Theor Appl Genet (1985) 69:625–629

Estimating single gene effects on quantitative traits

1. A diallel method applied to Est 6 in *D. melanogaster*

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Received July 30, 1984; Accepted August 5, 1984
Communicated by J. S. F. Barker

Summary. A modified diallel cross is used to estimate effects of alleles at the *esterase 6* locus, relative to strain and environmental variance, in *Drosophila melanogaster*. Three strains homozygous for *Est* 6^S and three homozygous for *Est* 6^F were crossed in all 36 combinations. Male progeny were scored for mating speed, copula duration and esterase 6 enzyme activity, and all progeny for developmental time. These alleles show a significant additive effect on mating speed, but not on the other traits. Copula duration, developmental time and enzyme activity show additive strain genetic variance. Enzyme activity and developmental time also have maternal or X-chromosome strain variance, and these two traits are significantly correlated. This modified diallel method is generally useful because it permits the partition of trait variance into additive and dominant locus, background genetic and environmental components.

Key words: *Drosophila melanogaster* – Esterase 6 – Mating behavior – Population genetics – Quantitative genetics

Introduction

Two effects of a single locus on quantitative traits can be estimated, for the case of two alleles: an additive effect (*a*) determined from the comparison of the two homozygotes, and a dominance effect (*d*) from the comparison of the heterozygote with the average homozygote value. Estimation of such locus effects is often based on comparisons among strains which are homozygous for alleles at a locus (Aslund and Rasmuson 1976; Clarke 1975; Gilbert and Richmond 1982). Any such comparison must account or control for general genetic differences among the strains to provide valid estimates of locus effects.

In the locus-strain diallel method described here, additive and dominant locus effects are estimated relative to residual, between-strain additive and dominant genetic variance. The complete diallel design is the cross of several strains in all possible combinations, and measurement of traits in the F_1 generation. This design permits estimation of additive and dominant genetic variance which is tested for significance relative to environmental or experimental variance. The precise estimation procedure depends on assumptions used in the diallel, and the degree of strain inbreeding (Hayman 1954; Griffing 1956). If the strains have been derived randomly from a natural population, the diallel analysis permits a valid estimate of genetic variance in that population. The locus-strain diallel extracts a third level of variance, the single locus variance contributed by additive and dominant variance between strains homozygous for different alleles.

This method is applied to test for effects of alleles at the *esterase 6* locus (*Est 6*) in *Drosophila melanogaster*. Putative functions of the esterase 6 enzyme include male reproduction (Richmond et al. 1980; Gilbert et al. 1981a) and larval nutrition (Danford and Beardmore 1980). The experimental design of these studies has been comparison of nearly co-isogenic strains differing in *Est 6* alleles. This report uses the diallel method to test for *Est 6* effects on the reproductive traits of mating speed and copula duration, on developmental time (a function of larval nutrition, c.f., Robertson 1960), as well as on esterase 6 enzyme activity. The utility of this diallel method for detecting locus effects is discussed in relation to other methods. Statistical properties of this and four other designs for detecting locus effects are analyzed in Gilbert (1985).

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Analysis of the locus-strain diallel

When the usual diallel cross of n strains is modified to include m strains homozygous for one allele and the other m homozygous for a second allele ($2m = n$), the between-allele components of variance can be partitioned out of the overall additive and dominant genetic variances, as indicated in Table 1. Significance of the locus effects is tested by the variance ratio of total variance (strain + locus) over the residual strain variance. A significant locus effect is established when the between-allele comparisons contribute significantly more variance than expected for a comparison between two arbitrary strains.

The analysis of variance for a locus-strain diallel is a straightforward derivation of the standard diallel analyses (Hayman 1954). The diallel values are summed over each of four locus types, the two homozygotes and two reciprocal heterozygotes. The same sums of squares (SS) are calculated from these locus sums as for the strains values, dividing the SS by the number (m^2) of F_1 types per locus type. The calculation of these SS and the F ratio tests of significance are shown in Table 1. For this analysis, the environmental error is estimated from the reciprocal SS remaining after between-strain reciprocal SS ('maternal' effect) is subtracted. An error term obtained from replicated diallel crosses may also be used. The additive (σ^2_A) and dominant (σ^2_D) strain variances contained in components 1–4 of the expected means squares (Table 1) are the components termed general and specific combining abilities by Griffing (1956a). The additive (a) and dominant (d) locus effects contained in components 1 and 3 are the additional variance terms introduced by partitioning strains by allele type.

Materials and methods

Three strains homozygous for the *Est 6*^{S2} and three homozygous for the *Est 6*^{F2} electrophoretic-thermal stability alleles were each derived from single third chromosomes extracted from a natural population (Cochrane and Richmond 1979). Male and female parents from these six strains were paired in a full diallel of 36 crosses. Eclosion times were recorded for the F_1 generation and F_1 males were mated to a standard female type to measure male effects on mating speed and copula duration. Another set of F_1 males was assayed for esterase 6 enzyme activity levels.

Drosophila strains

The six strains of *Drosophila melanogaster* used were derived from a Bloomington, Indiana, natural population by Cochrane and Richmond (1979). These strains were produced by extracting third chromosomes over a balanced lethal (TM3), making each chromosome with its *Est 6* allele (at 3–36.8) homozygous. The other chromosomes are wild-type, derived from the balancer stock and the extracted genome. Three strains had the *Est 6*^{S2} allele and three the *Est 6*^{F2} allele (S = 1.00, F = 1.10 in the nomenclature of Cochrane and Richmond), and are denoted here as S1, S2, S3, F4, F5, and F6.

Progeny collection

A single virgin female and male from each parental strain were paired, with two replicates, in the 36 combinations for the diallel. These pairs were kept in vials containing yeasted cornmeal-molasses-agar medium for 7 days. Eclosing progeny were collected using ether and counted at 12 h intervals for 36 h, starting 216 h (9 day) from pairing. Male progeny were saved on yeasted media until 3–4 days, at which time they were either mated or frozen for enzyme assay.

Table 1. Analysis of variance for the locus-strain diallel

Source	d.f.	Sums of squares	F ratio	Expected mean square
1. Additive locus	1	$SS_1 = T_1 - 2\,T_0$	MS_1/MS_2	$\sigma^2_E + 2\,r\,\sigma^2_D + 2\,n\,\sigma^2_A + 2\,m^2 a^2$
2. Additive stain	$n-2$	$SS_2 = T_2 - 2\,T_0 - SS_1$	MS_2/MS_4	$\sigma^2_E + 2\,r\,\sigma^2_D + 2\,n\,\sigma^2_A$
3. Dominant locus	1	$SS_3 = T_3 - T_0 - SS_1$	MS_3/MS_4	$\sigma^2_E + 2\,r\,\sigma^2_D + m^2 d^2$
4. Dominant strain	$\frac{1}{2}n(n-1)-1$	$SS_4 = T_4 - T_0 - SS_1 - SS_2 - SS_3$	MS_4/MS_6	$\sigma^2_E + 2\,r\,\sigma^2_D$
5. Maternal effect	$n-1$	$SS_5 = T_5$	MS_5/MS_6	$\sigma^2_E + 2\,\sigma^2_M$
6. Remainder	$\frac{1}{2}(n-1)(n-2)$	$SS_6 = T_6 - SS_5$		σ^2_E

Partial sums:

$T_0 = \ldots Y^2 \ldots / n^2$

$T_1 = \Sigma_i (_i.Y.. + .{}_iY..)^2/n^2$

$T_2 = \Sigma_{ik} (_i.Y_k. + .{}_iY._k)^2/2\,n$

$T_3 = \Sigma_{ij} (_{ij}Y.. + {}_{ji}Y..)^2/n^2$

$T_4 = \Sigma_{ijkl} (_{ij}Y_{kl} + {}_{ji}Y_{lk})^2/4$

$T_5 = \Sigma_{ijk} (_i.Y_k. - .{}_iY._k)^2/2\,n$

$T_6 = \Sigma_{ijkl} (_{ij}Y_{lk} - {}_{ji}Y_{lk})^2/4$

where m strains are homozygous for one of two alleles, with $n = 2\,m$ total strains; $_{ij}Y_{kl}$ is the phenotype of the ith maternal and jth paternal allele, the kth maternal and lth paternal strain; with i, j = [1, 2], k, l = [1 . . m]. Dots represent summation over that subscript. Based on the Hayman (1954) diallel analysis of variance. See Griffing (1956) for fuller derivations of strain expected mean square components: a, additive locus effect; d, dominant locus effect; σ^2_A, additive strain variance; σ^2_D, dominant strain variance; σ^2_M, reciprocal parental (maternal) variance; σ^2_E, environmental variance; $r = (n-1)/n$

Trait measures

The average eclosion time per cross is used as developmental time in the analysis. Male progeny were mated singly to Oregon-R strain females; mating speed (time to copulation) and copula duration were recorded. The average speed and duration of five males per cross is used in the analysis. Esterase 6 enzyme activity was measured by methods described in Sheehan et al. (1979). The average activity per male for two homogenates of 5–10 males per cross is used for analysis.

Results

Mating speed

The square root of minutes to mating is analyzed, to reduce skew toward early mating times and make variances homoscedastic. The strain means of mating speed are presented in Table 2. This table provides values for the 36 crosses, as well as the maternal (row) and paternal (column) sums used for the analysis of variance. Below this is the average response per locus type. Inspection of row and column sums, which contain the additive genetic effects, shows all S strain sums to be larger than F strain sums. In the analysis, this difference appears as a significant additive locus effect. There is no locus dominance associated with this apparent *Est 6* effect on mating speed. Neither is there significant additive genetic variance among these strains beyond that accounted for by the *Est 6* locus, but there is significant dominance. Maternal effects are not significant.

Table 2. Locus-strain diallel for mating speed ($\sqrt{\text{min}}$/male)

Cross means:

Maternal strain	Paternal strain S1	S2	S3	F4	F5	F6	Sum
S1	3.50	2.24	3.46	2.79	2.42	2.87	17.28
S2	2.79	5.10	2.75	2.61	2.61	2.24	18.10
S3	3.71	2.24	3.12	2.61	2.24	3.66	17.58
F4	3.05	3.57	2.42	2.24	2.56	2.42	16.26
F5	2.24	2.24	2.42	2.42	2.61	2.24	14.17
F6	3.12	2.61	2.56	2.42	2.24	2.42	15.37
Sum	18.41	18.00	16.73	15.09	14.68	15.85	98.76

Locus means:

	S	F
S	3.21	2.67
F	2.69	2.40

Analysis of variance:

Component		d.f.	SS	F ratio	Estimate
Additive	– locus	1	2.9931	25.12**	a = 0.400
	– strain	4	0.4767	0.23	σ_A = 0.0
Dominant	– locus	1	0.1344	0.25	d = 0.0
	– strain	14	7.4196	4.02*	σ_D = 0.489
Maternal effect		5	0.3224	0.49	σ_M = 0.0
Remainder		10	1.3181		σ_E = 0.363

Levels of significance: * $P<0.05$, ** $P<0.01$

Table 3. Locus-strain diallel for copula duration (min/male)

Cross means:

Maternal strain	Paternal strain S1	S2	S3	F4	F5	F6	Sum
S1	18	18	20	19	17	17	109
S2	19	20	19	24	20	21	123
S3	17	20	12	19	17	14	99
F4	24	25	20	24	22	21	136
F5	17	21	16	24	20	17	115
F6	16	21	15	20	17	18	107
Sum	111	125	102	130	113	108	689

Locus means:

	S	F
S	18.1	18.7
F	19.4	20.3

Analysis of variance:

Component		d.f.	SS	F ratio	Estimate
Additive	– locus	1	22.22	0.42	a = 0.0
	– strain	4	210.23	14.69***	σ_A = 2.020
Dominant	– locus	1	0.25	0.07	d = 0.0
	– strain	14	50.10	1.92	σ_D = 1.013
Maternal effect		5	4.83	0.52	σ_M = 0.0
Remainder		10	18.67		σ_E = 1.367

Level of significance: *** $P<0.001$

Copula duration

The diallel for copula duration is shown in Table 3. There is highly significant additive strain variance, but no other significant effects on this trait.

Developmental time

Table 4 gives the diallel results for mean developmental times. Row and column sums give no indication of an *Est 6* effect. The analysis confirms this, but shows significant additive and maternal strain variance.

Enzyme activity

The diallel for esterase 6 enzyme activity is shown in Table 5. Its analysis indicates no locus effect, but significant additive strain variance and a significant maternal effect.

Correlations among traits

The correlations calculated from the 36 diallel cells for each pair of traits are listed in Table 6. Only one pair,

Table 4. Locus-strain diallel for developmental time (h – 200/fly)

Cross means:

Maternal strain	Paternal strain						Sum
	S1	S2	S3	F4	F5	F6	
S1	61.8	52.3	68.8	85.8	72.2	38.8	380.7
S2	89.6	101.4	81.0	91.4	97.0	70.4	530.8
S3	79.9	88.3	75.8	76.8	80.9	85.8	487.5
F4	92.3	87.2	85.8	71.0	75.0	73.6	484.9
F5	86.8	87.2	68.1	84.3	89.9	65.9	482.2
F6	86.1	95.9	75.5	88.8	90.9	57.3	494.5
Sum	496.5	512.3	455.0	498.1	505.9	392.8	2860.6

Locus means:

	S	F
S	77.7	77.8
F	85.0	77.4

Analysis of variance:

Component		d.f.	SS	F ratio	Estimate
Additive	– locus	1	0.27	0.00	a = 0.0
	– strain	4	1,701.5	4.84*	σ_A = 5.30
Dominant	– locus	1	133.8	1.52	d = 2.26
	– strain	14	1,230.4	1.03	σ_D = 1.20
Maternal effect		5	2,157.2	5.05*	σ_M = 13.15
Remainder		10	854.9		σ_E = 9.25

Level of significance: * $P<0.05$

Table 5. Locus-strain diallel for esterase 6 activity (10^{-8} M β-napthol/male)

Cross means:

Maternal strain	Paternal strain						Sum
	S1	S2	S3	F4	F5	F6	
S1	214	274	212	133	230	228	1,291
S2	74	51	88	102	144	137	596
S3	100	80	133	99	126	96	634
F4	126	132	127	128	121	119	753
F5	137	102	104	124	145	142	753
F6	121	71	103	107	95	193	691
Sum	772	710	767	692	862	916	4,719

Locus means:

	S	F
S	136	144
F	114	130

Analysis of variance:

Coponent		d.f.	SS	F ratio	Estimate
Additive	– locus	1	1.4	0.02	a = 0.0
	– strain	4	299.1	8.67***	σ_A = 2.35
Dominant	– locus	1	1.9	0.22	d = 0.0
	– strain	14	120.7	0.84	σ_D = 0.0
Maternal effect		5	304.5	5.91**	σ_M = 5.03
Remainder		10	103.1		σ_E = 3.21

Levels of significance: ** $P<0.01$, *** $P<0.001$

Table 6. Correlations among traits

Trait	Copula duration	Development time	Enzyme activity
Mating speed	–0.068	+0.208	–0.148
Copula duration		+0.287	–0.133
Development time			–0.742***

Level of significance: *** $P<0.001$

developmental time and enzyme activity, show significant association. To determine if covariation between these traits could account for the significant additive and maternal effects on enzyme activity, the 36 cross values of activity were adjusted to remove developmental time covariance, with the regression of activity on developmental time. Locus-strain analysis of these adjusted values gives the same significant effects of additive strain and maternal components. As well, dominant strain variance is significant. Locus effects on esterase 6 activity remain insignificant.

Discussion

The locus-strain diallel analysis developed here has proved useful in distinguishing between effects of a single locus and general strain genetic variance in the traits measured. The power of this design for detecting true but small locus effects will increase with the number of strains analyzed (Gilbert 1985); use of only six strains in this report has limited the chance for detecting small *Est 6* effects. Any residual genetic variation within strains will also reduce the chance of detecting locus effects by increasing the error variance. The estimates of additive and dominant strain variance in Tables 2–5 are strictly applicable only to the experimental strains, since these strains include genes on the X and II chromosomes from the inbred balancer stock as well as from the wild genomes. They are only approximate estimates of the background variance in the Bloomington population. The diallel method, as well as other methods for estimating locus effects, is subject to possible error due to linkage disequilibria between the loci examined and unknown loci. Significant locus effects should be interpreted with caution where linkage disequilibria cannot be ruled out.

The *Est 6* effect on mating speed has been found by Aslund and Rasmuson (1976), and Gilbert and Richmond (1982) as well as in this experiment, all using different strains and presumably different *Est 6* alleles. The possibility that other loci in linkage disequilibrium with *Est 6* are the cause of this mating speed – locus association cannot be ruled out. Its occurrence for independently derived strains suggests that, if linked loci are responsible, they must be commonly linked, and in the same manner such that $Est\,6^F$ alleles appear to cause faster mating, in natural populations of *Drosophila*

melanogaster. Further evidence which strongly implicates *Est 6* specifically comes from the biochemical and physiological studies of its function in male reproduction (Richmond et al. 1980; Gilbert 1981; Gilbert and Richmond 1982). Recent evidence indicates that this enzyme metabolizes the male lipid cis-vaccenyl acetate, whose alcohol product acts as a mating pheromone (Mane et al. 1983).

Alleles at the *Est 6* locus may have additive effects on copula duration which was, however, not significant in this experiment; the mean square is large relative to all but additive strain variance (Table 3). Other evidence also suggests that this locus affects copula duration (Gilbert and Richmond 1982). This diallel analysis uncovered strain variance for copula duration and mating speed comparable to that found in other reports (Fulker 1966; Parsons 1964; Parsons et al. 1967).

Locus effects for development time and enzyme activity show no indication of approaching significance. The enzyme activity correlation with development time may be the result of slower esterase 6 production in the later developing males, as esterase 6 activity increases gradually in maturing males (Sheehan et al. 1979). However, the reverse cause, greater esterase 6 activity leading to faster development, cannot be excluded. This latter hypothesis is consistent with that developed by Beardmore (Danford and Beardmore 1980) that esterase 6 is involved in larval nutrition. The lack (or minor nature) of locus effects on esterase 6 enzyme activity has been verified in other experiments (Tepper et al. 1982). This is in contrast to loci such as *Adh* where the alleles or closely linked modifiers have a distinct effect on enzyme activity (Birley et al. 1980; McDonald et al. 1980). The significant maternal effect on enzyme activity in the male progeny is likely due to esterase 6 modifiers on the X-chromosome contributed by their mothers (Richmond and Tepper 1983).

The main favorable feature of this diallel design, in comparison with other designs for detecting locus effects (Gilbert 1985), is its simultaneous estimation of locus and genomic components of variation. Thus the relative importance of a locus effect can be determined, as with the results for copula duration. This method may be applied to a wide range of organisms where necessary homozygous lines can be bred.

Acknowledgements. I wish to thank Rollin Richmond for providing support, encouragement and suggestions for this work, which was completed while I was a doctoral student in his lab. W. T. Starmer, Dan Sulzbach, Eliot Spiess and anonymous reviewers provided useful comments on earlier drafts. Therese Collins performed the enzyme assays with expertise.

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