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The nature of 2-locus epistatic interactions in animals: evidence from Sewall Wright's guinea pig data

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Summary. The nature of epistatic interactions affects covariance between relatives and the expression of heterosis in various crossbred genotypes. The investigation of these interactions for metric traits requires large data sets of a suitable type. Data from Sewall Wright's early work with guinea pigs are used to compare the goodness-of-fit of seven biological models of 2-locus interaction for the six out of eleven traits in which epistatic effects are apparent. The model equivalent to additive × additive epistasis gives the best general fit over traits, with an average transformed R^2 value significantly greater than that of the next best fitting model ($P < 0.05$). This result is compatible with results from the one other study in this area, using data from mice. It is concluded that, based on results available to date, the additive × additive 2-locus model of epistatic interaction appears most suitable for reduced genetic models.

Key words: Epistasis – Heterosis – Genetic models – Gene interactions

Introduction

The theory of quantitative genetics is largely based on a simple one-locus model of inheritance, and those components of this theory which are used in practice are almost totally based on this model. Yet it seems quite unrealistic to assume that the interaction of gene products is not important – the co-ordination of life processes is much too complex for that.

How do gene products interact in the biochemical processes which affect quantitative traits? If gene products could be identified, this question could be tackled directly. An insight to how this might be approached is found in Kacser and Burns (1981) and Sedcole (1981), who discuss the kinetic organisation of enzyme systems. However, very little is known about gene products of importance for traits such as growth rate and litter size.

Where gene products of importance are unknown and inheritance is apparently polygenic, this question must be tackled at the population level, by estimating parameters which relate to epistatic gene interaction.

This paper contains analysis of the results of Sewall Wright's experiments with guinea pigs (Wright 1922a, b) as the design of these experiments is well suited to estimating parameters which relate to 2-locus epistatic gene interaction.

Materials and methods

From 1906 to 1919, an extensive guinea pig breeding program was conducted successively by R. H. Carr, E. H. Riley, F. R. Marshall and S. Wright. Inbred lines were developed from a single base population for a study of decline in vigour and differentiation among lines (Wright 1922a). Some of these lines were then crossed to produce a number of crossbred genotypes, including F_1, F_2 and F_3 crosses, three breed crosses and four breed crosses. Wright (1922b) used results from this work to study the effects of heterozygosity on heterosis.

The experimental design of the second phase of this program is suitable for an investigation of the nature of 2-locus epistatic interactions, but not higher order interactions. However, 2-locus interactions may be of more importance than higher order interactions (Kinghorn 1982).

None of the raw data from the second phase of the program remain (Wright, personal communication via J. F. Crow), but fortunately Wright (1922b) gives an extensive description of experimental design and many data summaries. From this information, it was possible to analyse the summarised data using a generalised least squares approach.

The nature of Wright's summarised data

Wright (1922b) presents many tables of counts and means which he uses to derive a summary of corrected data (his

Table 1. Percentage superiority of each crossbred genotype for each trait. See text for a description of genotypes and traits. Extracted from Table 2 of Wright (1922b)

Genotype	Trait no.										
	1	2	3	4	5	6	7	8	9	10	11
CO	+0.5	+11.2	+ 9.2	+ 1.7	+2.5	+13.2	+ 9.0	− 1.8	+ 0.8	− 1.0	+ 9.5
CA	+2.7	+12.4	+10.4	+ 3.6	+3.6	+11.7	+ 8.6	+ 3.6	+19.4	+23.6	+47.4
AC	+7.6	+11.8	+14.7	+ 8.8	+8.1	+11.9	+10.5	+28.3	+ 9.3	+40.2	+63.7
CC	+6.9	+12.0	+14.6	+12.9	+9.2	+21.0	+16.5	+14.3	+36.1	+55.4	+82.5
C1	+9.0	+ 9.3	+13.9	+10.7	+7.1	+15.9	+12.5	+10.2	+33.3	+46.9	+73.1
C2	+3.8	+ 7.9	+ 8.9	+ 6.7	+4.5	+12.7	+ 9.8	+ 4.0	+25.0	+29.9	+43.7

Table 2). [Hereafter, all references to Wright's tables relate to Wright (1922b).] This information is given in Table 1 for the six crossbred genotypes of interest in this paper. For a total of 11 reproduction and growth traits, this table gives the percentage superiority of each crossbred genotype over contemporary inbred stock − or heterosis expressed. The 11 traits are here numbered for later reference as follows:

1. Per cent born alive
2. Per cent of young born alive raised to 33 days
3. Per cent of all young born raised to 33 days
4. Birthweight in g for all young born
5. Birthweight in g for young raised to 33 days
6. Gain in g per day from birth to 33 days
7. Weight in g at 33 days
8. Litter size
9. Litters per year
10. Young born dead or alive per year
11. Young raised to 33 days per year.

Traits 1 to 7 are corrected for litter size. Wright carried this out by giving relative weights of 1, 3, 3 and 1 to data from litters of size, 1, 2, 3 and 4, respectively.

The genotypes of relevance here are as follows, using Wright's nomenclature:

1) Inbred: This 'genotype' includes five highly inbred lines. From Table 1 of Wright (1922a), these lines appear to have been bred for an average of 11.53 generations of full sib mating, corresponding to a predicted inbreeding coefficient of about 0.92. These five lines were selected from 17 such lines, partly because of their contrasting characteristics. A sixth group completes this 'genotype'. This group is termed 'Other' and is presumably a collection of lesser represented inbred lines.

2) CO: This genotype is made up of a large number of F_1 crosses between parental inbred lines included in the Inbred genotype.

3) CA: Inbred females were mated to CO males derived from two different lines. This is analogous to a range of three breed crosses with crossbred sires.

4) AC: Inbred males were mated to CO females derived from two different lines. This is analogous to a range of three breed crosses with crossbred dams.

5) CC: This genotype was generated by crossing between different F_1 crosses with no parental genotype in common. This is analogous to a range of four breed crosses.

6) C1: This genotype was generated by brother-sister mating among CO genotypes. If parental inbred lines are considered to be fully homozygous, this genotype can be considered as a range of F_2 individuals.

7) C2: This genotype was generated by brother-sister mating among C1 genotypes. If parental inbred lines are considered to be fully homozygous, this genotype can be considered as a range of F_3 individuals.

Table 2. The model of dominance expression. The degree of expression is shown for both single locus configurations, where K and L depict genes originating from different pure lines. Expression in linecrosses is obtained by multiplying the frequency and degree of expression for each configuration and summing over configurations

	Single locus configuration		Linecross				
	$\frac{K}{K}$	$\frac{K}{L}$	Inbred	CO	CA & AC	CC	C1 & C2
Degree of expression	0	1	0	1	1	1	1/2
Frequency of configuration							
Inbred	1	0					
CO	0	1					
CA & AC	0	1					
CC	0	1					
C1 & C2	1/2	1/2					

The representation of each parental line within each genotype was shown by Wright to be similar. Any corrections which might be made to account for different patterns of representation would be small in comparison with genotype differences.

Seasonal environmental effects were accounted for by expressing the value for each genotype as a deviation from contemporary inbred stock. This correction takes proper account of the pattern of data collection for each genotype over time.

Any confounding effects related to age of dam and sex of progeny were shown by Wright to be of little importance.

Wright appears to use the above genotype classes to refer to progeny performance, rather than maternal performance, for Traits 8−11. For example, litter size in genotype CO refers to litters containing CO progeny from inbred parents.

Wright's Table 30 appears to contain one error. The entry for genotype CO, trait "Per cent born alive", is given as +1.5. Inspection of previous tables shows this should be +0.5, and those previous tables appear to be correct. The value +0.5 is used for this paper.

The 1923 U.S.D.A. Experimental Station Record incorrectly abstracts Wright's Table 28 as "a summary of corrected

Table 3. Models of epistasis expression for unlinked loci. The degree of expression is shown for each 2-locus configuration under each of seven biological models. K, L, M and N depict genes originating from different pure lines. Expression in linecrosses is obtained by multiplying the frequency and degree of expression for each configuration and summing over configurations

	2-locus configuration							Linecross				
	K K / K K	K L / K L	K L / K K	K L / K M	K K / L L	K K / L M	K M / L N	Inbred	CO	CA & AC	CC	C1 & C2
Degree of expression under model shown												
No model	0	?	?	?	?	?	1	0	?	?	?	?
Model 1	0	1	1/2	1	1/2	3/4	1	0	1/2	5/8	3/4	1/2
Model 2	0	1	1/4	1	0	1/2	1	0	0	1/4	1/2	1/4
Model 3	0	1	1/2	1	0	1	1	0	0	1/2	3/4	3/8
Model 4	0	0	1/3	1/3	1	1	1	0	1	1	1	5/12
Model 5	0	1	0	1	0	0	1	0	0	0	1/4	1/8
Model 6	0	1	0	1	−1	0	1	0	−1	−1/2	0	−1/8
Model 7	0	1	3/4	1	7/8	15/16	1	0	7/8	29/32	15/16	23/32
Frequency of configuration												
Inbred	1	0	0	0	0	0	0					
CO	0	0	0	0	1	0	0					
CA & AC	0	0	0	0	1/2	1/2	0					
CC	0	0	0	0	1/4	1/2	1/4					
C1 & C2	1/8	1/8	1/2	0	1/4	0	0					

data". Table 28 is in fact the mean value of inbreds contemporary with each genotype for each trait.

Genetic models

Wright shows that any differences between genotypes in parent line representation are of little importance. This means that only parameters relating to heterotic effects need be included in genetic models for analysis.

In analysing this type of data, the genetic model for intralocus dominance effects is quite trivial. Homozygosity (whether allelic, or with respect to breed/line of origin) gives rise to no expression of the dominance effect (d), and heterozygosity gives rise to full expression of the dominance effect. The basic model is all-or-none in nature, and there is no need to consider the biological nature of dominance. The coefficient of dominance expression for a given genotype depends on expected degree of heterozygosity alone, as shown in Table 2.

Epistatic interactions are not so simple in nature. There are two approaches to development of a *general model of epistasis* for describing these 2-locus interactions. The models of Hayman and Mather (1955), Jakubec and Hyanek (1982) and Hill (1982) are based on classical statistical interaction using the F2 generation of a cross between two lines as a reference population. This involves the familiar interaction terms additive × additive, additive × dominance, and dominance × dominance interaction – or their equivalent.

However, these models are limited to involvement of two parental lines, and Wright's data needs a model which can accommodate at least four parental lines. The logical extension of these models to cover more lines requires so many parameters that estimation and prediction become impossible (Eberhart and Gardner 1966). An alternative approach is used in the general model of Kinghorn (1980), which is not limited by number of parental lines. This model is used in Table 3 to show that the basis of 2-locus interaction is not all-or-none in nature.

Where all four genes at two loci come from the same line, no expression of the epistatic effect (e) is expected, as shown in Table 3. Where the four genes come from four different lines, full expression of the epistatic effect is expected. [This argument ignores line-specific effects, as discussed by Kinghorn (1982). Elzo and Famula (1985) consider line specific effects, but not for all possible 2-locus configurations.] However, for the five intermediate gene configurations shown in Table 3, the degree of expression of the epistatic effect depends on the biological nature of the interaction. Fitting a data set to Kinghorn's general model involves *estimating* this biological nature via the degree of expression of e for each 2-locus configuration. However, Wright's data do not involve sufficient genotypes to do this.

The approach taken in this paper is to present seven hypotheses about the biological nature of 2-locus interactions, and to test these for goodness of fit to Wright's data. Kinghorn's general model can be used as a framework to describe these seven *specific models of epistasis*, by showing the expected degree of expression of e for each 2-locus configuration, under each model (Table 3).

The approach to translating this into expected degree of expression in a given linecross genotype is the same for both dominance and epistatic effects. In each case, expression in the cross in expected to be:

$$\sum_i F_i \cdot E_i \qquad \begin{array}{l} i = 1, 2 \text{ for dominance} \\ i = 1 \text{ to } 7 \text{ for epistasis} \end{array}$$

– where F_i is the expected frequency of the i-th gene configuration, and E_i is the expected degree of expression under the i-th gene configuration, as shown in Tables 2 and 3 for dominance and epistasis, respectively. Frequencies of the gene configurations under each linecross genotype were calculated under the assumption of no genetic linkage.

The biological bases of the seven specific models are as follows:

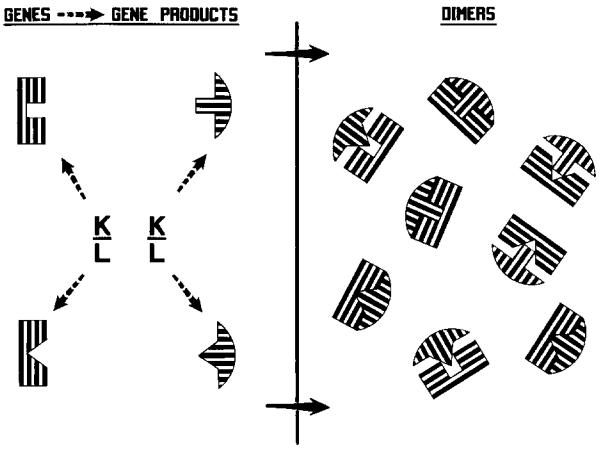

Fig. 1. An illustration of Model 1. K and L represent alleles derived from different lines. In this illustration both loci shown are heterozygous for line of origin. Products from the two loci combine to form dimers. Where coadaptation has taken place within lines, products derived from the same line are more compatible than products derived from different lines. For the genotype configuration shown, half the dimers are expected to involve a difference in line of origin of component gene products, such that half the epistatic effect is expressed

Model 1. This model considers simple or one-to-one interaction between gene products from two loci – as might be the case when two sub-units combine to form a dimeric enzyme. Where the two products are derived from different lines, the epistatic effect is expressed, as illustrated in Fig. 1. From Table 3 it can be seen that the degree of expression under any gene configuration is equal to the probability of randomly choosing one gene from each of the two loci, and finding that they differ in line of origin. This model is hypothesis X of Kinghorn (1980) and is equivalent to additive × additive epistasis.

Model 2. This model considers a two-step biochemical pathway, with gene products from two loci acting as enzymes at each step. The epistatic effect is expressed at the level of a single gene product. If this product is not complemented by a product derived from the same line acting at the other step, then it may be of no value. The epistatic effect itself may take this, or another form. From Table 3 it can be seen that the degree of expression under any gene configuration is equal to the probability of randomly choosing one gene and finding that its line of origin is not represented at the other locus. This model is hypothesis Y of Kinghorn (1980).

Model 3. This model assumes that each parental line is homozygous dominant at loci coding for enzymes acting at each step of a biochemical pathway – a different pathway for each line. Thus in a first cross two pathways are complete, and merit (or other epistatic effect) in a first cross is expected to be twice that in a parental line. This is the 'parental epistasis' model of Sheridan (1981). Under the restriction $d = -e$ it is described by the coefficients shown in Table 3, for 2-locus interaction.

Model 4. This model considers a two-step biochemical pathway, with gene products from two loci acting as enzymes at each step. Where both loci are homozygous with respect to line of origin, as in a pure line, there is only one 'route' to a final product. Where both loci are heterozygous there are four routes. This is the biochemical pathway model of Andresen and Christensen (1981) who propose that merit is related to the number of such routes which can be established. Firstly, the number of routes in excess of the single route in pure lines is found for each of the seven gene configurations. These numbers are (0, 0, 1, 1, 3, 3, 3). These numbers are then scaled, by dividing by 3, to give the degree of expression of the effect for each gene configuration, as shown in Table 3.

Model 5. This model also considers a two-step biochemical pathway, with gene products from two loci acting as enzymes at each step. The epistatic effect is expressed when no route can be established with both steps controlled by enzymes derived from one pure line, as shown in Table 3.

Model 6. This model also considers a two-step biochemical pathway, with gene products from two loci acting as enzymes at each step. However, in this case the degree of expression of the effect is related to the number of routes which can be established with both steps controlled by enzymes derived from one pure line. These numbers are (1, 0, 1, 0, 2, 1, 0) for the seven gene configurations. The degree of expression of the effect is the number of routes less than the single route found in pure lines. Where more than one route can be established, the degree of expression is thus negative, as shown in Table 3 for one of the seven configurations.

Model 7. This model is similar to Model 1, but differs in that two gene products derived from each locus combine to form a functional tetramer. A familiar example of this is the haemoglobin molecule, which is constructed from two alpha chains and two beta chains. For each of the seven 2-locus gene configurations, Table 4 shows the expected frequency of each of the seven possible configurations of tetramer. The model is described at the level of the tetramer – the epistatic effect is expressed whenever all four subunits are not derived from the same breed of origin. The frequencies shown in Table 4 are used to translate these values into the degree of expression under each gene configuration. These in turn are translated into degree of expression under various linecrosses in Table 3, as for previous models.

Parameters relating to the direct and maternal sub traits were included for all models. There were insufficient genotypes to fit the paternal sub trait, but Wright (1922b) indicates that this may be of some importance in trait 9 alone. The seven full genetic models used here can all be described by a single equation:

$$GM_i = X_{i,1} \cdot \mu + X_{i,2} \cdot D_d + X_{i,3} \cdot D_m + X_{i,4} \cdot E_d + X_{i,5} \cdot E_m$$

where

- GM_i is the genotype mean of the trait of interest for the i^{th} genotype
- μ is the overall mean value for the trait
- D_d is the effect of intra-locus line dominance for the direct subtrait (i.e. due to the genotype itself). Intra-locus dominance is assumed to be an effect operating independently from the biological models of epistasis described above
- D_m is the effect of intra-locus line dominance for the maternal subtrait (i.e. due to the dam genotype)
- E_d is the effect of 2-locus line epistasis for the direct subtrait
- E_m is the effect of 2-locus line epistasis for the maternal subtrait
- $X_{i,1}$ to $X_{i,5}$ are coefficients of expression of effects μ to E_m. The two dimensional matrix of X is the statistical design matrix which will be referred to in the section on statistical techniques.

Table 4. Models of epistasis conceived at the level of a tetramer such as haemoglobin, with two subunits derived from each of two gene loci (two α's and two β's). The degree of epistasis at this level is hypothesised under two Models. The first Model turns out to be equivalent to Model 1, and is named thus. Here expression of the epistatic effect is the proportion of interactions between subunits of different type (α or β) which involve a difference in breed of origin (as indicated by subscripts k, l, m, and n to α and β). For Model 7 the effect is expressed whenever the tetramer contains subunits of any type which are derived from more than one breed. The frequency of each tetramer configuration is shown for each gene configuration. The degree of expression of the effect under each gene configuration is the sum of products of these frequencies and the degree of expression under each tetramer configuration

Tetramer configuration	Degree of expression		Gene configuration						
	Model 1	Model 7	$\frac{K\ K}{K\ K}$	$\frac{K\ L}{K\ L}$	$\frac{K\ L}{K\ K}$	$\frac{K\ L}{K\ M}$	$\frac{K\ K}{L\ L}$	$\frac{K\ K}{L\ M}$	$\frac{K\ M}{L\ N}$
$\frac{\alpha_k\ \beta_k}{\alpha_k\ \beta_k}$	0	0	1	0	1/4	0	1/8	1/16	0
$\frac{\alpha_k\ \beta_l}{\alpha_k\ \beta_l}$	1	1	0	1	1/4	1/2	1/8	3/16	1/4
$\frac{\alpha_k\ \beta_l}{\alpha_k\ \beta_k}$	1/2	1	0	0	1/2	0	1/2	1/4	0
$\frac{\alpha_k\ \beta_l}{\alpha_k\ \beta_m}$	1	1	0	0	0	1/2	0	1/4	1/2
$\frac{\alpha_k\ \beta_k}{\alpha_l\ \beta_l}$	1/2	1	0	0	0	0	1/4	0	0
$\frac{\alpha_k\ \beta_k}{\alpha_l\ \beta_m}$	3/4	1	0	0	0	0	0	1/4	0
$\frac{\alpha_k\ \beta_m}{\alpha_l\ \beta_n}$	1	1	0	0	0	0	0	0	1/4
Degree of expression		Model 1	0	1	1/2	1	1/2	3/4	1
		Model 7	0	1	3/4	1	7/8	15/16	1

The values of $X_{i,1}$ to $X_{i,5}$ depend on genotype, and, for $X_{i,4}$ and $X_{i,5}$, on which of the seven specific models of epistatic interaction is currently assumed. Table 5 shows these values for each of the genotypes concerned and for each specific model of epistasis.

Crossbreeding is expected to cause a breakdown of any favourable coadaptation found in parental lines. The extent of such a breakdown for the i^{th} crossbred genotype depends on the nature of epistatic interactions, and is described by $X_{i,4}$ (or $X_{i,5}$ for the maternal genotype) for each model in Table 5. Of course, where there has been no selection for the trait concerned, there may be no coadaptation in parent lines, and no predictable direction of the epistatic effect.

Calculation of effective observations per cell

In order to be able to carry out a generalised least squares analysis of Wright's summarised data, it is necessary to calculate an effective number of observations ($NE_{i,j}$) for each genotype ($i = 1$ to 7) and each trait ($j = 1$ to 11). This is necessary because Wright only presents probable errors (his Table 31, giving 50% confidence limits) for uncorrected means (his table 29). In calculating effective numbers contributing to corrected means (his Table 30), two factors are taken as objectives:

a) To account for the numbers of animals scored in each cell, and for the numbers of inbred contemporaries used for correction purposes,

b) To account for differences between genotypes in within-genotype variance, wherever possible.

The procedure used to calculate NE values is presented in a stepwise manner in the appendix. NE values are presented in Table 6.

Statistical techniques

The objective of the present generalised least squares analyses of Wright's summarised data is to compare the goodness-of-fit of the seven genetic models under consideration. This is done here by first considering ratios of model sum of squares to group (genotype class) sum of squares, for each model to be tested. These ratios are equivalent to the square of the correlation between observed and predicted genotype means, with suitable weighting by effective numbers per genotype. They are referred to here as R^2 values.

These R^2 values are then corrected for model degrees of freedom (dfm = 4) and genotype group degrees of freedom (dfg = 6) in order to give a null hypothesis value of zero:

$$R_c^2 = R^2 - \frac{\text{dfm}\,(1 - R^2)}{\text{dfg} - \text{dfm}}.$$

Parameters for the j^{th} trait are estimated by the vector:

$$b_j = (X' V_j^{-1} X)^{-1} X' V_j^{-1} Y_j$$

where X is the design matrix for the model under consideration (referred to in the previous section); V_j is a 7×7 diagonal matrix with diagonal elements equal to the reciprocals of the effective number of observations of the 7 genotypes for the j^{th} trait (i.e. of $NE_{1,j}$ to $NE_{7,j}$); and Y_j is the column vector of genotype means for the j^{th} trait.

Table 5. Values for design coefficients $X_{i,1}$ to $X_{i,5}$. The first three coefficients are the same for each specific model of epistasis

				Specific model of epistasis													
	Mean	Dominance		Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
i[th] Genotype	$X_{i,1}$	$X_{i,2}$	$X_{i,3}$	$X_{i,4}$	$X_{i,5}$	$X_{i,4}$	$X_{i,5}$	$X_{i,4}$	$X_{i,5}$	$X_{i,4}$	$X_{i,5}$	$X_{i,4}$	$X_{i,5}$	$X_{i,4}$	$X_{i,5}$	$X_{i,4}$	$X_{i,5}$
Inbred	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CO	1	1	0	0.5	0	0	0	0	0	1	0	0	0	− 1	0	0.875	0
CA	1	1	0	0.625	0	0.25	0	0.5	0	1	0	0	0	− 0.5	0	0.906	0
AC	1	1	1	0.625	0.5	0.25	0	0.5	0	1	1	0	0	− 0.5	− 1	0.906	0.875
CC	1	1	1	0.75	0.5	0.5	0	0.75	0	1	1	0.25	0	0	− 1	0.938	0.875
C1	1	0.5	1	0.5	0.5	0.25	0	0.375	0	0.416	1	0.125	0	− 0.125	− 1	0.719	0.875
C2	1	0.5	0.5	0.5	0.5	0.25	0.25	0.375	0.375	0.416	0.416	0.125	0.125	− 0.125	− 0.125	0.719	0.719

The model sum of squares is:

$$b' X' V^{-1} Y_j$$

and the sum of squares due to genotype class is

$$\sum_i NE_{i,j} (GM_{i,j} - \text{mean})^2$$

where $GM_{i,j}$ is the genotype mean for the i^{th} genotype for the j^{th} trait, and

$$\text{mean} = \sum_i GM_{i,j} \cdot NE_{i,j} / \sum_i NE_{i,j}.$$

As the inbred genotype acts as control, all $GM_{i,j} = 0$ with zero standard error, and all $NE_{i,j} = \infty$ (see "Appendix"). Thus the first elements of all V_j^{-1} have the value infinity, giving rise to computational difficulties. However, as $X_{1,1}$ to $X_{1,5}$ have the values (1, 0, 0, 0, 0), these infinite elements define the value of the first parameter (μ) to equal the mean value of the first genotype (inbred). An equivalent model is therefore got by eliminating the first row and column of both X and V and the first element of Y. Equivalence was tested empirically by comparing this reduced model with an unreduced model where the first element of V^{-1} was set sufficiently large (10^6).

Results

Table 7 presents results from the analyses. Of initial interest is evidence for the existence of epistasis after fitting the dominance effects. This is shown in Table 7 by the significance levels P_1, P_2 and P_3 shown to the nearest percentage. These relate to the joint significance of the two epistatic parameters, Ed and Em, calculated from the appropriate F ratio described by Kinghorn (1983).

P_1 relates to Model 1, and this is presented because Model 1 is later shown to give the best overall fit.

P_2 relates to the best fitting model for the trait concerned. This significance levels is biassed as it does not account for the effect of selecting the best fitting model out of seven.

P_3 accounts for this bias: $P_3 = 1 - (1 - P_2)^7$. However, P_3 itself is biassed upwards (i.e. P_3 is conservative) as it does not account for the intra-trait correlation of significance levels – a value which cannot be calculated.

On the basis of $P_3 < 10\%$, traits 4, 6, 7, 9, 10 and 11 are identified as showing reasonable evidence of epistasis, and these traits alone will be considered in the comparison of models.

The overall goodness-of-fit of models 1 to 7 are shown by R_c^2 values in Table 7. In order to estimate the significance levels of these values, the analyses were each repeated 10,000 times using a different randomly generated data set for each repeat. Genotype means were distributed independently as $N(0, \sqrt{1/NE_{i,j}})$, where $NE_{i,j}$ is the effective number of observations for the i^{th} genotype for the j^{th} trait. The mean values of

Table 6. Within genotype standard deviation (SD), and effective number of observations in each genotype, for traits 1 to 11 (see text for description of traits and genotypes, and the appendix for methods of calculation). The effective number of observations for the inbred genotype is infinity for all traits

	Trait no.										
	1	2	3	4	5	6	7	8	9	10	11
SD	39.3	40.4	47.3	14.8	13.6	1.29	49.5	1.01	1.14	3.05	2.49
Genotype CO	859	785	913	922	582	582	582	454	117	144	136
CA	356	411	373	304	226	226	226	110	63	29	31
AC	490	471	463	352	271	271	271	103	54	33	34
CC	490	385	449	420	278	278	278	165	94	38	41
C1	576	386	480	447	297	297	297	173	100	40	44
C2	197	165	183	163	97	97	97	68	39	14	15

R^2 and R_c^2 from these analyses were 66.44 percent and -0.68 percent, respectively. This agrees well with expected values of dfm/dfg = 2/3 and 0, respectively. Rank ordering of the randomly generated R_c^2 values was used to test the significance of the R_c^2 values from Wright's data.

R_c^2 values are generally very high (overall average 88.96%) indicating that the genetic models used give a good description of genotype means. It should be noted that R_c^2 values used here relate to the proportion of genetic group variance described by models. Significant levels of variance remain undescribed within genetic groups.

As for any study of this nature, the results presented in Table 7 do not give a suitable basis for testing the relative goodness of fit of each model within a single trait. R_c^2 values and other descriptors of fit are error correlated within traits to an unknown extent.

However, this latter effect can be overcome in a simultaneous analysis across all traits, fitting trait as a main effect. This permits inferences to be made about the overall goodness of fit of models, across traits.

Due to skewness in R_c^2 values, a suitable transformation was carried out before analysis – uncorrected R^2 values are proportions, and so $\arcsin\sqrt{R^2}$ values were used as observations.

The transformed R^2 values were treated as a randomised complete block design (Snedecor and Cochran 1967), with models as treatments, and traits as replicates. Use of this method requires some caution in interpretation, as the traits are not fully independent, some having been calculated from overlapping pools of raw data (Wright 1922 b).

Results shown in Table 8 indicate that Model 1 is the best fitting overall. It is significantly better fitting than the mean of the remaining six models ($P < 0.001$).

The standard errors of model means, got from the randomised block analysis, were used in a two-tailed Student's-*t*-test to describe the significance of difference between the mean transformed R^2 for Model 1 and the mean for each of Models 2–7 (Table 8). Model 2 is the second best fitting model and Model 1 is better fitting than Model 2 with apparent significance ($P = 0.046$). It it is considered that if, for example, dependence among the six traits was such that there were only three "effective" traits, degrees of freedom for error would be reduced from $(7-1)\cdot(6-1) = 30$ to $(7-1)\cdot(3-1) = 12$. Under this circumstance, the significance of the difference between Models 1 and 2 still remains quite high ($P = 0.06$).

Parameter estimates

Parameter estimates under Model 1 are presented in Table 7. These estimates are very similar to those from an ordinary least squares analysis which gives equal weighting to each genotype, including inbred. Large differences might only be expected where the genetic model is inappropriate for the genotypes involved, and as is the case here, where the data are considerably unbalanced (Table 6).

The most notable parameter estimates relate to traits 9, 10 and 11 (Table 7). Although these traits would appear to involve maternal rearing ability, it is the estimates relating to the progeny (or direct) subtrait which are important. It may be that the demands of progeny have had a significant effect on maternal performance as expressed in these traits. Direct dominance is large and negative while direct epistasis is very large and positive. However, these two genetic effects will largely cancel in the expression of genetic value – the expressions of these effects are highly correlated across genotypes, and the dominance effect is more fully expressed in most genotypes (see Table 5).

Discussion

To estimate parameters of epistasis, or to test models of epistasis with accuracy requires a larger data set

Table 7. Results from generalised least squares analyses. For each trait, the following are given: parameter estimates μ – Em in percentage units under Model 1 (see text for details), joint significance of epistatic parameters P_1, P_2 and P_3 (to nearest percentage, see text for definitions), and R_c^2 values for Models 1 to 7

Trait	1	2	3	4	5	6	7	8	9	10	11
μ	0	0	0	0	0	0	0	0	0	0	0
Dd	− 5.73	4.65	1.78	− 8.88 *	− 0.70	− 6.72	− 4.48	21.17	− 87.68 **	− 69.46 **	− 96.77 **
Dm	8.73	0.06	6.43	8.92 *	6.04	2.79	3.62	21.01	17.90	45.74	67.93
Ed	11.15	12.41	13.87	20.97 **	6.70	35.83 *	24.68 *	− 40.55	175.05 **	140.61 **	217.65 **
Em	− 7.25	− 1.33	− 4.27	− 7.61	− 3.04	− 6.51	− 4.22	6.37	− 55.27	− 57.09	− 101.41
P_1	78	75	87	1	65	3	4	23	0	1	1
P_2	67	60	70	1	56	1	1	5	0	1	1
P_3	100	100	100	9	100	5	9	29	0	6	5
R_{c1}^2	94.46 *	98.60 **	97.63 *	98.72 **	99.51 **	91.94	95.30 *	56.01	98.39 **	96.30 **	97.76 *
R_{c2}^2	90.94	97.28 *	96.32 *	97.33 *	99.95 **	87.02	91.73	46.37	76.86	97.00 *	94.42 *
R_{c3}^2	91.18	97.27 *	96.33 *	95.47 *	99.77 **	86.10	90.67	50.39	70.62	97.09 *	94.96 *
R_{c4}^2	96.13 *	99.64 **	99.72 **	92.52	98.31 *	90.84	93.38 *	67.43	59.42	80.34	83.27
R_{c5}^2	91.23	97.27 *	96.31 *	98.04 *	99.07 **	94.87 *	97.09 *	66.29	73.86	84.60	85.17
R_{c6}^2	90.92	97.25 *	96.51 *	96.26 *	99.81 **	86.36	91.09	47.51	69.15	94.78 *	92.03
R_{c7}^2	96.68 *	99.84 **	99.85 **	93.13 *	98.31 *	91.94	94.26 *	69.22	65.01	80.91	84.65

* $P < 0.05$; ** $P < 0.01$

than that used in this paper. Moreover, error correlations between descriptors of fit within traits makes significance testing unreliable. The approach taken here has been to pool information over a number of traits and use this to make inferences about the nature of epistatic interactions.

Conclusions reached using this approach must be interpreted appropriately – they aim to define a working null hypothesis to be used in the absence of good information relating to specific traits, or species.

The main conclusion is that of the seven models tested, Model 1 (additive × additive interaction) gives the best general fit to Wright's data. This result is not due to a statistical artifact of the design matrix for this model. Statistical theory predicts that this would be the case, because the design matrix for each model is non-singular. The doubting author supported this prediction by fitting the models to several thousand randomly generated data sets, with equal goodness-of-fit.

Models equivalent to Models 1, 2 and 3 (which ranked first, second and fourth on mean transformed R^2 in the present paper) were tested for goodness-of-fit to data from 155 genotypes generated from three inbred lines of mice (Kinghorn 1983). The additive × additive epistasis model fitted best for all of the five traits analysed.

Experimental studies aimed at comparing models of epistasis are never likely to be very powerful. However, the apparent consistency in results to date gives a measure of confidence that models including additive × additive epistasis are the most generally suitable when analysing crossbreeding data, or when predicting the value of untested crossbred genotypes.

This paper has considered epistatic interaction solely as mediated through gene products. There is increasing evidence of cis-acting interaction at the DNA level, between closely linked regulatory and structural genes. However the closeness of this linkage is sufficient to make this an unlikely source of observed interaction in the present study, where only a few generations of breeding are involved. Nevertheless, there is evidence that genetic linkage may be of some importance (Kinghorn 1983), and this may have some effect on results from the present data set, which was too narrow to test for linkage effects.

It would be naive to predict that all, or even most epistatic interactions are additive × additive in nature. Biochemical processes are too diverse in nature for that and the differences between models in goodness-of-fit do not appear to be large. However, a trend for interaction between single gene products is predicted by the superiority of the additive × additive model. As indicated in Table 4, this type of interaction can be expressed in situations which are more complex than one-to-one interaction of isolated gene products.

Table 8. Comparisons of goodness-of-fit of models 1 to 7 for traits with significance $P_3 < 10\%$. The significance of difference between Model 1 and each other model is given (see text for details)

	Model						
Trait no.	1	2	3	4	5	6	7
Mean R_c^2	96.44	90.73	89.15	83.23	88.94	88.23	84.98
Mean arcsin $\sqrt{R^2}$	1.467	1.406	1.393	1.345	1.394	1.384	1.356
Significance	–	0.046	0.018	0.000	0.018	0.008	0.001

The goodness-of-fit of Model 1 is compatible with the fact that 2-locus covariance between generations is expected to be additive × additive in nature (Falconer 1981). This expectation ensures that selection operates in a manner which favours the development of biological systems based on this type of interaction. It is interesting to speculate that these systems may in fact be suboptimal in terms of individual fitness. Biological mechanisms based on other types of epistatic interaction may give rise to greater fitness, but these cannot be developed and maintained under a simple pattern of selection in diploids.

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Appendix

Procedure used to calculate effective number of corrected observations for each genotype (i = 1 to 7) for each trait (j = 1 to 11)

1. Calculate standard deviations within genotype/trait cells ($SD_{i,j}$). For Traits 4–7 the results of Wright (1922b, page 14) are used. Data from his Tables 14–17 were used for all other traits.

2. For traits j = 1 to 11 calculate overall within genotype standard deviations (SD_j, presented in Table 6). These are used hereafter as the standard deviations of the 11 traits.

3. Account for heteroscedasticity by transforming actual observations per cell ($NA_{i,j}$, extracted from a number of Wright's tables) to intermediate effective numbers, $NB_{i,j}$:

$$NB_{i,j} = NA_{i,j} \cdot (SD_j/SD_{i,j})^2 .$$

4. Calculate the effective number of inbred control animals, $NC_{i,j}$, acting as a control for each cell. This is done from information given in Wright's Tables 22–26. (These tables are set up to show the time span of data collection for each genotype and to make contemporary comparison of crossbred genotypes with inbred controls. Five tables are sufficient to cover the 11 traits.)

$$\frac{1}{NC_{i,j}} = \frac{1}{\left(\sum_k NXB_{i,j,k}\right)^2} \cdot \sum_k \frac{NXB_{i,j,k}^2}{NINB_{j,k}}$$

– where:

$NXB_{i,j,k}$ is the number of animals of the i^{th} crossbred genotype (i = 2 to 7) scored for the j^{th} trait in the k^{th} 3-month time period

$NINB_{j,k}$ is the number of inbred control animals scored for the j^{th} trait in the k^{th} 3-month time period.

These numbers include animals from litters of all sizes, whereas the summarised data includes results from litters up to size 4 only. As relatively few litters were greater than size 4, this is not expected to significantly affect either Wright's correction factors or the present results.

5. Correct $NC_{i,j}$ according to the standard deviations for each trait in the inbred control line (ISD_j). This assumes that standard deviations in the inbred control were constant over time. The result of this is:

$$ND_{i,j} = NC_{i,j} \cdot (SD_j/ISD_j)^2 .$$

6. Combine the effective number of uncorrected observations $NB_{i,j}$ with the effective number of control observations $ND_{i,j}$ to give the effective number of corrected observations, $NE_{i,j}$:

$$1/NE_{i,j} = 1/NB_{i,j} + 1/ND_{i,j} .$$

This is done under the assumption that uncorrected and control observations are unrelated except through environmental effects. Of course this is only true for genotypes other than the inbreds, as the latter act as the control. Observations on the inbred genotype covary fully with themselves giving corrected values of precisely zero (as $GM_{l,j} - GM_{l,j} = 0$, where GM is genotype mean). For any finite standard deviation this corresponds to an effective number of infinity (i.e. all $NE_{l,j} = \infty$).

Values of $NE_{i,j}$ derived as above are presented in Table 6.