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Epistatic Contributions to Quantitative Traits in *Tribolium castaneum*
II. Traits Closely Related to Fitness

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Summary. Triple-testcross experiments were used to analyze epistatic contributions to % hatchability of eggs, age of pupation, number of eggs laid in 24-hour period, and survival from hatching to day 35. Seven diverse inbred lines and the F_1 produced by crossing the two tester lines were examined for the presence of epistasis. There was evidence of epistasis for each of the 4 traits in at least one of the 8 lines tested. Epistasis was a major source of variation in survival in all of the lines tested.

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

Introduction

Goodwill and Walker (1977) presented evidence for epistasis contributing to larva weight, pupa weight, pupa width and adult weight in *Tribolium castaneum*. The presence of epistasis among the genes determining a trait introduces a bias in the estimates of additive genetic variance, dominance variance and consequently estimates of heritability. If heritability is quite low, as it often is for traits associated with fitness, then this bias could be particularly important in a breeding program when a population is approaching a selection plateau because additive genetic variance is nearly exhausted.

The 4 traits examined in the first paper of this series (Goodwill and Walker, 1977) are undoubtedly associated with fitness to some degree, but they are not generally considered components of fitness. In this paper, 4 traits which are components of fitness are analyzed for the presence of epistasis. These traits are: % hatchability of eggs, days to pupation, survival from hatching to day 35, and the number of eggs laid in 24 hours.

Experimental Methods

Triple-Testcross Experiment

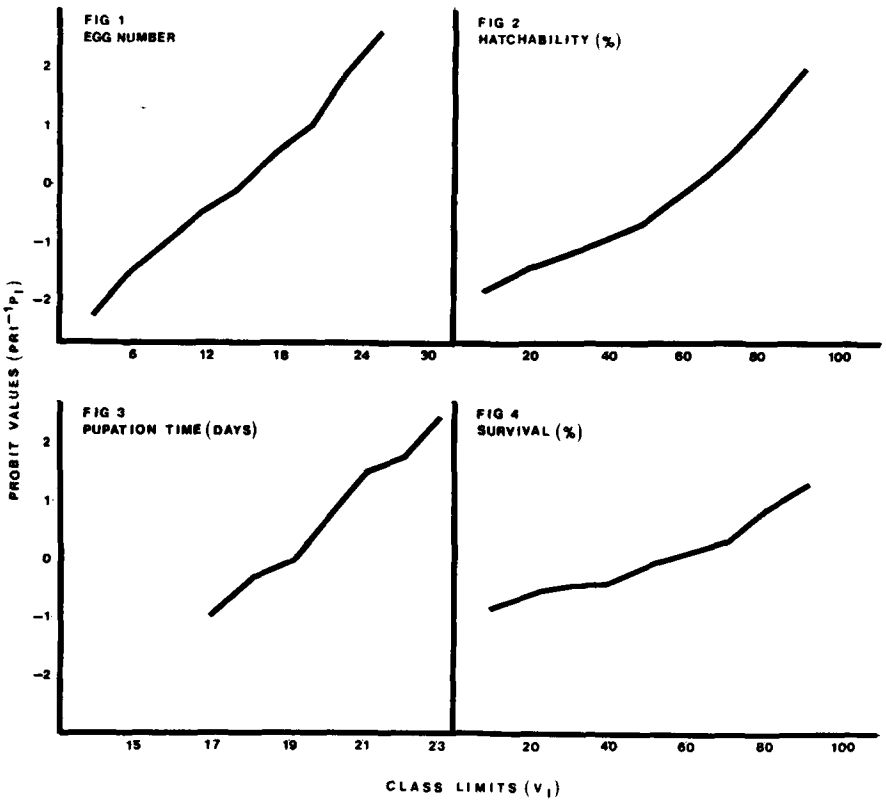
A detailed description of the procedures and design of the triple-testcross experiments was presented by Goodwill and Walker, 1977. Only the details unique to the study of the 4 traits to be discussed in this paper will be specified here.

Males from 8 lines (E2, E1, PE, PK, PV, CS, PM and F_1) were each mated to 3 tester-lines of females (E2, PM and F_1). The F_1 line is the progeny produced by crossing E2 and PM lines. Following mating, the females were placed into egglaying for 24 hours. Ten groups of 20 eggs were collected for each of the 24 mating types. These procedures were completely replicated twice. All eggs were examined daily for hatching. Hatchability was measured as the percentage of eggs which hatched by day 5 (day of egg-collection was day 0). On the day of hatching, larvae from the same line × tester cross, hatch day and replication were placed in treatment groupings. Larva were checked daily for pupation. On the day of pupation, pupae were sexed and stored separately by sex. Pupation time was measured as the days from egg-collection until pupation. Survival was measured on the treatmentgroups hatching on day 4 (the majority of eggs hatched on day 4), as the percentage of those hatching that were still living, as adults on day 35.

The number of eggs laid in a 24-hour period was considered an aspect of the female's phenotype. Time and facilities made it impossible to observe this trait for all 24 types of females thus 20 mature females (adults for at least 14 days) from each line × tester combination for the E2, PM, PV and F_1 male lines were mass mated to 10 males of the E2 line. Males from the most fertile inbred line (E2) were used with all females to equalize sire effects. After a 24-hour mating period, each female was placed in a separate beaker for 3 consecutive 24-hour egglaying periods, and the number of eggs laid in each period was recorded. These procedures were replicated twice.

Statistical Analasis

Both survival and hatchability were measured as percentages. Egg number and age of pupation are traits



Figs. 1-4. The plots of probit values versus class limits for (Fig. 1) egg number, (Fig. 2) % hatchability, (Fig. 3) pupation time in days and (Fig. 4) % survival

which may have a threshold. Thus, all 4 traits may not approach a normal distribution on the scale of measurement. This possibility was examined using procedures described by Wright (1969). First, a probit analysis for class limit percentiles war performed for each trait. In this procedure, a normal distribution is indicated by linearity in the plot of probit values ($pri^{-1}p_i$) versus class limits (V_i). In the probit, P_i is the probability of values lower than X_i, where X_i is the class limit (V_i) expressed in the form of a standard normal deviate. Values of $pri^{-1}p_i$ were obtained from a table of areas under a normal curve (Arkin and Colton, 1963). In addition, the means and standard deviations were calculated for each group of measurements which had male lines, female testers and replications in common. Then, the standard deviations were regressed upon the means for each trait to test for a linear relationship between the mean and standard deviation.

Pupation time was adjusted for day of hatch, and analysis of variance was performed on the data for each trait as described by Goodwill, 1975. The linear models for the analyses of variance along with the construction and genetic interpretations of the linear and quadratic contrasts among testers within male lines was described by Goodwill and Walker (1977). In the analysis of egg number each egg-collection period was considered a replication.

Results and Discussion

Checks for Normal Distribution

The graphs of the probits ($pri^{-1}p_i$) versus class limits (V_i) for the distributions of egg number, hatchability, pupation time and survival are presented in figures 1-4, respectively. Wright (1969) explains that if a linear relationship is exhibited between probit values and class limits, a normal distribution is indicated. He also states that little weight should be put on deviations from linearity where $pri^{-1}p_i$ exceeds 2.6 in absolute value, unless numbers are very large. A visual examination of the plots between probit values of -2.6 and 2.6 indicates a linear relationship is present for all traits. If a linear relationship between probit values and class limits does not exist, the mean and standard deviation of a trait will have a linear relationship and the trait will not have a nor-

Table 1. Least squares means for % hatchability (HB), pupation time in days (PT), % survival (S) and egg number (E) for each line × tester combination for the PM, PV, F_1 and E2 lines

Trait	Tester	Line PM	PV	F_1	E2
HB	E2	34.50	55.25	82.00	78.75
	PM	22.00	49.25	65.25	61.18
	F_1	14.75	52.50	65.00	82.25
PT ♂	E2	18.93	18.71	19.43	20.08
	PM	21.00	18.71	20.62	19.42
	F_1	20.75	18.60	19.73	19.93
PT ♀	E2	19.33	18.41	19.35	20.00
	PM	21.00	19.00	19.94	19.52
	F_1	20.50	18.37	20.00	19.98
S	E2	63.38	72.79	84.00	89.47
	PM	62.50	77.78	89.44	90.98
	F_1	30.40	24.73	46.95	58.74
E	E2	15.52	18.25	14.63	13.16
	PM	10.65	19.22	14.52	15.52
	F_1	14.52	16.93	14.38	14.63

Standard errors of the means were approximately 2.8%, 0.11 days, 3.4% and 1.02 for hatchability, pupation time, survival and egg number, respectively

Table 2. Least squares means for % hatchability (HB), pupation time in days (PT) and % survival (S) for each line × tester combination for the PK, E1, CS and PE lines

Trait	Tester	Line PK	E1	CS	PE
HB	E2	65.75	76.75	70.75	72.50
	PM	52.50	65.69	63.24	63.86
	F_1	55.00	72.00	69.75	70.75
PT ♂	E2	18.27	19.61	18.81	18.15
	PM	18.75	19.50	18.96	18.12
	F_1	18.80	19.48	18.71	18.79
PT ♀	E2	18.20	19.43	18.97	18.28
	PM	19.05	19.28	19.36	18.75
	F_1	18.50	19.84	19.13	18.69
S	E2	86.74	84.41	79.66	89.50
	PM	85.92	84.76	83.08	87.44
	F_1	18.39	45.79	58.54	37.60

Standard errors of the means were approximately 2.8%, 0.11 days and 3.4% for hatchability, pupation time and survival, respectively

mal distribution. Another check for the normal distribution, then, is to regress standard deviations on means for measured groups. The appropriate regression coefficients for hatchability, survival, egg number and pupation time were 0.00, 0.09, -0.03 and 0.04, respectively. None of these regression coefficients were significantly different from zero (P=.05). Therefore, it was concluded that all of these traits were normally distributed.

Triple-Testcross Experiments

The least-squares means in each of the line × tester crosses are presented for all four traits in tables 1 and 2. The analysis of variance for hatchability, pupation time and survival are summarized in table 3. The summary for the analysis variance for egg number is summarized in table 5. The sources of variation of particular interest in tables 3 and 5 are Lines and Tester (lines). The first one (Lines) contains a component of variance resulting from additive gene action while the latter (Tester (lines)) contains the variance components due to non-additive gene action. Both of these sources of variation are significant for all four traits. If epistasis is present the component due to additive gene action may be biased. The linear and quadratic contrasts among testers within each line (E2-PM and E2-$2F_1$ + PM, respectively) are presented for hatchability, pupation time and survival in table 4 and for egg number in table 6.

Table 3. The summary of the analysis of variance for pupation time, hatchability and survival

Source	df	Pupation time	Hatchability	Survival
Lines	7	11.02**	1,672**	505.29**
Tester(Lines)	16	2.16**	114*	1,289.37**
Replications (Rep)	1	6.21**	271**	0.64
Lines × rep	7	0.16	105**	63.63*
Tester(Lines) × rep	16	0.10	44**	22.89
Error	a	0.07	13	-

a Error degrees of freedom for hatchability and pupation time were 423 and 4049, respectively. Since survival was not measured on individuals the tester(lines) × rep is the error term for this trait

*,** Denote significance at .05 and .01 level, respectively

Table 4. Pertinent mean squares with the linear and quadratic contrasts among testers within male lines for hatchability, pupation time and survival

Source	d.f.	Hatchability	Pupation Time	Survival
Tester(Line)	16	114.60*	2.16**	1,289.37**
Tester(PK) (L)	1	175.56*	1.35**	0.67
(Q)	1	22.69	0.01	6,154.46**
Tester(PM) (L)	1	156.25	25.53**	0.77
(Q)	1	243.00*	0.07	1,411.80**
Tester(PV) (L)	1	36.00	0.83**	24.90
(Q)	1	0.08	0.15	3,407.74**
Tester(F_1) (L)	1	280.56*	4.79**	29.59
(Q)	1	99.19	0.00	2,108.87**
Tester(E2) (L)	1	318.62**	0.67**	2.28
(Q)	1	205.84*	0.38*	1,321.74**
Tester(E1) (L)	1	140.30	0.81**	0.12
(Q)	1	1.83	0.13	2,006.74**
Tester(CS) (L)	1	61.70	0.02	11.70
(Q)	1	13.46	0.09	649.95**
Tester(PE) (L)	1	70.56	0.07	4.24
(Q)	1	8.00	0.43*	3,450.34**
Rep × Tester (Line)	16	44.27**	0.10	22.89
Error	a	13.16	0.07	-

* Denotes significance at the .05 level
** Denotes significance at the .01 level
a Error degrees of freedom were reported in table 3

Table 5. The summary of the analysis of variance for egg number

Source	df	M.S.
Replications	5	101.04**
Lines	3	74.03**
Tester(Lines)	8	14.02**
Lines × Rep	15	2.29
Rep × Tester(Line)	40	2.24
Error	1160	2.25

** Denotes significance at .01 level

Table 6. Pertinent mean squares with the linear and quadratic among testers within male lines for egg number

Source	df	M.S.
Tester(Line)	8	14.02**
Tester(PM) (L)	1	71.05**
(Q)	1	8.22
Tester(PV) (L)	1	2.80
(Q)	1	12.96**
Tester(F_1) (L)	1	0.04
(Q)	1	0.15
Tester(E2) (L)	1	16.57**
(Q)	1	0.34
Rep × Tester(Line)	40	2.24
Error	1160	2.25

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

Hatchability

In the analysis of hatchability, the Rep × Tester (line) was significant and therefore used to test the significance of the linear and quadratic contrasts. In the PK and F_1 lines only the linear contrasts were significant. This suggests the presence of dominance but no evidence for epistasis in these lines. In the PM and E2 lines the quadratic contrasts are significant, indicating the presence of a significant epistatic component. In addition, the linear contrast is significant for the E2 line but this may be biased by epistasis. Neither contrast was significant for the PE, CS, E1 and PV lines.

Pupation Time

In the analysis of pupation time the error term was used to test the linear and quadratic contrasts since the Rep × Tester (lines) interactions were not significant. Only the linear contrasts were significant for the PK, PM, PV, F_1 and E1 lines. The quadratic contrasts were significant for the PE and E2 lines while neither contrast was significant for the CS line. Thus, there are indications of dominance in 5 of the lines, while epistasis is indicated in 2 lines for pupation time.

Survival

In the analysis of survival, the quadratic contrasts are significant for all the lines. Indeed the quadratic contrasts account for most of the tester (line) source of variation. Thus, for this trait, epistasis appears to be a very important source of variation.

Egg Number

There are indications of dominance among the genes affecting egg number in the PM and E2 lines. Epistasis is indicated only in the PV line.

Table 7. Detected epistatic effects, within lines, for hatchability, pupation time, survival, and egg number

Line	Hatch-ability	Pupation Time	Survival	Egg Number
PK	4.13	0.07	67.93**	
PM	13.50*	0.16	32.54**	-1.43
PV	-0.24	0.24	50.55**	1.80*
F_1	8.62	-0.03	39.77**	0.19
E2	-12.42*	-0.38*	31.48**	0.29
E1	-1.17	-0.22	38.79**	
CS	-3.18	0.19	22.83**	
PE	-2.45	-0.40*	50.87**	

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

The detected epistatic effects, calculated as described by Goodwill and Walker (1977), are presented for each trait and line in table 7. There are both positive and negative effects suggested for egg number, hatchability and pupation time, although only the positive effects for egg number and only the negative effects for pupation time are statistically significant. The detected epistasis for survival are all positive.

Discussion and Conclusions

In the previous paper (Goodwill and Walker, 1977) 4 traits not closely associated with fitness were examined for indications of epistasis. In this paper, 4 traits which might be considered components of fitness were examined in a similar manner. Overall, evidence for epistasis was observed for 7 of the 8 traits. In the one case where there was no evidence of epistasis, the tests for epistasis were weakened by the presence of significant Rep × Tester (line) interactions (Goodwill and Walker, 1977). Epistasis apparently is a common feature of the genes in *Tribolium castaneum*.

Among the 8 lines of *Tribolium* examined, the incidence of epistasis for each trait varied. The incidence of epistasis for hatchability, pupation time and egg number was about the same (2 out of 8 lines for the first 2 traits and 1 out of 4 lines for the latter). The incidence was somewhat greater for pupa weight (3 out of 8 lines), pupa width (4 out of 8 lines) and adult weight (5 out of 8 lines). Only in the case of survival, was there evidence for epistasis in all 8 lines. If these differences in the incidence of epistasis are real, they may reflect differences in complexity of the various traits and presumably the number of loci contributing to them.

The importance of this epistasis relative to other types of gene action is still an unanswered question. However, the data do indicate that this is a question that merits further investigation, particularly, if *Tribolium* is not unique among animal species with respect to the occurrence of epistasis. In an animal or plant breeding context, there are 2 issues that have a direct bearing on the relative importance of epistasis.

The first issue relates to the nature of the epistasis. For example, is it multiple peak epistasis? If multiple peak epistasis is important, the usual selection and breeding programs may not have the maximum limits possible. The data from these experiments do not address this issue. The second issue relates to the magnitude of the epistatic effects (variances) compared to additive or diminance effects. If epistatic effects are relatively large, a breeding program utilizing epistatic effects might be most efficient. Since the estimates of the variance components would be based on very few degrees of freedom in these experiments, it is hazardous to make comparisons among them. However, there was only one trait, survival, for which epistatic effects appeared approach the magnitude of the additive effects. In this instance it appears possible much of the genetic variation is due to epistasis.

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