FURTHER STUDIES ON FACTOR INTERACTION IN *DROSOPHILA VIRILIS*

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INTRODUCTION

A character is a result of the cooperative action of all genes, although some genes alone, and in case of "factor interaction," more than one gene, are chiefly concerned in the development of a particular character. Interacting genes, as well as modifiers, may be specific in their effect, since they can act only in the presence of a particular genetical environment (BRIDGES 1919), and non-specific, when their action is not confined to a particular gene.

The present paper deals with the inheritance of thorax protrusions, curling of bristles and other characteristics which appear in various phenotypical expressions as a result of the interaction of certain genes with non-specific modifiers.

The following five genes are involved in the interaction here studied:

Ruffled (*ru*), a fifth chromosome recessive gene. In ruffled flies the tips of the dorso-central bristles and hairs near them are curled forward, and the thorax may be slightly telescoped (figure 1a).

Rounded (*R*), a second chromosome dominant gene. Rounded flies have truncate wings, short and bushy arista (figure 1d).

Clipped (*Cl*), a fourth chromosome dominant gene, lethal when homozygous. In clipped flies the posterior end of the wings are cut and the majority have two dorso-central bristles slightly curled (figure 1e).

Shaggy (*sh*), a fifth chromosome recessive gene. In shaggy flies the abdominal hairs are directed irregularly.

Beaded (*Bd*), a fifth chromosome dominant, lethal when homozygous. In beaded flies the wing margins are scalloped and one or two of the dorso-central bristles may become slightly curled, depending upon condition of the food and age of the flies.

The following are new characters which have resulted from the interaction between some of the above described genes:

Vortex-1, two well-developed horn-like protrusions on the thorax, located lateral to and midway between the anterior and posterior dorso-central bristles. The microchaetae or small hairs around these protrusions are arranged in a whorl.

Vortex-2, protrusions are only slightly developed, and in extreme cases leaving only two brown pigmented spots surrounded by whorled hairs.

FIGURE 1.—(a) *ru/ru*, (b) *ru/+R/+*, (c) wild-type, (d) *R/R*, (e) *Cl/+*, (f) *ru/ru Cl/+*, (g) *ru/ru R/R* and *ru/ru R/+*, (h) *ru/ru sh/sh* and *ru/ru sh/+*, (i) *R/R Cl/+* and *R/+Cl/+*, (j) *Cl/+ sh/sh* and *Cl/+sh/+*, (k) *ru/ru R/+Cl/+*, (l) *ru/+R/+Cl/+*. (Drawings by Miss E. DEG. MCKEE.)

Vortex-3, the pigmented spots lie in the wall of an indentation or funnel extending more or less deeply into the thorax. Very often two pairs of intrusions are present, which may be arranged either in pairs on the anterior and posterior parts of the thorax, or in a row across the thorax.

Curled bristles, tips of dorso-central bristles are curled forward, hairs near them being normal.

Extremely ruffled, dorso-central bristles and hairs near them are almost perpendicular to the thorax.

INTERACTION OF RUFFLED AND ROUNDED GENES

The ruffled (*ru*) gene becomes incompletely dominant with rounded (*R*). The change in dominance is accompanied by the appearance of a new characteristic called roofed in *ru/+R/+* flies (figure 1b), and by an exaggeration of both rounded and ruffled characteristics as well as by the appearance of the new character vortex-3 in *ru/ru R/R* and *ru/ru R/+* flies (figure 1g) (LEBEDEFF 1932).

Further studies (LEBEDEFF 1933) revealed that these characteristics, as well as others, also result from interaction of clipped with either ruffled or rounded.

INTERACTION OF RUFFLED AND CLIPPED

From thirteen crosses between ruffled (*ru/ru*) and clipped (*Cl/+*) flies, 375 wild-type and 428 clipped flies were obtained. The majority of the clipped flies from the cross had curled bristles and vortex-2 characteristic

TABLE 1
The variability of Cl/+ and Cl/+ru/+ flies.

	STRAIGHT BRISTLES (PERCENT)	CURLED BRISTLES (PERCENT)	CURLED BRISTLES VORTEX-2 (PERCENT)	NO. FLIES EXAMINED
<i>Cl/+</i>	11.75	77.45	10.8	315
<i>Cl/+ru/+</i>	1.2	33.7	65.1	428

of the thorax. The detailed studies of *Cl/+* and *Cl/+ru/+* flies, as shown in table 1, show that the percent of flies having vortex-2 protrusions is 10.8 in *Cl/+* flies and 65.1 in *Cl/+ru/+* flies. This indicates that the vortex-2 characteristic is a result of interaction between heterozygous ruffled and clipped genes.

From the backcross of F_2 *Cl/+ru/+* flies to ruffled (*ru/ru*) four phenotypically distinct classes were obtained approximately equal in numbers (table 2a). There are two classes of clipped flies in the backcross population. One class (546 in number) is represented by flies having ruffled

bristles and vortex-2 characteristics. These flies resemble the F_1 clipped parent and evidently are genetically $Cl/+ru/+$. The other group (483) of clipped flies have the dorso-central bristles and adjacent hairs curled to a greater extent than the ruffled flies; the thorax of these flies has two very marked protrusions—vortex-1 (figure 1f). It is assumed that the vortex-1 character and the extreme ruffling of bristles and hairs is caused by the interaction of the homozygous ruffled and clipped genes, and that these flies are genetically $Cl/+ru/ru$. Wild-type (493) flies from the backcross have normal wings and straight bristles and hairs; genetically they are of $+/+ru/+$ constitution. Ruffled (499) flies have the dorso-central bristles and adjacent hairs ruffled and have a somewhat telescoped thorax, and their genetical constitution is assumed to be $+/+ru/ru$.

TABLE 2
Summary of backcross and F_2 data from $Cl/+ \times ru/ru$ cross.

	NUMBER OF CULTURES	CLIPPED $Cl/+/+$	CLIPPED RUFFLED VORTEX-2	CLIPPED EXTREMELY RUFFLED VORTEX-1	WILD-TYPE $+/+ru/+$ OR $+/+/+$	RUFFLED $+/+ru/ru$
			$Cl/+ru/+$	$Cl/+ru/ru$		
(a) $Cl/+ru/ru$						
$\times ru/ru$	26		546	483	493	499
Calculated			505	505	505	505
(b) $Cl/+ru/+$						
$\times Cl/+ru/+$	16	203	394	217	295	95
Calculated		200.6	401.2	200.6	300.9	100.3

In the F_2 from the cross between clipped (Cl) and ruffled (ru/ru) flies, five classes appeared in a 2:4:2:3:1 ratio (table 2b). This ratio is a result of the interaction of the dominant clipped gene, which is lethal when homozygous, with recessive ruffled. The clipped curled vortex-2, clipped extremely ruffled vortex-1, wild-type and ruffled F_2 flies are the same as the corresponding classes in the backcross progeny. The additional clipped class of flies in F_2 are genetically $Cl/+ +/+$ constitution.

GENETICAL TEST OF F_2 PHENOTYPES

There are two classes in the F_2 progeny from the $Cl/+ \times ru/ru$ cross, which phenotypically overlap each other: clipped and clipped ruffled vortex-2. As is shown in table 1, about 35 percent of $Cl/+ ru/+$ flies phenotypically cannot be distinguished from $Cl/+ +/+$ flies. Therefore the possibility of errors in classification of flies of these two classes cannot be excluded. In fact, out of the nine clipped ruffled vortex-2 F_2 flies which were tested, one proved to be clipped ($Cl/+ +/+$), since from the cross with ruffled (ru/ru) it gave 43 clipped ruffled vortex-2 and 39 wild-type flies. The other eight clipped ruffled vortex-2 flies gave 149 clipped ruffled

vortex-2, 135 clipped extremely ruffled vortex-1, 124 ruffled and 134 wild-type flies and thus proved to be $Cl/+ \text{ } ru/+$.

Four clipped extremely ruffled vortex-1 F_2 flies, when crossed with ruffled flies, gave 134 clipped extremely ruffled vortex-1, and 156 ruffled flies, and thus proved to be of $Cl/+ \text{ } ru/ru$ constitution.

From three crosses between F_2 ruffled flies only ruffled were obtained, indicating that ruffled flies are double recessive $+/+ \text{ } ru/ru$.

These genetical tests support the assumption that the ruffled vortex-2 characters are the result of an interaction of heterozygous clipped and heterozygous ruffled genes, and that the extreme ruffling of bristles and vortex-1 characters are the result of the interaction of heterozygous clipped and homozygous ruffled genes.

INTERACTION OF ROUNDED AND CLIPPED GENES

When it was found that ruffled interacts with rounded, as well as with clipped, it was of interest to determine whether rounded and clipped genes interact with each other. From eight crosses between rounded (R/R) and clipped ($Cl/+$) flies 281 rounded and 270 flies with rounded clipped extremely ruffled, vortex-1, and spread short roofed wings were obtained (figure 1i).

In order to test whether the ruffled gene was involved in this cross, since $R/+ \text{ } Cl/+$ flies closely resemble $ru/ru \text{ } R/+$, a cross was made between $R/+ \text{ } Cl/+$ and wild-type flies. From this cross four distinct classes of equal numbers were obtained (182:177:171:189). The 182 rounded, clipped, extremely ruffled vortex-1, with spread, roofed short wing flies phenotypically resemble the $R/+ \text{ } Cl/+$ parent and genetically are of the same constitution. The 177 rounded and the 171 clipped flies resemble the corresponding flies from stock cultures, and the 189 wild-type flies resemble their wild-type parent. This result shows that the ruffled gene was not involved in the cross, because if ruffled were present the rounded flies would be rounded, ruffled and roofed while the majority of clipped flies would have vortex-2, which was not the case. These data indicate that rounded and clipped genes interact to produce the characters vortex-1, extremely ruffled dorso-central bristles and adjacent hairs, and spread short roofed wings.

To find out what the phenotypical effect would be when R , Cl and ru genes are combined, F_1 flies from the cross $R/+ \text{ } Cl/+ \times ru/ru$ were back-crossed to ru/ru . From the $R/+ \text{ } Cl/+ \times ru/ru$ cross three cultures were obtained, in which flies were of four classes approximately equal in number: (1) 64 flies were $R/+ \text{ } Cl/+ \text{ } ru/+$ —rounded clipped vortex-1 extremely ruffled with spread short roofed wings (figure 11); (2) 60 were $R/+ \text{ } +/+ \text{ } ru/+$ —rounded ruffled roofed; (3) 77 were $+/+ \text{ } Cl/+$

ru/+—clipped vortex-2 ruffled and (4) 70 were *+/+ +/+ ru/+*—wild-type flies. The *R/+ Cl/+ ru/+* flies differ phenotypically from *R/+ Cl/+* flies only by having a deep furrow on the tip of the thorax. On the other hand, *R/+ Cl/+ ru/ru* flies, obtained together with other seven classes from *R/+ Cl/+ ru/+ × ru/ru* cross, differ markedly from *R/+ Cl/+* and *R/+ Cl/+ ru/+* flies in having a deep furrow on the thorax extending almost half its length, resulting in a hump in the middle of the thorax; the wings of these flies are also greatly reduced, leaving only vestiges (figure 1k).

INTERACTION OF SHAGGY WITH RUFFLED AND CLIPPED

From nine crosses between shaggy (*sh*) and clipped (*Cl*) flies 396 clipped ruffled vortex-1 (figure 1j), and 389 wild-type flies were obtained. From seven cultures from the backcross of *Cl/+ sh/+ × sh/sh* four classes appeared in approximately equal numbers: (1) 103 flies were *Cl/+ sh/sh*—clipped shaggy vortex-1 ruffled (figure 1j); (2) 97 were *Cl/+ sh/+* clipped vortex-1 ruffled; (3) 101 were *+/+ sh/sh*-shaggy and (4) 111 were *+/+ +/+*—wild-type.

The *Cl/+ sh/+* and *Cl/+ sh/sh* flies from this cross resemble each other as far as the ruffling of the dorso-central bristles and adjacent hairs, and vortex characteristics of the thorax are concerned. This indicates that clipped and shaggy interact with each other to produce vortex-1 and ruffled bristles and hairs on the thorax. The presence of *sh* in homozygous condition does not intensify this interaction.

A study of *ru/ru sh/sh* flies from a stock culture revealed that all have the vortex-3 character (figure 1h). From various crosses it was found that vortex-3 appeared also in *ru/ru sh/+* flies, which indicates that vortex-3 appears also from interaction of homozygous ruffled with homo- or heterozygous shaggy.

INTERACTION OF BEADED WITH CLIPPED AND ROUNDED

Beaded (*Bd*) also can be added to this series of interacting genes. The interaction of this gene with the others is not conspicuous, since the bristle characteristics in beaded flies are affected by condition of food and by age. *F*₁ flies (*Bd/+ Cl/+*) from crosses between clipped and beaded were found to have curled bristles and vortex-2 thorax. But *F*₁ flies (*Bd/+ R/+*) from crosses between beaded and rounded were found to have curled bristles. No detailed statistical studies, however, were made either of *Bd/+ Cl/+* or *Bd/+ R/+* flies.

DISCUSSION AND SUMMARY

The interrelationship of five genes makes it possible to trace the role played by each gene in production of different characteristics. As it is

shown in the diagram (figure 2), the vortex-1 character appeared from the following combinations: $Cl/+ sh/sh$, $Cl/+ sh/+$, $Cl/+ ru/ru$, $Cl/+ R/R$ and $Cl/+ R/+$, indicating that the clipped gene is primarily responsible for the vortex-1 character, but it does not appear unless in the presence of an intensifier, which can be either homozygous ruffled, or heterozygous shaggy or rounded genes. Similarly, vortex-2 also is due to the clipped gene, when intensified by heterozygous beaded or ruffled. However, vortex-3

FIGURE 2.—New characteristics appearing in crosses involving five genes as the result of the interaction between these genes.

appears from $ru/ru sh/sh$, $ru/ru sh/+$, $ru/ru R/R$ and $ru/ru R/+$ combinations, indicating that the ruffled gene is primarily responsible for the vortex-3 character when it is intensified either by shaggy or rounded. Spread short roofed wings appeared in $R/R Cl/+$, $R/+ Cl/+$, $R/R ru/ru$ and $R/+ ru/ru$ flies suggesting that the rounded gene is responsible for this characteristic but appears only when rounded is modified by either clipped or ruffled. The extreme ruffling of bristles and hairs cannot be assigned to any particular gene, since it appeared in $Cl/+ ru/ru$, $ru/ru R/R$, $ru/ru R/+$, $Cl/+ R/R$ and $Cl/+ R/+$ flies, showing that any two of clipped, ruffled, and rounded genes can produce it.

These studies also revealed the important role played by genetic environment upon the phenotypical expression of the gene. In a particular environment the ruffled gene, for example, produces only curling of dorso-central bristles and adjacent hairs, while under other conditions the same gene may produce various types of vortex protrusions on the thorax, exaggerated curling of bristles and hairs, and short spread and roofed wings.

LITERATURE CITED

- BRIDGES, C. B., 1919 Specific modifiers of eosin eye color in *Drosophila melanogaster*. J. Exp. Zool. 28: 337-384.
 LEBEDEFF, G. A., 1932 Interaction of ruffled and rounded genes of *Drosophila virilis*. Proc. Nat. Acad. Sci. Washington 18: 343-349.
 1933 Studies on factor interaction in *Drosophila virilis*. Amer. Nat. 68: 68-69.