Mutations and Linkage Groups in Japanese Strains of the Housefly¹⁾

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It has been generally recognized that the extensive use of synthetic insecticides for pest control has resulted in the development of insecticide-resistance in insects of medical and agricultural importance. In order to overcome such a serious problem, co-operative investigation of the fundamentals is necessary from the practical standpoint. Genetical studies on insecticide-resistance in houseflies and mosquitoes by several pioneer workers have been based on the results obtained from reciprocal crosses between resistant and susceptible strains or from backcrosses of the F_1 hybrids to their parental strains. These studies have been skillfully reviewed by Milani (1956-7), Crow (1957) and Brown (1958). The deficiencies of the early work on the genetic mechanism of insecticide-resistance were mainly due to the lack of knowledge of basic genetics in the housefly. Therefore there is a present and urgent need to integrate fundamental information on housefly genetics to form a basis for further investigations on insecticide-resistance.

The first report on the formal genetics of the housefly appeared in the form of lists of several mutants contributed by Sarkissian and by Serebrovsky to the 15th issue of the *Drosophila* Information Service in 1941, though these reports were not considered as formal publication. Unfortunately, no extensive study was subsequently developed by these Russian investigators. However, investigation of the formal genetics of the housefly has been reopened by several insect geneticists in Italy, Japan and the U. S. A.

Milani (1954) has described 22 mutants of the housefly in Italy, and some of them were employed in the subsequent genetic analyses of DDT-resistance in this species. Knockdown-resistance to DDT was found to be inherited as a single recessive gene, *kdr*, which was linked with *bwb* (*brown-body*) and *dv* (*divergent*) but not with *ocra* (*ocra*) (Milani, 1955, 1958; Milani and Travaglino, 1957). At the University of Kansas, U. S. A., the housefly genetics has also been initiated. Some mutant types and aberrant forms of the housefly were reported by Sullivan and Hiroyoshi (1960). Recently, Hiroyoshi (1960) has studied 17 mutants of American houseflies and clas-

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sified them into 5 linkage groups which are assumed to represent all the autosomes of the housefly.

Several years ago, study of the housefly genetics was concurrently attempted in Japan, and a part of the work was reported by Tsukamoto *et al.* (1957). Since 1958, isolation of mutant strains and performance of linkage tests with them have proceeded on a large scale in this laboratory. After the Japanese linkage groups had been established, certain important marker strains were obtained from the University of Kansas and compared with the Japanese strains for allelism and/or linkage in order to avoid further confusion or synonymy.

Materials and Methods

Samples taken in the field in 1958 from Hikone and from Akashi were used as major source of mutations. Inbreeding of these strains for several generations resulted in the discovery of various kinds of morphological aberrations. Adult flies of each generation were anaesthetized with CO₂ and ether, and examined with a binocular dissecting microscope for any visible aberrant characters which appeared useful as mutant markers. To establish pure mutant strain, continuous selections and inbreedings were performed for these mutant-like flies, and the mode of inheritance and linkage relationships of thus established mutants were studied by examining the segregation among the offspring from crossing experiments. In addition, a white-eyed mutant strain was obtained from Mr. Y. Washizuka, Ihara Agric. Chem. Laboratory, Shimizu, and a yellow-eyed strain from Dr. S. Asahina, National Institute of Health, Tokyo, who had originally obtained it from the 406th Medical General Laboratory, Sagamihara.

For the crossing experiments, newly emerged virgin females were separated from males within 8 hours after the breeding jar had been cleared of any adult flies already emerged. Powdered milk and water were separately fed to adult flies. Mating, feeding and oviposition were obtained in cages measuring 10×10×12 cm³ or 20×20×20 cm³. The larval medium consisted of a mixture of 2 parts wheat bran, 1 part powdered fish, 1 part powdered mouse biscuit and 3 parts water; this was placed in a glass jar of about 650 ml. capacity and the eggs were inoculated. The jar was then covered with a piece of cotton cloth and placed in a rearing room maintained at 25°C, at which temperature each jar yielded 100–500 flies by the 10th to 13th day.

Results and Discussions

Mutant List

Large numbers of morphologically aberrant flies were found to appear spontaneously in the field-collected strains. During the subsequent course of inbreeding and selection, many of them failed to become established because of their poor viability, penetrance and expressivity. However, 12 relatively good mutants have been isolated

and established successfully in this laboratory. From the yellow-eyed strain, two eye-color mutants were separated and proved to be allelic to the American *ge* (*green*) mutant and to the Italian *ocra* mutant, respectively. The white-eyed mutant was also allelic to the American *w⁵⁷* (*white-57*) gene. Other eye-color mutants from Japanese strains, *bu* (*brunette*) and *do* (*dark orange*), were not allelic to other American eye-color mutants examined, *rb* (*ruby*), *car* (*carnation*) and *cm* (*carmine*). Other mutants affected the bristles, size or shape of wing, wing venation, shape of legs, etc. The following is a list of those mutants which became sufficiently established for use in linkage tests:

- Abr*: *Abrupt* Vein L_5 shortened, usually just near posterior crossvein. Posterior wing margin smoothed without definite notch and/or slight spine at a point opposite the tip of L_5 (Fig. 2). Semidominant; expressivity and penetrance erratic.
- acv*: *anterior-crossveinless* Anterior crossveins entirely absent (Fig. 3). Posterior crossvein normal. Veins L_3 and L_4 often interrupted at middle part. Shape of scutellum not triangular but rather rectangular, occasionally with the posterior margin flattened or incised medially as in the mutant *en* (*engrailed*) of *Drosophila melanogaster*. Penetrance, expressivity and viability good.
- bu*: *brunette* Eye color translucent reddish brown. Combination of *do*; *bu* gives more dilute eye-color than *do* or *bu*. Expressivity and penetrance good.
- do*: *dark orange* Eye color translucent dark orange or light reddish brown. Slightly lighter than *bu*, but difficult to distinguish from it. Expressivity and penetrance good.
- fs*: *fasciculate* Vein L_4 with a knot of vein, extra vein or often fascicles at a posterior region of the vein after swinging forward (Fig. 5). Usually recessive, but often a small portion of the heterozygotes expresses the character as in the homozygotes.
- ge²*: *green-2* Eye color diluted to greenish yellow at emergence, darkening with age to pale yellowish brown. Found as *ge²*; *ocra^e* flies from a yellow-eyed stock. Allelic to the original *ge*. Expressivity and penetrance good.
- ocra^e*: *ocra-crimson* Eye color translucent deep red or crimson. Found in the yellow-eyed stock with *ge²*. Allelic to the original *ocra*. Expressivity and penetrance good.
- pcv*: *posterior-crossveinless* Posterior crossveins absent or broken (Fig. 4). Anterior crossvein normal. Expressivity variable but penetrance complete.
- rd*: *reduced* Bristles and hairs of abdominal tergites and sternites reduced in number, particularly at the fourth and/or fifth sternites. Expressivity and penetrance not complete.
- Rl*: *Rolled* In homozygous condition, wings rolled up intensely (Fig. 7); body size somewhat smaller and body surface less polished than wild type. In heterozygous condition, expressivity rather irregular, wings curled up, turned up at tips,

Figs. 1—5: Wing venations of wild and mutant strains of the housefly.

Figs. 6—9: Other mutants.

- Fig. 1. + (Wild type).
 3. *acv* (anterior-crossveinless).
 5. *fs* (fasciculate).
 7. *Rl* (Rolled wings).

2. *Abr* (Abrupt).
 4. *pcv* (posterior-crossveinless).
 6. *Sn* (Singed bristles).
 8. *sht* (short tarsi).
 9. *tw* (twisted fore-legs).

wavy, curved downward, or somewhat approaching the wild type; body size and body surface normal. Homozygotes viable and fertile, but less viable than heterozygotes or homozygous wild type.

sht: *short* Tarsi two-jointed instead of normal five-jointed, hence legs shortened; wing venation extremely irregular and consequently bubbled, blistered or occasionally unexpanded (Fig. 8). Expressivity and penetrance good.

Sn: *Singed* In homozygous condition, all bristles and hairs of the body extremely singed (Fig. 6); expressivity and penetrance excellent; female almost sterile, male fertile. In heterozygous condition, only the tip of bristles slightly gnarled and both sexes fertile; expressivity and penetrance not good.

ss: *spineless* Bristles on the thorax reduced in length. Expressivity and penetrance not complete.

tw: *twisted* Femur and tibia of the fore-legs, and occasionally of the hind-legs also, fused with each other and/or twisted at the joint, giving an appearance reminiscent of a praying mantis (Fig. 9). Expressivity, penetrance and viability variable.

w³: *white-3* Eye color pale grayish white. Isolated by Y. Washizuka and H. Ike-moto from the Takatsuki Laboratory strain in February, 1959. Allelic to the mutant *w⁵⁷* from the University of Kansas. Expressivity, penetrance, fertility and viability good.

Linkage Relationships

The chromosome number of the housefly is 12 or 6 pairs in diploid (Stevens, 1908; Perje, 1948), and hence 6 linkage groups were to be expected in this species. However, evidence for only 5 autosomal linkage groups has been obtained from the present crossing experiments, as follows:

1. X and Y (sex chromosomes, unmarked)
2. *ge²*, *pvc*, *Sn*, *w³*
3. *ocra^c*
4. *acv*, *do*, *fs*, *Rl*, *sht*, *ss*
5. *bu*, *rd*, *tw*
6. *Abr*

The establishment of the linkage groups is based on the evidence that no or few crossing-over, if any, takes place in the male as in *Drosophila melanogaster*. The numbering of the linkage groups is in accordance with that proposed by Hiroyoshi (1960). Perje (1948) had observed cytologically that most parts of the sex chromosomes were heterochromatic and hence predicted that sex-linked genes might be few or altogether lacking in the housefly. The fact no sex-linked mutant has yet been found in both Japanese and foreign strains supports her prediction.

The genes *ge²* and *w³* are located on the second chromosome because they are

allelic to the American mutant genes already associated with that chromosome. Similarly, it is concluded that *ocra*^c belongs to the third chromosome. When the mutant *acv* was crossed with the fourth chromosomal mutant *ct* (*cut*) from America, no double recessive phenotype, *acv ct*, was observed in the F₂ generation. This evidence indicates that *acv* is linked with *ct* and belongs to the fourth chromosomal group. The genes of Japanese origin assigned to the fifth and the sixth chromosomes were also located by similar crossing experiments with American mutants already associated with these chromosomes.

The order of arrangement of these mutant genes on each chromosome and the map-distances between them will be reported in a later publication.

Sex-limited Inheritance

Although no sex-linked mutant was found, an interesting case of sex-limited inheritance was observed in a certain strain. When males of a particular strain bearing the marker gene *bu* on the fifth chromosome were crossed with females carrying the marker gene *w*³ on the second chromosome, no *w*³-type males appeared in the F₂ generation. On the other hand, when *bu* females were crossed with *w*³ males, the normal segregation was demonstrated in the F₂ generation. Such evidence suggests that the Y-chromosome of the *bu* strain has become translocated or attached to the second chromosome carrying *w*⁺ gene. Similar crosses with another *bu* strain did not demonstrate such an abnormal segregation, thus furnishing proof that the phenotypically "sex-limited" inheritance observed in the original *bu* strain was not due to the *bu* gene itself but to the effect of the translocated Y-chromosome.

The "sex-linked" mutant *t* (*ala tumoscita*) described by Sarkissian (1941) as follows: "blisters on wings; sex linked, lethal in homozygous condition in females", might equally well be a case of translocated Y-chromosome also. Instances of sex-limited inheritance have been reported by Milani and Franco (1959a) and Sullivan (in press) in other strains. It is of interest that as far as we know such phenomena take place only between the Y-chromosome and the second chromosome.

It has been reported by Milani and Franco (1959b) that the genes for DDT-resistance in both the Italian and American strains of the housefly, which are not allelic to each other, are linked with *bwb* and thus located on the second chromosome. More recently a case of sex-limited inheritance of the DDT-resistance character has been reported by Kerr (1960) in an Australian strain. From the above consideration, it could appear probable that in his Australian strain the DDT-resistance factor is also located on the second chromosome and that the Y-chromosome has become attached to it.

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Summary

Fifteen visible mutants of the housefly have been isolated from Japanese populations and developed in marker strains suitable for further genetic investigation.

Although 6 linkage groups were to be expected from the known chromosome number, no sex-linked visible mutants were found, all the mutants being assignable to one or other of the 5 autosomal linkage groups.

In one strain the characteristic w^+ of the second chromosome behaved as if it was sex-limited, it was suggested that the Y-chromosome was occasionally translocated to the second chromosome.

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