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Estimation of maternal, sex-linked and additive × additive epistatic gene effects for body size of *Tribolium**

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Summary. The genetic structure of two quantitative traits, 13-day larval weight and pupal weight, in two unselected populations of *Tribolium castaneum* was investigated by the genetic model of Carbonell et al. (1983). The variability among two and three-way crosses was analyzed into components due to: general and specific combining abilities, maternal, sex-linkage, specific reciprocal and additive-by-additive epistasis. Also, indirect evidence of higher order epistasis was studied. It is concluded that the heterotic trait larval weight is highly affected by sex-linked genes and by non-additive gene action with additive-by-additive as well as higher order epistasis playing major roles. Pupal weight, on the other hand, is determined mostly by additive gene action although epistasis is also a significant source for genetic variability. Both traits are significantly influenced by maternal effects.

Key words: A×A epistasis – Maternal effects – Sex-linkage – *Tribolium* – Body weight

Introduction

Following the classical contributions of Fisher (1918) and Wright (1920), many investigators have attempted to quantify the relative importance of hereditary and environmental effects and their interactions. For prediction purposes, it is essential to subdivide the total hereditary variation into additive, dominance, and epistatic effects including correlations among the various effects. Estimation procedures for additive effects are well known today, as are those for dominance, while quantitative epistatic, sex-linked and maternal effects have been considered in fewer cases.

Epistasis has been estimated using procedures suggested by Eberhart and Gardner (1966) and Kearsey and Jinks (1968), but their models do not account for the reciprocal differences found in many animal breeding experiments. On the other hand, several authors have described methods to obtain estimates of the maternal effects (e.g. Foulley 1978; Foulley and Leport 1978; Robinson et al. 1981), but epistasis was not included in their models. Dickerson's (1969) concept of "recombination loss" recognizes the importance of epistasis in crossbreeding systems as does Sheridan's (1980) "parental epistasis", but neither can be equated unequivocally to generalized gene effects. More recently, Kinghorn (1982) has presented statistical models for the estimation of epistatic effects in animal populations, but he ignored sex-linked effects; and Eisen et al. (1983) describe the theoretical framework underlying the genetic interpretation of statistical parameters estimated from diallel animal crosses, but they assume no epistatic or sex-linked effects. Lately, Carbonell et al. (1983) have provided a model for the estimation of maternal, sex-linked and additive × additive epistatic effects plus an indirect estimation of higher order epistasis.

In the present paper, the contributions of maternal effects, sex-linked genes, and additive-by-additive epistasis in addition to the usually considered additive and dominance gene effects are demonstrated by applying a modification of the Carbonell-Nyquist-Bell Model to a heterotic trait (13-day larval weight) and a non-heterotic trait (pupal weight) in unselected *Tribolium* populations.

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Materials and methods

The genetic material for this study originated from two unrelated, heterogeneous and unselected laboratory populations of the flour beetle, *Tribolium castaneum*, which have been used extensively for model experiments in quantitative genetics (Bell 1982). The Purdue Black Population is genetically marked with the partially dominant black body color gene, *b*, and the Purdue Pearl Population is marked with the recessive pearl eye color gene, *p*. Both populations had been reproduced each generation since their formation in 1956 by mass mating of approximately 200 adults.

A random sample of 100 inbred lines was initiated from each population by single pair full-sib matings with continued full-sibbing for six generations. At this time there were 81 surviving inbred lines from the Black Population and 84 from Pearl. Random inbred lines were expanded for use in appropriate crosses. The mating design involved twelve independent sets of four inbred lines, with two random Black and two random Pearl Lines constituting a set. The four lines in a set were arbitrarily coded 1, 2 for the two Black Lines and 3, 4 for the two Pearl Lines. A balanced set of 12 two-way and 24 three-way crosses (Table 1), avoiding backcrosses but including reciprocal crosses, were made among the four lines within each set. A second replication of each set was made. In addition, four random crosses were made twice or repeated within each replication in each set in order to have an estimate of the experimental error. A 24-h egg collection in a 3/4 ounce creamer containing 4 g of standard medium (whole wheat flour enriched with 5% dried brewers' yeast) was taken from a mass mating of four males and four females from the appropriate lines. This egg collection was cultured for 13 days in an environmental chamber controlled at approximately 33 °C and 70% relative humidity. At that time the larvae were screened and a group weight of 10 random larvae per mating was recorded in decamicrograms. All larvae from each mating were returned to the environmental chamber until most had pupated, at which time a group weight of 10 random pupae per mating was recorded. If a mating produced only 10 or fewer offspring, all were weighed.

Statistical model

The statistical model used to analyze the data for each set was

$$Y_{pqr} = \mu + R_p + \delta_{(p)} + C_q + RC_{pq} + E_{r(pq)}$$

where Y_{pqr} is the observed value of the analyzed trait (larval or pupal weight); μ is the overall mean; R_p is the effect (random) of the pth replication (p = 1, 2); $\delta_{(p)}$ is the restriction error (random) due to the restriction in the randomization (Anderson 1970); C_q is the effect (fixed) of the qth cross (q = 1 ... 36), $E_{r(pq)}$ is the within error.

The sum of squares due to crosses can be broken down in its genetic components using the following models for two and three-way crosses as suggested by Carbonell et al. (1983).

$$C_{ii'} = {}^*\mu + {}^*g_{i'} + {}^*g_{i'} - m_i + m_{i'} + {}^*s_{ii'} + r_{ii'} + a\,a_{ii'}$$

$$C_{i.i'i''} = {}^*\mu + {}^*g_i + 1/2\,({}^*g_{i'} + {}^*g_{i''}) - m_i + 1/2\,(m_{i'} + m_{i''}) + 1/2\,({}^*s_{ii'} + {}^*s_{ii''}) + 1/2\,(r_{ii'} + r_{ii''}) + 1/4\,a\,a_{i'i''} + 1/2\,(a\,a_{ii'} + a\,a_{ii''})$$

$$C_{i'i''.i} = {}^*\mu + {}^*g_i + 1/2\,({}^*g_{i'} + {}^*g_{i''}) + m_i - 1/2\,(m_{i'} + m_{i''}) - 1/4\,L_{i'}^{\varphi} + 1/4\,L_{i''}^{\varphi} + 1/2\,({}^*s_{ii'} + {}^*s_{ii''}) - 1/2\,(r_{ii'} + r_{ii''}) - 1/4\,LL_{ii'} + 1/4\,LL_{ii''} + 1/4\,a\,a_{i'i''} + 1/2\,(a\,a_{ii'} + a\,a_{ii''})$$

where $C_{ii'}$ is the single cross between lines i (male) and i' (female), $C_{i.i'i''}$ is the three way cross between male line i and female single cross i' i'', $C_{i'i''.i}$ is its reciprocal, and

$${}^*\mu = \mu - \bar{a}\,\bar{a}^h$$

$${}^*g_i = g_i - a\,a_i^h$$

$$L_i^{\varphi} = 1/2\,(a_i^{s\varphi} + d_i^s) + h_i^s$$

$${}^*s_{ii'} = s_{ii'} - a\,a_{ii'}^h$$

$$LL_{ii'} = h\,h_{ii'}^s \quad \text{and}$$

$$h_{ii'}^s = \bar{h}^s + h_i^s + h_{i'}^s + h\,h_{ii'}^s$$

$$a\,a_{ii'} = \bar{a}\,\bar{a}^h + a\,a_i^h + a\,a_{i'}^h + a\,a_{ii'}^h,$$

Furthermore, g_i and $s_{ii'}$ are defined as general and specific combining abilities for autosomal genes, and m_i and $r_{ii'}$ as general and specific maternal effects (Cockerham and Weir 1977); $L_{i'}^{\varphi}$ and $LL_{ii'}$ have the same genetic meaning as ${}^*g_i$ and ${}^*s_{ii'}$ but applied to sex-linked genes. The $a\,a_{ii'}$ is the additive × additive epistatic effect present when two lines are crossed as defined by Eberhart and Gardner (1966). As they indicate, $a\,a_{ii'}$ can be subdivided into its components, the average effect $\bar{a}\,\bar{a}^h$, the two general effects for both lines $a\,a_i^h$ and $a\,a_{i'}^h$, and the specific effect $a\,a_{ii'}^h$. The additive and dominance effects of sex-linked genes as defined by Carbonell et al. (1983) are denoted $a_i^{s\varphi}$ and d_i^s for these respective effects when expressed in females. The sex-linked heterotic effect, $h_{ii'}^s$, arises when the two populations i and i' are crossed and the sex-linked dominance contribution of the cross deviates from the mean of the sex-linked dominance contributions of both parental populations, i.e. it represents the heterosis due to sex-linked genes. This effect can be subdivided in a similar fashion to that of $a\,a_{ii'}$ in its components $\bar{h}^s$, h_i^s, $h_{i'}^s$ and $h\,h_{ii'}^s$ representing the average, general and specific heterotic effects. The general maternal effect of any crossed dam is assumed to be the average of that of the grandmaternal lines. The difference between the sum of squares due to the models and that for crosses will provide an indirect estimation of higher order epistasis and/or linkage.

As noted in the "Materials and methods" section, the offspring from the various crosses were unsexed when the two analyzed traits (larval weight and pupal weight) were observed. Therefore, those parameters peculiar to each sex ($a_i^{s\delta}$, $a_i^{s\varphi}$, d_i^s and $h_{ii'}^s$) were not obtainable. Those parameters that are common to both sexes represent the average of each parameter for the two sexes.

Since only 24 out of the 36 possible three-way crosses per replication and set were actually made, the variation due to all effects could not be estimated independently. To estimate the contribution of each genetic effect, a general least squares regression computer program was used. The general model was fitted sequentially to the unweighted mean larval and pupal weights for the crosses within each set. A coding procedure assigning dummy values to the effects in the model was used in order to partition the total sum of squares.

Table 1. The two-way and three-way crosses made among the four inbred lines of each set. Inbred lines 1 and 2 from the Black Population and lines 3 and 4 from the Pearl Population were randomly selected for each set of crosses. The reciprocal crosses, $C_{i'i}$ and $C_{i'i''.i}$, were made but are not listed here

Two-way crosses ($C_{ii'}$)	Three-way crosses ($C_{i.i'i''}$)	
1×2	1×(3×2)	3×(1×2)
1×3	1×(2×4)	3×(1×4)
1×4	1×(3×4)	3×(2×4)
2×3	2×(3×4)	4×(1×2)
2×4	2×(4×3)	4×(3×1)
3×4	2×(1×4)	4×(3×2)

Results and discussion

The mean larval and pupal weights for the two-way and three-way crosses within each set are presented in Tables 2 and 3. For the heterotic trait, 13-day larval weight, the three-way crossses were consistently larger than the corresponding two-way crosses. But the same was not true for pupal weight with no significant over-all difference between the two types of crosses.

The fact that one-half of the three-way crosses involved F_1 dams, while all 2-way crosses had inbred dams, accounts for a part of the better over-all performance for the three-way crosses, due possibly to heterotic maternal effects. However, this fact is not the sole cause of the better performance. Among the four lines involved in each set, two originated from Purdue Black and two were from the unrelated Purdue Pearl Population. When genetically diverse lines are crossed, one would expect more heterosis on the average than when crosses are made among lines with more similar genetic origins. In the three-way crosses one of the lines was always from a different base population than the other two lines, whereas only 2/3 of two-way crosses involved lines with diverse genetic backgrounds. Larval weight, being more heterotic than pupal weight, provides sharper contrasts for the two effects under consideration. Furthermore, the mean of the two-way crosses can be shown to be $*\mu + \frac{2}{12}(aa_{12} + aa_{13} + aa_{14} + aa_{23} + aa_{24} + aa_{34})$ and that of the three-way crosses to be equal to $*\mu + \frac{2 \cdot 5}{12}(aa_{12} + aa_{13} + aa_{14} + aa_{23} + aa_{24} + aa_{34}) - \frac{1}{24}(L_1^{\circ} + L_3^{\circ})$. Hence, if the "aa" contribution is positive and larger than the negative contribution of sex-linked effects, the three-way crosses will have higher values than two-way crosses. This finding is in agreement with that observed by Stuber et al. (1973) with plants. They found three-way crosses to have larger values than two-way crosses. They pointed out that the mean of all possible single crosses from an array of random homozygous lines derived from a population in linkage equilibrium is expected to equal the mean of all possible three-way crosses, when no maternal or sex-linked effects are present. Similar equalities are expected if epistasis is negligible even in the absence of linkage equilibrium. In our model the extra components L_1° and L_3° come from the fact that the present study had a partial set of three-way crosses.

The analyses of variance among the various crosses according to the above model are given in Table 4. Since these analyses are based on many observations a substantial number of degrees of freedom are associated with the various effects. Consequently, tests of significance are very powerful and rather small F ratios are sufficient to declare statistical significance. Therefore, the number of sets listed in Table 4 with significant effects at 5% level (N+) and 1% level (N**) out of a total of 12 sets are more indicative of the relative importance of effects. In addition, relative measurements of variation based on the percentages of the sums of squares attributable to each of the different genetic effects are given in Table 5. It is evident from Tables 4 and 5 that the epistatic effects are much more important for larval weight than for pupal weight.

Table 2. Larval weight (dμg) for two and three-way crosses by sets with a comparison of within and between population two-way crosses and inbred versus F_1 dams for three-way crosses

Set	Two-way crosses*		Three-way crosses*	
	Within	Between	F_1 dam	Inbred dam
1	104.3	113.7	155.2	143.1
2	120.5	156.3	187.1	167.7
3	145.4	164.0	188.4	178.3
4	141.0	140.8	165.5	154.8
5	153.7	182.1	217.0	196.7
6	121.6	128.5	150.2	128.2
7	131.8	170.0	172.1	173.3
8	91.8	98.8	132.5	120.6
9	102.7	113.3	157.2	122.7
10	113.7	128.2	197.0	153.8
11	106.6	109.8	126.7	135.0
12	109.4	166.7	194.1	166.7
Overall mean	120.2	139.3	170.2	153.4

* SE = 1.6 to 3.2 (dμg)

Table 3. Pupal weight (dμg) for two and three-way crosses by sets with a comparison of within and between population two-way crosses and inbred versus F_1 dams for three-way crosses

Set	Two-way crosses*		Three-way crosses*	
	Within	Between	F_1 dam	Inbred dam
1	250.9	237.8	246.1	247.5
2	264.9	282.0	269.1	275.0
3	263.7	262.8	266.4	268.0
4	271.9	273.1	264.8	266.1
5	278.3	280.0	272.7	271.9
6	240.7	239.3	248.5	241.8
7	242.8	249.5	246.5	243.4
8	263.5	267.4	262.0	262.3
9	262.1	259.2	258.7	254.0
10	257.7	257.1	252.7	256.9
11	249.6	252.5	248.7	252.3
12	232.5	236.0	244.7	233.8
Overall mean	256.5	258.1	256.7	256.1

* SE = 1.2 to 1.6 (dμg)

Table 4. Analyses of variance for genetic effects influencing larval and pupal weights, pooled over sets

Source	d.f.	Mean squares and no. of significant sets					
		Larval wt			Pupal wt		
		MS	N^+	N^{**}	MS	N^+	N^{**}
Reps/Sets	12	7,272.0			698.1		
Crosses	420	2,396.1**	12	12	376.3	11	9
*g	36	9,500.7**	12	12	1,945.8**	12	12
L	36	1,373.1**	6	2	181.1	1	0
*s	24	2,147.8**	5	4	244.2+	3	1
LL	24	1,259.8**	3	2	88.5	0	0
m	36	2,904.7**	8	8	680.4**	8	6
r	36	729.1	1	1	195.8+	2	0
aa	72	3,451.1**	11	11	218.8**	2	0
dev	156	986.2**	7	4	168.0+	2	1
RC	420	491.8	0	0	128.6	0	0
Residual	96	493.7			131.8		

+ and ** denote significance at the 5% and 1% level, respectively

Table 5. Relative variation attributable to genetic effects

Effect	% of total variation among crosses	
	Larval wt	Pupal wt
*g (autosomal additive)	33.99	44.32
L (sex-linked additive)	4.91	4.12
*s (autosomal heterosis)	5.12	3.71
LL (sex-linked heterosis)	3.00	1.34
m (general maternal)	10.39	15.50
r (specific maternal)	2.61	4.46
aa (A×A epistasis)	24.69	9.97
dev (higher order epistasis and/or linkage)	15.29	16.58
Total	100.00	100.00

If epistasis were negligible, the *g effects would be primarily additive and intraline dominance effects, and the *s effects would be primarily heterotic effects as a result of the cross of two lines. Also, the m effects would be due to differences between a line entering as a paternal or maternal parent in a cross. And, the reciprocal effects not accounted for by m effects would be included in the r effects.

The results of this study indicate that the epistatic effects were declared significant in many instances. Consequently, mean squares and statistical tests for *g, and *s may be biased because our definition of these effects includes an epistatic component that does not sum to zero over all lines. Nevertheless such comparisons are indicative of the genetic structure of these traits in the base populations. The effects in the model are sums of an unknown number of genes and may be close to zero due to cancellation when averaging both positive and negative effects.

For both larval and pupal weight, the general combining ability was highly significant ($P < 0.01$) in all twelve sets whereas specific combining ability was relatively much more important in larval weight (*g effects were 4.5 times greater than *s for larval weight and 8 times for pupal weight). Relatively, additivity was higher for pupal weight (44.32 vs. 33.99) whereas heterosis was more important for larval weight (5.12 vs. 3.41), reflecting the nonadditive nature of larval weight (Bell 1969).

Sex-linked effects were relatively unimportant for pupal weight, but this type of gene action was a significant source of genetic variation for larval weight. These findings could have some practical implication in connection with the correlated response to selection of one of the traits when selection is based on only one sex.

Maternal effects were important for both traits. Larval weight showed more highly significant sets but the relative measure of this genetic effect (Table 5) was a little higher for pupal weight, implying that maternal effects present in early stages of development still are important for pupal weight. Bondari et al. (1978), using the estimation procedure suggested by Willham (1963), found specific maternal effects to be very small for both traits. However, they observed only 2000 offspring for which the estimates had large standard errors as found by the analysis of Thompson (1976).

Epistasis in our study seems to be more important for larval weight than for pupal weight. Eleven out of twelve sets showed highly significant additive by additive epistasis in larval weight and seven of them were significant ($P < 0.05$) for deviations due to higher order epistasis. Furthermore, the relative importance of additive × additive epistasis for larval weight was

2.5 times higher than for pupal weight. Values were very similar in both traits for deviations due to higher order epistasis and/or linkage.

Using the triple-test cross analysis of Kearsey and Jinks (1968), epistatic gene effects for *Tribolium* pupal and larval weights were observed by Goodwill and Walker (1974) and Goodwill (1975). In a later study (Goodwill and Walker 1978), they did not find significant epistasis for larval weight in any of the eight lines observed. However, the lack of significance in their studies was really due to either significant interactions between replicates and tester lines, as they indicated, or to the small number of degrees of freedom associated with the analysis (1 and 16 for the F test). This problem of low power analysis when investigating epistasis was pointed out by Stuber and Moll (1971). They indicated that most studies have utilized small numbers of lines which were not representative of any random population or variety. That may account for the contradictory results found in the literature regarding the importance of epistasis. Obviously, a large sample of lines from each population is essential in order to make general inferences regarding epistatic effects. Concern on this point led us to include twelve sets of four inbred lines and two replicates per population.

It should be pointed out that epistasis estimated by the triple-test cross method is the within-line epistasis due to segregating alleles which are different in the two tester lines and cannot cope with the same allele showing different dominance properties in different populations. As Kearsey and Jinks (1968) indicate, such situations would almost certainly be detected as epistasis but it would not in fact be a type of epistasis found within a population. Crosses between populations exhibit a kind of non-additive variation not present within a population and this type of epistatic action is what we are estimating in the present paper in contrast to the intra-line estimate of Goodwill and Walker. Hence, our model estimates a different kind of epistasis than that of Kearsey and Jinks. Furthermore, it breaks down epistasis to the additive × additive component and allows indirect estimation of higher order epistasis. In addition, maternal and sex-linked effects can be investigated and their contributions evaluated. On the other hand, our method has the drawback that when many lines are to be investigated the number of three-way crosses increases very rapidly.

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