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Epistatic Contributions to Quantitative Traits in *Tribolium castaneum*
I. Traits not Closely Related to Fitness*

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Summary. Triple-testcross experiments were used to analyze epistatic contributions to larva weight, pupa weight, pupa width and adult weight in *Tribolium castaneum*. Seven diverse inbred lines and the F_1 produced by crossing the two tester lines were examined for indications of epistasis. Larva weight was the only trait for which no significant epistasis was detected. There was significant epistasis for pupa weight in three of the inbred lines; for pupa width in four of the inbred lines; for adult weight in five of the inbred lines. Only one inbred line and the F_1 line failed to exhibit significant epistasis for any trait. Each inbred line had a unique pattern of epistasis, suggesting that a number of different loci were contributing to the detected epistasis.

Key words: *Tribolium* - Epistasis - Fitness - Weight

Introduction

An assumption basic to the analysis of a quantitative character is that phenotypic variation can be partitioned into components reflecting the magnitude of the environmental effects, additive genetic effects and deviations from additivity resulting from dominance or epistasis. The theory for this partitioning is now well developed, and in general, it has provided a satisfactory basis for predicting short-term response to selection of for making decisions about breeding programs.

However, the quantitative geneticist has a special interest in learning whether non-allelic interactions (epistasis) are present and important. This interest relates to the utility of the theory which has been developed, ignoring epistasis for the sake of easier derivation. The usual methods of partitioning phenotypic variation often maximize the additive genetic component (Jana 1971, Jana 1972, Barker 1974). These methods then are not satisfactorx for describing the nature of the genetic variation.

Various methods for detecting epistasis have been developed. Barker (1974) pointed out that results using these methods have not been very encouraging relative to determining the importance of epistasis. In recent years, data have been accumulating which indicate epistasis is a common phenomenon in many plant species. But even this kind of information is limited for animal species. Jana (1971; 1972) and Barker (1974) have suggested that non-additive genes may be more important than previously recognized by quantitative geneticists.

It has been reported previously (Goodwill, 1975) that epistasis made significant contributions to the variation of pupa weight in certain populations of *Tribolium castaneum*. The present study was designed with two major objectives: 1) to determine, for a number of quantitative traits in *Tribolium castaneum*, whether there was a significant amount of epistasis and 2) if epistasis was found, to localize the source of epistasis of specific inbred lines. Eight different characters in *Tribolium castaneum* were examined. In this paper, only the data for four characters, (larva weight, pupa weight, pupa width, and adult weight) which are not generally considered components of fitness, will be reported. The four remaining characters which are more closely related to fitness will be discussed in a subsequent paper.

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Table 1. Origins, generations of inbreeding, and contributors of the inbred lines

Line	No. Generations Full Sib Mating	Origin	Contributor
E1	38	Edinburgh	Boylan (Minn.)
E2	37	Edinburgh	Boylan (Minn.)
PE	121	Chicago	Bell (Purdue)
PK	99	Kansas	Bell (Purdue)
PM	67	McGill (Canada)	Bell (Purdue)
PV	46	Virginia	Bell (Purdue)
CSI-10*	60	California	Enfield (Minn.)

* Hereafter known as CS

Experimental Methods

Origin and Characterization of Strains

Seven highly inbred lines of *Tribolium castaneum* with diverse geographical origins were obtained for this study (Table 1). After receipt, these strains were maintained separately by mass matings. An experiment, to characterize these inbred lines, was conducted with 12 single pair matings from each line in each of 2 replications. After a 24-hour mating period, each mating pair was put into fresh media for 3 consecutive 24 hour egg-collection periods. The following metric traits were measured in the resulting progeny; egg hatchability, 13-day-larva weight, 21-day-pupa weight, and 35-day adult weight. Hatchability was measured as the percentage of eggs which hatched. Ages were determined by counting the day of egg-collection as day zero.

Culturing Conditions and Equipment

All populations were maintained in a growth chamber with regulation of temperature to 31 ± 1°C and relative humidity to 65 ± 5%. The lines were kept in stoppered half-pint milk bottles, containing an ample supply of standard media (95% whole wheat flour and 5% dried brewers yeast). Mass matings or treatment groupings of progeny were kept in 100 ml. tri-pour beakers. Eggs were collected with a 60-mesh screen and placed in plastic petri dishes which had been painted with a flat black paint to reduce static electricity and make the eggs more visible. Weighings were made on a Cahn electrogram balance. A Zeiss particle size analyzer TGZe was used to measure pupa width from enlarged (6X) photographs.

Triple-testcross Experiments

A group of approximately 50 males was collected from each inbred line and the F_1 line from the cross of E2 × PM. Each of these eight groups of males was mass-mated to a set of three tester-line (E2, PM, F_1) females. There were 100 females per tester-line in each set. This procedure was replicated twice. The females remained in egg laying for 24 hours and 10 groups of 20 eggs were collected from each of the mating types. Eggs were observed daily for hatching and all larvae were removed after each observation. Individuals from the same line × tester cross, hatch day and replication were maintained in separate treatment groupings throughout the study. On the 13th day following eggcollection, the larvae within each treatment grouping were weighed in groups not exceeding five. On the day of pupation, all pupae within a treatment grouping were separated by sex and weighed in groups not exceeding 5. The sexes were separated within treatment groups for the remainder of the study. After weighing, the pupae were placed ventral side up on a piece of graph paper and photographed in groups of 40 or 50. These photographs were enlarged (6X), and the width of the abdominal segment just posterior to the wing structure was measured with a particle size analyzer. Adults were weighed on day 35 in groups not exceeding five within each treatment grouping.

Statistical Analysis

Each of the four traits was adjusted for the effects of different day of hatch. In addition, pupa weight and pupa width were adjusted for the effect of age at pupation. Analyses of variance were performed on the data for pupa weight, pupa width, and adult weight using the following model:

$$Y_{ijkno} = u + L_i + T(L_i)_j + R_k + S_n + (LR)_{ik} + (LS)_{in} + \\ + T(L_i)R_{jk} + T(L_i)S_{jn} + (RS)_{kn} + (LRS)_{ikn} + \\ + T(L_i)RS_{jkn} + E_{ijkno}.$$

where Y_{ijkno} is the measurement of a trait on the o^{th} individual, E_{ijkno} is the random error associated with this measurement and u is the overall mean of the trait. The effects due to replications (R_k) were considered random. While the effects due to male lines (L_i), sex (S_n) and tester lines within males lines $T(L_i)_j$ were considered fixed. The same model was used for larva weight except the effect of sex and any associated interaction was deleted. Due to the unequal subclass numbers the data were analyzed as described by Goodwill, 1975.

Results and Discussion

Characterization of Inbred Lines

The means with standard errors for the seven inbred lines with respect to % hatchability, 13-day-larva weight, 21-day-pupa weight and adult weight are given in Table 2. Owing to late pupation there were no 21-day female pupa in the PM line. The E2 line had the highest weights and % hatchability. Therefore, it was chosen as high tester line for the triple-testcross experiments. The PM line had the lowest % hatchability, very low larva and pupa weights and was the slowest to pupate. Therefore, the PM line was chosen to be the low tester parent.

Table 2. Means and standard errors of the seven inbred lines, with respect to % hatchability, 13-day-larva weight, 21-day-pupa weight, and adult weight. All weights are in micrograms

Line	% Hatchability	Larva Weight	Pupa Weight		Adult Weight
			Males	Females	
E2	82 ± 2	948 ± 43	2570 ± 15	2751 ± 19	2194 ± 15
PV	47 ± 2	545 ± 18	2279 ± 47	2407 ± 55	1959 ± 41
CS	54 ± 3	489 ± 30	2245 ± 76	2527 ± 90	1989 ± 34
E1	31 ± 2	510 ± 33	1658 ± 70	1860 ± 72	1713 ± 54
PE	78 ± 3	824 ± 31	2378 ± 40	2534 ± 30	2123 ± 16
PM	11 ± 2	485 ± 40	2278 ± 43		2124 ± 64
PK	45 ± 3	355 ± 16	2033 ± 17	2425 ± 125	1861 ± 26

Genetic Interpretation of the Triple-testcross

The genetic interpretations of data from a triple-testcross experiment have been discussed by Kearsey and Jinks (1968), Jinks et al. (1969) and Jinks and Perkings (1970). This experimental design is an extension of the North Carolina Design III (Comstock and Robinson, 1952). Table 3 presents the degrees of freedom and expected mean squares for the pertinent sources of variation. As in the case of the North Carolina Design III, the variance component due to differences among male lines (σ^2_L) estimates 1/8 the additive genetic variance. The variance component due to differences among testers within male lines estimates non-additive genetic variance. Two contrasts among the tester lines (linear and quadratic) can be constructed for each male line from the tester-within-lines sum of squares. The linear contrast (E2-PM) provides an estimate of 1/4 the dominance variance. This is identical to the σ^2_{ml} in the North Carolina Design III (Jinks et al., 1969). Both σ^2_L and σ^2_{ml} may be biased by epistasis (Comstock and Robinson, 1952; Enfield et al., 1969, Jinks et al., 1969). The quadratic contrast among testers (E2-2F_1 + PM) in this triple testcross can detect the presence of epistasis. However the proportion of the epistatic variance present (σ^2_i) in this contrast is dependent recombination rates among the loci (Walker, 1974).

Table 3. The expected mean squares of the sources of variation pertinent to the genetic interpretation of the triple testcross

Source	df	Expected mean squares
Lines	7	$\sigma^2_e + 6\sigma^2_{LR} + 12\sigma^2_L$
Tester (lines)	16	$\sigma^2_e + 2\sigma^2_{T(L)R} + 4\sigma^2_{T(L)}$
Lines × Rep.	7	$\sigma^2_e + 6\sigma^2_{LR}$
Tester (line) × Rep.	16	$\sigma^2_e + 2\sigma^2_{T(L)R}$
Error	a	σ^2_e

a - Error degrees of freedom for pupa weight, pupa width, adult weight and larva weight were 979, 3683, 767, and 844, respectively

Results of the Triple-testcross Experiments

The least square means for the four traits in each of the line × tester crosses are presented in Tables 4 and 5. The analyses of variance are summarized in Table 6.

There were highly significant differences between the sexes for pupa weight, pupa width and adult weight.

Table 4. Least squares means for larva weight (L) and pupa weight (P) measured in micrograms for each line × tester combination

Trait	Tester	PK	PM	PV	F_1	E2	E1	Cs	PE
L	E2	1267	1183	1203	1037	974	1016	1230	1265
	PM	1204	585	1061	780	1060	1041	1215	1262
	F_1	1218	925	1174	869	921	1090	1222	1169
P ♂	E2	2601	2683	2680	2628	2591	2502	2725	2645
	PM	2507	2321	2511	2503	2718	2543	2732	2549
	F_1	2404	2688	2462	2517	2617	2512	2756	2623
P ♀	E2	2617	2796	2847	2705	2638	2588	2902	2948
	PM	2583	2484	2816	2569	2826	2750	2938	2855
	F_1	2492	2519	2673	2607	2610	2582	2944	2854

Standard erros of means were on the order of 24 and 39 µg., for larva weights and pupa weights, respectively

Table 5. Least squares means for pupa width (PW) expressed in mm. × 10^3 and adult weight expressed in micrograms (μg.) for each line × tester combination

Trait	Tester	PK	PM	PV	F_1	E2	E1	Cs	PE
PW	E2	1119	1132	1137	1112	1120	1090	1132	1113
♂	PM	1102	1053	1116	1096	1125	1105	1107	1102
	F_1	1081	1112	1088	1110	1113	1094	1112	1117
PW	E2	1126	1175	1152	1132	1131	1109	1131	1129
♀	PM	1138	1065	1134	1097	1159	1128	1146	1124
	F_1	1010	1154	1120	1117	1126	1102	1146	1119
A	E2	2088	2180	2180	2156	2078	2070	2156	1057
♂	PM	2025	1915	2091	2003	2137	2085	2091	1984
	F_1	2013	2212	2089	2069	2075	2031	2139	2128
A	E2	2110	2154	2199	2221	1933	2068	2337	2251
♀	PM	2134	1789	2264	2168	2142	2198	2376	2253
	F_1	2098	1993	2252	2234	1988	2027	2338	2298

Standard errors of the means were on the order of 7 mm. × 10^3 for pupa width and 25 μg. for adult weight

The females were heavier and wider than males. In addition, replications and a few of the interactions involving replications or sex were significant. Lines and testers within lines were significant sources of variation for each trait. This is an indication that both additive and non-additive gene action may be present.

The pertinent mean squares with the linear and quadratic contrasts for each line are presented in Table 7. The error term was used to test the linear and quadratic contrasts whenever the tester (line) × rep interaction was not significant (P = .05).

In the case of larva weight, the non-additive gene effects were due to the presence of dominance (significant linear contrasts) in the PM, PV and F_1 lines. Significant epistatic effects are indicated for pupa weight in the PK, PV and E2 lines; for pupa width in the PK, PM, PV and E2 lines; for adult weight in the PM, PV, E2 and E1 lines.

The detected epistatic effects for these traits are given in Table 8. These effects were calculated from the mean squares of the quadratic comparisons (Table 7) by use of the formula for orthogonal comparison mean squares (Snedecor and Cochran, 1968). The signs of the epistasis estimates were determined by using the means of the testcross progeny in the linear function: E2 + PM - 2(F_1). These two methods are equivalent when there are equal numbers contributing to each mean in the contrast. The three significant epistatic effects for pupa weight were all positive. However for pupa width and adult weight, there are both positive and negative significant epistatic effects. In the case of pupa width, the negative and positive effects cancel each other in the analysis over all lines. While additive genetic variance and dominance variance may be estimated, in those instances where epistasis is present these estimates will be biased.

Conclusions

Each of the inbred lines had a unique pattern of epistasis (Table 8). Only the CS line failed to exhibit epistasis for any of the traits. In addition, there were both

Table 6. The summary of analysis of variance for larva weight, pupa weight, pupa width and adult weight

Source	df	Larva weight	Pupa weight	Pupa width	Adult weight
Lines	7	114,238**	123,010**	51.48*	59,579*
Tester (lines)	16	30,152**	28,627**	5.88**	22,639**
Replications (rep)	1	65,860**	83,722*	18.87**	218,218**
Sex	1		368,901**	44.76**	141,297**
Lines × rep	7	4,532**	8,518*	9.24**	10,081**
Lines × sex	7		28,616**	13.21**	47,312**
Tester (line) × rep	16	3,682*	5,933	1.95**	2,194
Tester (line) × sex	16		6,681	3.00	5,957
Rep × sex	1		10,437	0.63	3,614
Line × rep × sex	7		3,820	0.89	3,423
Tester (line) × rep × sex	16		5,075	1.33**	5,497**
Error	9	1,407	3,812	0.77	1,641

a - Error degrees of freedom for pupa weight, pupa width, adult weight and larva weight were 979, 3683, 767, and 844, respectively
* - Denotes significance at .05 level
** - Denotes significance at .01 level

Table 7. Pertinent mean squares of the nested analyses for larva, pupa, and adult weight, and pupa width

Source[a]		df	Larva Weight	Pupa Weight	Pupa Width	Adult Weight
Tester (Line)		16	30,152**	28,627**	6.88**	22,639**
Tester (PK)	(L)	1	4,096	4,950	0.32	200
	(Q)	1	867	48,510**	19.06**	4,704
Tester (PM)	(L)	1	337,561**	168,200**	20.07**	199,712**
	(Q)	1	15,408	4,988	15.96*	40,344**
Tester (PV)	(L)	1	19,460*	18,625*	12.65*	392
	(Q)	1	2,380	43,011**	11.45*	9,126*
Tester (F_1)	(L)	1	65,792**	32,513**	5.49	22,155**
	(Q)	1	1,752	4,108	0.32	247
Tester (E2)	(L)	1	7,310	54,615**	6.02	34,322**
	(Q)	1	13,267	24,130*	7.59*	5,891*
Tester (E1)	(L)	1	100	22,685*	4.93	11,250**
	(Q)	1	1,680	10,004	1.26	15,403**
Tester (CS)	(L)	1	441	465	0.01	181
	(Q)	1	3	2,420	0.03	43
Tester (PE)	(L)	1	25	18,625*	4.91	3,003
	(Q)	1	12,288	182	0.01	15,251**
Rep X Tester (Line)		16	3,682*	5,933	1.95*	2,194
Error		a	1,407	3,812	0.77	1,641

* Denotes significance at the .05 level
** Denotes significance at the .01 level
[a] Line, rep, and sex effects, and error degrees of freedom are reported in Table 6

positive and negative epistatic effects for each of the traits. Though only the positive epistatic effects were significant for pupa weight. These observations suggest that more than just a few loci were participating in epistatic interactions.

Three of the four traits examined in this study were affected significantly by epistasis. Only larva weight appeared to be free of epistatic interactions. However, the significant interactions between replications and lines severely weakened the test for epistasis for this trait. Additional investigations may revela that epistasis makes a significant contribution to larva weight, also. Admittedly, the four traits examined in this study are somewhat related. However, the data do suggest that a significant amount of epistasis is not an unusual phenomenon in Tribolium. If this is true for animals in general then perhaps more consideration should be given to utilization of epistatic genetic variation in breeding programs. This type of genetic variation may be of particular importance in populations nearing selection plateaus or for traits with low heritability such as traits related to reproductive fitness.

Table 8. Detected epistatic effects, within lines, for larva, pupa, and adult weight, and pupa width

Line	Larva Weight (μg.)	Pupa Weight (μg.)	Pupa Width (mm. $\times 10^3$)	Adult Weight (μg.)
PK	25.50	134.88**	2.76**	42.00
PM	-107.50	-43.25	-2.45*	-123.00**
PV	-42.25	127.01**	2.07*	-58.50*
F_1	36.25	39.25	-0.35	-9.62
E2	99.75	95.13*	1.69*	47.00*
E1	-35.50	61.25	0.69	76.00**
CS	1.50	-30.13	-0.10	4.02
PE	96.00	8.26	-0.07	-75.62**

* Denotes significance at the .01 level
** Denotes significance at the .05 level

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