largest is $(x + 2s)^n$. So $x = \sqrt[n]{L_L}$, $x + 2s = \sqrt[n]{L_U}$ and $s = \frac{1}{2}\left(\sqrt[n]{L_U} - \sqrt[n]{L_L}\right)$.

For the height example, with 10 loci and 3 alleles, say,

$$x = \sqrt[10]{141} = 1.64 \qquad s = \frac{1}{2}\left(\sqrt[10]{185} - \sqrt[10]{141}\right) = \frac{1}{2}(1.685 - 1.64) = 0.0225$$

and an individual who is heterozygous at all loci for alleles 2 and 3 has a genotypic value of

$$\left(x + \frac{3}{2}s\right)^{10} = 1.67375^{10} = 172.5$$

Finally, suppose that locus effects are multiplicative and alleles exhibit dominance. Then an individual at the upper extreme has genotypic value $\left[x + \left(\frac{2k-3}{k-1}\right)s\right]^n$ while an individual at the lower extreme has genotypic value x^n.

Thus, $x = \sqrt[n]{L_L}$, $x + \left(\frac{2k-3}{k-1}\right)s = \sqrt[n]{L_U}$

and $s = \left(\sqrt[n]{L_U} - \sqrt[n]{L_L}\right)\left(\frac{k-1}{2k-3}\right)$

Using the height example, with 10 loci and 3 alleles, an individual homozygous for the recessive allele (number 1) at all loci has genotypic value 141, one heterozygous at all loci for alleles 1 and 2 has value $\left(x + \frac{1}{2}s\right)^{10} = (1.655)^{10} = 154.3$ as does an individual homozygous at all loci for allele 2 and one heterozygous at all loci for alleles 2 and 3 has the value 185. It should be noted that an individual will not usually have the same combination of alleles at each locus; these have been used for illustrative purposes only.

2.4 Simulations and Results

A FORTRAN 77 program was written to run on a VAX 11/780 computer. All aspects of the program were verified by printing out intermediate results and performing hand calculations for small examples. This program implemented the model described in section 2.3 by simulating genotypes for 5000 individuals, each with 10 loci controlling a quantitative trait.

Upper and lower extremes of female height, as previously described, were used to delineate the trait. No environmental variance was included and so genotypic and phenotypic values for an individual were equal.

Allelic frequencies were chosen for several reasons. Equal frequencies of all alleles represented a selective model, while one allele having a much higher frequency than other alleles represented a neutral model. These frequencies were used by Mani (1988). The set of frequencies $\{0.78, 0.2, 0.02\}$ was used extensively in the 3-allele model since these values were calculated by Santachiara-Benerecetti (1970) for the Babinga pygmies. The set $\{0.60, 0.35, 0.05\}$ represented a less extreme version of the above frequencies.

Each simulation was repeated 50 times and the heterozygosity class means were averaged over the 50 runs, while the heterozygosity class variances were pooled. These pooled variances were plotted against heterozygosity score. The plots for two allele models are shown in figure 2-1, 2-2, 2-3 and 2-4. As was predicted by Chakraborty and Ryman (1983), the variances for additive allelic effects and additive locus effects, showed a steady decrease with increasing heterozygosity. The same was also true when allelic effects exhibited dominance; this was predicted by my calculations in section 2.2 Two further models with multiplicative loci, one with dominant alleles and the other with additive alleles, both showed decreasing variances with increasing heterozygosity. When the frequencies of the two alleles were very different, the decrease in the variances was very shallow.

For three alleles, when all effects were additive, the results were similar to those obtained by Mani (1988) (see figure 2-5), i.e. there was an upward trend in variance when one allele was much more frequent than the other two. This trend became steeper as the two rare alleles became less frequent. There was a downward trend in variance when all alleles had similar frequencies.

However, this pattern was not maintained when allelic and locus effects were not both additive. For instance, figure 2-6 shows that if alleles exhibited dominance, while locus effects remained additive, allele frequencies representing selective models, i.e. all equal, caused the variance to remain stationary with increasing heterozygosity. On the other hand, if the allele frequencies were very different $\{0.2, 0.02, 0.78\}$, the variance decreased or increased

depending on whether the least frequent allele had one of the extreme effects or a moderate effect. Although the means have not been plotted, they increased with increasing heterozygosity for all of these frequency combinations.

The reasons for this pattern can be illustrated by considering one locus only. If allele one is recessive with effect 0 and alleles two and three are codominant with effects ½s and s respectively, then the mean and variance for the locus effect for heterozygotes are given in table 2-1a. The mean and variance for the locus effect for homozygotes are given in table 2-1b. The variance of the genotype values for an individual locus is highest among the homozygotes when allele two has the lowest frequency and at the same time is lowest among the heterozygotes. So one can see that each additional heterozygous locus would decrease the variance. The converse is true when one of the extreme alleles is least frequent.

When an extreme example of neutral theory was considered, namely allelic frequencies of $\{0.9, 0.05, 0.05,\}$ then the variances always increased regardless of which allele was least frequent, but the increase was steepest when the most frequent allele had a moderate effect (figure 2-7).

The trends in variance for additive allelic effects and multiplicative locus effects were similar to those found for additive alleles and additive loci (figure 2-8). These trends agreed with Mani's results.

Finally, when alleles exhibited dominance and loci had multiplicative effects, many results were similar to those for dominant alleles with additive locus effects (fig. 2-9). However, when allelic frequencies were all equal, the variances increased after an initial dip, with increasing heterozygosity. This was a direct contradiction of previous findings for equal allelic frequencies.

An attempt was made to see whether similar patterns emerged when the number of alleles was increased to four. When allelic frequencies were equal, allelic effects exhibited dominance and locus effects were additive, the variances increased with increasing heterozygosity. With these same effects, if one allele was much less frequent than the other three, then the trend in variances depended on which allele was the rare one (figure 2-10). If one of the two middle alleles was rare, the variances tended to decrease and then increase, i.e.

almost a quadratic trend, with increasing heterozygosity. On the other hand, if one of the extreme alleles was the rare one, the variance increased with increasing heterozygosity.

Using a set of allelic frequencies similar to those used for the three allele case, namely $\{0.05, 0.05, 0.6, 0.3\}$, the trend in variance depended on the particular permutation of frequencies. The variance decreased with increasing heterozygosity except when the two middle alleles were the rare ones (figure 2-11). The reasons would be similar to those discussed for the three allele situation with frequencies $\{0.2, 0.78, 0.02\}$.

2.5 Conclusions

While Chakraborty and Ryman (1983) and Chakraborty (1987) believed that decreasing phenotypic variability with increasing heterozygosity was caused by additivity of allelic effects over all the loci controlling the polygenic trait, Mani (1988) pointed out that this argument did not necessarily apply when there were more than two possible alleles at each locus. This argument was that phenotypic variability decreased or increased with increasing heterozygosity depending on whether allelic frequencies were indications of neutral theory or selective theory.

I have shown that when there are only two possible alleles per locus, the interactions between loci may be multiplicative or additive and the interactions between alleles may be additive, dominant/recessive or additive with some heterosis. Whatever the situation,phenotypic variability will decrease with increasing heterozygosity.

I have also shown using simulations that when there are more than two possible alleles at each locus, one cannot resort solely to the neutral versus selection argument to explain increasing and decreasing trends in phenotypic variability. Evidence of this is the fact that different trends in variability can be obtained by permuting the alleles having the different frequencies when alleles interact with dominance.

Thus Mani's conclusions will only apply if there is no dominance and Chakraborty and Ryman's conclusions will only apply if there are no more than two alleles per locus. Perhaps these restrictions are the reasons for some of the conflicting results indicated in Chapter 1.

Table 2-1 Mean and variance of genotypic value for a single locus with three alleles; the first of which is recessive, while the other two are dominant to the first and overdominant between themselves.

genotype	locus genotypic value	allelic frequencies (.2,.02,.78)	(.02,.2,.78)	(.2,.78,.02)	(.78,.2,.02)
a. heterozygotes					
12	$\frac{1}{2}s$	0.008	0.008	0.312	0.312
13	s	0.312	0.0312	0.008	0.0312
23	$\frac{3}{2}s$	0.0312	0.312	0.0312	0.008
	mean	1.0330s	1.4328s	0.6002s	0.5672s
	variance	$0.0268s^2$	$0.0405s^2$	$0.0845s^2$	$0.0405s^2$
b. homozygotes					
11	0	0.04	0.0004	0.04	0.6084
22	$\frac{1}{2}s$	0.0004	0.04	0.6084	0.04
33	s	0.6084	0.6084	0.0004	0.0004
	mean	0.9377s	0.9686s	0.4695s	0.0314s
	variance	$0.0580s^2$	$0.0150s^2$	$0.0145s^2$	$0.0150s^2$

Figure 2-1. Genotypic variance versus heterozygosity. Two alleles per locus with additive allelic effects and additive locus effects. Allelic frequencies:

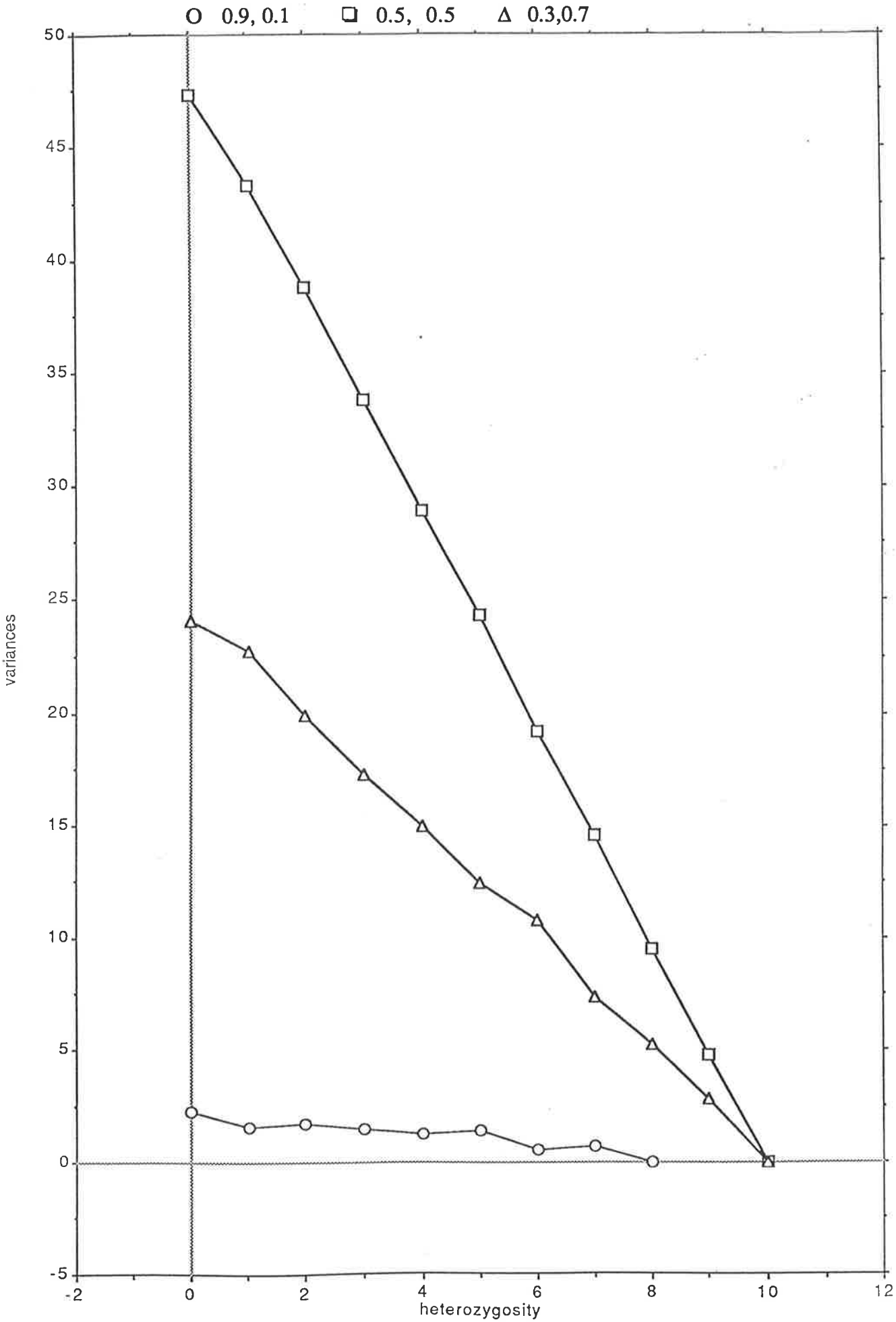


Figure 2-2. Genotypic variance versus heterozygosity. Two alleles per locus with allele 2 dominant to allele 1 and additive locus effects. Allelic frequencies:

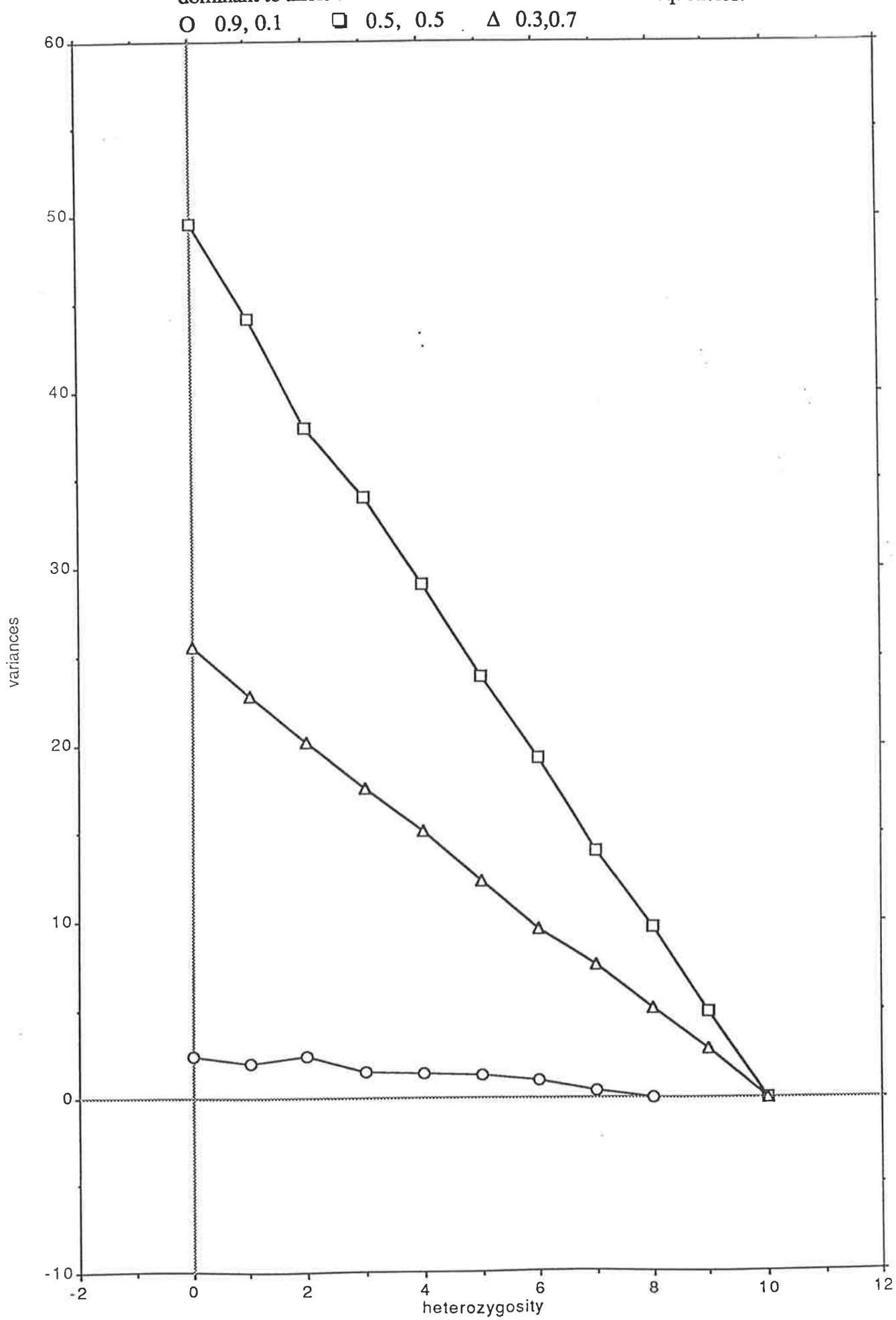


Figure 2-3. Genotypic variance versus heterozygosity. Two alleles per locus with additive allelic effects and multiplicative locus effects. Allelic frequencies:

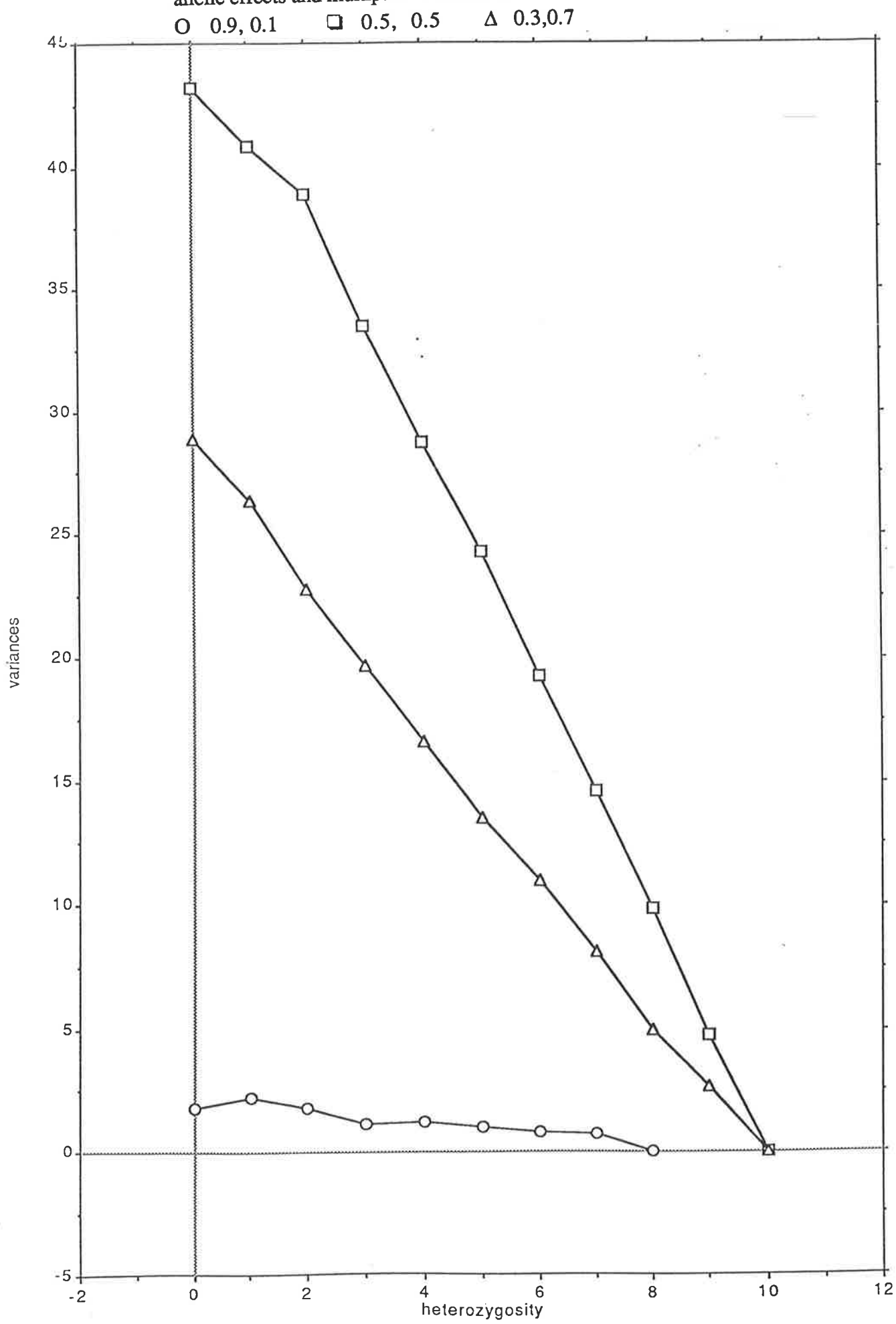


Figure 2-4. Genotypic variance versus heterozygosity. Two alleles per locus with allele 2 dominant to allele 1 and multiplicative locus effects. Allelic frequencies:

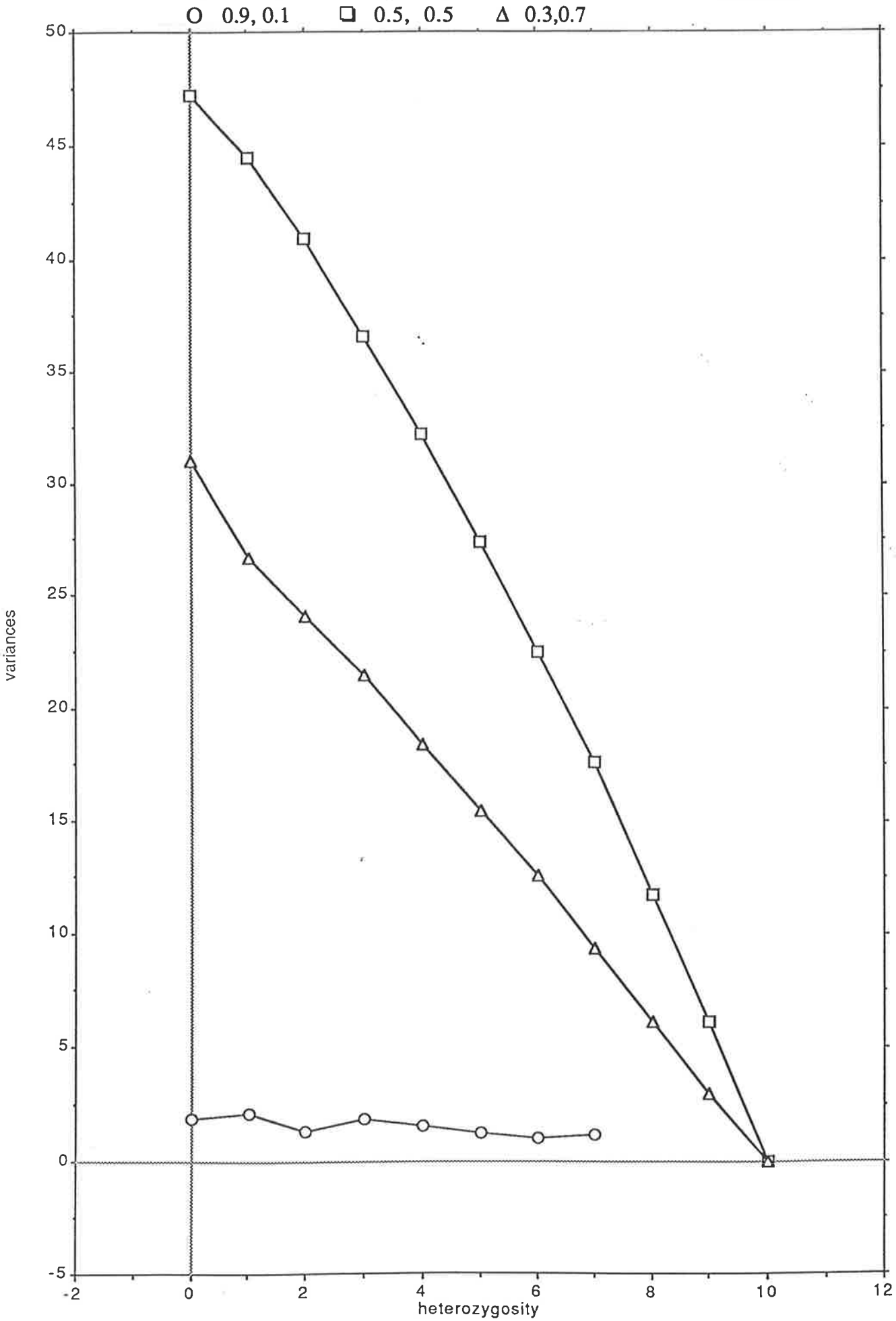


Figure 2-5. Genotypic variance versus heterozygosity. Three alleles per locus with additive allelic effects and additive locus effects. Allelic frequencies:
O 0.33,0.33,0.34 ❑ 0.1,0.1,0.8 Δ 0.8,0.1,0.1 ◊ 0.05,0.05,0.9

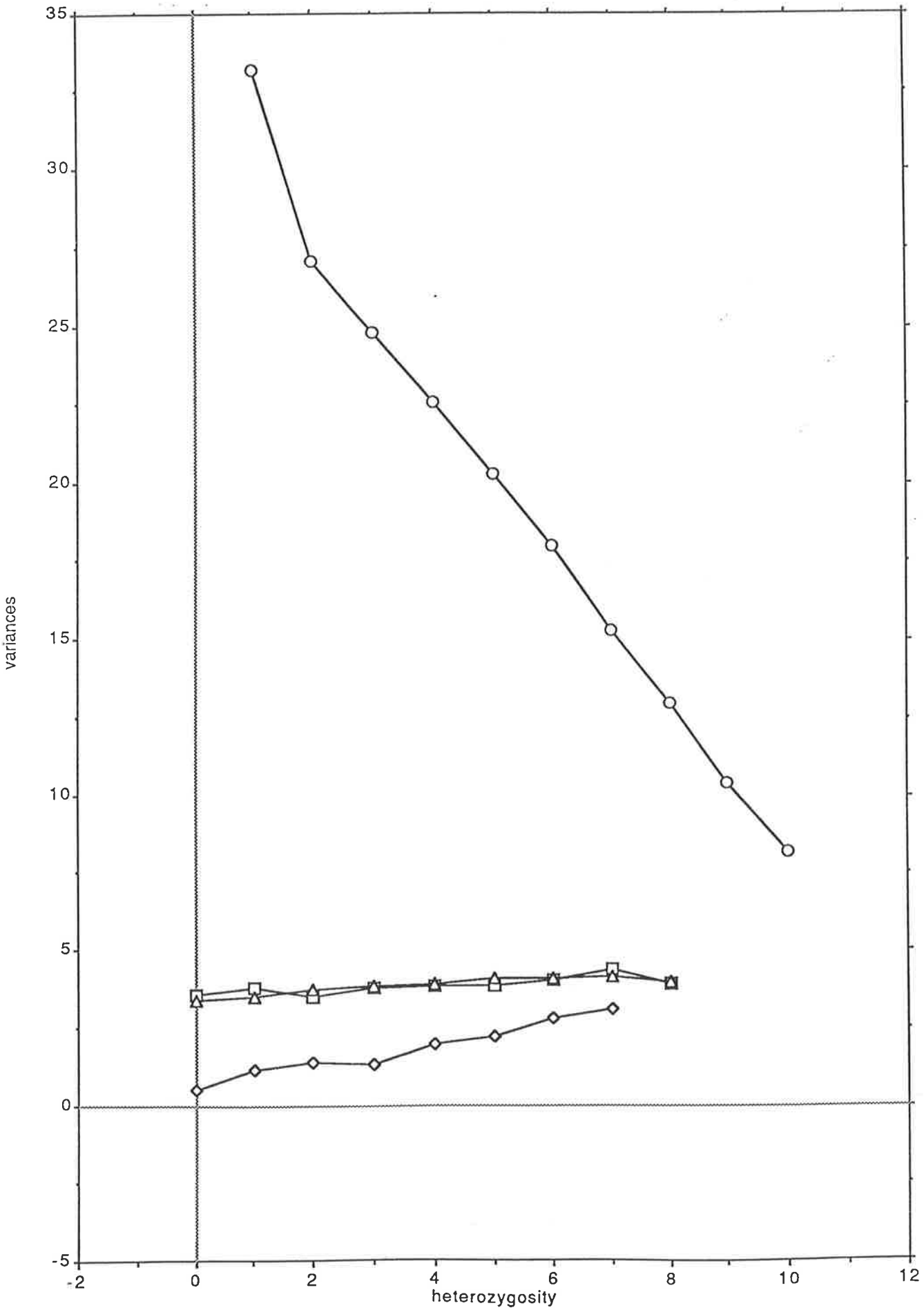


Figure 2-6. Genotypic variance versus heterozygosity. Three alleles per locus, alleles 2 and 3 acting additively and both dominant to allele 1, with additive locus effects. Allelic frequencies:

● 0.33,0.33,0.34 O 0.2,0.02,0.78 ❑ 0.02,0.2,0.78 Δ 0.2,0.78,0.02

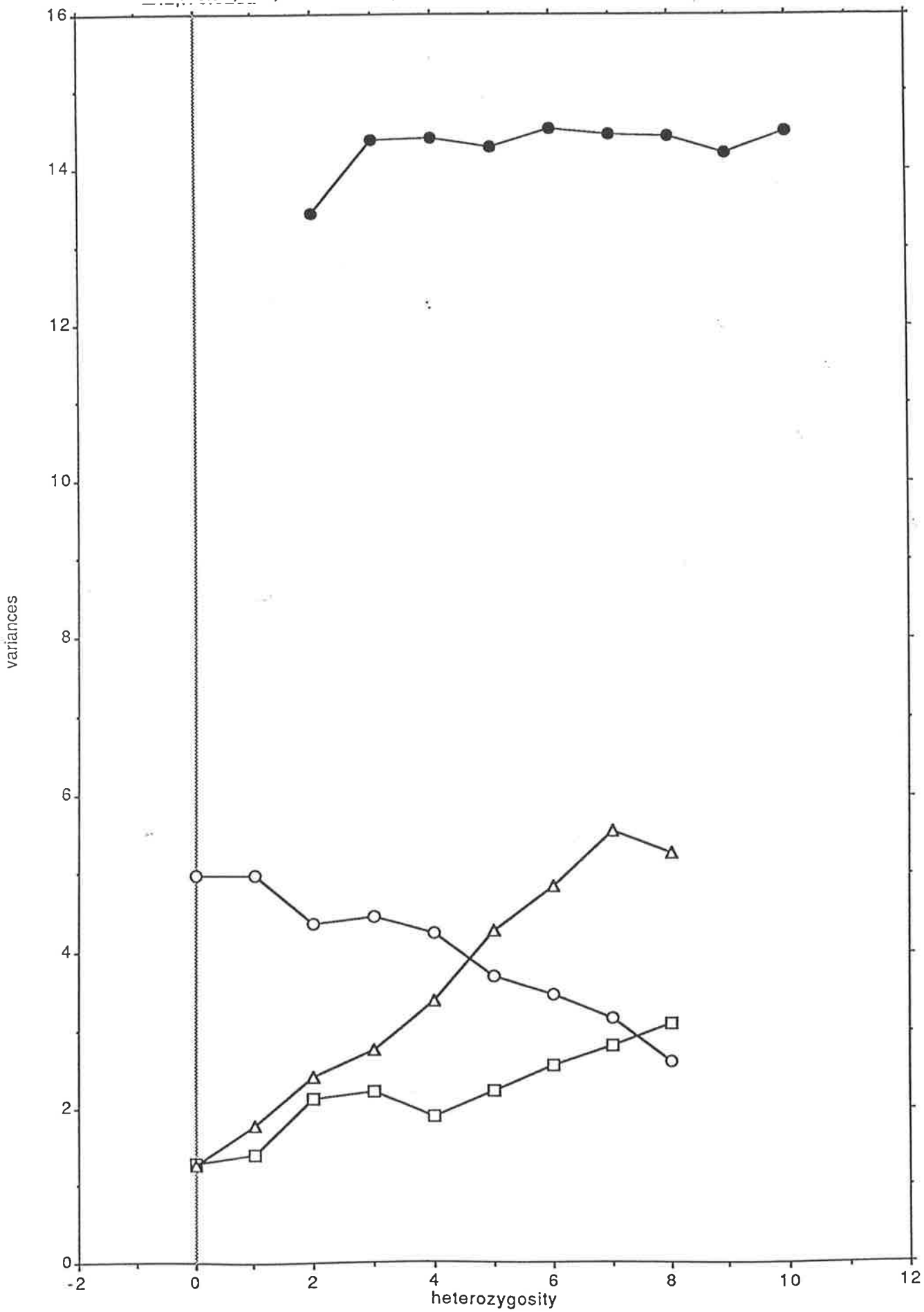


Figure 2-7. Genotypic variance versus heterozygosity. Three alleles per locus, alleles 2 and 3 acting additively and both dominant to allele 1, with additive locus effects. Allelic frequencies:
● 0.05,0.05,0.9 O 0.05,0.9,0.05 ❑ 0.9,0.05,0.05

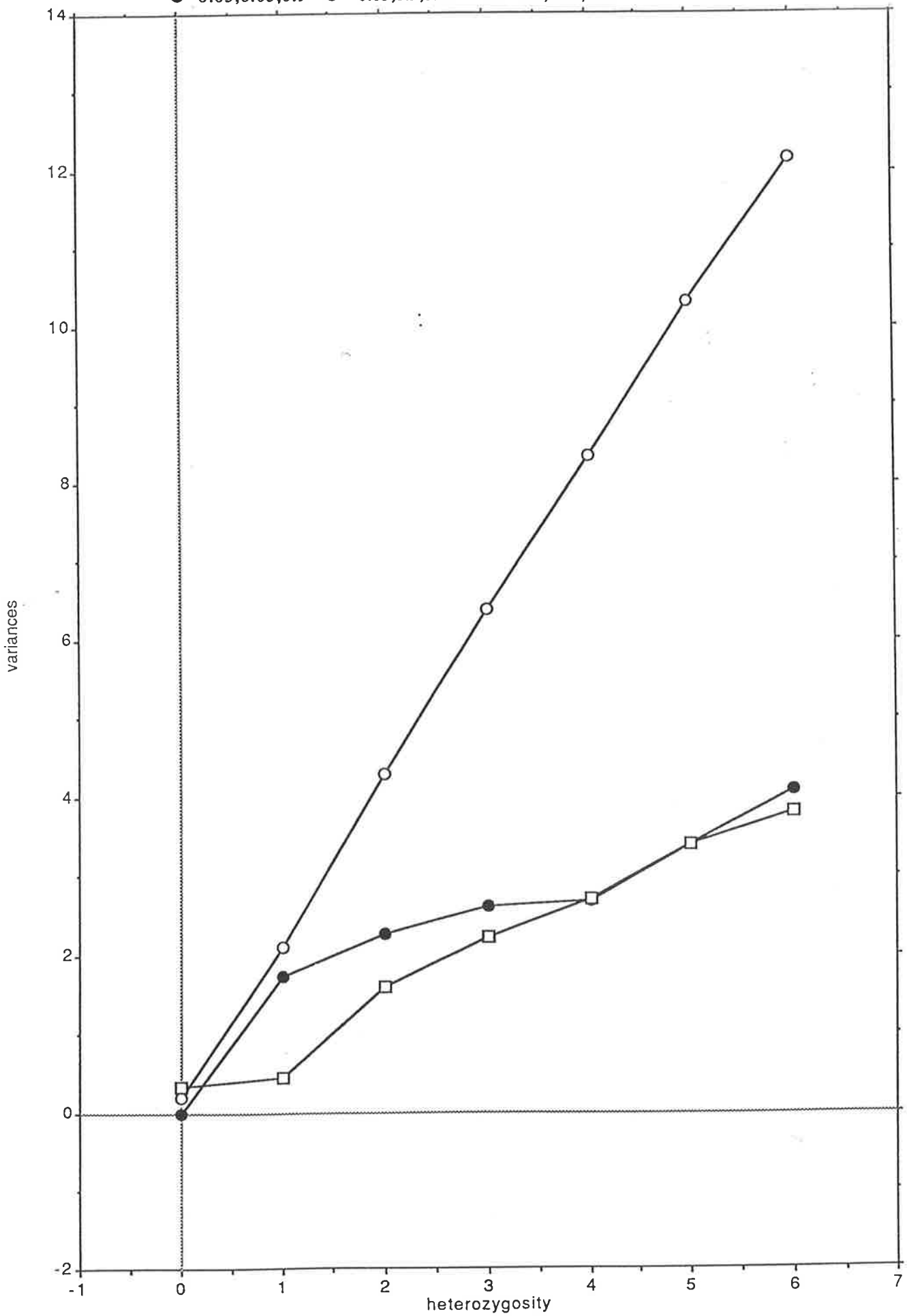


Figure 2-8. Genotypic variance versus heterozygosity. Three alleles per locus with additive allelic effects and multiplicative locus effects. Allelic frequencies:
O 0.33,0.33,0.34 ❑ 0.1,0.1,0.8 Δ 0.02,0.2,0.78
◊ 0.05,0.05,0.9 + 0.05,0.6,0.35

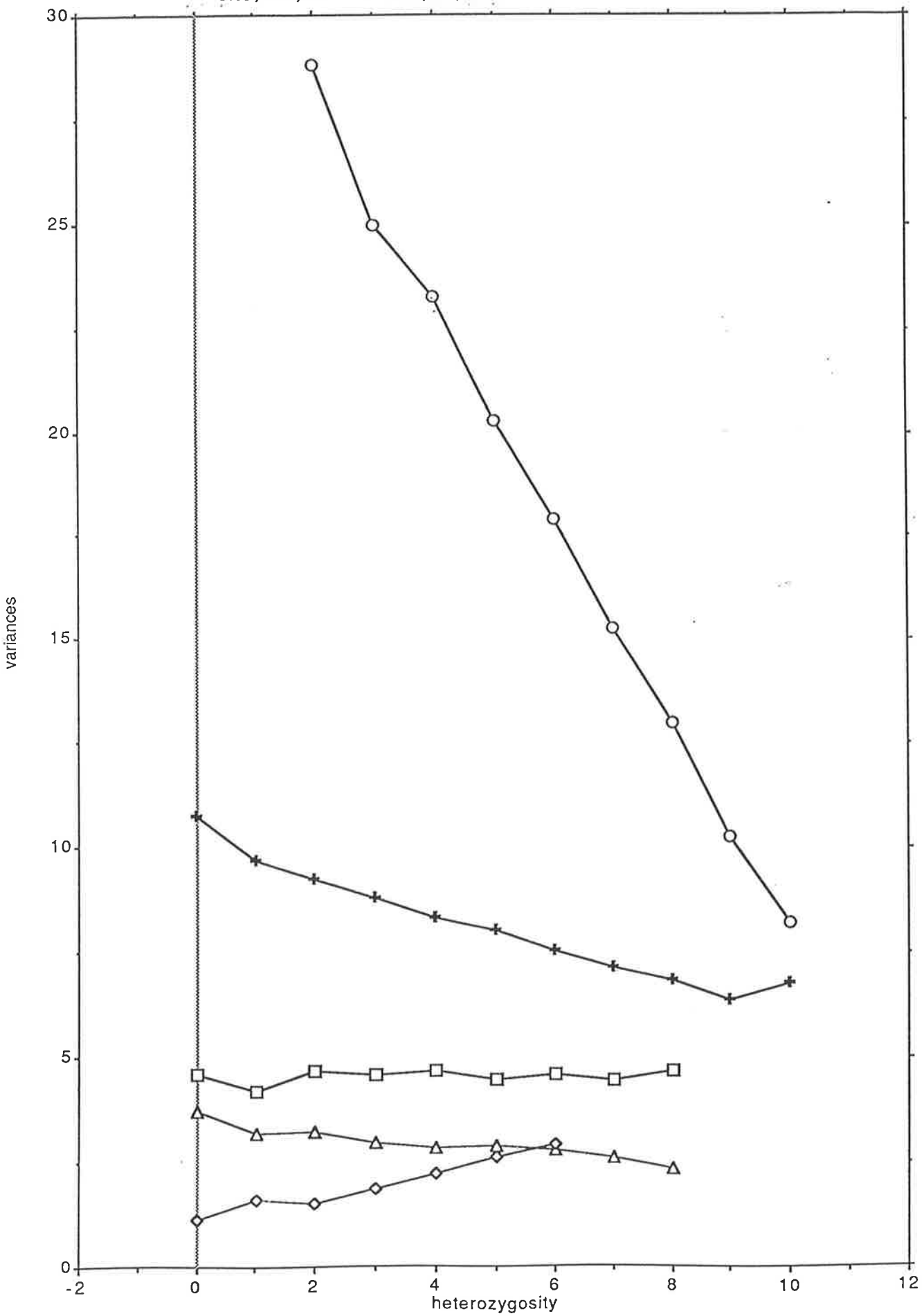


Figure 2-9. Genotypic variance versus heterozygosity. Three alleles per locus, alleles 2 and 3 acting additively and both dominant to allele 1, with multiplicative locus effects. Allelic frequencies:

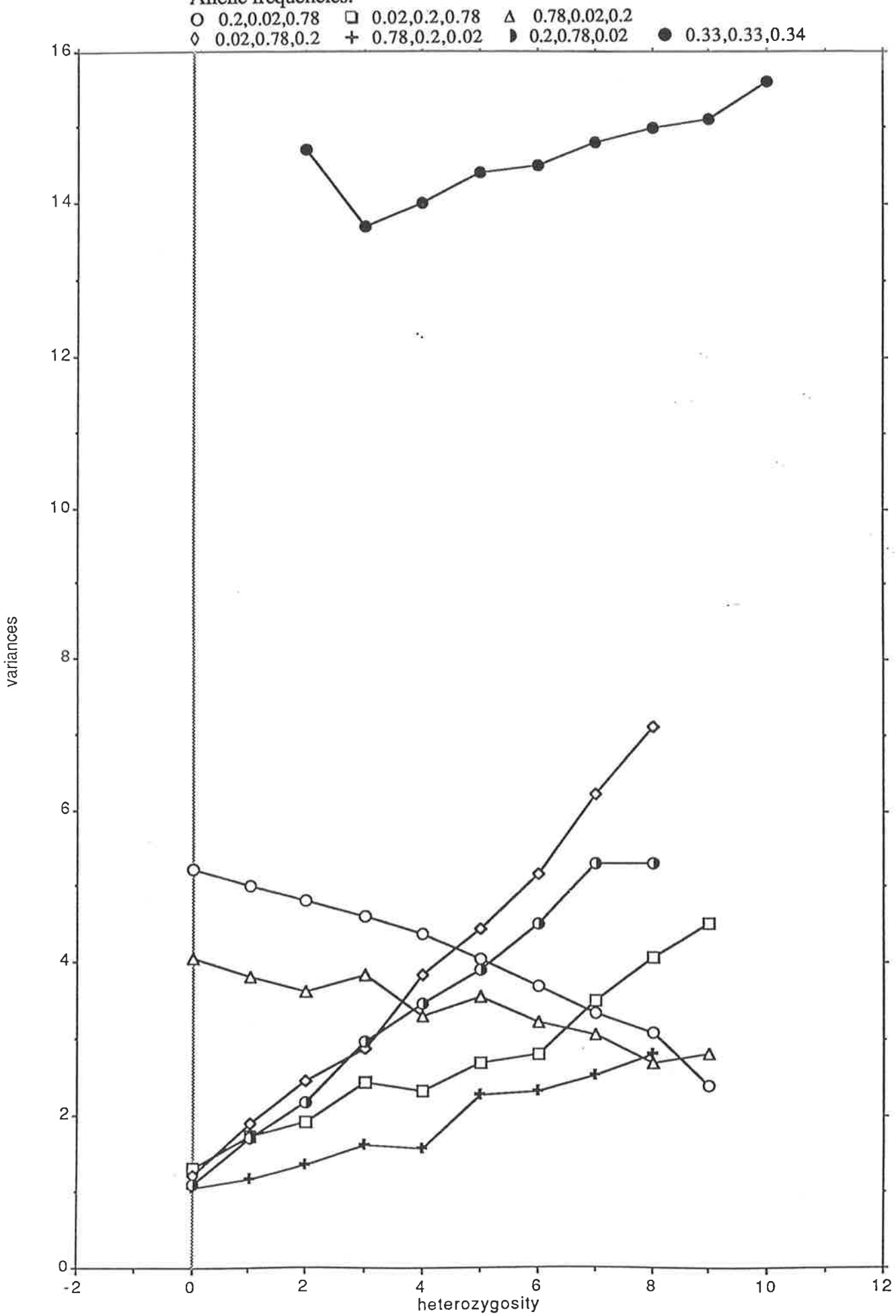


Figure 2-10. Genotypic variance versus heterozygosity. Four alleles per locus, alleles 2, 3 and 4 acting additively and all dominant to allele 1, with additive locus effects. Allelic frequencies:

O 0.32,0.32,0.32,0.04 ❑ 0.32,0.32,0.04,0.32

Δ 0.32,0.04,0.32,0.32 ◊ 0.04,0.32,0.32,0.32

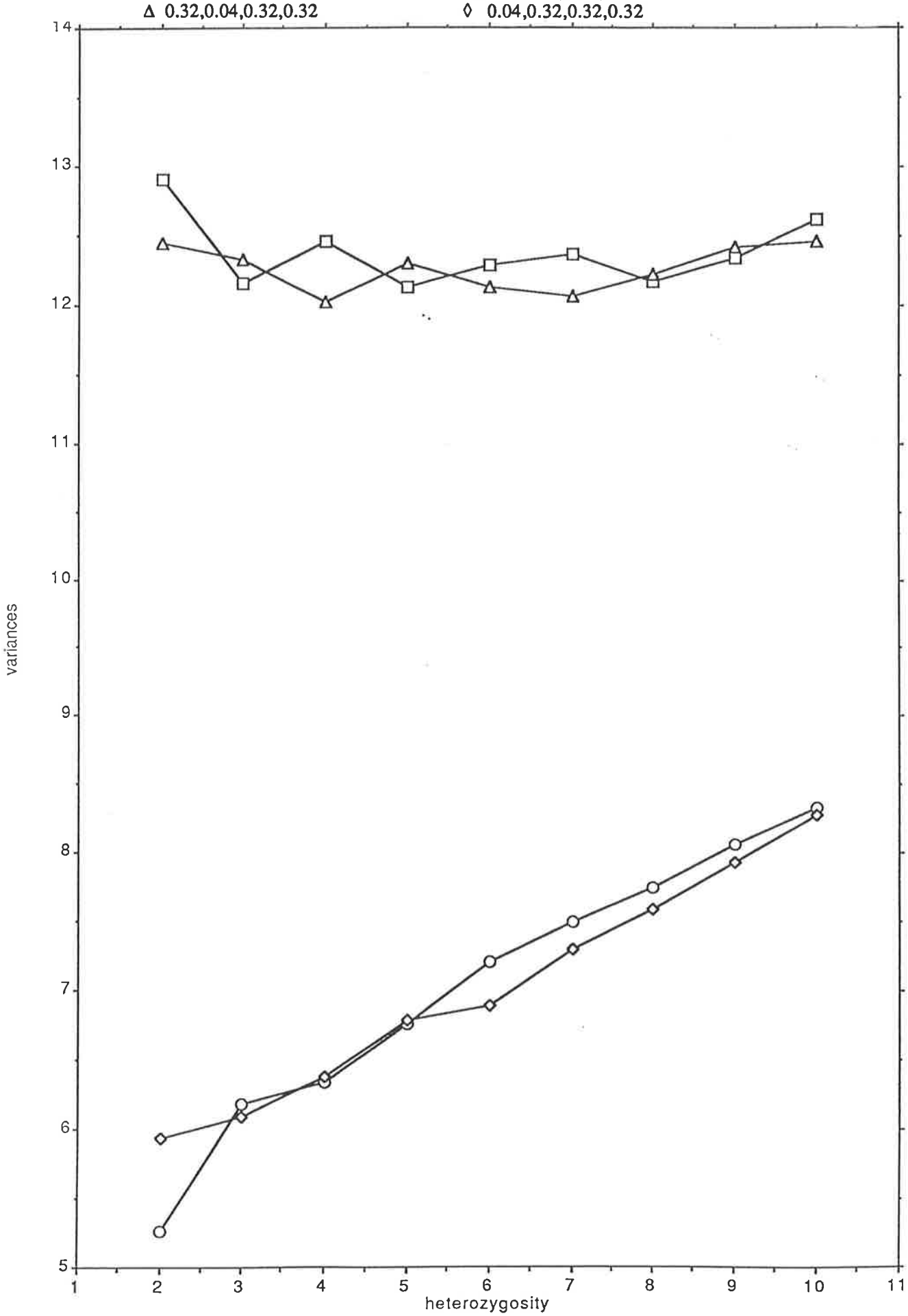
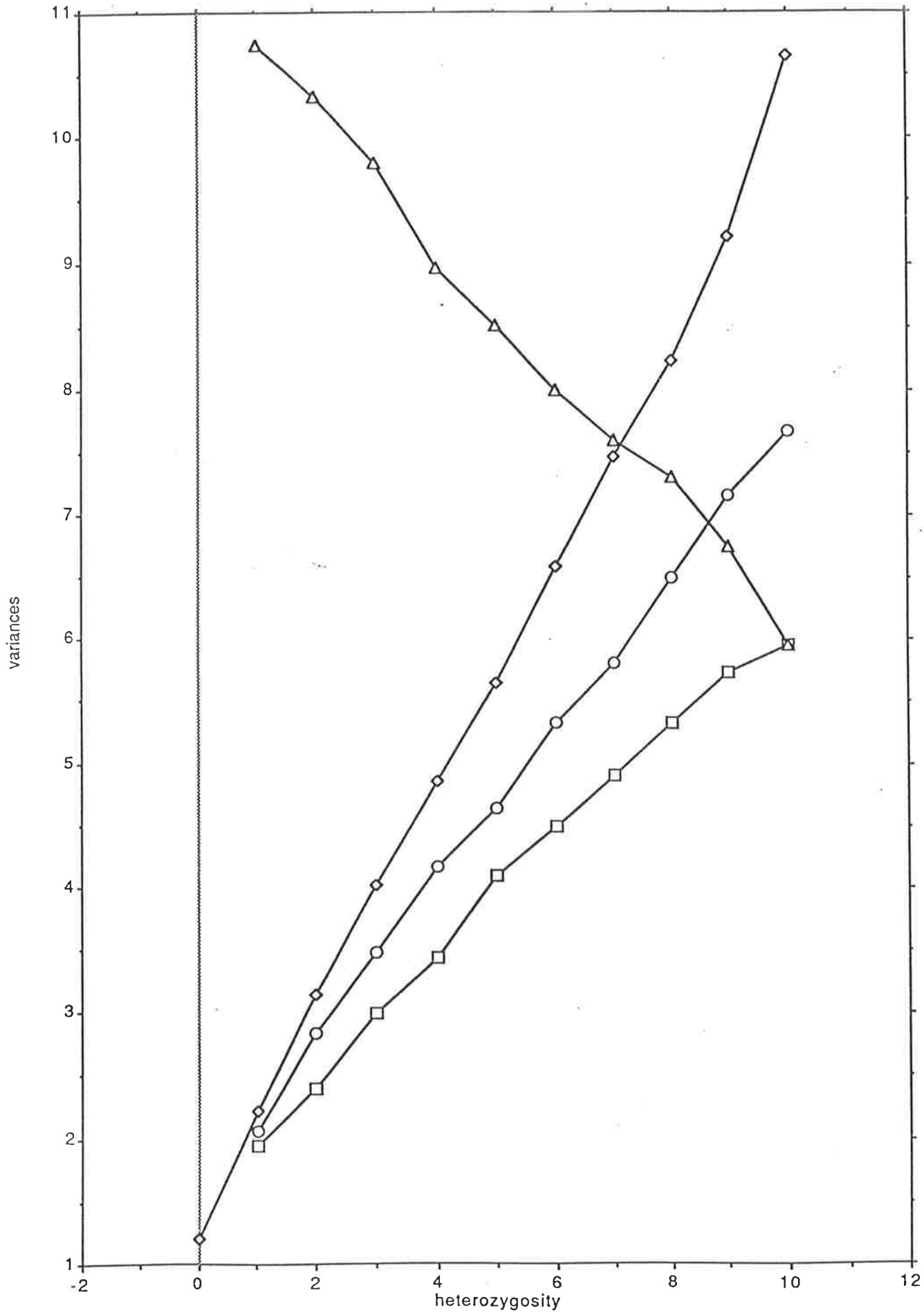


Figure 2-11. Genotypic variance versus heterozygosity. Four alleles per locus, alleles 2, 3 and 4 acting additively and all dominant to allele 1, with additive locus effects. Allelic frequencies:

O 0.6,0.3,0.05,0.05
Δ 0.3,0.05,0.05,0.6
❑ 0.05,0.6,0.3,0.05
◊ 0.05,0.05,0.6,0.3



3. ANALYSES OF MOTHER-BABY DATA

3.1 Description

One of the problems encountered when looking for evidence for homeostasis among humans is to find suitable data. The data must consist of one or more quantitative traits, which in some way reflect the fitness of the individual, together with genotypes for several loci. One such set was provided by Professor L. Beckman. The data comprised measurements made on 414 mothers and their newborn infants, some of which have been published by Beckman, Beckman and Magnusson (1971). The measurements used were nine quantitative traits: acid phosphatase in mother's serum (ACFM), heat-stable and heat-sensitive alkaline phosphatases in mother's serum (PLFM and LFM), acid and alkaline phosphatases in child's serum (ACFB and BFB), birth weight (BW), placental weight (PW), baby's length (L) and length of gestation (GL).

Genotypes had been ascertained for ABO blood groups and red cell acid phosphatase for both mother and baby, and for placental alkaline phosphatase and mother's haptoglobin. The three most common alleles for the red cell acid phosphatase locus, viz. ACP_1^A, ACP_1^B and ACP_1^C and the three most common alleles for placental alkaline phosphatase, viz. PL^s, PL^f and PL^i were all present in these data as were the two common haptoglobin alleles, Hp_1 and Hp_2. Thus analyses involving polymorphisms at these loci could be contemplated. It should be noted that when analyses by genotype were performed, some genotypes were omitted because they were only represented by a very small number of individuals. These were genotype CC for ACP_1, both mothers and infants, and genotype ii for the PL locus.

Mitton and Grant (1984) suggested a number of conditions which would favour a positive relationship between an individual's fitness and heterozygosity. One was that a character should be examined at a stage of the life cycle when any surplus energy would be used in the expression of that character. Length and weight at birth and placenta weight are good examples of this property.

Another was that characters should not be physically constrained in their development and a third was that a range of environments should be considered. Obviously there are some

constraints on a baby's size at birth, but they are not so rigid as to allow little variation. Biochemical measurements will also be loosely constrained. Since the data were collected in a large city hospital in Sweden, mothers would have come from a range of socioeconomic backgrounds, but there would have been some degree of uniformity of environments.

Phenotypic stability among heterozygotes if it occurs could be attributed to partial inbreeding (Lande and Schemske, 1985). Although the data were collected in a large city hospital, significant effects could be the result of local inbreeding followed by local outbreeding on account of increased poulation mobility.

Finally, Mitton and Grant (*ibid*) stated that characters with low canalization and small coefficients of variation would be most likely to show the relationship between fitness and heterozygosity. Since canalized characters are those whose development is normally unaffected by environmental stresses and underlying genetic variability (Rieger, Michaelis and Green, 1976), characters to be studied should be affected by these factors.

Beckman, Beckman and Magnusson (1971) discuss environmental and genetic factors which influence the levels of alkaline phosphatases in maternal and cord sera, indicating that these are not canalized characters.

Ulizzi and Terrenato (1987) present data which indicate that there was a reduction in birth weight variance all over the world during the 1970's. They attribute this reduction to modifications in environmental conditions. Although the data used in this chapter were collected before this period, other data which Ulizzi *et al.* (*ibid*) present imply that the trend in variance reduction had started well before this time. The coefficients of variation for birth weight which they tabulate are reasonably low, between 0.17 and 0.20. Mayo (1983) comments that human birth weight is influenced by maternal genotype, maternal age and parity. So birth weight appears to be moderately canalized, with a low coefficient of variation.

One can see that the quantitative traits measured in this data set have several points to recommend them for studying the question of heterozygote advantage.

Many researchers analyse heterozygosity data by adding up the number of loci for which an individual is heterozygous and thus giving that individual a score for heterozygosity. The classes generated by these scores are then used as the basis for the analysis. Smouse

(1986) criticises this approach when developing his "adaptive distances" model. His model shows that although individuals who are heterozygous at all loci are the fittest and those who are homozygous at all loci are the least fit, there is no strict order of fitness for intermediate numbers of heterozygous loci.

If one regards the loci, for which genotypes have been ascertained, as representative of the whole genome, then it may be reasonable to pool loci and obtain a heterozygosity score. However, this is a hypothesis about which, as discussed in chapter 1, there is some contention. It is also very unlikely that the very small sample of loci measured in this data set would be representative of the whole genome (Chakraborty, 1981) and so for most of the analyses genotypes have been kept separate. Where appropriate, heterozygosity scores have also been used to see how they compare with results for separate loci.

3.2 Univariate Tests

Examination of the rank correlations in table 3-1 reveals strong associations among all of the anthropometric measurements and also among several of the biochemical measurements, but no associations between the two groups.

These associations indicate that in analyses of the data, the biochemical measurements should be considered as a group as should the anthropometric measurements, rather than on an individual variable basis. However, initially, individual traits were considered in order to obtain a 'feel' for the data. Tables 3-2, 3-3, 3-4 and 3-5 show the mean and variance for each of the nine traits by genotype for the placental alkaline phosphatase, mother's haptoglobin, mother's red cell acid phosphatase and baby's red cell acid phosphatase loci respectively. While the number of individuals in each genotypic class is indicated, there were sometimes missing values for some traits.

Bartlett's test for homogeneity of variance across genotypes was performed for each trait in each polymorphism. All of the biochemical measurements except placental alkaline phosphatase in mother's serum exhibit skewness and kurtosis and since Bartlett's test is not robust under such conditions, Layard's test (1973) was also used, as implemented in GENSTAT IV (1983). The result was that none of the differences among the variances for each trait in any polymorphism was significant. Nor was there any tendency for heterozygotes

at any of the loci to have lower variances than homozygotes for any of the traits. The exceptions to this lack of trend were the traits, acid phosphatase in the child's serum and alkaline phosphatase in the child's serum. Both exhibited variances lower among heterozygotes than homozygotes, although not significant, at the mother's haptoglobin locus. The former also showed the postulated trend at the mother's red cell acid phosphatase locus, but this was not significant.

3.3 Multivariate Tests and Discussion

A different picture emerges when groups of traits are analysed together. For instance, the multivariate coefficients of variation (van Valen, 1974) tabulated at the bottom of each of tables 3-2, 3-3, 3-4 and 3-5, show a tendency to be greater for homozygotes than for heterozygotes at the mother's haptoglobin and mother's red cell acid phosphatase loci. If separate multivariate coefficients of variation are calculated for the biochemical traits and for the anthropometric traits it appears that the former group has a much greater effect on the combined coefficient than the latter. This is obviously due to scaling. For instance baby's length has means of approximately 50 and variances of approximately 5, i.e. 10% of the mean, whereas red cell acid phosphatase in the mother's serum has means of approximately 90 and variances of approximately 1000, i.e. more than 1100% of the mean. There is still an obvious scaling problem among the biochemical traits and so trends among BFB, LFM and PLFM are likely to overshadow those among ACFB and ACFM.

The multivariate coefficient of variation for all traits, CV_9, and the coefficient for biochemical traits only, CV_5, exhibit lower values for heterozygotes than for homozygotes at both the mother's red cell acid phosphatase locus and the mother's haptoglobin locus. The coefficient for anthropometric traits only, CV_4, also exhibits this trend at the mother's red cell acid phosphatase locus.

As a matter of interest, these multivariate coefficients of variation were compared with the results obtained from Handford's extension to Levene's test using all nine traits (see chapter 1 for a description of this test). This test was used on raw data and on log-transformed data; the aim of the latter was to combat some of the scaling problems. The results are presented in table 3-6.

As for CV_9, the mean values of the raw data were lower for heterozygotes than for homozygotes at the mother's haptoglobin and the mother's red cell acid phosphatase loci, although these means were not significantly different. When the data were log-transformed the trend at the mother's haptoglobin locus was maintained and remained non-significant. However, the means for the log-transformed data at the mother's red cell acid phosphatase locus were very significantly different, which was due to a high value for genotype *AA*, while all other genotypes had similar means. Thus removing some of the scaling effects also removed some of the trend towards lower variances among heterozygotes. The raw data means for the genotypes of placental alkaline phosphatase were significantly different mainly because of the high value for genotype *ff*; all other genotypes had similar values. The trend was maintained but the significance was lost when the data were transformed.

Some of the results for the modified Levene's test were similar to the trends exhibited by the multivariate coefficients of variation, although the latter cannot be tested for significance. It must be stressed that the statistical properties of the modified Levene's test are unknown and so not much weight should be attached to these results.

Now let us turn to a multivariate test which is scale-free, viz. a multivariate non-parametric test for differences in dispersion as described in chapter 1. This test was particularly appropriate for the biochemical traits since they were not distributed normally. A MINITAB program to implement this test was written (see Appendix B). Its validity was checked by hand calculations at representative points throughout the program.

As was mentioned earlier, the patterns in the variances of the different traits for any set of genotypic classes are usually similar to the patterns exhibited by the univariate coefficients of variation. The patterns do vary for placental alkaline phosphatase genotypes. Thus for the other three loci, it is reasonable to assume that location vectors of the biochemical measurements are equal across genotypic classes. Hence we can test the hypothesis, $H_0^{(1)}$, of Puri and Sen, referred to in section 1.4. This means that the dispersion matrices for the different genotypic classes may be tested for equality assuming that the location vectors are

equal. The results for the mother's haptoglobin, mother's red cell acid phosphatase and baby's red cell acid phosphatase loci are presented in tables 3-8, 3-9 and 3-10 respectively. $J(u) = \sqrt{12}(u-\frac{1}{2})$ was used to form score matrices as in the example in chapter 1.

The assumption of equal location vectors is not so easily applied to the genotypic classes of the placental alkaline phosphatase locus and so $H_0^{(2)}$ referred to in section 1.4 has been tested, i.e. the dispersion matrices were tested for equality without assuming that the location parameters were equal. In this case $J(u) = \mathcal{E}\left[\Phi^{-1}(u)\right]$ was used. The results are presented in table 3-7.

The alternative hypotheses for all of these tests were that the dispersion matrices were not equal and in fact the null hypothesis was definitively rejected for each locus. There are no tests to determine which elements of the various dispersion matrices contribute significantly to the overall result. However, attention should be given to the diagonal elements of each matrix since these correspond to the dispersions of the traits either for a particular genotypic class or for the whole data set.

In general, these results do not point to an inverse relationship between heterozygosity and variance, although there are a few exceptions. For instance, ACFM has higher dispersions for the two homozygote classes of baby's red cell acid phosphatase than the three heterozygote classes, although it is hard to see what the causal relationship between this locus and this trait would be. Other examples are the traits ACFB and BFB which have higher dispersion for the homozygotes than the heterozygotes at the mother's haptoglobin locus. A similar trend can be seen for PLFM at the placental alkaline phosphatase locus.

Most of the differences among dispersion matrices are not due to trends of this type, but to differences of individual elements. For instance the co-dispersion of ACFM and LFM is much higher for genotype *ff* at the placental alkaline phosphatase locus than for all other genotypes at that locus.

So the conclusion is that, although this test indicates differences in dispersion among genotypes at all loci, it does not lead us any further along the road to heterozygote advantage.

Tiku and Balakrishnan (1985) proposed a robust statistic, T_R^2, to test the equality of covariance matrices from two populations. They compared T_R^2 with L_N, the rank-order statistic used here and found that T_R^2 was much more powerful than L_N when comparing two dispersion matrices. Their tests were conducted on equal sample sizes of 30 and they also found that the χ^2 approximation to the null distribution of L_N was inadequate for these sample sizes. This point should not be of too much concern for the mother-baby data since most sample sizes are larger than 30 (sample sizes are given in tables 3-2, 3-3, 3-4 and 3-5). Except for normal distributions, L_N was more robust than Hotelling's T^2 and λ_1^* (see section 1.4) but probably less powerful than the former.

If only two dispersion matrices were to be compared, T_R^2 would be a superior statistic. Thus if one felt confident that all heterozygotes were homogeneous and all homozygotes were homogeneous, the differences in dispersion for the two could be compared using T_R^2. However, the test has not been extended to include several matrices and so L_N is preferable in this case and should be adequate in view of the large sample sizes.

Of the remaining traits, gestation length was negatively skewed (–1.765 with standard error 0.123) and leptokurtic (9.55 with standard error 0.25) and thus was clearly not normally distributed. The other three traits were distributed normally and it was considered appropriate to use a parametric test statistic, λ_1^*. Since the parametric test for equality of dispersion matrices described in chapter 1 is sensitive to departures from normality, gestation length was omitted from the subsequent analyses.

The genotypic covariance matrices and the pooled covariance matrix for each of placental alkaline phosphatase, mother's haptoglobin, mother's red cell acid phosphatase and baby's red cell acid phosphatase are shown in tables 3-11, 3-12, 3-13 and 3-14 respectively. The covariance matrices for placental alkaline phosphatase genotypes are significantly different. Inspection reveals that all of the elements of matrices for the two homozygous genotypes, *ff* and *ss*, are greater than or equal to the corresponding elements of the matrices for the two heterozygous genotypes, *fs* and *si*. The heterozygous genotype, *fi*, does not fit this pattern. However, this genotype only contains nine members and when the analysis was repeated, omitting this genotype, the four remaining matrices were still very significantly different. Thus

there is some indication that heterozygotes are less variable than homozygotes for these three anthropometric traits.

The same cannot be said of the covariance matrices for mother's red cell acid phosphatase. While the five covariance matrices are significantly different, there is no particular pattern of lower variances among the heterozygotes than the homozygotes. The covariance matrices of anthropometric traits for the other two loci are not significantly different.

So we are led to conclude that the anthropometric traits are less variable among heterozygotes than among homozygotes for the placental alkaline phosphatase locus. No such trend can be observed at other loci or among the group of biochemical traits. The inconclusiveness of these results is in agreement with other findings on human populations and could have several explanations in addition to those problems already discussed.

First, not all polymorphisms need be subject to natural selection. Furthermore, even if most polymorphisms were at times subject to selection, this might not be the case in any given population at any given time. Thus, most polymorphisms may be selectively neutral most of the time (Kimura, 1983).

Second, not all selection is centripetal, stabilizing or normalizing. Much selection is directional, so that homozgotes are favoured, and a balance among opposing directional forces will not necessarily favour heterozygotes. Further, consistent directional selection, such as has been observed for human birth weight (Mayo, 1983) can overcome simultaneous stabilizing selection (Mayo and Hancock, 1985).

Third, a small number of polymorphic electrophoretic loci is not an appropriate predictor of genomic heterozygosity (Chakraborty, 1987). Unless the postulated advantage of heterozygotes is consistent over all loci, different samples of a few loci may be expected not to produce concordant results. Smouse (1986) supports the view that heterozygote advantage is not consistent over all loci. His adaptive distances model assumes interactions among loci and that some homozygotes are more fit than others. The latter point certainly appears to be true for these data. Smouse's model will be discussed in the next section.

3.4 Adaptive Distances Model

Smouse (1986) argues that the loci which have actually been examined, or some others closely linked to them, are responsible for the observed differences in fitness for different numbers of heterozygous loci. To test this hypothesis he develops a multiplicative overdominance model for unlinked loci. He assumes that each locus has only two possible alleles; if a locus has multiple alleles then these can be pooled to produce a pair of allelic classes. He also assumes that if k loci are being used in the model, it must be possible to determine relative fitnesses of all genotypes by reference to the optimal genotype, the k-locus heterozygote. The model employs a measure of adaptive distance between any homozygote and the optimal genotype; this measure is zero for a heterozygote and the inverse of the frequency of the allele for a homozygote.

The model fitted is

$$\log (W_j) = - \alpha X_A - \beta X_B - .... - \kappa X_K + \varepsilon_j$$

where W_j is the fitness of individual j;

$\alpha, \beta, ... \kappa$ are the intensities of selection for the k loci;

X_A is a measure of adaptive distance for the A-locus genotypes;

$X_B, ... X_K$ are similar to X_A for the other loci;

ε_j is the error term for individual j, incorporating differences at unmeasured loci, environmental influences and measurement error.

This model was fitted to the mother-baby data. Smouse (*ibid*) recommends estimating the gene frequencies from a set of data other than that being used to fit the model. Kirk (1968) provides the results of four Swedish studies of haptoglobin and two of these, from populations similar to the mother-baby data set, provided estimates of the frequency of Hp_1 as 0.386 and Hp_2 as 0.614.

Fitnesses of the haptoglobin genotypes (HP) were estimated as follows:

	Hp11	*Hp12*	*Hp22*
frequency f	p_1^2	$2p_1p_2$	p_2^2
fitness w	1-s	1	1-t
f x w	$(1-s)p_1^2$	$2p_1p_2$	$(1-t)p_2^2$

$\bar{w} = (1-s)p_1^2 + 2p_1p_2 + (1-t)p_2^2$

At equilibrium $w_1 = w_2 = \bar{w}$

$w_1 = p_1 (1-s) + p_2$ (1) $w_2 = p_1 + p_2 (1-t)$ (2)

substitute $p_1 = 0.386$, $p_2 = 0.614$ (Kirk, 1968) into (1) and (2) and solve:

$$s = 1.59t$$

Thus if $t = 0.01$, $s = 0.0159$

The values for adaptive distances are calculated by:

$$\text{when HP} = \begin{cases} Hp_{11} \\ Hp_{12} \\ Hp_{22} \end{cases} \qquad \text{then } X_{HP} = \begin{cases} 1/p_1 = 2.591 \\ 0 \\ 1/p_2 = 1.629 \end{cases}$$

For the mother's red cell acid phosphatase locus, (RCAP) there are three alleles, A,B and C. Beckman (1972) presents red cell acid phosphatase gene frequencies for various populations including the mother-baby data being used in this analysis. The first 26 entries in the table relate to populations of European origin and these have been used to calculate the following gene frequencies :

$p_A = 0.336$ $p_B = 0.604$ $p_c = 0.060$

Fitnesses of the red cell acid phosphatase genotypes were calculated as follows :

genotype	*AA*	*BA*	*CA*	*BB*	*CB*	*CC*
fitness, w	1-s	1	1	1-t	1	1-r
frequency, f	p_A^2	$2p_Ap_B$	$2p_Ap_C$	p_B^2	$2p_Bp_C$	p_C^2
f x w	$p_A^2(1-s)$	$2p_Ap_B$	$2p_Ap_C$	$p_B^2(1-t)$	$2p_Bp_C$	$p_C^2(1-r)$

$\bar{w} = p_A^2(1-s) + 2p_Ap_B + 2p_Ap_C + p_B^2(1-t) + 2p_Bp_C + p_C^2(1-r)$ is the average fitness

and $w_A = p_A(1-s) + p_B + p_C$ (1)

$w_B = p_B(1-t) + p_A + p_C$ (2)

$w_C = p_C(1-r) + p_A + p_B$ (3)

At equilibrium $w_A = w_B = w_C = \bar{w}$

Substituting $p_A = 0.336$, $p_B = 0.604$ and $p_C = 0.060$ into (1), (2) and (3) we get

$s = 1.798t$

$r = 10.067t$

and since $0 \leq r, s, t \leq 1$

$t \leq 0.0993$

Problems of choosing values of t and thence of r and s will be discussed later in this section, but possible values to try would be t = 0.01, s = 0.018, r = 0.101

or t = 0.001, s= 0.0018, r = 0.0101, corresponding to different intensities of selection. Since there are three alleles at this locus, two must be pooled to form two allelic classes altogether. Smouse (*ibid*) recommends pooling the rarer alleles to ensure nontrivial frequencies for the calculations. Thus alleles A and C were pooled to give the following results :

genotype	*BB*	*B(AC)*	*(AC) (AC)*	
frequency, f	0.365	0.478	0.157	
fitness, w	0.99	1	0.982	if t = 0.01
fitness, w	0.999	1	0.996	if t = 0.001

$$w_{B(AC)} = \frac{2p_B p_A w_{AB} + 2p_B p_C w_{BC}}{2p_B p_A + 2p_B p_C}$$

$$= \frac{0.604 \times 0.336 \times 1 + 0.604 \times 0.060 \times 1}{0.604 \times 0.336 + 0.604 \times 0.060} = 1.0$$

$$w_{(AC)(AC)} = \frac{p^2_A w_{AA} + 2p_A p_B w_{AC} + p_C^2 w_{CC}}{(1-p_B)^2}$$

$$= \frac{0.113 \times 0.982 + 2 \times 0.336 \times 0.060 \times 1 + 0.0036 \times 0.899}{0.157}$$

$$= 0.982 \quad \text{if} \quad t = 0.01$$

If t = 0.001

$$w_{(AC)(AC)} = \frac{0.113 \times 0.9982 + 2 \times 0.336 + 0.060 \times 1 + 0.0036 \times 0.9899}{0.157}$$

$$= 0.9959$$

For the adaptive distances when

$$RCAP = \begin{cases} BB \\ B(AC) \\ (AC)(AC) \end{cases} \qquad \text{then } X_{RCAP} = \begin{cases} 1/p_B = 1.656 \\ 0 \\ 1/p_{(AC)} \end{cases}$$

$$\text{and } \beta = \frac{s_{RCAP}\, t_{RCAP}}{s_{RCAP} + t_{RCAP}} = \frac{0.01 \times 0.018}{0.01 + 0.018} = 0.0064 \quad \text{if } t = 0.01$$

The third locus to be included in this analysis was mother's ABO blood group (ABO). Beckman, Cedergren, Collinder and Rasmuson (1972) provide ABO blood group data from northern Sweden for 60,000 individuals in 14 regions for the period 1871 to 1967. A marked change in inbreeding occurred between 1940 and 1960 and so data collected during the last seven years only of the study have been used to estimate gene frequencies as follows :

$p_A = 0.289$, $p_B = 0.092$, $p_i = 0.619$

Fitnesses were then calculated :

genotype	ii	I^Ai	I^Bi	I^AI^A	I^AI^B	I^BI^B
frequency, f	0.383	0.358	0.114	0.084	0.053	0.008
fitness, w	1-s	1	1	1-t	1	1-r
f x w	0.383(1-s)	0.358	0.114	.084(1-t)	0.053	.008(1-r)

$$\bar{w} = p_i^2 (1-s) + 2p_ip_A + 2p_ip_B + p_A^2 (1-t) + 2p_Ap_B + p_B^2 (1-r)$$

$$w_i = p_i (1-s) + p_A + p_B \quad (1)$$

$$w_A = p_A (1-t) + p_i + p_B \quad (2)$$

$$w_B = p_B (1-r) + p_i + p_A \quad (3)$$

At equilibrium $w_i = w_A = w_B = \bar{w}$

Substituting values for p_A, p_B and p_i into (1), (2) and (3)

gives : $t = 2.142\, s$

$$r = 6.728\, s$$

since: $0 \le r,s,t, \le 1, \quad s \le 0.1486$

If $s = 0.001$ then $t = 0.0021$ and $r = 0.0067$

Some alleles had to be pooled to obtain two allelic classes. If alleles I^B and i were pooled, the resulting allelic classes would be : $\{I^AI^A\}$, $\{I^Ai, I^AI^B\}$, $\{ii, I^Bi, I^BI^B\}$. Since the data did not distinguish between I^AI^A and I^Ai and since these two genotypes would be in different allelic classes, 179 mothers with blood group A would have to be omitted from the analysis. Alternatively alleles I^A and I^B could be pooled, obtaining classes $\{ii\}$, $\{I^Ai, I^Bi\}$ and $\{I^AI^B, I^AI^A, I^BI^B\}$. The data did not distinguish between I^BI^B and I^Bi either and so 223 mothers would have to be omitted from the analysis. Consequently, the first scheme was adopted.

genotype	I^AI^A	I^AI^B, I^Ai	I^BI^B, I^Bi, ii
frequency	0.084	0.411	0.505
fitness	1-t	1	[0.008 (1-r) + 0.383(1-s) + 0.114] / 0.505

If $s = 0.001$, $t = .0021$ and $r = 0.0067$, the values for the fitnesses are 0.9979, 1 and 0.9981 respectively. The values for the adaptive distances are :

$$\text{when} \quad \text{ABO} = \begin{cases} I^AI^A \\ I^A_i \text{ or } I^AI^B \\ I^BI^B, I^Bi \text{ or } ii \end{cases} \qquad \text{then} \quad X_{\text{ABO}} = \begin{cases} 3.46 \\ 0 \\ 1.406 \end{cases}$$

$$\text{and} \quad \gamma = (0.0021 \times 0.0019) / 0.0040 = 0.000998$$

Table 3-15 displays the genotypic classes for the three loci, the number of mothers in each class, the log fitness, Y_j, for each class and the number of heterozygous loci for each class. Spearman's rank correlation coefficients between log fitness and each of the nine quantitative traits were found. These values are tabulated in table 3-16. There was a significant positive

correlation between mother's serum acid phosphatase and log fitness; all other correlations were not significant.

There are at least three possible causes for this lack of correlation. Y_j is a measure of the fitness of each genotype but the quantitative traits themselves may not reflect the fitness of an individual and so they will be uncorrelated with Y_j. Alternatively, the quantitative traits may reflect fitness but not in a linear manner. For instance, a trait may have an optimum value for survival which is somewhere in the middle of the range of possible values for that trait. Since a correlation coefficient is a measure of linear association, it would not detect such a situation.

Finally, the parameters used to calculate the Y_j values may not have been well estimated. This problem and others will be discussed later in this section.

Fitnesses for genotypes at three loci of the babies were then considered. The ABO locus and the red cell acid phosphatase locus (PL) were treated similarly to the mothers. The third locus for the babies was placental alkaline phosphatase. Beckman (1972) gives two sets of data from Sweden relating to this locus and the resulting gene frequencies are:

$p_f = 0.255$, $p_i = 0.087$ and $p_s = 0.646$ while the gene frequency of other rare alleles is 0.012. These rare alleles were ignored in the following analysis and so the frequency values of the common alleles were increased to 0.258, 0.088 and 0.654 so that they would sum to 1.0.

genotype	ff	fs	fi	ss	si	ii
fitness, w	1-2	1	1	1-t	1	1-r
frequency, f	0.0666	0.3375	0.0454	0.4277	0.1151	0.0077

$$\bar{w} = 0.0666\,(1-s) + 0.498 + 0.4277\,(1-t) + 0.0077\,(1-r)$$

$$= 1 - 0.0666s - 0.4277t - 0.0077r.$$

$$w_f = 0.258\,(1-s) + 0.654 + 0.088 = 1-0.258s \qquad (1)$$

$$w_s = 0.654\,(1-t) + 0.258 + 0.088 = 1-0.654t \qquad (2)$$

$$w_i = 0.088\,(1-r) + 0.258 + 0.654 = 1-0.088r \qquad (3)$$

At equilibrium $w_f = w_s = w_i = \bar{w}$ and so $s = 2.5435t$, $r = 7.432t$

Suppose $t = 0.001$, then $s = 0.0025$ and $r = 0.0074$.

The alleles f and i, being less frequent, were combined to give the following genotypic classes:

genotype	*ss*	*s(fi)*	*(fi)(fi)*
frequency, f	0.4277	0.4526	0.1197
fitness, w	0.999	1	0.998

The adaptive distances were calculated as follows :

$$\text{when PL} = \begin{cases} ss \\ fs \text{ or } si \\ ff,\ ii \text{ or } fi \end{cases} \qquad X_{PL} = \begin{cases} 1.529 \\ 0 \\ 2.890 \end{cases}$$

and $\alpha = s_{PL}\, t_{PL} / (s_{PL} + t_{PL}) = 0.001 \times 0.002 / 0.003$

$= 0.00067$

$Yj = \log(W_j) = -0.00067\, X_{PL} - 0.000804\, X_{RCAP} - 0.000998\, X_{ABO}$

are tabulated for each genotypic class, together with class frequency and heterozygosity score in table 3-17. Correlations between log fitness and each of the nine quantitative traits are shown in table 3-18. None of these correlations was significant.

In summary, the adaptive distance model is limited in its ability to explain any of the variation in the quantitative variables in the mother-baby data set, with the exception of mother's serum acid phosphatase activity. The model, in the form in which it has needed to be used here, has several shortcomings; better data would overcome some of these.

First, it is assumed that measures of fitness for each genotype are available. These can be estimated if one assumes that the population is at equilibrium. However, the estimates obtained are only relative, e.g. $s = 2.535t$ and $r = 7.432t$ with the value of t being open to guesswork. The intensities of selection are used to weight the loci and the guessed value of t is crucial to this weighting.

For instance, suppose for the PL locus, t is set to 0.01 i.e. increased tenfold, then s and r will also be increased tenfold and this results in a tenfold increase in α, the selection intensity. The log fitness for the genotype *B(CA)*, *ss* and $(I^B i)$, say, would change from - 0.0024218 to -0.01149, which is substantial.

Second, the lumping together of genotypes in the three-allele case means that information about heterozygosity is lost. For instance, at the PL locus, when alleles f and i were combined, genotype fi was then regarded as a homozygote.

Third, since the actual genotypes of ABO blood groups A and B were not available much information was lost.

Fourth, in addition to the first and second problems already discussed, the PL locus posed an additional problem in that several rare alleles had to be ignored. Thus selection coefficients were probably poorly estimated.

Finally, the fact that the loci measured for mothers and babies were not the same made any direct comparison of the two difficult. One could make comparisons based on the two loci common to both mothers and babies, but since the ABO locus has suffered such a loss of information, it would be little better than a direct comparison of the red cell acid phosphatase locus.

3.5 Heritability Estimates

This section does not relate directly to the question of heterozygote advantage but while that question was being studied with reference to the mother-baby data, some other interesting points emerged.

Spencer, Hopkinson and Harris (1964) demonstrated that serum acid phosphatase activity differs among the genotypes, detected by electrophoresis, of the ACP_1 locus. To determine the effect of genes, other than the major gene in question, on the activity of the enzyme, one usually carries out analyses within genotypic groups and then pools the results across these groups. Such a procedure assumes that there is no interaction between the major gene and any other genes which may have an effect. Therefore pooling may not always be statistically valid.

It is not always necessary to pool. For instance, Nance and Grove (1972), when estimating the heritability of percentage sickle-cell haemoglobin, used only heterozygotes for HbS in their analysis. However, that is a clear-cut situation since the sickle-cell gene is deleterious in the homozygous state. The case of serum acid phosphatase activity, and also

other enzymes, is not so clear-cut. Accordingly, there is a need for a simple method of estimation of genetic parameters which allows for differences between genotypic classes. Multivariate analysis of variance (MANOVA) provides such a method.

MANOVA (Anderson, 1984) partitions the matrix of sums of squares and cross products for a set of traits into various meaningful components. In the present context these components would be ACP_1 genotypes for mothers and babies, while the set of traits would be mother's and baby's serum acid phosphatase activity. So the data have the following form:

genotype pair	1 (AA,BA)		2 (BA,AA)		.....	k	
mother-baby pair							
1	Y_{1m1}	Y_{1b1}	Y_{2m1}	Y_{2b1}		Y_{km1}	Y_{kb1}
2	Y_{1m2}	Y_{1b2}	Y_{2m2}	Y_{2b2}		.	.
	.	.	.	.		.	.
	.	.	.	.		.	.
	Y_{1mn_1}	Y_{1bn_1}	Y_{2mn_2}	Y_{2bn_2}		Y_{kmn_k}	Y_{kbn_k}

where Y_{imj} is the value of the trait for the jth mother in genotypic group i;

Y_{ibj} is the value of the trait for the jth baby in genotypic group i.

The number of mother-baby pairs in each genotypic combination varies; hence $n_1, n_2 \ldots n_k$. The covariance matrix for $(Y_{imj}, Y_{ibj})'$ is partitioned by MANOVA into a component attributable to differences among the genotypic groups and a residual component. This second component is of the form $\begin{pmatrix} \sigma_{mm} & \sigma_{mb} \\ \sigma_{bm} & \sigma_{bb} \end{pmatrix}$ where σ_{mm} and σ_{bb} are the variances of serum acid phosphatase for mothers and babies respectively and $\sigma_{mb} = \sigma_{bm}$ is the covariance between mothers and babies, after adjusting for differences between genotypic groups. σ_{mb} estimates $\frac{1}{2} V_A$ while σ_{mm} and σ_{bb} are two separate estimates of V_P, the total phenotypic variance in the trait (Falconer, 1982). Heritability can be estimated by V_A/V_P i.e. $h^2 = 2\,\sigma_{mb} / \sigma_{mm}$ and its variance is estimated by $4(\sigma_{mm}\,\sigma_{bb} - \sigma^2_{mb}) / df(\sigma_{mm})\,\sigma^2_{mm}$.

It should be noted that other pairs of relatives could be used in this type of analysis. In chapter 4 the results of a similar analysis using dizygous twin pairs will be discussed.

The mothers and babies were divided into genotypic classes according to the ACP_1 locus. Some genotype combinations were poorly represented in the data and so only those

combinations with at least 19 observations were used in the analyses which follow. The numbers in the genotypic combinations are displayed in table 3-19. For some genotypes, serum acid phosphatase exhibited skewness and kurtosis. Box-Cox power transformations (1964) were examined using a macro of GENSTAT IV. As a result it was decided to use the logarithmic transformation of both mother's and baby's serum acid phosphatase.

MANOVA was performed using the seven genotypic combinations as seven levels of a factor. GENSTAT IV was used for the analysis. Wilks' Λ indicated that there was no significant difference among the genotypic combinations. Nor were the two estimates of V_P significantly different from one another. The covariance matrix and estimate of heritability are shown in table 3-20a.

Since the more usual method of estimating heritability is to use the simple linear regressions of offspring on parent within genotypic classes, this technique was used as a comparison with the MANOVA results.

Simple linear regression of the log of baby's serum acid phosphatase versus the log of mother's serum acid phosphatase was performed for each of the seven genotype combinations with at least 19 observations as indicated in table 3-19. One can see from table 3-21 that one group *(CB,CB)* had a negative regression coefficient, although this was not significantly different from zero. The pooled regression coefficient was 0.422.

The 19 *(CB,CB)* pairs were omitted and the MANOVA was repeated. The revised estimates are shown in table 3-20b. Thus the estimate of $h^2 = 0.912$ can be compared with the estimates from the regressions, ranging from 0.4122 to 1.268 with a pooled value of approximately 0.9.

Thus there is good agreement between the traditional way of estimating heritability and this new method. The advantage of the new method is that we can feel confident that pooling over genotypic classes is valid since differences among them have already been allowed for. Many statistical packages have MANOVA and so it is straightforward to use.

There are two interesting results from these calculations. First, the heritability of serum acid phosphatase activity is high even after allowing for the effects of the ACP_1 locus. Since this locus, as well as ACP_2 and ACP_3, controls red cell acid phosphatase activity it is not

surprising that it has little effect on serum acid phosphatase activity. The regulation of the latter is uncertain.

Second, the two estimates of phenotypic variance, $\sigma_{mm} = 34.948$ and $\sigma_{bb} = 26.90$ are significantly different at the 5% level. This is an indication of the low environmental effect on newborn serum acid phosphatase and a possible cause of high heritability.

Table 3-1 Rank correlations among the nine quantitative traits.
*A correlation, r_s, is significant at the 5% level if $|r_s| \geq 0.1$.

	ACFM	PLFM	LFM	ACFB	BFB	BW	PW	GL
PLFM	0.210*							
LFM	0.231*	0.048						
ACFB	0.438*	0.092	0.028					
BFB	0.122*	0.040	0.187*	0.223*				
BW	–0.015	0.076	0.009	0.032	–0.038			
PW	0.030	0.085	0.071	–0.030	–0.010	0.619*		
GL	–0.019	0.079	0.013	0.075	–0.032	0.452*	0.200*	
L	0.009	0.030	0.059	0.024	0.015	0.775*	0.439*	0.430*

ACFM = acid phosphatase in mother's serum; PLFM = placental alkaline phosphatase in mother's serum; LFM = heat-sensitive alkaline phosphatase in mother's serum; ACFB = acid phosphatase in child's serum; BFB = alkaline phosphatase in child's serum; BW = birth weight; PW = placenta weight; GL = length of gestation; L = baby's length.

Table 3-2. Means, variances and coefficients of variation of quantitative traits for different genotypes of placental akaline phosphatase (all given to 3 significant figures). Numbers in brackets are the numbers of mothers with those genotypes.

	GENOTYPE									
Trait	*ff* (29)		*fs* (144)		*ss* (177)		*si* (48)		*fi* (13)	
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
ACFB	164	8900	157	2770	149	1760	163	3290	155	535
ACFM	102	1150	93.3	1060	89.2	753	102.8	2060	88.2	856
BFB	527	25600	470	29500	481	24000	474	22600	482	88900
LFM	398	70400	280	23000	314	26700	340	37700	369	72700
PLFM	557	43900	535	43800	503	40400	430	38100	346	6140
Birth Weight (kg)	3.52	0.293	3.47	0.234	3.52	0.253	3.53	0.205	3.36	0.311
Placenta Weight (kg)	0.580	.0118	0.548	.0104	0.547	.0130	0.545	0.49	0.555	.0117
Baby's Length (cm)	50.8	4.72	50.6	4.14	50.8	5.57	51.1	4.78	49.8	5.06
Gestation Duration	279	133	280	201	280	142	282	125	280	127
CV_9 (all 9 traits)	40.8		37.4		36.6		40.6		40.5	
CV_5 (first 5 traits)	42.8		39.7		38.9		43.4		44.6	
CV_4 (last 4 traits)	4.20		5.06		4.32		3.93		3.18	

The *ii* genotype has been omitted since it only contained 3 observations.

Table 3-3 Means, variances and multivariate coefficients of variation of quantitative traits for different genotypes of mother's haptoglobin (all given to 3 significant figures). Numbers in ( ) are the numbers of mothers with those genotypes.

	GENOTYPE					
Trait	Hp_{11}(46)		Hp_{12}(200)		Hp_{22}(158)	
	Mean	Variance	Mean	Variance	Mean	Variance
ACFB	156	2420	150	1850	157	2820
ACFM	85.9	901	94.7	1100	93.8	1180
BFB	502	32700	459	22700	490	28600
LFM	311	26000	309	27300	314	36700
PLFM	459	46100	511	43100	511	42100
Birth Weight (kg)	3.55	0.197	3.47	0.236	3.50	0.258
Placenta Weight (kg)	0.567	0.0178	0.546	0.0114	0.547	0.0101
Baby's Length (cm)	50.8	3.94	50.6	4.95	50.9	5.14
Gestation Duration	278	74.2	280	213	281	118
CV_9 (all 9 traits)	39.9		37.6		39.0	
CV_5 (first 5 traits)	42.4		40.0		41.4	
CV_4 (last 4 traits)	3.15		5.21		3.86	

Table 3-4. Variances, means and multivariate coefficients of variation of quantitative traits for different genotypes of mother's red cell acid phosphatase (3 significant figures). Numbers in ( ) are the numbers of mothers with these genotypes.

	GENOTYPE									
Trait	*AA*(35)		*AB* (144)		*BB* (155)		*CB* (43)		*CA* (26)	
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
ACFB	152	1210	154	2280	162	4290	140	926	149	1650
ACFM	81.3	774	93.7	890	96.6	1400	95.1	717	87.6	2050
BFB	474	37500	475	25400	495	32400	448	15400	463	26000
LFM	235	23300	311	31800	320	31900	332	29400	312	30000
PLFM	572	92000	504	33500	508	42100	471	43800	441	30700
Birth Weight (kg)	3.49	0.196	3.52	0.175	3.45	0.294	3.61	0.272	3.58	0.273
Placenta Weight (kg)	0.508	0.009	0.554	0.010	0.552	0.012	0.564	0.015	0.555	0.006
Baby's Length (cm)	50.6	3.01	50.7	4.29	50.7	5.40	51.4	5.30	50.5	5.6
Gestation Duration	282	173	281	162	279	173	281	84.0	277	137
CV_9 (all 9 traits)	45.9		35.3		39.2		37.5		38.0	
CV_5 (first 5 traits)	48.7		37.5		41.5		40.1		40.7	
CV_4 (last 4 traits)	4.66		4.55		4.74		3.18		4.34	

The genotype for this locus was not obtained for 7 mothers and the *CC* genotype has been omitted since it only contained 4 observations.

Table 3-5 Means variances, and multivariate coefficients of variation of quantitative traits for different genotypes of baby's red cell acid phosphatase (3 significant figures). The numbers in ( ) are the numbers of babies with these genotypes.

	GENOTYPE									
	AA (43)		*BA* (136)		*BB* (144)		*CB* (44)		*CA* (37)	
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
ACFB	153	1020	157	3490	161	3530	140	835	140	1310
ACFM	94.8	1240	96.7	1430	92.6	1140	91.0	631	86.7	519
BFB	495	24300	491	40800	485	21600	485	18100	405	19200
LFM	290	39800	308	29500	323	31000	334	34900	276	21400
PLFM	506	26600	501	53000	513	37600	488	66500	489	24300
Birth Weight (kg)	3.51	0.104	3.47	0.262	3.48	0.267	3.56	0.298	3.54	0.190
Placenta Weight (kg)	0.545	0.007	0.546	0.010	0.545	0.014	0.581	0.012	0.539	0.010
Baby's Length (cm)	50.6	2.15	50.5	5.31	50.8	5.10	51.2	5.73	50.9	3.87
Gestation Duration	280	85.3	279	171	281	199	281	97.0	279	129
CV_9 (all 9 traits)	36.5		41.4		36.4		41.1		33.8	
CV_5 (first 5 traits)	38.8		43.9		38.6		43.6		36.3	
CV_4 (last 4 traits)	3.32		4.75		4.99		3.57		4.07	

The genotype for this locus was not obtained for 6 babies and the *CC* genotype has been omitted since it only contained 4 observations.

Table 3-6 Extensions to Levene's test using $Y_a^{(k)} = \left[\sum_i (X_{ai}^{(k)} - X_{.i}^{(k)})^2\right]^{1/2}$ where $X_{ai}^{(k)}$ is the value of case a of trait i of genotype class k.
Where indicated, $X_{ai}^{(k)}$ has been log-transformed.

Means of genotypic classes						Variance ratio	Degrees of freedom
Placental alkaline phosphatase							
Genotype:	*ff*	*fs*	*ss*	*si*	*fi*		
Raw data	358.6	272.2	272.5	249.2	236.4	2.503*	4,373
Log data	0.922	0.917	0.875	0.813	0.865	0.54	4,368
Baby's red cell acid phosphatase							
Genotype:	*AA*	*BA*	*BB*	*CB*	*CA*		
Raw data	273.8	294.5	277.1	289.6	230.0	1.42	4,370
Log data	0.922	0.917	0.875	0.813	0.865	0.54	4,368
Mother's haptoglobin							
Genotype:	Hp_{11}	Hp_{12}	Hp_{22}				
Raw data	289.4	270.6	288.3			0.699	2,376
Log data	0.911	0.866	0.908			0.43	2,374
Mother's red cell acid phosphatase							
Genotype:	*AA*	*BA*	*BB*	*CB*	*CA*		
Raw data	318.1	265.0	294.2	258.8	241.5	1.913	4,370
Log data	1.18	0.863	0.863	0.792	0.887	4.86**	4,368

Values tabulated are means, $Y^{(k)}$, of genotypic classes and the variance ratios from one-way analysis of variance. * indicates significance at 5% level, ** significance at 1% level.

Table 3-7 Non-parametric dispersion matrices of biochemical measurements for placental alkaline phosphatase types, using the normal scores function for J.

	ACFM	PLFM	LFM	ACFB	BFB	ACFM	PLFM	LFM	ACFB	BFB
	Genotype *ff*					Genotype *fs*				
ACFM	0.79	0.14	0.74	0.34	–0.04	0.98	0.13	0.18	0.57	0.12
PLFM		0.91	0.39	0.02	–0.03		0.88	–0.03	0.15	–0.03
LFM			2.10	0.04	0.18			0.83	–0.01	0.18
ACFB				0.95	–0.10				1.10	0.30
BFB					0.85					1.07
	Genotype *ss*					Genotype *si*				
ACFM	0.89	0.27	0.21	0.35	0.09	1.52	0.26	0.30	0.60	0.15
PLFM		1.05	0.04	0.07	0.14		0.87	–0.04	0.24	0.10
LFM			0.91	0.04	0.09			0.88	0.18	0.22
ACFB				0.95	0.18				0.97	0.22
BFB					0.94					0.72
	Genotype *fi*					Pooled matrix				
ACFM	0.85	0.24	0.03	–0.08	0.22	0.99	0.21	0.25	0.44	0.11
PLFM		0.28	0.03	–0.13	0.01		0.99	0.02	0.10	0.06
LFM			1.46	0.32	1.05			0.99	0.04	0.00
ACFB				0.37	0.65				0.99	0.21
BFB					2.05					0.99

$\chi^2_{60} = 115.9 \quad P < 0.001$

Table 3-8 Non-parametric dispersion matrices of biochemical measurements for mother's haptoglobin types, using $J(u) = \sqrt{12}\,(u-\frac{1}{2})$

	ACFM	PLFM	LFM	ACFB	BFB	ACFM	PLFM	LFM	ACFB	BFB
	Genotype Hp_{11}					Genotype Hp_{12}				
ACFM	1.08	0.38	0.40	0.51	0.10	1.00	0.21	0.24	0.44	0.22
PLFM		0.93	0.18	0.16	0.28		1.06	0.05	0.12	0.08
LFM			0.91	0.08	0.16			0.98	0.07	0.17
ACFB				1.02	–0.03				0.95	0.20
BFB					0.99					0.97
	Genotype Hp_{22}					Pooled matrix				
ACFM	0.96	0.15	0.17	0.43	0.03	1.00	0.21	0.23	0.44	0.12
PLFM		0.93	0.00	0.03	–0.07		1.00	0.04	0.09	0.04
LFM			1.06	–0.05	0.21			1.00	0.02	0.00
ACFB				1.05	0.30				1.00	0.22
BFB					1.01					1.00

$\chi^2_{30} = 59.9 \quad P < 0.001$

Table 3-9 Non-parametric dispersion matrices of biochemical measurements for mother's red cell acid phosphatase types, using $J(u) = \sqrt{12}\,(u-\frac{1}{2})$.

	ACFM	PLFM	LFM	ACFB	BFB
	Genotype *AA*				
ACFM	0.91	0.04	0.27	0.43	–0.07
PLFM		1.06	–0.24	0.06	–0.106
LFM			1.08	0.09	0.09
ACFB				0.90	–0.04
BFB					1.32
	Genotype *BB*				
ACFM	1.02	0.21	0.22	0.55	0.24
PLFM		0.93	0.05	0.08	0.03
LFM			1.02	0.02	0.23
ACFB				1.12	0.33
BFB					1.00
	Genotype *CA*				
ACFM	0.86	0.01	–0.04	0.36	0.07
PLFM		1.09	0.41	–0.25	–0.05
LFM			1.07	–0.44	–0.19
ACFB				1.11	0.29
BFB					1.09

	ACFM	PLFM	LFM	ACFB	BFB
	Genotype *BA*				
ACFM	0.99	0.27	0.21	0.44	0.07
PLFM		0.98	0.03	0.15	0.10
LFM			0.95	0.05	0.16
ACFB				0.94	0.19
BFB					0.98
	Genotype *CB*				
ACFM	0.97	0.32	0.29	0.09	–0.05
PLFM		1.16	0.20	0.04	–0.02
LFM			0.85	0.24	0.35
ACFB				0.76	0.06
BFB					0.76
	Pooled matrix				
ACFM	1.00	0.21	0.23	0.44	0.12
PLFM		1.00	0.04	0.08	0.04
LFM			1.00	0.03	0.00
ACFB				1.00	0.22
BFB					1.00

$\chi^2_{60} = 105.9 \quad P < 0.001$

Table 3-10 Non-parametric dispersion matrices of biochemical measurements for baby's red cell acid phosphatase types, using $J(u) = \sqrt{12}\,(u-\frac{1}{2})$.

	ACFM	PLFM	LFM	ACFB	BFB
	Genotype *AA*				
ACFM	1.14	0.17	0.35	0.30	–0.07
PLFM		0.91	–0.01	0.13	0.05
LFM			1.08	–0.21	–0.01
ACFB				0.74	0.25
BFB					1.06
	Genotype *BB*				
ACFM	1.06	0.26	0.28	0.54	0.18
PLFM		0.96	0.07	0.10	–0.02
LFM			1.01	0.04	0.21
ACFB				1.21	0.26
BFB					0.84
	Genotype *CA*				
ACFM	0.81	0.09	0.10	0.15	–0.01
PLFM		0.98	0.14	0.29	0.07
LFM			0.99	–0.36	–0.02
ACFB				0.85	0.15
BFB					0.98

	ACFM	PLFM	LFM	ACFB	BFB
	Genotype *BA*				
ACFM	0.98	0.18	0.20	0.56	0.16
PLFM		1.05	–0.02	0.09	0.08
LFM			1.01	0.22	0.23
ACFB				0.96	0.28
BFB					1.18
	Genotype *CB*				
ACFM	0.91	0.26	0.17	0.22	0.03
PLFM		1.09	0.17	–0.14	0.17
LFM			0.86	–0.04	0.17
ACFB				0.73	–0.01
BFB					0.85
	Pooled matrix				
ACFM	1.00	0.20	0.23	0.45	0.12
PLFM		1.00	0.04	0.10	0.03
LFM			1.00	0.03	0.00
ACFB				1.00	0.23
BFB					1.00

$\chi^2_{60} = 103.2 \quad P < .001$

Table 3-11 Covariance matrices of anthropometric measurements for placental alkaline phosphatase types.

	BW	PW	L	BW	PW	L
	Genotype *ff*			Genotype *fs*		
BW	0.290			0.233		
PW	0.044	0.012		0.031	0.011	
L	0.114	0.016	0.050	0.079	0.009	0.040
	Genotype *ss*			Genotype *si*		
BW	0.261			0.183		
PW	0.033	0.012		0.027	0.010	
L	0.087	0.010	0.057	0.076	0.012	0.050
	Genotype *fi*			Pooled matrix		
BW	0.400			0.247		
PW	0.068	0.014		0.033	0.011	
L	0.120	0.022	0.049	0.085	0.010	0.049

$\rho = 0.9456 \quad f = 24$

$-2\rho \log \lambda_1^* = 49.06 \qquad (P < 0.01)$

If genotype *fi* is omitted from the analysis, f = 18 and

$-2\rho \log \lambda_1^* = 44.05$ $(P < 0.001)$

p,f and λ_1* are defined in section 1.4.

Table 3-12 Covariance matrices of anthropometric measurements for mother's haptoglobin types.

	BW	PW	L	BW	PW	L
	Genotype Hp_{11}			Genotype Hp_{12}		
BW	0.205			0.239		
PW	0.041	0.015		0.031	0.012	
L	0.075	0.013	0.040	0.081	0.009	0.050
	Genotype Hp_{22}			Pooled matrix		
BW	0.264			0.245		
PW	0.032	0.010		0.033	0.011	
L	0.092	0.012	0.050	0.085	0.010	0.049

$\rho = 0.9814 \quad f = 12.$

$-2\rho \log \lambda_1^* = 17.72 \qquad (P > 0.10)$

Table 3-13 Covariance matrices of anthropometric measurements for mother's red cell acid phosphatase types.

	BW	PW	L	BW	PW	L
	Genotype *AA*			Genotype *BA*		
BW	0.204			0.182		
PW	0.032	0.009		0.025	0.010	
L	0.060	0.008	0.030	0.058	0.007	0.044
	Genotype *BB*			Genotype *CB*		
BW	0.294			0.250		
PW	0.039	0.012		0.044	0.015	
L	0.106	0.013	0.053	0.087	0.015	0.054
	Genotype *CA*			Pooled matrix		
BW	0.273			0.241		
PW	0.012	0.005		0.032	0.011	
L	0.120	0.004	0.062	0.084	0.010	0.049

$\rho = 0.9680$ $f = 24$

$-2\rho \log \lambda_1^* = 45.15$ $(P < 0.01)$

Table 3-14 Covariance matrices of anthropometric measurements for baby's red cell acid phosphatase types.

	BW	PW	L	BW	PW	L
	Genotype *AA*			Genotype *BA*		
BW	0.109			0.266		
PW	0.010	0.007		0.031	0.010	
L	0.028	0.002	0.022	0.087	0.010	0.054
	Genotype *BB*			Genotype *CB*		
BW	0.257			0.305		
PW	0.040	0.014		0.036	0.012	
L	0.096	0.013	0.052	0.104	0.011	0.058
	Genotype *CA*			Pooled matrix		
BW	0.202			0.246		
PW	0.030	0.010		0.033	0.011	
L	0.070	0.010	0.040	0.085	0.010	0.049

$\rho = 0.9743$ $f = 24$

$-2\rho \log \lambda_1^* = 34.60$ $(P > 0.05)$

Table 3-15 Values for $Y_j = - 0.000615\ X_{HP} - 0.000804\ X_{RCAP} - 0.000998\ X_{ABO}$, log fitnesses for mothers, for each genotypic class. The frequency of each class and the number of heterozygous loci are also shown.

genotype		Y_j	frequency	no. heterozyous loci
Hp_{12} B(AC)	$I^A(I^Bi)$	0.0	5	3
Hp_{22} B(AC)	I^AI^B	- 0.0010025	2	2
Hp_{12} BB	I^AI^B	- 0.0013313	5	2
Hp_{12} B(AC)	(I^Bi)	- 0.0014025	44	2
Hp_{12} (AC)	I^AI^B	- 0.0020299	1	2
Hp_{22} BB	I^AI^B	- 0.0023338	3	1
Hp_{22} B(AC)	(I^Bi)	- 0.0024049	39	1
Hp_{12} BB	(I^Bi)	- 0.0027338	34	1
Hp_{11} BB	I^AI^B	- 0.0029251	2	1
Hp_{11} B(AC)	(I^Bi)	- 0.0029963	8	1
Hp_{22} (AC)	I^AI^B	- 0.0030324	1	1
Hp_{12} (AC)	(I^Bi)	- 0.0034324	16	1
Hp_{22} BB	(I^Bi)	- 0.0037362	29	0
Hp_{11} BB	(I^Bi)	- 0.0043276	10	0
Hp_{22} (AC)	(I^Bi)	- 0.0044348	14	0
Hp_{11} (AC)	(I^Bi)	- 0.0050262	3	0

Table 3-16 Spearman's rank correlation coefficients between mother's log fitness, Y_j, and each of nine quantitative traits.

trait	r_s	trait	r_s
ACFM	0.1514 *	birth weight	0.0078
PLFM	- 0.0697	placenta weight	0.0932
LFM	- 0.0417	gestation length	- 0.0694
ACFB	0.0205	baby's length	- 0.0038
BFB	-0.0759		

* significant at the 5% level.

Table 3-17 Values for Y_j = - 0.000667 X_{PL} - 0.000804 X_{RCAP} - 0.000998 X_{ABO}, log fitnesses for babies, for each genotypic class. Class frequencies and heterozygosity scores are also shown.

genotype			Y_j	frequency	heterozyosity
s(fi)	B(CA)	$I^A(I^Bi)$	0.0	4	3
ss	B(CA)	$I^A(I^Bi)$	- 0.0010193	5	2
s(fi)	BB	$I^A(I^Bi)$	- 0.0013313	4	2
s(fi)	B(CA)	(I^Bi)	- 0.0014025	35	2
(fi)	B(CA)	$I^A(I^Bi)$	- 0.0019267	3	2
ss	BB	$I^A(I^Bi)$	- 0.0023506	1	1
ss	B(CA)	(I^Bi)	- 0.0024218	34	1
s(fi)	BB	(I^Bi)	- 0.0027338	37	1
ss	(CA)	$I^A(I^Bi)$	- 0.0030492	2	1
(fi)	B(CA)	(I^Bi)	- 0.0033292	9	1
s(fi)	(CA)	(I^Bi)	- 0.0034324	11	1
(fi)	(CA)	$I^A(I^Bi)$	- 0.0039566	1	1
ss	BB	(I^Bi)	- 0.0037531	21	0
ss	(CA)	(I^Bi)	- 0.0044517	22	0
(fi)	BB	(I^Bi)	- 0.0046604	6	0
(fi)	(CA)	(I^Bi)	- 0.0053591	3	0

Table 3-18 Spearman's rank correlation coefficients between baby's log fitness, Y_j, and each of nine quantitative traits.

trait	r_s	trait	
ACFM	- 0.0120	Birth weight	- 0.0695
PLFM	- 0.0190	Placenta weight	- 0.0291
LFM	- 0.0539	gestation length	- 0.0442
ACFB	0.0087	baby's length	- 0.0764
BFB	0.1134		

Table 3-19 Frequencies of mother-baby pairs determined by genotype at the ACP, locus for mothers and babies.

	Baby's genotype.						
mother's genotype	*AA*	*BA*	*BB*	*CB*	*CA*	*CC*	total
AA	9	* 20	0	0	6	0	35
BA	* 28	* 53	* 43	9	11	0	144
BB	0	* 51	* 95	9	0	0	155
CB	0	7	6	* 19	9	2	43
CA	5	5	0	6	8	2	26
CC	0	0	0	1	3	0	4
total	42	136	144	44	37	4	407

* classes used in the analyses.

Table 3-20 Error covariance matrix of serum acid phosphatase (log transformed).

	a.		b.	
	mother	baby	mother	baby
mother	36.213	15.739	34.948	15.932
baby	15.739	27.629	15.932	26.900
	b = 15.739/36.213 = 0.435		b = 15.932/34.948 = 0.456	
	h^2 = 0.87 se (h^2) = 0.087		h^2 = 0.91 se (h^2) = 0.089	

a = 309 pairs including *(CB,CB);* b = 290 pairs excluding *(CB,CB)*

Table 3-21 Regression of acid phosphatase in baby's serum on acid phosphatase in mother's serum (log tranformed).

mother's red cell acid phosphatase genotype	*AA*	*BA*	*BA*	*BA*	*BB*	*BB*	*CB*
baby's red cell acid phosphatase genotype	*BA*	*AA*	*BA*	*BB*	*BA*	*BB*	*CB*
regression coefficient	0.363	0.206	0.53	0.395	0.634	0.484	-0.153
standard error	0.119	0.110	0.136	0.118	0.098	0.083	0.18
p.	<0.01	>0.05	<0.001	<0.01	<0.001	<0.001	>0.05

4. ANALYSES OF TWIN DATA

4.1 Description

Dr. N.G. Martin provided two sets of twin data for which various quantitative traits and the genotypes for several loci had been measured.

The first set has been described by Martin (1975a, 1975b) and Martin and Martin (1975). The subset used in this study comprised 45 same-sex twin pairs, of which 16 pairs were monozygous females, 10 were monozygous males, 12 were dizygous females and 7 were dizygous males. These were the twin pairs for which genotypes at six polymorphic loci were available as well as height and IQ measurements. The six loci were MN, Ss, haptoglobin, the C and E loci of the Rhesus system and Jk.

The necessary conditions suggested by Mitton and Grant (1984) for a successful study of heterozygote advantage were outlined in chapter 3. Do the variables measured in this data set meet these conditions?

The twins studied were all adolescents and so surplus energy would certainly have been used in the expression of both height and IQ for this age group. Constraints on these two traits would be minimal.

Smith (1975) states that the mean IQ of a population is arbitrarily set to 100 while the standard deviation is usually about 15 and so the coefficient of variation is reasonably low at 0.15. He also says that there are excesses of individuals in both tails of the IQ distribution and presents data on IQ measurements for various family groupings indicating the presence of genetic, environmental and genotype by environment interaction components in the variance of IQ. Thus IQ does not appear to be canalized.

Kark, Friedlander and Stein (1986) tabulated heights of 17-year-old boys in Jerusalem. Their data were divided into eight ethnic groups which had very low coefficients of variation of approximately 0.04. Smith (1975) gives a figure for the heritability of adult height as 0.85 indicating that this trait varies with genotype. It is well known that nutrition, an environmental factor, affects height. Thus, height is not canalized, either.

For non-human species, one would expect to find that traits with high heritabilities such as morphological characters would have low coefficients of variation. The reverse would be

true for life history characters and physiological and behavioural characters would fall somewhere between these (Mousseau and Roff, 1987). They conclude from their review that fitness components generally possess lower heritabilities than traits unconnected to fitness. However, it is often difficult to unravel the connection that a particular character has with fitness. For instance, in ectotherms fecundity and development time are highly correlated with body size, a morphological character. If such a character followed the usual pattern, it would have high heritability, low coefficient of variation and be a good indicator of fitness. However, this does not appear to be true of most characters with high heritability and low coefficient of variation. Perhaps Mitton's and Grant's (1984) condition of low coefficient of variation for investigating the relationship between fitness and heterozygosity is not appropriate.

The twin data were collected in Adelaide, South Australia, and all the participants had sat for a particular public examination in one of two years. Thus it is unlikely that a large range of environmental stresses was at work. However, the probability of any phenotypic stability among heterozygotes being attributable to local inbreeding followed by local outbreeding, as was thought possible for the mother-baby data, is small, since the Australian population has experienced considerable population mobility since 1788.

The objective was to see whether the variance of height or IQ was lower among heterozygotes than among homozygotes. Clearly, the data could not be treated as 90 individual sets of observations for several reasons. Monozygous twins shared all genes which might have influenced height and IQ. They had also shared common environments since they had been reared together and the effect of common environments was confounded with the effect of shared genotypes. Dizygous twins shared, on average, half their genes and they had also shared common environments.

A suitable way of analysing these data was to treat every twin pair as a pedigree of size 2 and perform a pedigree analysis. A method of calculating variance components by the scoring method in a pedigree analysis was described by Lange, Westlake and Spence (1976). Hopper and Mathews (1982) extended this method to include, as a component of variance, the additive effect of a marker locus.

4.2 Basic Model

In this section the basic model of Lange *et al.* (1976) will be outlined using pedigrees of size 2 consisting of either monozygous or dizygous twin pairs.

Suppose that some quantitative trait has been measured for each individual of the pedigree and suppose also that the mean of this trait may differ between males and females. It is assumed that the trait follows a multivariate normal distribution with separate male and female means, μ_M and μ_F respectively, and with a covariance structure:

$$\Omega = 2\sigma_a^2 \Phi + \sigma_d^2 \Delta + \sigma_e^2 \mathbf{I}$$

where Φ is a kinship matrix;

Δ is a matrix of Jacquard's condensed coefficient of identity, Δ_7 (Jacquard, 1974);

$\mathbf{I}$ is the identity matrix;

σ_a^2 is additive genetic variance;

σ_d^2 is dominance genetic variance;

and σ_e^2 is environmental variance peculiar to an individual.

To understand coefficients of kinship and identity, consider two individuals of known relationship, A and B, each with two genes at a given locus. The two individuals each receive one gene from their respective mothers and one from their respective fathers. Δ_7 is the probability that one gene of A is identical by descent to one gene of B and that the other gene of A is identical by descent to the other gene of B. On the other hand, the coefficient of kinship, ϕ_{AB} is the probability that a gene taken at random from A is identical with a gene at the same locus taken at random from B (Jacquard, *ibid*).

Some examples will make these concepts clearer. For instance $\phi_{AA} = \frac{1}{2}$ and $\phi_{AB} = 0$ if A and B are unrelated. If A and B are siblings, $\phi_{AB} = \frac{1}{4}$ and if A is the mother of B, $\phi_{AB} = \frac{1}{4}$. If A and B are siblings,

Δ_{AB} = prob (A and B have same gene from mother) x prob (A and B have same gene from father)

$= \frac{1}{2} \cdot \frac{1}{2} = \frac{1}{4}$.

Δ_{AA} = prob (A has the same genes as itself) = 1

If A is the mother of B, and B's father is unrelated to A, then

Δ_{AB} = p (B has one of her mother's genes) x p (B's father's gene is same as A's father'sgene)
= 1.0 = 0

For monozygous twins $\Phi = \begin{pmatrix} \frac{1}{2} & \frac{1}{2} \\ \frac{1}{2} & \frac{1}{2} \end{pmatrix}$ and $\Delta = \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix}$

while for dizygous twins $\Phi = \begin{pmatrix} \frac{1}{2} & \frac{1}{4} \\ \frac{1}{4} & \frac{1}{2} \end{pmatrix}$ and $\Delta = \begin{pmatrix} 1 & \frac{1}{4} \\ \frac{1}{4} & 1 \end{pmatrix}$

Let $\mathbf{X} = (X_1, X_2)'$ be a random variable of the quantitative trait for twin 1 and twin 2. Then

$$\text{cov}(X_1, X_2) = 2\phi_{12}\sigma_a^2 + \Delta_{12}\sigma_d^2$$

$$= \begin{cases} \sigma_a^2 + \sigma_d^2 \text{ for monozygous twins} \\ \frac{1}{2}\sigma_a^2 + \frac{1}{4}\sigma_d^2 \text{ for dizygous twins} \end{cases}$$

Hopper and Mathews (1982) extended this model by adding a term to the covariance structure,

viz. $\sigma_{ma}^2 \Psi_a$

$$\text{where } \psi_{aij} = \begin{cases} 1 \text{ when } i = j \\ 1 \text{ when i and j share both haplotypes} \\ \frac{1}{2} \text{when i and j share one haplotype} \\ 0 \text{ when i and j share no haplotype} \end{cases}$$

σ_{ma}^2 is the additive genetic effect of a polymorphic marker locus.

Thus for monozygous twins, $\Psi_a = \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix}$

while for dizygous twins Ψ_a may be $\begin{pmatrix} 1 & \frac{1}{2} \\ \frac{1}{2} & 1 \end{pmatrix}$ or $\begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix}$ or $\begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$

4.3 Extended model.

However, neither the basic model nor the marker locus extension can answer the question of whether heterozygotes at some locus are less variable than homozygotes at that locus. Thus a different extension to the basic model will now be proposed.

Suppose the locus in question has two alleles *Y* and *y*. Then if homeostasis is operating, the environmental variance peculiar to an individual will be influenced by whether or not the individual is homozygous or heterozygous at the locus. Thus environmental variance cannot be estimated by a single parameter, σ_e^2, for all individuals but instead will have to be broken into three parts, σ_{YY}^2, σ_{Yy}^2 and σ_{yy}^2. These represent the environmental variances for individuals with genotypes *YY*, *Yy* and *yy* respectively.

So the covariance structure now becomes:

$$\Omega = 2\sigma_a^2 \Phi + \sigma_d^2 \Delta + \sigma_{YY}^2 \mathbf{Q} + \sigma_{Yy}^2 \mathbf{R} + \sigma_{yy}^2 \mathbf{S}$$

where $q_{ii} = \begin{cases} 1 \text{ if twin i has genotype } YY \\ 0 \text{ if twin i has some other genotype} \end{cases}$

$q_{ij} = 0.$

The elements of R and S are defined similarly, substituting *Yy* and *yy* for *YY* respectively.

Thus Q, R and S can be $\begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}$ or $\begin{pmatrix} 0 & 0 \\ 0 & 0 \end{pmatrix}$ or $\begin{pmatrix} 1 & 0 \\ 0 & 0 \end{pmatrix}$ or $\begin{pmatrix} 0 & 0 \\ 0 & 1 \end{pmatrix}$.

A vector of seven parameters $\vartheta = \left(\mu_M, \mu_F, \sigma_a^2, \sigma_d^2, \sigma_{YY}^2, \sigma_{Yy}^2, \sigma_{yy}^2\right)'$ must be estimated for this model. This is done by maximizing the log likelihood, which is given by:

$$\log L = -\tfrac{1}{2} \sum_{i=1}^{k} \log |\Omega_i| - \tfrac{1}{2} \sum_{i=1}^{k} (X_i - \mu_i)' \Omega^{-1} (X_i - \mu_i) - \text{constant}$$

where μ_i is $\left(\mu_M, \mu_M\right)'$ or $\left(\mu_F, \mu_F\right)'$ depending on the sex of twin pair i;

Ω_i is covariance matrix for twin pair i

and k is the total number of twin pairs.

4.4 Fitting the extended model

Initially, the basic model was fitted to the heights of the 45 twin pairs by minimizing minus the log likelihood using a quasi-Newton minimization routine, ZXMIN, from the IMSL library (1984). The vector of parameters to be estimated was $\vartheta = \left(\mu_M, \mu_F, \sigma_a^2, \sigma_d^2, \sigma_e^2\right)'$ Since all the variance parameters had to be non-negative, a modified vector of unconstrained parameters, $\vartheta_M = \left(\mu_M,\mu_F,\sigma_a,\sigma_d,\sigma_e\right)'$ was used when fitting the model. The unconstrained estimates were squared to give non-negative variance estimates which were used in calculating Ω and the information matrix. The FORTRAN 77 program which I wrote to perform this model fitting is listed in Appendix C. The program was tested by performing hand calculations for a simple example at all stages of the program except the minimization routine itself.

The information matrix was calculated from the formula, $\mathcal{E}\left[\frac{-\partial^2 l}{\partial\vartheta_i\partial\vartheta_j}\right]_{\vartheta=\hat{\vartheta}}$ where $\mathcal{E}$ is the expectation operator, l is the loglikelihood and $\hat{\vartheta}$ is the vector of estimates. The covariance matrix of the estimates was found by inverting the information matrix.

As can be seen in table 4-1, the fitted value for dominance variance was very close to zero. This was not surprising given the small sample size. Martin, Eaves, Kearsey and Davies (1978) showed that for the extreme case of 90% heritability, complete dominance and no differences among family environments, 3330 twin pairs would be required to distinguish between the model with dominance variance and the model without in 95% of cases. This number increased tenfold when dominance was intermediate. Martin *et al. (ibid)* discussed other methods for determining dominance variance using smaller numbers of twins, but they were not appropriate to the model used here.

A reduced model was then fitted omitting the term $\sigma_d^2\,\Delta$ from the variance structure. As anticipated this made no difference to the other estimates, nor to the value of the log likelihood, but due to the very large negative covariance of σ_a^2 and σ_d^2 there was a very slight shift in the estimate of σ_a^2. The estimated variance of σ_a^2 is of course very much improved.

The extended model was then fitted, omitting the dominance variance, for each locus in

turn. The parameter vector was then $\vartheta_E = \left(\mu_M, \mu_F, \sigma^2_a, \sigma^2_{YY}, \sigma^2_{Yy}, \sigma^2_{yy}\right)'$ where, for instance, σ^2_{YY}, σ^2_{Yy} and σ^2_{yy} correspond to σ^2_{11}, σ^2_{12} and σ^2_{22} for the haptoglobin locus and to σ^2_{MM}, σ^2_{MN} and σ^2_{NN} for the MN locus. The results are presented in table 4-2. Minus twice the difference between the log likelihood for any particular extended model and the reduced model of table 4-1 represents the improvement of the extended model over the reduced model. Since there are two more parameters in the extended model, this quantity will be asymptotically distributed as chi-square with two degrees of freedom. A significant departure ($p < 0.05$) from this distribution would indicate that the three genotypic variances are not all equal. There was no significant departure for the first four loci of table 4-2. The genotypes of the Jk locus were not measured for two twin pairs and so the results for this locus are not directly comparable with the reduced model of table 4-1. The reduced model was fitted to the data, omitting these two twin pairs and a value of 169.9 was obtained for –l, thus giving 1.2 in the final column of table 4-2.

From these results we can say that the variance of height is not smaller among heterozygotes than among homozygotes for any of these loci. In fact, only the MN locus shows even a trend in this direction.

The analysis was repeated for IQ measurements, but this time a further reduction was afforded by pooling means for males and females. The results of fitting the basic model and two reduced models are shown in table 4-3. It can be seen that use of the pooled mean makes very little difference to the log likelihood. As before, the problems of small sample size surfaced, since IQ is believed to have a reasonable degree of dominance (Eaves, 1973, 1975).

Once again the reduced model, this time with pooled means, was extended to include parameters for each genotype of a major locus. The results for the six loci are presented in table 4-4. As before, the extended model did not offer any significant improvement over the reduced model. This time the variance of the heterozygotes was lower than both the variances for homozygotes at the MN and the haptoglobin loci, but not significantly so. Other loci showed contradictory trends. Note that minus the log likelihood for the reduced model, using the 43 twin pairs who had been typed at the Jk locus, was 238.3 and this value was used for

calculating minus twice the difference in log likelihood for the improvements afforded by the Jk locus model.

One school of thought suggests that instead of considering heterozygosity at individual loci, all measured loci should be pooled to give a total heterozygosity score (for discussion, see section 3.1). Since six loci had been typed, possible scores for heterozygosity ranged from 0 to 6. A possible model would be

$$\Omega = 2\sigma_a^2\Phi + \sigma_0^2 Q + \sigma_1^2 R + \sigma_2^2 S + \sigma_3^2 T + \sigma_4^2 U + \sigma_5^2 V + \sigma_6^2 W$$

where σ_i^2 represents the environmental variance for individuals with heterozygosity score i.

Such a model would mean that eight variance parameters would have to be estimated; this is too many given that the data set is not large. Instead, the data were grouped into three heterozygosity classes, L = {0,1}, M = {2,3}, H = {4,5,6}, and so the model became:

$$\Omega = 2\sigma_a^2\Phi + \sigma_L^2 Q + \sigma_M^2 R + \sigma_H^2 S$$

where L indicates low heterozygosity, M is medium and H is high.

Only 43 twin pairs were used for this model since the Jk genotypes had not been ascertained for two twin pairs. Thus the value for minus the log likelihood should be compared with 169.92 for height and 238.3 for IQ. Results are presented in table 4-5. These components did not contribute significantly to the overall variance. It is interesting to note that the variance for the highly heterozygous individuals was relatively high for height and relatively low for IQ.

Hopper and Matthews (1982) suggested testing the adequacy of the fit of the model by examining the quantities

$$F_i = (\mathbf{X}_i - \hat{\mu}_i)' \ \hat{\Omega}_1^{-1} (\mathbf{X}_i - \hat{\mu}_i)$$

for all twin pairs i = 1, ... k.

F_i should be distributed as χ^2 with two degrees of freedom when the pedigrees consist of twin pairs. These F_i can be further transformed to

$F_i^{(1)} = \sqrt{2F_i} - \sqrt{3}$ which has a standard normal distribution.

The $F_i^{(1)}$ can be plotted against normal scores. Any outliers from a straight line through the origin would indicate a discordant twin pair. Plots for the Ss locus model are shown in fig. 4-1. The fit is very good for the height data but less so for the IQ data, because the bottom left values deviate from the straight line. Twin pair 25 appears to be discordant for IQ and possibly twin pair 21 is discordant for height.

To examine the adequacy of fit for individuals, Hopper and Mathews (*ibid*) suggest calculating residuals, $r_i = X_i - \mu_i$ where X_i is either height or IQ for the ith individual and μ_i is the appropriate mean. Then for a pair of twins, $(r_1, r_2)'$ is the vector of residuals and if

$$\Omega^* = \Omega_{11} - \Omega_{21}^2 / \Omega_{22} \text{ and } \beta_{1.2} = \Omega_{21} / \Omega_{22},$$

$$\text{then } Q_1^* = (\hat{r_1} - \hat{\beta}_{1.2}\,\hat{r_2})^2 / \hat{\Omega}^* \text{ has a } \chi_1^2 \text{ distribution.}$$

Q_2^* for twin 2 in any given pair is calculated similarly and also follows a χ_1^2 distribution but the two distributions are dependent.

Raw data values, standardized residuals and Q_i^* values for discordant twins of a pair are shown in table 4-6. Twin pair 36 illustrates the effect of a discordant twin most clearly. Twin number 1 of this pair is at the lower extreme of the range of IQ values while twin number 2 is close to the average.

On the whole, the models seem to fit reasonably well, with few discordant values. The main problem in fitting the pedigree model is the small sample size. For instance, in the reduced model for height, the estimate of σ_a^2 is 41.1 with a standard deviation of 7.6 and the estimate of σ_e^2 is 1.70 with a standard deviation of 0.47. Hopper and Mathews (*ibid*) fit their model to 80 families consisting of a total of 617 individuals, a much larger sample. There will be further discussion of sample size with respect to heritability estimates in the next section.

The lack of relationship between variance of the two quantitative traits considered and heterozygosity might have been due to the small sample size, but there could have been other factors. Of the six loci considered, two pairs were closely linked (MNSs and the two Rhesus

loci) and such a small number would probably not reflect the heterozygosity of the whole genome (Chakraborty, 1987). The loci occurred on chromosomes 1, 2 4 and 16 (Shows, McAlpine and Miller, 1984) and so provided some opportunity for linkage disequilibrium with loci controlling height and IQ, but not enought since we do not know where the loci controlling height and IQ are situated. Similar arguments to those used in chapter 3 would also apply here.

4.5 Estimates of genetical parameters

The use of multivariate analysis of variance (MANOVA) to remove major effects and thus allow heritability to be estimated from the residual covariance matrix was discussed in section 3.4. In that section the method was used to make calculations of heritability from mother-baby pairs. In this section the same method will be used to estimate heritability from full sib pairs, in fact dizygous twin pairs.

The second data set, provided by Dr. N.G. Martin and described by Gibson, Martin, Oakeshott, Rowell and Clark (1983) where they report the results of a study of lung function in a sample of twins from Sydney, was used in this analysis.

The Pi genotype had been established for 36 pairs of dizygous females, 30 pairs of dizygous males and 33 pairs of opposite sex twins. Monozygous twins were not used in this analysis because the heritability estimate is formulated differently for them. The variable of interest was lung function, FEC, which was calculated from forced expiratory volume, FEV, corrected for age and height according to the formula described by Gibson *et al.* (ibid).

In order to perform a MANOVA, there must be more than one observation (twin pair) in any given class. Thus a number of twin pairs had to be omitted since they were the only ones of their particular genotypic combination. Frequencies for genotypic classes are shown in table 4-7. The error covariance matrices resulting from MANOVA for each set are shown in table 4-8. In each matrix, σ_{11} and σ_{22} were both estimates of V_P, the phenotypic variance. These two were used in the denominator to calculate the sib-sib correlation, t, where 2t is an upper limit for the heritability (Falconer, 1982).

Several worrying features emerge from these data. First, σ_{11} and σ_{22} are significantly different for the dizygous males; $0.9505 / 0.39 = 2.44$ ($F_{18,18} = 2.22$ at 5% level). It is

difficult to see why the variance of lung function would be different for two groups of male twins if the twins had been allocated randomly to position 1 or 2.

Second, the estimated upper limit to heritability for females is less than half the estimated limit for opposite sex pairs. Third, these values are substantially less than the estimates for heritability calculated by Gibson *et al* (ibid) and the standard errors are much greater. They give values of 0.56 ± 0.10 in males and 0.84 ± 0.04 in females, using a model which fits individual environmental variance for male and female and additive genetic variance for each sex. Admittedly, their results are based on the whole data set consisting of 203 twin pairs and so are likely to be more accurate.

The MANOVA method which worked so well for the mother-baby data, has failed here. There are several possible reasons for its failure. As can be seen from table 4-7, one genotypic class dominates all others in each set of twin pairs. Correlation coefficients were calculated for the largest class only and the values obtained were 0.484 for female pairs, 0.505 for male pairs and 0.582 for opposite pairs. Comparison of these values with those of table 4-7 reveals that male pairs were the only ones that were close. Thus the results cannot be explained so simply.

Lush (1949) defined heritability as the proportion of total phenotypic variance due to genetic causes. In the narrow sense this means additive genetic variation only, while in the broad sense it includes all genetic variation. However, it does assume that there is no genotype by environment interaction. Bishop, Mayo and Beckman (1987) comment on the low environmental effect on newborn serum acid phosphatase as a possible cause of high heritability. It also means that there was little room for genotype by environment interaction in the mother-baby data. One can see that there is much room for genotype by environment interaction when twins have reached adulthood.

Eaves (1982) discussed some of the difficulties of detecting genotype by environment interaction in twin studies. If monozygous and dizygous twin data were available for two different environments, within pair variance and phenotypic correlation would follow certain patterns in the presence of interaction. In an experiment it would be possible to allocate twin pairs to particular environments but when studying life data, not only can this not be done but there is also the possibility that genes and environments are not independent. Hewitt (1987)

discusses examples of this with particular reference to intelligence, but a parallel argument could be developed for lung function.

Another shortcoming of the data set is its small size. Elston and Bonney (1984) discussed the sample sizes required to estimate a heritability of 0.2 with a standard error of 0.1 and concluded that 390 sib pairs (or dizygous twin pairs) would be required. If the standard error were to be halved the sample size would have to be increased fourfold. On the other hand if the value of the heritability were larger, the required sample size for sib pairs would not decrease. Thus a sample size as small as 20 twin pairs is clearly inadequate.

Thus it is reasonable to assume that the causes of failure of the MANOVA method for the twin data set were small sample size and the presence of genotype by environment interaction.

Table 4-1 Maximum likelihood estimates and covariance matrices of parameters of the basic and reduced pedigree models for height data.

	μ_M	μ_F	σ^2_a	σ^2_d	σ^2_e	–l
Basic	175.5	163.4	41.2	0.0	1.70	178.4
Reduced	175.5	163.4	41.1	-	1.70	178.4

Covariance matrix of basic model

μ_M	2.17				
μ_F	0.0	1.31			
σ^2_a	0.0	0.0	1025		
σ^2_d	0.0	0.0	–891	821	
σ^2_e	0.0	0.0	0.33	–0.50	0.22

Covariance matrix of reduced model.

μ_M	2.17			
μ_F	0.0	1.31		
σ^2_a	0.0	0.0	57.3	
σ^2_e	0.0	0.0	–0.21	0.22

Table 4-2 Maximum likelihood estimates of parameters of the extended pedigree model for height data and six polymorphic loci. The final column represents the improvement of each extended model over the reduced model of table 3-11.

Locus	Genotypes			μ_M	μ_F	σ^2_a	σ^2_{YY}	σ^2_{Yy}	σ^2_{yy}	–l	–2Δl
haptoglobin	11	12	22	175.5	163.4	41.6	2.18	2.07	0.52	176.4	4.0
MN	*MM*	*MN*	*NN*	175.5	163.4	41.0	1.88	1.51	1.78	178.3	0.2
Rhesus C	*CC*	*Cc*	*cc*	175.5	163.4	41.2	2.19	2.53	0.93	176.9	3.0
Rhesus E	*ee*	*Ee*	*EE*	175.5	163.4	41.4	2.03	0.78	0.18	177.3	2.2
Jk	*aa*	*ab*	*bb*	175.5	163.1	42.2	1.76	2.22	1.06	169.3*	1.2
Ss	*SS*	*Ss*	*ss*	175.5	163.4	41.3	3.01	1.18	1.49	177.6	1.6

* Only 43 twin pairs were measured at this locus. See text for explanation.

Table 4-3 Maximum likelihood estimates of parameters of the basic and two reduced pedigree models for IQ data.

	μ_M	μ_F	σ^2_a	σ^2_d	σ^2_e	–l
Basic	111.7	109.0	97.4	0.0	27.0	248.1
Single mean	110.0		98.8	0.0	26.9	248.5
Reduced	110.0		99.0	-	27.0	248.5

Estimated Covariance Matrices

Basic model

μ_M	5.84				
μ_F	0.0	3.53			
σ^2_a	0.0	0.0	9590		
σ^2_d	0.0	0.0	–8850	8600	
σ^2_e	0.0	0.0	79.9	–122	56.0

Single mean model

μ	2.23			
σ^2_a	0.0	9840		
σ^2_d	0.0	–9060	8800	
σ^2_e	0.0	79.0	–121	55.0

Reduced single mean model.

μ	2.23		
σ^2_a	0.0	508.0	
σ^2_e	0.0	–45.0	54.0

Table 4-4 Maximum likelihood estimates of parameters of the extended pedigree model for IQ data and six polymorphic loci. The final column represents the improvement of each extended model over the reduced model of table 3-13.

Locus	Genotypes			μ	σ^2_a	σ^2_{YY}	σ^2_{Yy}	σ^2_{yy}	–l	–2Δl
Haptoglobin	11	12	22	110.0	95.7	25.8	18.8	44.8	247.5	2.0
MN	*MM*	*MN*	*NN*	110.0	109.4	23.9	16.4	35.4	248.0	1.0
Rhesus C	*CC*	*Cc*	*cc*	110.0	84.3	72.5	41.1	16.0	246.6	3.8
Rhesus E	*ee*	*Ee*	*EE*	110.0	99.2	26.1	29.6	0.02	248.3	0.4
Jk	*aa*	*ab*	*bb*	110.0	100.7	56.4	20.3	15.4	236.3*	4.0
Ss	*SS*	*Ss*	*ss*	110.1	85.7	19.5	60.3	21.0	247.0	3.0

* only 43 twin pairs were measured at this locus.

Table 4-5 Maximum likelihood estimates of parameters of the pedigree model extended to include heterozygosity scores.

IQ						
μ	σ_a^2	σ_L^2	σ_M^2	σ_H^2	$-l$	$-2\Delta l$
109.9	112.0	22.7	34.4	6.45	236.9	2.8
Height						
μ_M	μ_F	σ_a^2	σ_L^2	σ_M^2	σ_H^2	$-l$	$-2\Delta l$
175.5	163.1	42.1	1.66	1.56	2.30	169.8	0.24

Table 4-6 Observed values, standardized residuals and approximate χ_1^2 variables for three different twin pairs.

twin pair	twin	height	$\hat{r}_i / \hat{\sigma}$	Q_i^*
10	1	150.3	-1.89	5.95
	2	154.8	-1.19	3.71
twin pair	twin	IQ	$\hat{r}_i / \hat{\sigma}$	Q_i^*
32	1	130	1.85	4.6
	2	99	-0.97	3.05
36	1	80	-2.81	6.26
	2	106	-0.36	0.17

$\chi^2_{1,0.05} = 3.84$

Table 4-7 Frequencies of twin pairs in genotypic classes for the Pi locus.

a. Dizygous females

twin 2 \ twin 1 genotype	M_1M_1	M_1M_2	M_1S	
M_1M_1	16			
M_1M_2	2	4		
M_1S			4	
M_2S	2			
				28

b. Dizygous males

twin 2 \ twin 1 genotype	M_1M_1	M_1M_2	
M_1M_1	13	3	
M_1M_2	3	3	
			22

c. Opposite sex pairs

male \ female genotype	M_1M_1	M_1M_2	
M1M1	11	3	
M1M2	3	3	
			20

Table 4-8 Error covariance matrices of FEC after allowing for genotypic effects of the Pi locus.

a. Dizygous females

	sister 1	sister 2
sister 1	0.6713	0.0803
sister 2	0.0803	0.5895

$t = 0.0803 / \sqrt{(0.6713)(0.5895)} = 0.128$

$h^2 < 0.255$ se $(h^2) = 0.410$

b. Dizygous males

	brother 1	brother 2
brother 1	0.39	0.2937
brother 2	0.2937	0.9505

$t = 0.2937 / \sqrt{(0.39)(0.9505)} = 0.482$

$h^2 < 0.964$ se $(h^2) = 0.362$

c. Opposite sex pairs

	sister	brother
sister	0.6990	0.1849
brother	0.1849	0.5744

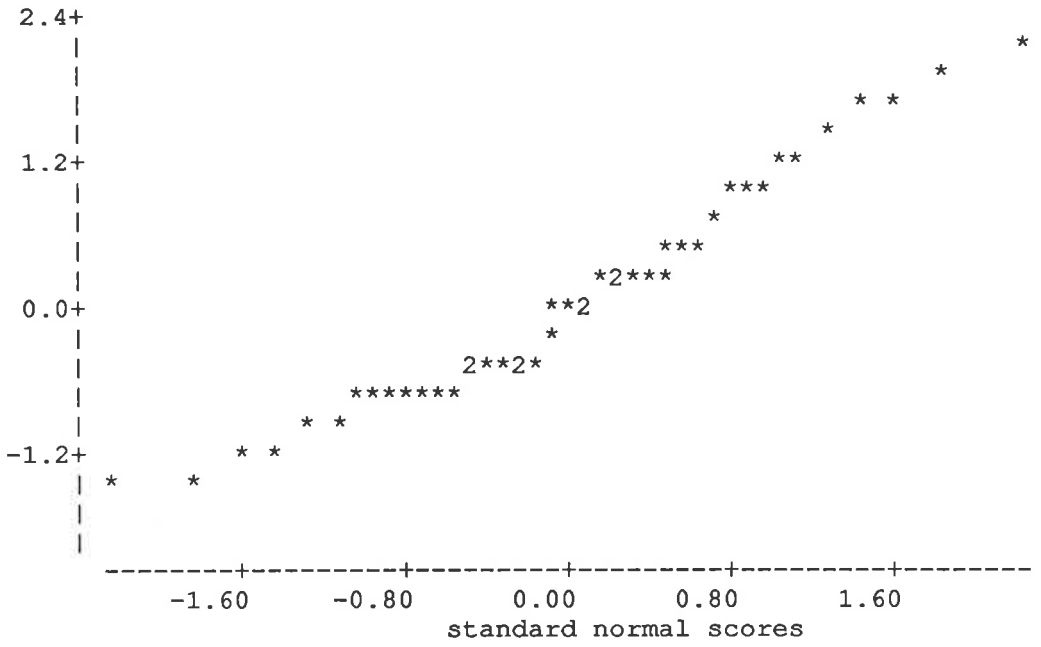
$t = 0.1849 / \sqrt{(0.699)(0.5744)} = 0.292$

$h^2 < 0.584$ se $(h^2) = 0.472$

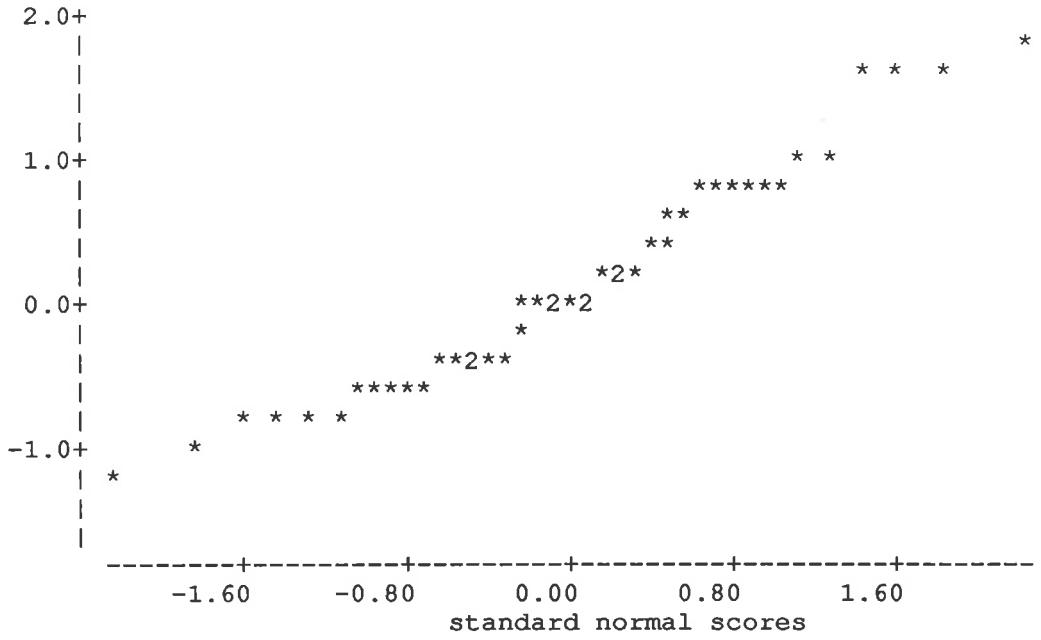
N.B. $\sigma^2_{h\,2}$ is calculated from the formula $4(1+t)^2(1-t)^2$ / d.f.

Figure 4-1 Plots of $F_i^{(1)}$ against normal scores for the Ss locus.

a. Height data



b. IQ data



5. CONCLUSIONS

The question of heterozygote advantage in humans has been tackled in this thesis by considering a number of biochemical polymorphisms, a variety of quantitative traits, several statistical mehods for analysing data and some mathematical models to describe the phenomenon. Several interesting results have emerged to shed some light on the conflicting results previously reported in the literature.

Prior to the research done for this thesis, there was theoretical reasoning to support the view that if a number of loci were having a direct effect on a quantitative trait, then adding their effects together would result in a decreasing variance with increasing heterozygosity, without there being any heterozygote advantage *per se* (Chakraborty and Ryman, 1983). Further theoretical reasoning stated that the trend in variance would depend on the frequencies of alleles at each locus (Mani, 1988). Both of these results depended on assumptions of additivity of allelic effects and also of locus effects.

It has been shown in this thesis that, when there are only two possible alleles per locus, it is irrelevant whether the interactions between loci are multiplicative or additive or whether the interactions between alleles are additive or dominant/recessive. It has also been shown that it does not matter what the allelic frequencies are. Whatever the situation, the phenotypic variance of a quantitative trait which is directly affected by the loci will decrease with increasing heterozygosity.

It has also been shown using simulations that when there are more than two alleles at each locus, the trends in phenotypic variance cannot be neatly categorised according as allelic frequencies are all reasonably similar or one is much smaller then the others. In fact, different trends in variance may be obtained by allowing the least frequent allele to be recessive, dominant with a small effect or dominant with a large effect.

If we choose, for investigation, loci which are known to have an effect on the quantitative trait, then, if they are two-allele loci, we will get decreasing variance with increasing heterozygosity, regardless of whether the heterozygotes afford any advantage. On the other hand, if each locus

has more than two alleles, decreasing variance with increasing heterozygosity is more likely to indicate heterozygote advantage. However, we cannot expect heterozygosity at each locus to have the same effect on the variance and so we can expect fluctuations in this downward trend. Environmental variance will also cause fluctuations.

One way of dealing with the different locus contributions to variance is to use the adaptive distances model (Smouse, 1986). In its current state this model has the shortcoming of being able to deal with only two alleles per locus. However, an extended model is being developed (Smouse, personal communication). The adaptive distances model's other major shortcoming is that it requires measures of fitness for each genotype. More accurate estimation of fitness than has been used in this thesis may be possible by extending the analyses of Hed (1986). However, the fitness for the different genotypes of a locus will always be relative and cause some problems in the use of this model. One possible way to minimise the problem is to obtain estimates from a different data set to the one being used to fit the model.

The pedigree model is a very powerful tool for fitting variance components but unfortunately no results of any consequence were obtained using this model because of the small numbers of twin pairs available for analysis.The number of mother-baby pairs was large but these data did not lend themselves to a pedigree model. Since only a small number of loci were available for the latter set, the data were analysed by considering each locus individually and grouping the quantitative traits into anthropometric and biochemical. Dispersion matrices for the different genotypes were compared but the results were inconclusive.

Taken as a whole, the results seem to indicate that the degree of heterozygote advantage differs among loci. In particular, there does seem to be some effect of heterozygosity at the placental alkaline phosphatase locus on the variance of newborns' anthropometric traits. The more loci we examine for a wide variety of quantitative traits the more we are likely to see trends emerging.

```
      IF(R.LT.CRIT(1)) THEN
           ILOC(L,J)=1
           GO TO 30
      ENDIF
      DO 35 K=2,NAL
       IF(R.GE.CRIT(K-1).AND.R.LT.CRIT(K))THEN
           ILOC(L,J)=K
           GO TO 30
      ENDIF
35     CONTINUE
30     CONTINUE
C***** If the two allelesat the locus are different, increment heterozygosity
      IF(ILOC (L,1).NE.ILOC (L,2))IHET(I)=IHET(I)+1
25     CONTINUE
C*****  Invoke appropriate model depending on whether allelic effects(ALEF)
C      are additive or dominant and whether locus effects(GEF) are additive or
C      multiplicative.
       IF(ALEF.EQ.'a'.AND.GEF.EQ.'m')THEN
       CALL ADDMUL(I,EF(I))
       ELSE IF(ALEF.EQ.'a'.AND.GEF.EQ.'a')THEN
           CALL ADDADD(I,EF(I))
          ELSE IF(GEF.EQ.'m')THEN
              CALL DOMMUL(I,EF(I))
            ELSE
              CALL DOMADD(I,EF(I))
      ENDIF
20     CONTINUE
C*****  Calculate numbers in each heterozygosity class.Keep counts in NHET.
      DO 44 J=1,NJ
      NHET(J)=0
      XM(J)=0
      SS(J)=0
44    CONTINUE
      DO 45 I=1,INDIV
      J=IHET(I)+1
      NHET(J)=NHET(J)+1
      XM(J)=XM(J)+EF(I)
      SS(J)=SS(J)+EF(I)**2
45     CONTINUE
C*****Calculate mean and variance of each heterozygosity class
      DO 50 J=1,NJ
C*****  First increment grand mean classes
      XBAR(J)=XBAR(J)+XM(J)
      NHT(J)=NHT(J)+NHET(J)
      RN=REAL(NHET(J))
       IF(RN.GT.0)DF(J)=DF(J)+RN-1.0
       IF(RN.LE.1.0)THEN
         VAR(J)=0
         GO TO 50
      ENDIF
      XM(J)=XM(J)/RN
       VAR(J)=(SS(J)-RN*XM(J)**2)/(RN-1.0)
C*****  Increment grand variance classes
      VBAR(J)=VBAR(J)+(RN-1.0)*VAR(J)
50     CONTINUE
```

```
19    CONTINUE
C***** Calculate pooled variance and grand mean for each class.
      DO 60 J=1,NJ
      RNJ=REAL(NHT(J))
      IF(DF(J).LE.0.0)THEN
        VBAR(J)=0
        XBAR(J)=XBAR(J)
      ELSE
        XBAR(J)=XBAR(J)/RNJ
        VBAR(J)=VBAR(J)/DF(J)
      ENDIF
60    CONTINUE
      WRITE(2,65)
      WRITE(2,55)(XBAR(J),VBAR(J),DF(J),NHT(J),J=1,NJ)
5     FORMAT(4I10,2A1/2F10.0,I5/(8F10.0))
6     FORMAT(5X,'allelic effects ',A1,'  genotypic effects ',A1/
     +5X,'allelic freqs',10F6.3)
16    FORMAT(8E10.3)
21    FORMAT(5X,'RUN',I5)
31    FORMAT(E12.4,2I5)
40    FORMAT(5(I5,E12.5))
55    FORMAT(5X,3E12.5,I10)
65    FORMAT(5X,'Averages over all runs')
      END

      SUBROUTINE ADDMUL(I,F)
      COMMON ILOC (10,2),RLL,RUL,NAL,NLOCI,CRIT(10)
C***** Calculate the genotypic effect for individual i when allelic effects are
C     additive and locus effects are multiplicative
      F=1.0
C***** Use the range of possible values of the trait to determine base value(X)
C     and increment(S) for each gene.
      ZNL=1.0/REAL(NLOCI)
      X=RLL**ZNL
      Y=RUL**ZNL
      S=0.5*(Y-X)
C***** For individual,I, calculate the genotypic effect.
      AL=REAL(NAL)
      DO 10 L=1,NLOCI
      GEN=S*(ILOC(L,1)+ILOC(L,2)-2.0)/(AL-1.0)+X
      F=F*GEN
10    CONTINUE
1     FORMAT(3E12.5)
      RETURN
      END

      SUBROUTINE DOMMUL(I,F)
      COMMON ILOC (10,2),RLL,RUL,NAL,NLOCI,CRIT(10)
C***** Alleles exhibit dominance and locus effects are multiplicative.
C     If there are more than two alleles, the first one is recessive and
C     the others are codominant and additive.
      F=1.0
      ZNL=1.0/REAL(NLOCI)
      X=RLL**ZNL
      Y=RUL**ZNL
```

```
      AL=REAL(NAL)
      S=(Y-X)*(AL-1.0)/(2.0*AL-3.0)
C***** For individual,I, calculate the genotypic effect.
      DO 10 L=1,NLOCI
      C=(ILOC(L,1)+ILOC(L,2)-2.0)/(AL-1.0)
      IF(ILOC(L,1).EQ.ILOC(L,2))C=0.5*C
      GEN=C*S+X
      F=F*GEN
10    CONTINUE
1     FORMAT(4E12.5)
      RETURN
      END

      SUBROUTINE ADDADD(I,F)
      COMMON ILOC (10,2),RLL,RUL,NAL,NLOCI,CRIT(10)
C***** This subroutine calculates the genotypic effect if the alleles are
C      additive and the locus effects are also additive.
      S=(RUL-RLL)/(2.0*REAL(NLOCI))
      X=RLL
      F=X
      AL=REAL(NAL)
      DO 10 L=1,NLOCI
      F=F+S*(ILOC(L,1)+ILOC(L,2)-2.0)/(AL-1.0)
10    CONTINUE
1     FORMAT(3E12.5)
      RETURN
      END

      SUBROUTINE DOMADD(I,F)
      COMMON ILOC (10,2),RLL,RUL,NAL,NLOCI,CRIT(10)
C***** Alleles exhibit dominance and locus effects are additive
C      If there are more than two alleles the first is recessive and
C      the others are codominant and additive.
      X=RLL
      AL=REAL(NAL)
      S=(RUL-RLL)/REAL(NLOCI)*(AL-1.0)/(2.0*AL-3.0)
      F=X
      DO 10 L=1,NLOCI
      C=(ILOC (L,1)+ILOC(L,2)-2.0)/(AL-1.0)
      IF(ILOC(L,1).EQ.ILOC(L,2))C=0.5*C
      F=F+C*S
10    CONTINUE
1     FORMAT(3E12.5)
      RETURN
      END
```

APPENDIX B - MINITAB program

```
NOECHO
NOTE *** Puri and Sen's test for equality of dispersion matrices.
NOTE *** Variables ACFM PLFM LFM ACFB BFB
NOTE *** J=sqrt(12)*(u-0.5) transforms ranks to scores,E.
NOTE *** Populations - mother's haptoglobin types (3 populations)
READ 'HAPT.DAT' C2 C3 C4 C5 C6 C1
NOTE *** C1 specifies the population for each set of variables
NOTE *** K1 is number of observations
NOTE *** K2 is number of variables
COUNT C1 K1
LET K2=5
NOTE *** Form ranks for each variable
RANK C2 C12
RANK C3 C13
RANK C4 C14
RANK C5 C15
RANK C6 C16
NAME  C12='R1',  C13='R2',C14='R3',C15='R4',C16='R5'
NOTE *** Raw data not required again. Erase and reuse C2-C6.
ERASE C2-C6
NOTE *** Form E values by transforming ranks
LET K3=SQRT(12)
LET  C2=K3*('R1'/(K1+1)-0.5)
LET  C3=K3*('R2'/(K1+1)-0.5)
LET  C4=K3*('R3'/(K1+1)-0.5)
LET  C5=K3*('R4'/(K1+1)-0.5)
LET  C6=K3*('R5'/(K1+1)-0.5)
NAME  C2='E1',C3='E2',C4='E3',C5='E4',C6='E5'
MEAN 'E1' K11
MEAN 'E2' K12
MEAN 'E3' K13
MEAN 'E4' K14
MEAN 'E5' K15
NOTE *** Find sums of squares of E vectors
SSQ 'E1' K16
SSQ 'E2' K17
SSQ 'E3' K18
SSQ 'E4' K19
SSQ 'E5' K20
NOTE *** Find products of E vectors
LET  C21='E1'*'E2'
LET  C22='E1'*'E3'
LET  C23='E1'*'E4'
LET  C24='E1'*'E5'
LET  C26='E2'*'E3'
LET  C27='E2'*'E4'
LET  C28='E2'*'E5'
LET  C30='E3'*'E4'
LET  C31='E3'*'E5'
LET  C33='E4'*'E5'
NOTE *** Find sums of products of E vectors
SUM C21 K21
SUM C22 K22
```

```
SUM C23 K23
SUM C24 K24
SUM C26 K25
SUM C27 K26
SUM C28 K27
SUM C30 K28
SUM C31 K29
SUM C33 K30
NOTE *** Calculate values for pooled S vector, S*.
LET K3=K1-1
LET K16=(K16-K1*K11*K11)/K3
LET K17=(K17-K1*K12*K12)/K3
LET K18=(K18-K1*K13*K13)/K3
LET K19=(K19-K1*K14*K14)/K3
LET K20=(K20-K1*K15*K15)/K3
LET K21=(K21-K1*K11*K12)/K3
LET K22=(K22-K1*K11*K13)/K3
LET K23=(K23-K1*K11*K14)/K3
LET K24=(K24-K1*K11*K15)/K3
LET K25=(K25-K1*K12*K13)/K3
LET K26=(K26-K1*K12*K14)/K3
LET K27=(K27-K1*K12*K15)/K3
LET K28=(K28-K1*K13*K14)/K3
LET K29=(K19-K1*K13*K15)/K3
LET K30=(K30-K1*K14*K15)/K3
STACK K16 K21-K24 K17 K25-K27 K18 K28 K29 K19 K30 K20 C17
NAME C17='S*'
NOTE *** K values and rank vectors not required again so erase.
ERASE K11-K30 C12-C16
NOTE *** Calculate squares of E vectors
LET C20='E1'**2
LET C25='E2'**2
LET C29='E3'**2
LET C32='E4'**2
LET C34='E5'**2
LET K6=20   #no. of column of first E**2 product
LET K5=1    # index for column K4
LET K3=20   # no. of column of second E**2 product
LET K4=35   # no. of first column where E**4 products are stored
EXECUTE 'OUTER' 15  # execute p(p+1)/2 times - p is no. variates
LET C35=C35/K1
LET C36=C36/K1
LET C37=C37/K1
LET C38=C38/K1
LET C39=C39/K1
LET C40=C40/K1
LET C41=C41/K1
LET C42=C42/K1
LET C43=C43/K1
LET C44=C44/K1
LET C45=C45/K1
LET C46=C46/K1
LET C47=C47/K1
LET C48=C48/K1
LET C49=C49/K1
```

```
NOTE *** Products of E vectors no longer required; so erase.
ERASE C20-C34
NOTE *** C35-C49 now contain the sums of products
NOTE *** Put into matrix 1 and then erase.
COPY C35-C49 M1
ERASE C35-C49
NOTE
NOTE *** Using the E values corresponding to each population in
NOTE    turn construct an S vector for each.
NOTE    Store S vectors in C7-C9
NOTE
COPY 'E1' 'E2' 'E3' 'E4' 'E5' C12-C16;
USE C1=0.
LET K4=1 #K4 is number of first population
LET K5=7 # K5 is column for first S vector
EXECUTE 'SVECTOR'
COPY 'E1' 'E2' 'E3' 'E4' 'E5' C12-C16;
USE C1=1.
EXECUTE 'SVECTOR'
COPY 'E1' 'E2' 'E3' 'E4' 'E5' C12-C16;
USE C1=2.
EXECUTE 'SVECTOR'
ERASE K5 K4
NAME C7='S0',C8='S1',C9='S2' #S vector for each population
PRINT 'S0' 'S1' 'S2' 'S*'
NOTE
NOTE *** Complete calculations for variance matrix and store in M4
NOTE
TRANSPOSE 'S*' M2
MULTIPLY 'S*' M2 M3
SUBTRACT M3 M1 M4
INVERT M4 M5
PRINT M4 M5
LET K3=0
LET K5=7      # column no. for S vector of first population
LET K4=1
NOTE *** Calculate chi-square in K3
EXECUTE 'PURILN' 3  # execute once for each population
PRINT K3
END

        macro OUTER

EXECUTE 'MULT' 15        # execute p(p+1)/2 times: p is number variates
LET K4=K4+1              #column no. for storing sums of products
LET K3=K3+1              #column for second E vector
LET K5=1            #reset pointer to position 1 of column K4
LET K6=20           # column for first E product
END

        macro MULT

LET C18=CK6*CK3          #multiply E vectors
SUM C18 K7
LET CK4(K5)=K7    #store sum of products at position K5 of col K4
```

```
LET K6=K6+1
LET K5=K5+1
END

        macro SVECTOR

NOTE *** Find sums and sums of squares of E values for a particular sample
SUM C12 K11
SUM C13 K12
SUM C14 K13
SSQ C12 K16
SSQ C13 K17
SSQ C14 K18
NOTE *** Find products of E values
LET C21=C12*C13
LET C22=C12*C14
LET C23=C12*C15
LET C24=C12*C16
LET C25=C13*C14
LET C26=C13*C15
LET C27=C13*C16
LET C28=C14*C15
LET C29=C14*C16
LET C30=C15*C16
NOTE *** Find sums of products of E values
SUM C21 K21
SUM C22 K22
SUM C23 K23
SUM C24 K24
SUM C25 K25
SUM C26 K26
SUM C27 K27
SUM C28 K28
SUM C29 K29
SUM C30 K30
COUNT C13 K10
LET C19(K4)=K10
NOTE *** Calculate elements of S vector
LET K16=(K16-K11*K11/K10)/(K10-1)
LET K17=(K17-K12*K12/K10)/(K10-1)
LET K18=(K18-K13*K13/K10)/(K10-1)
LET K19=(K19-K14*K14/K10)/(K10-1)
LET K20=(K20-K15*K15/K10)/(K10-1)
LET K21=(K21-K11*K12/K10)/(K10-1)
LET K22=(K22-K11*K13/K10)/(K10-1)
LET K23=(K23-K11*K14/K10)/(K10-1)
LET K24=(K24-K11*K15/K10)/(K10-1)
LET K25=(K25-K12*K13/K10)/(K10-1)
LET K26=(K26-K12*K14/K10)/(K10-1)
LET K27=(K27-K12*K15/K10)/(K10-1)
LET K28=(K28-K13*K14/K10)/(K10-1)
LET K29=(K29-K13*K15/K10)/(K10-1)
LET K30=(K30-K14*K15/K10)/(K10-1)
NOTE *** Stack elements into column K5
STACK K16,K21-K24,K17,K25-K27,K18,K28,K29,K19,K30,K20 CK5
```

```
LET K5=K5+1
LET K4=K4+1
ERASE C12-C16 K10-K30 C21-C30          #erase to make room for next vector
END

        macro PURILN

SUBTRACT 'S*' CK5 M6
TRANSPOSE CK5 M7
SUBTRACT M2 FROM M7 M8
MULTIPLY M8 M5 M9
MULTIPLY M9 M6 K6
LET K7=C19(K4)
LET K3=K3+K6*K7          #K3 contains test statistic
LET K5=K5+1
LET K4=K4+1
ERASE M6-M9 K6
END
```

APPENDIX C - Pedigree analysis program

```
      PROGRAM ATS
C***** This program performs a pedigree analysis for twin data. The
C     variance matrix includes components for additive variance and
C     dominance variance. The error variance is divided into 3 components,
C     depending on an individual's genotype at a major locus.
C***** Input Variables ************************************************
C     FY          family number
C     SEX          sex of twins(all twins are same sex)
C     ZYG          twins are MZ or DZ
C     HT          height for each twin
C     GE          genotype at major locus for each twin
C**********************************************************************
      COMMON PHI(45,3),DEL(45,3),Q(45,3),R(45,3),S(45,3),HT(45,2),SEX(
     +45),NF,HMF,HMM,X2(7),OMI(45,3),SSP(45),U(45,2),OM(45,3)
      DIMENSION FY(45),ZYG(45),GE(45,2),X(7),G(7),W(21),H(28),O1(3)
      DIMENSION P1(3),Q1(3),R1(3),S1(3),FNI(21),FI(21),WK(40),COV(21)
      DIMENSION OP(2,2),OQ(2,2),OR(2,2),OS(2,2),QS(45),RES(45,2)
      DIMENSION OMS(2),B(2),QA(45,2)
      EXTERNAL FUNCT
      OPEN(UNIT=1,FILE='MATS.DAT',STATUS='OLD')
      NF=45
C****  NF is number of families
      READ(1,10)(FY(I),SEX(I),ZYG(I),(HT(I,J),GE(I,J),J=1,2),I=1,NF)
      HMF=0
      HMM=0
      NFS=0
      NMS=0
      NCC=0
      NCL=0
      NLL=0
      DO 15 I=1,NF
C***** Find cumulative sum of heights for each sex; also number of each sex.
      IF(SEX(I).EQ.0)THEN
        HMF=HMF+HT(I,1)+HT(I,2)
        NFS=NFS+2
      ELSE
        HMM=HMM+HT(I,1)+HT(I,2)
        NMS=NMS+2
      ENDIF
C***** PHI,DEL,Q,R and S are symmetric 2*2 matrices containing kinship
C     coefficients, coeffs of identity and indicators of genotype for a
C     major locus.
      PHI(I,1)=0.5
      PHI(I,3)=0.5
      DEL(I,1)=1.0
      DEL(I,3)=1.0
      Q(I,2)=0
      R(I,2)=0
      S(I,2)=0
C***** Define first element of Q,R,S according to twin 1's genotype.
      IF(GE(I,1).EQ.'11')THEN
        Q(I,1)=1.0
        R(I,1)=0
```

```
        S(I,1)=0
        NCC=NCC+1
      ELSE IF(GE(I,1).EQ.'12')THEN
         Q(I,1)=0
         R(I,1)=1.0
         S(I,1)=0
         NCL=NCL+1
        ELSE
         Q(I,1)=0
         R(I,1)=0
         S(I,1)=1.0
         NLL=NLL+1
      ENDIF
C***** If twins are MZ third element =first element for Q,R,S  since
C      genotypes are the same.
      IF(ZYG(I).EQ.1)THEN
        PHI(I,2)=0.5
        DEL(I,2)=1.0
        Q(I,3)=Q(I,1)
        R(I,3)=R(I,1)
        S(I,3)=S(I,1)
      ENDIF
C***** If twins are dizygous, second twin's genotype may be different.
      IF(ZYG(I).EQ.2)THEN
        PHI(I,2)=0.25
        DEL(I,2)=0.25
         IF(GE(I,2).EQ.'11')THEN
          Q(I,3)=1.0
          R(I,3)=0
          S(I,3)=0
         ELSE IF(GE(I,2).EQ.'12')THEN
            Q(I,3)=0
            R(I,3)=1.0
            S(I,3)=0
         ELSE
            Q(I,3)=0
            R(I,3)=0
            S(I,3)=1.0
        ENDIF
      ENDIF
15     CONTINUE
C***** NCC,NCL,NLL are numbers of first twins with each of the three
C      genotypes.
      WRITE(2,53)NCC,NCL,NLL
C***** HMF and HMM are means for females and males respectively.
      HMF=HMF/NFS
      HMM=HMM/NMS
      MAXFN=500
      IOPT=2
      NSIG=2
      N=6
C***** Initial estimates of parameters
      X(1)=HMM
      X(2)=HMF
      X(3)=6.0
```

```
      X(4)=1.5
      X(5)=1.5
      X(6)=0.8
C***** Call the IMSL subroutine for minimizing a function.
       CALL ZXMIN(FUNCT,N,NSIG,MAXFN,IOPT,X,H,G,F,W,IER)
      WRITE(2,30)(X(K),K=1,N)
      WRITE(2,30)(X2(K),K=3,N)
      WRITE(2,31)(G(K),K=1,N)
      WRITE(2,32) F
      WRITE(2,33)IER
      WRITE(2,34)(W(K),K=1,3)
      CALL MATPR(H,N)
C***** Obtain covariance matrix by inverting information matrix calculated
C     at the maximum likelihood estimates.
      CALL FUNCT(N,X,F)
      NN=N*(N+1)/2
      DO 49 J=1,NN
      FI(J)=0
49    CONTINUE
C***** Calculate symmetric information matrix for each family
      DO 50 I=1,NF
      IF(SEX(I).EQ.0) THEN
        FNI(3)=OMI(I,1)+OMI(I,2)*2+OMI(I,3)
        FNI(1)=0
      ELSE
        FNI(3)=0
        FNI(1)=OMI(I,1)+2*OMI(I,2)+OMI(I,3)
      ENDIF
C***** In this data set sexes are always the same
      FNI(2)=0
C***** Expected 2nd derivative between mean and variance terms is always 0.
      FNI(4)=0
      FNI(5)=0
      FNI(7)=0
      FNI(8)=0
      FNI(11)=0
      FNI(12)=0
      FNI(16)=0
      FNI(17)=0
C***** Convert OMI,PHI,Q,R,S to 1-dimensional arrays for use in MULT
      DO 51 J=1,3
      O1(J)=OMI(I,J)
       P1(J)=PHI(I,J)
      Q1(J)=Q(I,J)
      R1(J)=R(I,J)
       S1(J)=S(I,J)
51    CONTINUE
C**** Calculate 2nd derivatives between variance terms
      CALL MULT(O1,P1,OP)
      DO 58 K=1,2
      DO 58 J=1,2
       OP(K,J)=2*OP(K,J)
58    CONTINUE
       FNI(6)=0.5*(OP(1,1)**2+2*OP(1,2)*OP(2,1)+OP(2,2)**2)
      CALL MULT(O1,Q1,OQ)
```

```
      FNI(10)=0.5*(OQ(1,1)**2+2*OQ(1,2)*OQ(2,1)+OQ(2,2)**2)
      CALL MULT(O1,R1,OR)
      FNI(15)=0.5*(OR(1,1)**2+2*OR(1,2)*OR(2,1)+OR(2,2)**2)
      CALL MULT (O1,S1,OS)
      FNI(21)=0.5*(OS(1,1)**2+2*OS(1,2)*OS(2,1)+OS(2,2)**2)
      FNI(9)=0.5*(OQ(1,1)*OP(1,1)+OQ(1,2)*OP(2,1)+OQ(2,1)*OP(1,2)
     1  +OQ(2,2)*OP(2,2))
      FNI(13)=0.5*(OR(1,1)*OP(1,1)+OR(1,2)*OP(2,1)+OR(2,1)*OP(1,2)
     1  +OR(2,2)*OP(2,2))
      FNI(18)=0.5*(OS(1,1)*OP(1,1)+OS(1,2)*OP(2,1)+OS(2,1)*OP(1,2)
     1  +OS(2,2)*OP(2,2))
      FNI(14)=0.5*(OQ(1,1)*OR(1,1)+OQ(1,2)*OR(2,1)+OQ(2,1)*OR(1,2)
     1  +OQ(2,2)*OR(2,2))
      FNI(19)=0.5*(OQ(1,1)*OS(1,1)+OQ(1,2)*OS(2,1)+OQ(2,1)*OS(1,2)
     1  +OQ(2,2)*OS(2,2))
      FNI(20)=0.5*(OR(1,1)*OS(1,1)+OR(1,2)*OS(2,1)+OR(2,1)*OS(1,2)
     1 +OR(2,2)*OS(2,2))
C***** Print information matrix for each family
      CALL MATPR(FNI,N)
C***** Add up information matrices for all families
      DO 50 J=1,NN
      FI(J)=FI(J)+FNI(J)
50    CONTINUE
      WRITE(2,52)
      CALL MATPR(FI,N)
C***** Invert information matrix to obtain covariance matrix
      CALL LINV2P(FI,N,COV,IDGT,D1,D2,WK,IER)
      WRITE(2,54)
      CALL MATPR(COV,N)
C***** Calculate Q statistics for goodness of fit tests.
      SUMQ=0
      SQ3=SQRT(3.0)
      DO 60 I=1,NF
      RES(I,1)=HT(I,1)-U(I,1)
      RES(I,2)=HT(I,2)-U(I,2)
      QS(I)=SQRT(2*SSP(I))-SQ3
      SUMQ=SUMQ+SSP(I)
      OMS(1)=OM(I,1)-OM(I,2)**2/OM(I,3)
      B(1)=OM(I,2)/OM(I,3)
      QA(I,1)=(RES(I,1)-B(1)*RES(I,2))**2/OMS(1)
      OMS(2)=OM(I,3)-OM(I,2)**2/OM(I,1)
      B(2)=OM(I,2)/OM(I,1)
      QA(I,2)=(RES(I,2)-B(2)*RES(I,1))**2/OMS(2)
60    CONTINUE
      WRITE(2,62)(QS(I),I=1,NF)
      WRITE(2,35)SUMQ
      WRITE(3,63)(RES(I,1),RES(I,2),QA(I,1),QA(I,2),I=1,NF)
10      FORMAT(F3.0,2X,2F2.0,4X,F6.1,1X,A2/13X,F6.1,1X,A2)
30    FORMAT(5X,'Parameter Estimates'/5X,6E12.5)
31    FORMAT(5X,'Gradient Estimates'/5X,6E12.5)
32    FORMAT(5X,'-log likelihood'/5X,E12.5)
33    FORMAT(5X,'IER',I10)
34    FORMAT(5X,'WORK SPACE'/5X,3E12.5)
35    FORMAT(5X,6E12.5)
37    FORMAT(5X,'VARY PARAMETER',I3/5X,10F8.2)
```

```
52    FORMAT(5X,'Information matrix')
54    FORMAT(5X,'Covariance matrix')
53    FORMAT(3I10)
62     FORMAT(5X,'Q STATISTICS'/(5X,6E12.5))
63    FORMAT(4F8.2)
      END


      SUBROUTINE FUNCT(N,X,F)
      COMMON PHI(45,3),DEL(45,3),Q(45,3),R(45,3),S(45,3),HT(45,2),SEX(
     +45),NF,HMF,HMM,X2(7),OMI(45,3),SSP(45),U(45,2),OM(45,3)
      DIMENSION X(7),G(7),XX(3),ZZ(2,2)
C****** This subroutine calculates -log likelihood in F and also the
C           derivative wrt each parameter of this function.
      F=0
C*****  X(1) estimates male mean, X(2) female mean.
      DO 10 I=1,NF
      IF(SEX(I).EQ.0)THEN
         U(I,1)=X(2)
         U(I,2)=X(2)
      ELSE
         U(I,1)=X(1)
         U(I,2)=X(1)
      ENDIF
C***** Variance estimates must be gt 0; so square the estimates.
      X2(3)=X(3)**2.
      X2(4)=X(4)**2.
      X2(5)=X(5)**2.
      X2(6)=X(6)**2.
C*****  OM is the variance matrix, omega
      DO 15 J=1,3
      OM(I,J)=2.0*X2(3)*PHI(I,J)+X2(4)*Q(I,J)+X2(5)*R(I,J)+X2(6)*
     +S(I,J)
15    CONTINUE
C***** Calculate the inverse of omega: put it in OMI
      DOM=OM(I,1)*OM(I,3)-OM(I,2)**2.0
      OMI(I,1)=OM(I,3)/DOM
      OMI(I,3)=OM(I,1)/DOM
      OMI(I,2)=-OM(I,2)/DOM
      H1=HT(I,1)-U(I,1)
      H2=HT(I,2)-U(I,2)
      SSP(I)=H1**2.0*OMI(I,1)+H2**2.0*OMI(I,3)+2*H1*H2*OMI(I,2)
C***** Calculate function value
      F=F+(LOG(DOM)+SSP(I))/2.0
10    CONTINUE
22    FORMAT(5X,6E12.5)
50    RETURN
      END

      SUBROUTINE MULT(A,B,C)
      DIMENSION A(3),B(3),C(2,2)
C*****  Multiply two symmetric 2x2 matrices stored as a vector.
      C(1,1)=A(1)*B(1)+A(2)*B(2)
      C(1,2)=A(1)*B(2)+A(2)*B(3)
```

```
      C(2,1)=A(2)*B(1)+A(3)*B(2)
      C(2,2)=A(2)*B(2)+A(3)*B(3)
10    FORMAT(5X,6E12.5)
      RETURN
      END

      SUBROUTINE MATPR(Y,N)
      DIMENSION Y(30)
C***** Print a matrix,Y, which has been stored in symmetric storage mode.
      KI=1
      DO 40 K=1,N
      KE=KI+K-1
      WRITE(2,35)(Y(L),L=KI,KE)
      KI=KE+1
40    CONTINUE
35    FORMAT(5X,6E12.5)
      RETURN
      END
```

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