GENETIC VARIATION IN CHAETAE NUMBER OF TRIBOLIUM¹

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GENETIC study of variation in bristle or chaetae numbers in *Drosophila* has yielded several interesting models of gene action. In addition to classical major genes such as scute (*sc*), Bristle (*Bl*), microchaete (*mc*), hairy (*h*), and Hairless (*H*), two distinct quantitative genetic systems have been reported. The number of abdominal or sternopleural hairs is a well known model for experimental studies concerned with normally distributed traits determined largely by additive genetic and environmental effects (e.g., MATHER and HARRISON 1949; CLAYTON, MORRIS and ROBERTSON 1957). In contrast, the number of scutellar bristles is essentially invariant in unselected populations and represents the best known example of WADDINGTON's (1957) theory of the canalization of developmental pathways. In extending this concept RENDEL (1967) presents evidence that a broad range of genetic variation is almost always canalized during development to produce the "optimal" phenotype of four scutellar bristles. Yet the canalized phenotype is subject to change by directional selection or by the introduction of major genes (e.g., scute) which control one or more of the developmental pathways.

Other than scutellar bristles of *Drosophila*, well documented cases of canalization are rare. Numerous studies of genetic variation for metric traits of *Tribolium* (BELL 1969) provide little evidence of canalization. However, noncanalized traits (body weight, fecundity, developmental time, etc.) were apparently chosen for these studies. Efforts to alter the number and distribution of bristles or chaetae of *Tribolium* have not been reported.

This paper describes the effects of selection in *Tribolium* on the number of pregenital chaetae, a trait showing some aspects of canalization. In addition, the genetic bases for unusual responses will be presented.

MATERIALS AND METHODS

The trait: Major genes are known which alter the number of chaetae in *Tribolium*. HOY (1966) reported that aureate (*au*) increased the number of chaetae in all developmental stages. LANGE (1967) observed that the gene grossly deformed (*gdf*) caused a variable reduction in chaetae of pupae. In addition, the recessive, finely scute (*sct*) was observed (LANGE 1968) to cause a reduction in size and number of chaetae in both larval and pupal stages.

The present investigation examines the number of pregenital chaetae on the eighth pupal

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FIGURE 1.—Composite drawing of the ventral side of the eighth and final pupal sternite of *Tribolium castaneum*. The right side is the male; the left side is the female.

- A. First Lateral Pregenital Chaetae
- B. First Medial Pregenital Chaetae
- C. Second Medial Pregenital Chaetae
- D. Second Lateral Pregenital Chaetae

sternite of *Tribolium castaneum*. In the wild type the male usually has three pairs—the first and second laterals and the first medials. Infrequently, a fourth pair, the small second medials, is observed for males (Figure 1). The wild-type female always possesses the first lateral and first medial pairs, but the second laterals are frequently absent. Second medials are never observed in wild females.

Base population: The basic genetic material chosen for this study was the Purdue Foundation b population, a large randomly mated control population genetically marked with the autosomal recessive gene *b* (black body). This stock was especially chosen because the marker gene darkens all chaetae and enhances their identification. Also, genetic equilibrium was reasonably assured since the population had been reproduced by random mating of about 200 parents each generation since it was constituted in 1960 from four laboratory stocks. The uniform culturing environment (33°C with 70% relative humidity in standard medium consisting of 95% wheat flour enriched with 5% nutritional *Torula* yeast) maintained over these years was continued throughout the present study.

Methods: The selection phase of this study was represented by two generations (—1 and 0) of no selection followed by nine generations of high–low mass selection. Initially, random samples of 357 males and 342 females were taken as pupae from the base population and were individually scored for total number of pregenital chaetae. This sample represented Generation —1. Fifty single-pair matings were made from randomly chosen males and females of Generation —1 seven days after the scoring of pupae. Seven days after the matings were made, a single 48-hr egg collection from each mating was taken in two grams of standard medium in $\frac{3}{4}$ oz. coffee creamers. After 18 days of incubation, pupae were screened from each collection at intervals of 2 days. A random sample of offspring (never exceeding ten of either sex) from each fertile mating was individually scored for total number of pregenital chaetae to represent Generation 0.

Selection for high and low chaetae number was initiated from Generation 0 by choosing, on individual phenotype, the 50 males and 50 females with the highest chaetae number to form the high selection line (H) while the 50 of each sex with the lowest chaetae number were taken to initiate the low selection line (L). Selected males and females were randomly mated in single pairs with progeny collected and scored for number of chaetae in the same manner as described for Generation 0. Divergent selection of the H and L lines continued for nine generations with the 50 superior (highest in H and lowest in L) individuals for each sex selected on individual score and randomly mated in pairs.

Beginning with the selected parents of the fourth generation reciprocal crosses between lines were made each generation. After obtaining the customary 48-hr egg collection from each mating for reproducing the lines (H and L), the mated pairs within lines were separated and individually combined with a mate from the other line: e.g., H males were mated to L females and L males to H females. Even though sperm from a second male is known to replace completely that of the first within 24 hr, the 48-hr egg collection from these reciprocal matings was delayed for a period of seven days.

No control or unselected line was maintained simultaneously with the H and L lines because our major interest was the divergence between lines and any possible divergence of sexes within lines. Information provided by a control line in this case would not have enhanced our primary objective. Even though a standard control line was not maintained, periodic observations made on the base population revealed the predominant developmental pattern to be stabilized at six chaetae as long as the population was cultured under the uniform conditions of this experiment. Preliminary studies had established that the trait varies directly, to a minor degree, with changes in the culturing temperature.

RESULTS

Selection response: The results of nine generations of selection for high and low number of chaetae are summarized by sex in Table 1 and Figure 2. In examining these results, attention is drawn to the divergence between lines and to the differential responses of sexes.

The divergence between high and low lines provides an interesting contrast to that usually observed for metric traits. In this experiment, meager response for both sexes during the early generations of selection was followed by enhanced rates of genetic change in later generations while the opposite is the usual case.

Even more surprising was the differential responses for sexes. While an upper limit of six chaetae prevailed for females, the male phenotype was only weakly canalized, if at all. In fact an enhanced response was evidenced for males in the later generations with individual males frequently possessing 10–12 chaetae.

The frequency of females possessing three pairs of chaetae increased slightly from 67% in the base population to 83% in the ninth generation of high selection. Even though females with a second medial or supernumerary chaetae were never

TABLE 1

Number of progeny and mean chaetae number for two unselected generations and nine generations of high-low selection

Generation of selection	Mean chaetae number by sex and line							
	Males				Females			
	High		Low		High		Low	
	No.	$\bar{X} \pm SE$	No.	$\bar{X} \pm SE$	No.	$\bar{X} \pm SE$	No.	$\bar{X} \pm SE$
-1	357	6.20 $\pm$.04	357	6.20 $\pm$.04	342	5.63 $\pm$.04	342	5.63 $\pm$.04
0	241	6.23 $\pm$.04	241	6.23 $\pm$.04	223	5.56 $\pm$.05	223	5.56 $\pm$.05
1	201	6.34 $\pm$.07	265	6.18 $\pm$.04	189	5.66 $\pm$.01	280	5.40 $\pm$.05
2	239	6.23 $\pm$.06	249	6.06 $\pm$.05	275	5.63 $\pm$.05	284	5.22 $\pm$.06
3	398	6.62 $\pm$.09	322	6.07 $\pm$.10	387	5.67 $\pm$.06	330	4.99 $\pm$.10
4	396	6.71 $\pm$.04	368	5.95 $\pm$.04	418	5.73 $\pm$.03	354	4.77 $\pm$.06
5	479	6.80 $\pm$.04	392	5.72 $\pm$.05	466	5.72 $\pm$.03	387	4.25 $\pm$.07
6	456	6.81 $\pm$.05	422	5.19 $\pm$.03	447	5.79 $\pm$.02	411	3.41 $\pm$.06
7	447	6.93 $\pm$.05	365	4.98 $\pm$.05	456	5.81 $\pm$.02	383	2.92 $\pm$.06
8	429	7.62 $\pm$.06	415	4.63 $\pm$.04	419	5.86 $\pm$.02	448	2.66 $\pm$.05
9	438	8.06 $\pm$.06	416	4.51 $\pm$.04	434	5.84 $\pm$.02	441	2.55 $\pm$.04

FIGURE 2.—Response by sex to nine generations of high-low selection for chaetae number in *Tribolium*.

observed in the base population, a few were observed in the last two generations of the study. Consequently, the canalized phenotype of six chaetae in females might have been on a threshold of response due to this new source of variation (second medial and supernumerary chaetae).

If an upper limit near six chaetae initially existed for the male phenotype (the slow initial response suggested this to be the case), this threshold was clearly broken by generation seven with sharp responses in subsequent generations. A definite pattern of response was observed. After the second laterals were established, a small pair of supernumeraries in the region cephalad and medial to the second medials became prevalent. Finally, other supernumeraries appeared further cephalad and medially.

The initial response of both sexes to selection for reduced chaetae numbers was slow, to suggest again that this trait was canalized for approximately six chaetae in the base population. However, continued selection yielded significant responses in both sexes, but that in females was nearly twice that observed for males (-3.08 vs. -1.69 chaetae).

The pattern for reduction in chaetae followed somewhat the reverse of that observed for high selection. That is, the supernumeraries were the first to disappear followed in order by the second medials, the second laterals, the first medials, and finally the first laterals. Unfortunately, the data were collected in a manner that does not allow anything to be said about any possible lateral asymmetry during selection. A barrier to the loss of the first lateral and medial chaetae was observed in the males (only 3% of the L males in the last two generations had any of these four chaetae missing). For L females only the loss of the final pair, the first laterals, appeared to constitute a barrier in low selection.

The overall pattern of response to selection on number of pregenital chaetae is suggestive of strong developmental control of the large chaetae and weak control of the small chaetae.

Although selection was practiced only upon the number of pregenital chaetae, other pupal chaetae were observed to have been affected in the same direction but to a lesser degree.

Reciprocal crosses between H and L lines: Since differential responses were observed for males and females in both H and L lines, reciprocal crosses between these lines were initiated at Generation 5 and repeated each generation thereafter in an effort to identify the genetic basis for these peculiar responses. The means, standard errors and number of offspring by sexes observed for five generations of these reciprocal crosses are listed in Table 2. These means are graphed by sex in Figure 3 in contrast with means from the pure lines ($L \times L$ and $H \times H$).

Two aspects of these results are noteworthy. First, both males and females of the reciprocal crosses are somewhat intermediate in chaetae number to the parental lines. The fact that females of the crosses were consistently above the mid-parental value suggests partial dominance in females of genes for high chaetae number, but such was not the case in males. In general it appears that a large share of the selection responses observed here resulted from additively acting genes.

FIGURE 3.—Chaetae number by sex in the selected lines and their reciprocal crosses over the last five generations of high-low selection.

The second point of interest, and a surprising one, is the fact that one cross ($L \times H$) is consistently higher for males than its reciprocal while the reverse is true for females. Since males in *T. castaneum* are known to be the heterogametic sex, this phenomenon cannot be attributed to sex linkage.

In view of the heterogeneous responses observed in the two sexes, the data for the reciprocal crosses were analyzed separately. The analysis chosen is an adaptation of the parallel line assay given by FINNEY (1964). The analyses as presented in Table 3 provide the following information:

TABLE 2

Number of progeny and mean chaetae number for reciprocal crosses between H and L lines for each of the last five generations of selection

Generation of selection	Mean chaetae number by sex and cross							
	Males				Females			
	L♀ × H♂		H♀ × L♂		L♀ × H♂		H♀ × L♂	
	No.	$\bar{X} \pm SE$	No.	$\bar{X} \pm SE$	No.	$\bar{X} \pm SE$	No.	$\bar{X} \pm SE$
5	304	6.52 ± .05	389	6.21 ± .04	288	5.20 ± .06	364	5.45 ± .04
6	337	6.28 ± .05	347	6.07 ± .04	333	4.97 ± .06	346	5.14 ± .05
7	276	6.42 ± .05	333	6.22 ± .04	267	4.82 ± .07	343	5.13 ± .05
8	344	6.44 ± .05	397	6.31 ± .05	333	4.87 ± .06	391	5.02 ± .05
9	342	6.68 ± .06	437	6.28 ± .03	325	4.73 ± .06	441	4.96 ± .05

1. Whether the reciprocal crosses differ in effect—tested by “mating type.”
2. Whether the regression of chaetae number on generations is linear—tested by “regression.”
3. Whether the regressions of chaetae number on generation for the two mating types are parallel—tested by “parallelism.”
4. Whether the deviations from linear regression are significant—tested by “linearity.”

For female offspring it is obvious from the significant mean square for “mating type” in Table 3 and the means given in Table 2 that the H × L cross was significantly higher in chaetae number than its reciprocal. Since the female offspring from these two crosses would have the same expected genotype, this difference signifies a maternal effect. In addition, the test of “parallelism,” which was non-significant, indicates that this maternal effect had become fixed during the initial four generations since no advance or increase in the magnitude of the effect occurred during the last five generations represented in this analysis.

The highly significant regression (Table 3) found for female offspring re-

TABLE 3

Analyses of variance in chaetae number by sex for progeny from five generations of reciprocal crossing between H and L lines

Source	Males			Females		
	df	M.S.	F	df	M.S.	F
Mating type	1	51.47	77.98**	1	40.37	38.08**
Regression	1	13.92	21.09**	1	78.74	74.28**
Parallelism	1	15.20	23.03**	1	0.04	<1
Linearity	6	2.58	3.91**	6	2.27	2.14
Between generations	9	10.67		9	14.75	
Error	3,501	.66		3,421	1.06	

** P < .01.

flects a decline in means of the reciprocal crosses between the H and L lines. Undoubtedly, this decline reflects the asymmetrical nature of the high-low selection responses and is not of particular interest to the present study.

The male offspring from the reciprocal crosses also differ significantly but in the opposite direction from that observed for females. Furthermore, the direction of this difference in males is opposite that expected from either maternal or sex-linked effects. In fact, the possibility of holandric or *Y* chromosome effects is clearly suggested. The significant mean squares for "regression," "parallelism," and "linearity" in the male analysis could reflect an asymmetrical response in the high direction due in part to an enhanced *Y* chromosome effect. Another possibility is that in analyzing this non-normal trait we rely heavily upon the statistical law of large numbers and the robustness of the *F*-test. In this case our error term may be too small for some reason.

Genetic tests for maternal and Y chromosome effects: A series of test crosses were made in order to test further the hypotheses of maternal and *Y* chromosome effects on pregenital chaetae number of *Tribolium*. In order to minimize genetic segregation in the tester stock, a highly inbred line, I, was used for these crosses. (The inbred line, coded B₁ in our laboratory, had undergone 76 generations of full-sib inbreeding and was derived from a wild stock collected at Bellview, Texas). The contrasting genetic material used was the High selection line at Generation seven. The reciprocal F₁ and critical testcrosses were made simultaneously and were maintained under identical environmental conditions.

The results obtained from parental, F₁ and critical testcrosses are summarized in Table 4. Since phenotypes were not normally distributed, large numbers (more than 300 of each sex) were classified in each category.

Before examining the critical testcrosses (Matings 5 *vs.* 6 for maternal effects and Matings 7 *vs.* 8 for *Y* chromosome effects), one notes that the inbred line (I × I) possesses fewer pregenital chaetae than even the base population. The results for reciprocal F₁ females (Matings 3 *vs.* 4) were surprising in that we expected a lower maternal contribution from I dams than from H dams. The opposite appears true in that a significantly higher number of chaetae was ob-

TABLE 4

*Chaetae number of progeny by sex from matings involved in the testing of maternal effects (Mating 5 *vs.* 6) and Y chromosome effects (Mating 7 *vs.* 8)*

Mating code	Mating genotype		Sons		Daughters	
	Dam	Sire	Number	Mean	Number	Mean
1	H	× H	447	6.93 ± .05	456	5.81 ± .02
2	I	× I	139	5.84 ± .04	127	4.20 ± .05
3	H	× I	416	6.25 ± .03	415	5.41 ± .04
4	I	× H	353	6.49 ± .04	367	5.59 ± .03
5	(H ♀ × I ♂)	× I	385	6.13 ± .02	368	4.94 ± .04
6	(I ♀ × H ♂)	× I	397	6.21 ± .03	404	5.06 ± .04
7	I	× (H ♀ × I ♂)	379	6.09 ± .02	413	5.01 ± .04
8	I	× (I ♀ × H ♂)	403	6.23 ± .03	407	5.02 ± .04

served for ($I \times H$) females than for ($H \times I$) females. The difference between reciprocal F_1 males is of little interest in that maternal, sex-linked and Y chromosome effects are all confounded.

The critical test for maternal effects comes from contrasting both males and females from Matings 5 and 6. These matings were made so that their male offspring would have the same expected average chromosomal constitution, including Y , X and autosomes. They differ in expectation only by the maternal cytoplasm (I vs. H). The same was true for female offspring. The observed difference was statistically significant in both cases (at the 10% level) and established the fact, not evident earlier, that the maternal effect on pregenital chaetae number affected males as well as females.

A question that needs to be answered is why the ($I \times H$) female progeny were higher than the reciprocal ($H \times I$) females. The I dams had a lower phenotype than the H females and we expected ($I \times H$) female progeny to have a lower phenotype. We are currently working on the assumption that there are two maternal effects involved in this trait: A direct genetic maternal effect caused by the action of the genotype of the dam upon her progeny's performance and a phenotypic maternal effect caused by the dam's phenotype. (The direct genetic maternal effect is not the dam's genetic contribution to her progeny but is some effect of her genotype upon the egg). This assumption is supported by the results of the testcrosses since the testcross dams have equal genetic effects but unequal phenotypes. The direction of the inequality in the progeny is the same as the dams. Further, the fixation of the size of the maternal effect in the reciprocal crosses indicates a genetic base, for if it were purely phenotypic the reciprocal females should have continued to diverge.

The results from the critical testcrosses (Matings 7 and 8) for Y chromosome effects are unequivocal. The male offspring from these two matings have the same expected maternal effects and average genotypes including cytoplasmic, autosomal, and sex-linked genes. The sole difference comes from their Y chromosomes in that all males from Mating 7 possessed I inbred Y chromosomes while all males from Mating 8 had H Line Y chromosomes. The female offspring from these two matings provide a contrast of sex-linked (X chromosome) genes in that females from Mating 7 would be heterozygous H/I while Mating 8 females would be I/I for sex-linked genes. Females from these two matings would otherwise have the same expected mean. The contrasting male offspring differed significantly while females from these two matings had similar means. Thus holandric genes affecting chaetae number in *Tribolium* do exist. Evidence for sex-linked genes was not found, but the test was not as critical as that made for Y chromosome effects.

DISCUSSION

The development of pregenital chaetae in *Tribolium* reflects a marked degree of canalization toward six in number. Wild-type males and females always possess two pairs, the first lateral and first medial. The males usually possess a third pair, the second laterals, with second medials and supernumerary chaetae rarely

observed. In females the second laterals are frequently absent and the second medials never appear.

Additional evidence of canalization was provided by the selection responses observed during nine generations of high-low selection. Developmental pathways are clearly different in males than in females and are subjected to different degrees of canalization. For example, nine generations of high selection failed to break through the six chaetae limit or barrier in females. The pattern of chaetae development for males in the base population suggested that six chaetae might be a weakly canalized upper threshold. Subsequent response to high selection confirmed the point in that the mean number of chaetae advanced even beyond eight without any sign of a second threshold.

While meager selection responses for reduced numbers of chaetae were initially observed for both sexes to suggest a mild degree of canalization, the thresholds were clearly passed by generations 4-5 and enhanced responses followed. In this case the female response was about twice that observed in males. A low threshold or limit of two chaetae, the first laterals, was clearly being approached in the late generations.

The physiological function and evolutionary significance of pregenital chaetae is not at all clear. Suggested functions such as sensory organs, developmental stabilizers or secondary sex characteristics are little more than conjecture. To state that a regular pattern for number and position of chaetae is canalized because it is part of the normal development hardly accounts for chaetae initially. The present study sheds no light on the origin or function of pregenital chaetae, but it does present two kinds of evidence regarding their developmental pattern. One points to the position and size of the chaetae as influencing the degree of canalization. For example, the larger chaetae were found to be more strongly canalized than the smaller ones. This agrees with the findings of FRASER (1963) and SCOWCROFT, GREEN and LATTER (1968) that some *Drosophila* chaetae may be more strongly canalized than others. The second and more interesting line of evidence revealed that the degree of canalization for chaetae was sex influenced. A case in point is the rigid upper limit or threshold of six chaetae observed for females but not for males. Could chaetae be related to different physiological processes in the two sexes? Different developmental pathways for males and females are evidenced not only by differing thresholds but by clearly established sex-limited genetic effects, e.g., holandric genes and possibly sex-dependent dominance. Genetic effects which modify the normal canalized developmental pattern uniquely in one sex merit further study as they relate to sexual dimorphism.

SUMMARY

Genetic variation in the number of pregenital chaetae was investigated in a population of *Tribolium castaneum*. Initially, both sexes invariably possessed two pairs of chaetae, the first lateral and medial pairs, with a third pair usually present. Additional chaetae were infrequently observed in males but never in females. High-low selection over nine generations revealed the degree of canalization in

the development of chaetae to be influenced by direction of selection, sex, and chaetae position. A rigid upper limit or threshold at five chaetae prevailed for females while the mean number in males gradually advanced to eight with no apparent limit in sight. The male phenotype was more strongly canalized in the negative direction with response in females being twice that observed for males. Genetic effects identified from reciprocal and testcross matings as responsible in part for these peculiar responses were holandric genes (*Y* chromosome effects) and a genetic maternal influence. Some evidence for sex-limited dominance was found. However, the major source of genetic variation revealed by selection was due to additively acting genes.

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