Biochemical Heterozygosity and Morphological Variability: Interpopulational versus Intrapopulational Analyses

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Abstract The literature is replete with articles suggesting the existence of a relationship between variability at biochemical loci and morphological variation in various animal populations, including humans. With few exceptions these previous studies have utilized an interpopulational approach by examining levels of heterozygosity between modal and extreme phenotypes, typically by use of analysis of variance. Here we consider these purported relationships in a midwestern Mennonite population ($n = 890$) by correlating individual biochemical heterozygosity and deviation from the mean for anthropometric traits. The results of this intrapopulational correlation indicate that (1) with protection for multiple tests, there are few significant correlations and these have low R^2 values, and (2) males and females show different patterns of correlation (males negative, females positive). Based on these findings, the results of earlier studies are in question because nonprotected alpha values are used for multiple tests and heterozygosity is calculated on the basis of a few highly heterozygous blood group systems and is assumed to be representative of the heterozygosity for the entire genome. In general, no evidence is found to support the concept of a direct relationship between biochemical heterozygosity and morphological variability.

For a trait controlled by polygenes individuals most proximal to the population mean are the most heterozygous for that trait (Falconer 1981). Such a relationship between heterozygosity and phenotypic distribution is a reflection of the additive nature of loci in a polygenic system (Elston 1980). This distribution may be enforced further by stabilizing selection that acts against the more homozygous individuals (Beardmore and Shami 1979; Falconer 1981; Soule 1979). The concept of stabilizing selection was originally utilized by Lerner (1954) and Waddington (1957) to support developmental canalization in heterozygotes. According to this theory, given environmental fluctuations, the more canalized

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heterozygotes should exhibit a higher degree of developmental homeostasis, that is, less variability (Lerner 1954; Vrijenhoek and Lerman 1982; Waddington 1957). Irrespective of its actual cause, the normal distribution typically associated with morphological variation has increasingly been assumed by many researchers to reflect underlying levels of heterozygosity.

It has been suggested that the heterozygosity expressed by an individual for simple Mendelian traits, such as protein polymorphisms in the blood, should reflect an equivalent level of heterozygosity in quantitative traits (Eanes 1978; Handford 1980; Kat 1982; Kobyliansky and Livshits 1983; Livshits and Kobyliansky 1984a,b; Mitton 1978). Specifically, attempts have been made to correlate heterozygosity in biochemical systems to the variance observed in quantitative morphological characters (Kobyliansky and Livshits 1983; Livshits and Kobyliansky 1984a, b; Schmitt et al. 1988). Although most of these studies have utilized a variety of organisms (Eanes 1978; Handford 1980; Mitton 1978), there has been only minimal research on this relationship in human populations (Chakraborty et al. 1986; Kobyliansky and Livshits 1983; Livshits and Kobyliansky 1984a,b; Schmitt et al. 1988). Most of these previous studies share a common methodological approach to the relationship between heterozygosity and morphological vaiability, namely, an interpopulational perspective. The present study differs because it not only focuses on humans but also examines the relationship from an intrapopulational level. Schmitt et al. (1988) used a similar approach. Our purpose here is to test the validity of the previously reported negative association between biochemical heterozygosity and morphological variability.

Materials and Methods

The sample for this study consists of 890 adult Mennonites (434 males, 456 females) ranging in age from 18 to 87 years and residing in three midwestern Mennonite communities (seven congregations). Of the total sample 121 males and 96 females are 40 years of age or younger. The three communities are a geographically isolated and genetically well-defined population as a result of their religious and social structure. We utilized both genotypic data for biochemical traits and anthropometric data for the quantitative traits for all individuals in the study sample.

We determined the phenotypes for 23 different erythrocytic antigens and serum proteins for all individuals who participated in this study. The blood group markers were Rhesus, MN, Ss, Kell, Kp, Kidd, Lewis, P, Duffy, and Lutheran, and the 13 red cell and serum proteins were hap-

toglobin (Hp), ceruloplasmin (Cp), group-specific component (Gc), properdin factor (Bf), adenylate kinase (AK), adenosine deaminase (ADA), 6-phosphogluconate dehydrogenase (6-PGD), acid phosphatase (AP), esterase D (EsD), phosphoglucomutase 1 (PGM1), phosphoglucomutase 2 (PGM2), glycoxalase (GLO), and isocitrate dehydrogenase (ICD). The methods used to identify the blood group and protein phenotypes are described by Crawford et al. (1989).

The means and standard deviations for 34 anthropometric traits and the transmissibilities (using the TAU path analytical model) for this population have been described by Devor et al. (1986a,b), who demonstrated that in Mennonites the linear traits tend to exhibit a higher genetic component than the circumferential measurements. Of the 34 anthropometric traits reported, we selected 19 measurements for use in this study. The traits selected reflect the basic dimensions of body height and width and have on average a relatively large genetic component (Devor et al. 1986b). The use of these traits maximizes comparisons on a genetic level while reducing environmental influences on the correlations. The traits are stature, weight, sitting height, iliospinal height, trochanteric height, biacromial width, chest breadth, chest width, bicristal width, bitrochanteric width, upper limb length, upper arm length, suprasternal height, leg length, head length, head width, minimum frontal width, bizygomatic breadth, and bigonial width.

We estimated individual heterozygosity by enumerating all heterozygous loci per individual and dividing this sum by the total number of available blood loci ($h = n_{het}/n$). We computed heterozygosity for three combinations of the blood genetic data: (1) all 23 loci; (2) 13 serum and red cell proteins; and (3) the MN, Ss, Rhesus (C and E loci), and Duffy blood groups. To compare the Mennonite data set with other studies of human populations (Kobyliansky and Livshits 1983; Livshits and Kobyliansky 1984a,b), we measured heterozygosity on the bases of only the MN, Ss, Rhesus (C and E loci), and Duffy antigens.

We subdivided the anthropometric data by sex and then calculated descriptive statistics for both the male and female samples for the 19 traits. We computed individual morphological variation (standard deviation) by subtracting each anthropometric measurement from the mean value for the appropriate sex ($d = |X - \bar{X}|$). This absolute value of the standard deviation provides a measure of the dispersal of each individual value around the mean. Following the calculation of the individual estimated heterozygosity (h) and the deviation from the mean (d) for metrical traits, we examined the normality of distribution of these traits by graphical means. We calculated multiple correlations (Pearson's product moment r score) between individual mean heterozygosity and the abso-

lute value of the individual deviation from the mean for anthropometric traits (Sokal and Rolf 1981). We computed these correlations for the heterozygosities estimated on all 3 combinations of blood genetic data and for all 19 anthropometric traits for total samples and for samples of individuals 40-years-old or younger for both sexes. We examined possible age-related changes in the variability of morphological traits by comparing genetic and morphological variation in individuals 40-years-old or younger. The reason for this subdivision by age was to avoid increases in individual variation from the population mean resulting from such age-related factors as decrease in stature.

To maintain an alpha value of 0.05 across all 19 correlations, we used Bonferroni's experiment-wise protected alpha (Wallenstein et al. 1980). This statistical procedure was necessary because the probability of committing a type I error increases with the number of tests conducted. Although Bonferroni's protection is a conservative approach, it is recommended that some type of experiment-wise protection be used when making multiple statistical tests of this nature (Wallenstein et al. 1980). With multiple tests at least 1 significant correlation out of 20 can be expected to be due to chance alone at the 0.05 level without the use of a protected alpha value. To ensure a 0.05 alpha across our entire experiment, we obtained the protected alpha value needed for significance of each individual test by dividing 0.05 by the number of tests being conducted. For our data an individual alpha of 0.002 is necessary to maintain an experiment-wise alpha of 0.05 for the 19 correlations.

Results

In all cases the average heterozygosity estimates, based on the three groupings of the genotypic data, provided similar results for both sexes (Table 1). We observed no major differences when the sample was restricted to those individuals less than or equal to 40 years of age. Average heterozygosity, calculated by means of all available biochemical loci versus serum and red cell proteins, shows little difference, with an average heterozygosity of 19–25%. However, the mean heterozygosity estimates calculated on the bases of the MN, Ss, Rhesus (C and E loci), and Duffy antigens gave higher estimates of 39–45%.

As shown in Table 2, no significant correlations exist in males when Bonferroni's experiment-wise protection is applied to all three estimates of heterozygosity. Also, with the application of Bonferroni's protection, for males 40 years of age or younger there are no significant correlations

Table 1. **Population Estimates of Mean Percent Heterozygosity Based on Three Combinations of Biochemical Loci**

Group	*23 Loci*	*13 Red Cell and Serum Proteins*	*Rhesus, MN, S, and Duffy*
All males	24.05	19.69	42.95
Males ≤ 40 years of age	25.09	20.22	44.96
All females	24.14	19.77	41.75
Females ≤ 40 years of age	24.81	21.07	39.79

between individual heterozygosity and morphological variability for all three estimates of heterozygosity. Without Bonferroni's protection we observed several significant negative correlations between individual heterozygosity and morphological variability for some of the width measurements.

Despite Bonferroni's experiment-wise protection, we found several significant correlations for females of all ages between individual heterozygosity estimated on all 23 loci and on the MN, Ss, Rhesus, and Duffy loci and morphological variability. For heterozygosity estimated on the bases of all 23 biochemical loci, there were significant positive correlations between heterozygosity and morphological variability for iliospinal height and upper arm length. Heterozygosity based entirely on the MN, Ss, Rhesus, and Duffy loci was significantly correlated with upper arm length.

For females less than or equal to 40 years of age there were no significant correlations between heterozygosity and morphological variability for all three estimates when Bonferroni's protection was employed (Table 3). Both female samples exhibited several significant correlations for all three estimates of heterozygosity without the Bonferroni's experiment-wise protection (see Tables 2 and 3). However, in all cases the coefficient of determination was too low to offer any significant explanation.

We tested the interrelationship of anthropometric traits through a series of multiple correlations and observed significant correlations between all height measurements and most of the width measurements. These findings concur with the results of Devor et al. (1986a), who performed principal component analyses of this same data set. For significant correlations between heterozygosity and morphological variability in the male sample, we noted a negative relationship associated with measurements of width when Bonferroni's protection was applied. For both female samples, when Bonferroni's protection was employed, all significant correlations between heterozygosity and morphological variability were positive and associated with measurements of height.

Table 2. **Correlations between Individual Mean Heterozygosity and Morphological Variation for All Adults[a]**

	Males		Females	
Trait	*r*	r^2	*r*	r^2
Heterozygosity estimated on 23 loci				
Stature			0.112	0.013
Iliospinal height			0.162[b]	0.026
Trochanteric height			0.121	0.015
Upper limb length			0.093	0.009
Upper arm length			0.183[b]	0.033
Suprasternal height			0.121	0.015
Leg length			0.140	0.020
Chest width	−0.100	0.010		
Bitrochanteric width	−0.100	0.010		
Bizygomatic breadth	−0.101	0.010		
Heterozygosity estimated on 13 serum proteins				
Stature			0.137	0.019
Iliospinal height			0.142	0.020
Trochanteric height			0.107	0.011
Upper limb length			0.100	0.010
Upper arm length			0.100	0.010
Suprasternal height			0.113	0.013
Leg length			0.111	0.012
Head length			0.126	0.016
Head width			0.089	0.008
Bicristal width	−0.125	0.016		
Bitrochanteric width	−0.140	0.020		
Heterozygosity estimated on the MN, S, Rhesus, and Duffy loci				
Iliospinal height			0.116	0.013
Trochanteric height			0.117	0.014
Chest width			−0.091	0.008
Upper arm length			0.192[b]	0.037
Suprasternal height			0.102	0.010

a. Significant values at the 0.05 level without Bonferroni's protection.
b. Significant with Bonferroni's corrected alpha for an experiment-wise error rate of 0.05 (individual test significant at the 0.002 level).

Table 3. **Individual Mean Heterozygosity and Morphological Variation for Adults Less than or Equal to 40 Years of Age**

	Males		*Females*	
Trait	*r*	r^2	*r*	r^2
Heterozygosity estimated on 23 loci				
Suprasternal height			0.228	0.052
Bicristal width	−0.186	0.035		
Heterozygosity estimated on 13 serum proteins				
Stature			0.199	0.040
Chest breadth			−0.234	0.055
Bicristal width	−0.203	0.041	−0.272	0.074
Bitrochanteric width	−0.191	0.036		
Heterozygosity estimated on the MN, S, Rhesus, and Duffy loci				
Iliospinal height			0.290	0.084
Chest width			−0.221	0.049
Upper limb length			0.250	0.063
Suprasternal height			0.237	0.056
Biacromial width	−0.187	0.035		

a. Significant values at the 0.05 level without Bonferroni's protection.
b. No significant correlations with the use of Bonferroni's protected alpha values.

Discussion

The validity of an estimate of genomic heterozygosity based on a limited number of loci must be questioned. Mitton and Pierce (1980) have suggested that estimations of heterozygosity from as few as a dozen randomly chosen loci correlate significantly with heterozygosity estimated from upward of a hundred biochemical loci. However, Chakraborty (1981, 1987) argues that estimates of heterozygosity based on a limited number of loci do not adequately reflect the heterozygosity of the system. This should be of no surprise, given that in humans the number of structural loci in the genome is estimated to be between 50,000 and 100,000 (Harris and Hopkinson 1976; McKusick 1976). Therefore estimates of heterozygosity based on 100 loci (a larger sample than has thus far been employed in humans) are sampling only one–one-thousandth of the total genome.

Although the estimations of individual heterozygosity in this study based on the 23 loci and the 13 red cell and serum proteins must be interpreted cautiously, they should provide a better estimate of genomic variability than the heterozygosities based on the MN, Ss, Rhesus, and Duffy loci alone. Kobyliansky and Livshits (1983) and Livshits and Kobyliansky (1984a,b) based their findings on estimates of heterozygosity using only these four blood group loci. Furthermore, they chose these four loci because of the high levels of variation exhibited by these blood markers. In this study we observed that higher heterozygosity values are obtained when these highly polymorphic loci are utilized. As a result, the correlations found by Kobyliansky and Livshits (1983) and Livshits and Kobyliansky (1984a,b) between heterozygosity and morphological variability must be considered biased as a result of inflated estimates of heterozygosity calculated from a small and nonrandomly selected sample of biochemical loci. An accurate interpretation of their findings is further confounded by the use of few data points in the calculation of correlations between population heterozygosity and coefficients of variation and other measures of populational morphological variability. In these earlier studies Kobyliansky and Livshits (1983) and Livshits and Kobyliansky (1984a,b) made no attempts to control for experimentwise error rates with protected alpha values, which leads to an increased chance of committing a type I statistical error across all tests conducted. Therefore the validity of their reported significance at the 0.05 level must be reevaluated.

Although several researchers (Eanes 1978; Handford 1980; Kobyliansky and Livshits 1983) have suggested a relationship between heterozygosity and morphological variability at the populational level, there appears to be little or no evidence for such a relationship when the analysis is intrapopulational. Our findings support the previous work of Chakraborty (1987), Chakraborty and Ryman (1983), Chakraborty et al. (1986), and Schmitt et al. (1988). We observed several significant correlations even with Bonferroni's protection, even though the coefficients of determination (r^2) were too low to adequately explain the relationships between heterozygosity for the biochemical loci and morphological variability. If, however, our results are interpreted as evidence for the existence of a relationship between heterozygosity and morphological variability, the apparent difference in this relationship between the sexes must be explained. Why should males exhibit a negative correlation between genetic and morphological variation and females a positive association?

Previous studies of a variety of organisms (Eanes 1978; Handford 1980; Kat 1982; Kobyliansky and Livshits 1983) have demonstrated the existence of a negative correlation between heterozygosity and

morphological variability, with higher heterozygosity correlated with decreased morphological variability. This relationship has been interpreted (Livshits and Kobyliansky 1984a) as reflecting an increased level of developmental homeostasis conferred on the heterozygote. If Bonferroni's protection and the low coefficients of determination are ignored, then this same pattern is seen in the results for the male Mennonites. At the same time, however, a pattern of positive correlations emerges for the females.

In Kobyliansky and Livshit's research males comprise the entire sample. As a result, the researchers' finding of a negative correlation between heterozygosity and morphological variation reflects a sex bias. In this study we did not observe significant differences in the individual estimates of heterozygosity between the sexes. However, we did note a difference between the type of anthropometric trait and the direction for which heterozygosity and morphological variability are correlated between the sexes. The males exhibit negative correlations with measurements of width, and the females exhibit positive correlations with measurements of length. If such a pattern is considered significant, then it is not possible to fit these results into the earlier explanations of the relationship of biochemical heterozygosity and morphological variation.

According to Soule (1979) much of the apparent disagreement between the findings in this and other studies (Kobyliansky and Livshits 1983; Livshits and Kobyliansky 1984a,b) may be due to differences in approach between interpopulational and intrapopulational analyses. The earlier studies primarily examined the variation between classes of heterozygotes. As a result, relationships between heterozygosity and morphological variability were analyzed at a broad interpopulational level. However, examination of this relationship at the intrapopulational level may provide too fine a resolution for any pattern to be readily apparent. Because quantitative traits are used in these analyses, environmental variation may be sufficiently large to obscure any genetic correlation for heterozygosity between biochemical and polygenic loci. Additional research is needed to examine the interaction of developmental homeostasis, heterozygosity, and morphological variability.

By utilizing an interpopulational approach, Livshits and Kobyliansky (1984a,b) and Kobyliansky and Livshits (1983) observed a negative correlation between heterozygosity and the coefficient of variation for a male sample. An examination of this purported association on an intrapopulational level in Mennonites revealed some negative correlations for males but positive correlations for females. Three out of 19 anthropometric traits for males and 7 out of 19 traits for females were significantly correlated with genetic heterozygosity. All the significant correlations in

the male sample were for width measurements, whereas the female significant correlations were limited to the highly heritable linear traits. With the application of a highly conservative Bonferroni's correction, all the significant correlations for the males became nonsignificant, whereas for the females only two significant correlations were observed.

There are three major reasons for these conflicting results: (1) the small number of blood loci used to estimate heterozygosity in previous studies; (2) the use of multiple statistical tests without employing a protected alpha value in significance testing; (3) small sample sizes. Livshits and Kobyliansky used only four highly polymorphic blood group loci to characterize organismic variation, which has been estimated to consist of at least 100,000 loci. As stated earlier, given 20 possible associations, chance will dictate that on average at least 1 significant association will be observed at the 0.05 level of significance. As few as five to seven male subdivisions (populations) were utilized by Livshits and Kobyliansky in their interpopulational correlation analysis. Thus it is not surprising that with so few data points any trend can result in a highly significant correlation.

In this Mennonite intrapopulational study the observation of positive significant correlations between heterozygosity and several linear (high heritability) traits is worthy of further investigation. These findings contradict the negative associations between heterozygosity and morphological variables observed by Kobyliansky and Livshits in a number of different populations. The results of this study raise some questions as to whether stabilizing selection operates on contemporary human populations with regard to all body dimensions.

The exact relationship of heterozygosity at biochemical loci and morphological variability has been questioned (McAndrew et al. 1982). The adequacy of estimating heterozygosity based on small samples of biochemical loci has been challenged as well (Mitton and Pierce 1980; Nei and Roychoudhury 1974). It can be seen from the results of this study that the number and type of loci used to estimate heterozygosity can have a pronounced effect on the outcome. The difference in the structure of variation at both the population and the individual level (Soule 1979) and perhaps even differences between the sexes must be considered. At present, the relationship between biochemical heterozygosity and morphological variability in human populations remains somewhat ambiguous and in need of further study.

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