NOTES AND COMMENTS

GENETIC ANALYSIS OF A POPULATION OF *TRIBOLIUM*. IV. GENE EXPRESSION MODIFIED BY CORN OIL AND RELATIVE HUMIDITY*

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1. Introduction

The phenotypic expression of a genotype may vary in heterogeneous environments. Indeed, this variable gene expression may yield information on the very nature of a biological system. In particular, Costantino, Bell and Rogler (1966, 1967, 1968) and Costantino, Mumma and Bruszewski (1970) have described a genotype in the flour beetle *Tribolium castaneum* whose phenotypic expression, growth pattern, has been quantitatively associated with the presence of the fatty acids oleic, linoleic and linolenic in the culturing medium. Our objective in this report is to characterise the impact of the environmental component relative humidity on the phenotypic expression of this unsaturated fatty acid sensitive mutant.

2. Materials and methods

Three populations of *T. castaneum* were used in this study: the Sensitive, genetically homozygous corn oil sensitive (cos/cos), the Purdue Foundation and Black Foundation populations. The history of the Sensitive population is presented in the earlier papers in this series. The Purdue strain is a wild-type population formed from a broad genetic base and maintained with no artificial selection and a minimum of inbreeding. The Black population is unrelated to the Purdue strain and is genetically marked with the partially dominant autosomal gene *black* (b). The latter two stocks were kindly provided by Professor A. E. Bell.

A completely randomised $3 \times 2 \times 2$ factorial design of experiment involving the three populations mentioned above, each cultured on diet 0 (percentage composition: wheat flour 90, dried brewers yeast 5, and glucose monohydrate 5) and diet 5 (percentage composition: wheat flour 90, dried brewers yeast 5, and corn oil 5) and maintained in controlled chambers at 61 ± 3 per cent. or 85 ± 3 per cent. relative humidity with a uniform temperature of $33 \pm 0{\cdot}25°$ C., was used. Relative humidity within each growth chamber was monitored with chamber air movement only.

Eggs from each population were collected and randomly distributed on the two diets and two levels of relative humidity. The incubators were sealed and not opened until 13 days later when 30 larvae were individually weighed for each treatment combination and then returned to their respective control chamber. At 16, 18, 20, 22 and 26 days after egg collection the distribution of larvae, pupae and adults was determined for each group.

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The above design of experiment was completely replicated and a total of 720 animals were evaluated.

3. Results and discussion

The statistical analysis of the 13-day larval weight data is summarised in table 1. All of the replication by main effect interactions were not

Table 1

Analysis of variance of 13-day larval weight

Source of variation	Degrees of freedom	Mean square	F-ratio
Relative humidity (RH)	1	184,757,020·9	108·1*
Oil (O)	1	60,610·0	< 1·0
Population (P)	2	30,311,041·5	17·7*
Replication	1	35,950,423·6	21·0*
RH × O	1	1,179,846·4	< 1·0
RH × P	2	490,956·1	< 1·0
O × P	2	11,552,762·2	6·8*
RH × O × P	2	4,380,495·3	2·6
Residual	707	1,709,163·0	

* Significant at the 0·05 level of probability.

statistically significant and were added to the residual source of variation. This basic estimate of error was used in all tests of significance. The main effects relative humidity, population and replication were statistically meaningful. Oil was not an important main effect. The oil by population interaction was significant. The three way interaction of relative humidity by oil by population was not statistically significant; however, the test was conservative and the F-ratio of 2·6 comparable to the critical value of 2·99. Each of the main effects can be viewed as a measure of the response of larval weight to changes, say, in relative humidity averaged over the three populations two levels of corn oil and two replications. Interaction is an additional effect due to the simultaneous influence of two or three factors.

The statistical analysis has identified and focused our attention on several factors, however, it is critical to further examine the data and note the

Table 2

Mean larval weight of three populations of T. castaneum *cultured on two levels of corn oil in chambers with different relative humidities*

Replication		Relative humidity 61 ± 3%		Relative humidity 85 ± 3%	
Dietary Oil		0%	5%	0%	5%
	Population:				
	Sensitive	701 ± 30*	236 ± 32	2235 ± 54	1141 ± 79
1	Black	1008 ± 60	1149 ± 69	1988 ± 56	2140 ± 82
	Purdue	1287 ± 80	1022 ± 58	2178 ± 88	2549 ± 53
	Sensitive	349 ± 26	159 ± 15	1205 ± 58	856 ± 64
2	Black	582 ± 34	670 ± 41	1269 ± 81	1735 ± 82
	Purdue	808 ± 53	904 ± 66	1453 ± 79	2282 ± 60

* Mean 13-day larval weight (μg.) ± standard error of mean; 30 observations per mean.

biological importance of the differential response of these populations to the experimental treatments. A listing of mean larval weights in both replications is thus presented in table 2. The Black and Purdue populations' responses were similar: larval weight was unaffected or even enhanced on the 5 per cent. corn oil diet as compared to diet 0 and larval weight increased approximately twofold in the 85 per cent. relative humidity environment. The Sensitive population's response was different: larval weight was markedly reduced on the 5 per cent. corn oil diet as compared to diet 0. This observation is consistent with our earlier phenotypic description of this mutant. The change in mean larval weight of the Sensitive population to relative humidity was qualitatively similar to the Black and Purdue populations, however, the magnitude of change is noteworthy. On diet 0 a threefold increase and on diet 5 a fivefold increase in mean larval weight was recorded in the 85 per cent. relative humidity environment.

To focus attention on the phenotypic expression of the cos/cos genotype as modified by corn oil and relative humidity the Sensitive population's mean larval weight in the diet 5, 61 per cent. relative humidity treatment combination was designated as the base phenotype, $\bar{w}$, and comparative phenotypic expressions were established (table 3). Interestingly, the

TABLE 3

Comparative phenotypic expression of the cos/cos genotype as measured by mean 13-day larval weight, $\bar{w}$

	Relative humidity	
Diet	61 ± 3%	85 ± 3%
0% oil	$3\bar{w}$	$9\bar{w}$
5% oil	$\bar{w}$	$5\bar{w}$

population's mean larval weight was greater in the presence of corn oil at high relative humidity, $5\bar{w}$, than it was in the absence of corn oil at low relative humidity, $3\bar{w}$. This generalisation was plausible for both replications and revealed the internal consistency of these experiments.

To see if the response of these populations to corn oil and relative humidity reflected a biologically meaningful change in their developmental growth patterns, the distribution of larvae, pupae and adults at 16, 18, 20, 22 and 26 days after egg collection was recorded (table 4). In general, the behaviour of the measured variable 13-day larval weight appears to be indicative of a real change in growth pattern and not merely increased incorporation of water. Twenty-six days after egg collection the animals in all treatment groups, with minor exceptions, were adults. However, the mutant genotype on diet 5 and 61 per cent. relative humidity (dotted line box) had no adults. In fact, in this group six animals were still larvae, 18 pupae and six had died, which is the only appreciable mortality noted in the entire experiment. Comparison of the remaining treatment combinations is similar to the larval weight data.

As a means of integrating the response of larval weight in the three populations to the two levels of corn oil and two levels of relative humidity the data from table 3, replication 1, are presented in fig. 1. The 13-day larval weight of an animal in the 61 ± 3 per cent. relative humidity environment is measured along the vertical axis and the 13-day larval weight of a

TABLE 4

Distribution of larvae, pupae and adults at 16, 18, 20, 22 and 26 days after egg collection

Dietary oil		0%					5%				
Day		16	18	20	22	26	16	18	20	22	26
	Population:										
R.H. 61±3%	Sensitive	29, 1, 0*	28, 1, 0	28, 1, 0	7, 22, 0	0, 10, 18	27, 0, 0	26, 0, 0	24, 0, 0	24, 0, 0	6, 18, 0
	Black	30, 0, 0	30, 0, 0	9, 21, 0	2, 28, 0	0, 4, 26	30, 0, 0	30, 0, 0	12, 18, 0	2, 28, 0	0, 2, 28
	Purdue	30, 0, 0	30, 0, 0	13, 17, 0	4, 26, 0	0, 3, 26	30, 0, 0	30, 0, 0	10, 20, 0	0, 30, 0	0, 0, 30
R.H. 85±3%	Sensitive	28, 2, 0	5, 25, 0	0, 30, 0	0, 6, 24	0, 0, 30	30, 0, 0	23, 7, 0	2, 28, 0	0, 27, 3	0, 0, 30
	Black	29, 1, 0	13, 17, 0	0, 30, 0	0, 18, 12	0, 0, 30	25, 5, 0	3, 27, 0	0, 26, 4	0, 8, 22	0, 0, 29
	Purdue	21, 9, 0	7, 23, 0	1, 25, 4	0, 14, 16	0, 3, 27	8, 22, 0	1, 29, 0	0, 11, 19	0, 2, 28	0, 0, 30

* Entry is number of : larvae, pupae, adults; Replication 2.

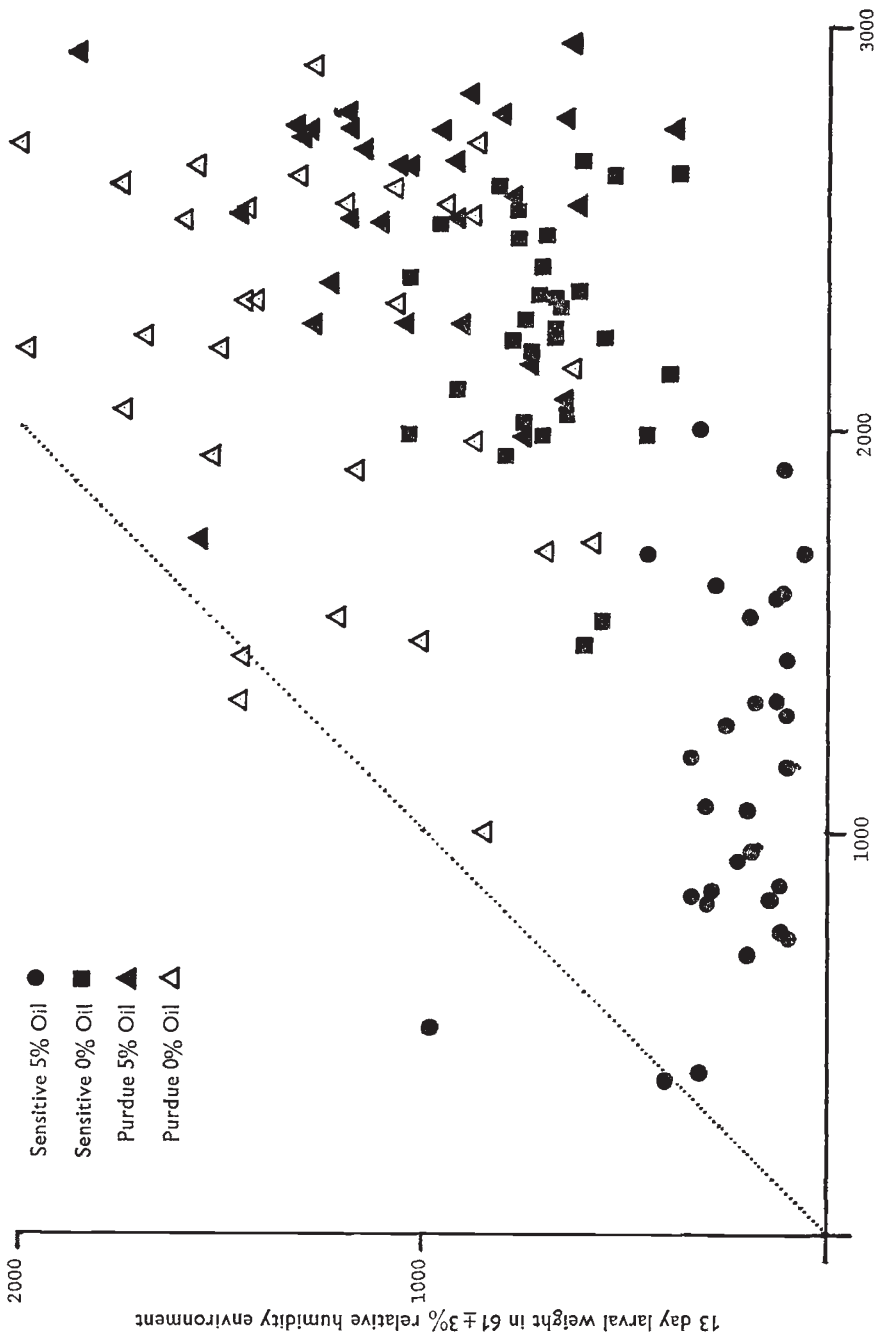

FIG. 1.—Visualisation of the larval weight data.

randomly chosen animal of the same population in the 85 ± 3 per cent. relative humidity environment is measured along the horizontal axis. This pairwise association of animals is somewhat artificial and hence no rigorous statistical analysis is imposed on this presentation but rather a visualisation of the data is offered. Each phenotype is then represented as a point in a two-dimensional relative humidity space and the patterns of points generated by population-oil treatment combinations can be compared. The effect of relative humidity is obvious from the figure, for in only four cases was an animal heavier in the low relative humidity environment than in the high. The Sensitive-5 per cent. oil combination is isolated in the lower central part of the graph and exhibited a unique response. The Sensitive-0 per cent. oil combination is above and to the right of the latter group and is thoroughly mixed with the Purdue population.

4. Summary

1. A population homozygous at the fatty acid sensitive locus (cos/cos) and two genetically heterogeneous populations, Black and Purdue, of *Tribolium castaneum* were studied in two separate experiments on diets with 0 and 5 per cent. corn oil in controlled chambers at 61 ± 3 and 85 ± 3 per cent. relative humidity. Thirteen-day larval weight and the distribution of larvae, pupae and adults at 16, 18, 20, 22 and 26 days after egg collection were recorded.

2. The measurement 13-day larval weight is a function of the particular population examined, the concentration of corn oil in the culturing medium and, a newly identified factor, the relative humidity of the growing environment.

3. The cos/cos genotype as measured by mean 13-day larval weight on the 5 per cent. corn oil diet, 61 per cent. relative humidity (r.h.) treatment combination was designated as the base phenotype and symbolised $\bar{w}$. The comparative phenotypic expressions are: $3\bar{w}$ on diet 0, 61 per cent. r.h.; $5\bar{w}$ on diet 5, 85 per cent. r.h.; and $9\bar{w}$ on diet 0, 85 per cent. r.h.

4. The distribution of larvae, pupae and adults of 16, 18, 20, 22 and 26 days after egg collection emphasised that the response of these insects to corn oil and relative humidity was a biologically meaningful change in their developmental growth patterns.

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