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Genetic Dissection of the *Drosophila* Nervous System by Means of Mosaics

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Abstract. Given a mutant having abnormal behavior, the anatomical domain responsible for the deficit may be identified by the use of genetic mosaicism. Individuals may be produced in which a portion of the body is mutant male while the rest is normal female. In such sex mosaics, or gynandromorphs, the division line between normal and mutant parts can occur in various orientations. Mutants of five different genes (cistrons) on the X-chromosome of *Drosophila melanogaster*, having various abnormalities in visual function, have been tested by this method. All of these have been found to be autonomous, i.e., a mutant eye always functions abnormally, regardless of the amount of normal tissue present elsewhere, indicating that the primary causes of the behavioral deficits in these mutants are within the eye.

The scalpel cleaves a biological system along anatomical lines. Gene mutations may dissect the system in other ways, for instance by deleting a particular enzyme in all of the cells. But genetics can also be used in a manner similar to the scalpel, by creating composite individuals. In this paper, we describe some applications of this approach to behavior in *Drosophila melanogaster*.

A behavior pattern may involve many parts of an organism. Thus, the movement of *D. melanogaster* toward light (phototaxis) requires receptor response, central nervous system integration, and motor output. A defect in any of these steps could lead to a deficit in phototaxis. Given a nonphototactic mutant, where is the responsible defect located? In some cases, anatomical study might reveal an abnormal structure. In others, electrophysiological study might reveal an abnormality in electrical events along the pathway from input to output. Our studies of nonphototactic mutants of *Drosophila* have indeed demonstrated both of these types. However, finding a local abnormality does not necessarily locate the primary cause, since the defect might be the secondary result of malfunction elsewhere. Suppose one has a mutant in which the eye does not function properly. The defect could be autonomous, i.e., inherent in the eye itself, but it could equally well result from lack of some circulating substance, as in the case of the vermilion eye color mutant, which becomes normal if kynurenine is supplied by other tissues.[1–3] In principle, this could be tested for by making composite flies by an exchange of mutant and normal parts, assuming that the surgery could be performed successfully, including regeneration of all connections.

In *Drosophila*, the production of such composite individuals by genetic methods is a well-known technique, and was the subject of a long article by Morgan and Bridges in 1919.[4] Sex mosaics (gynandromorphs) have been extensively used for tracing cell lineage during development.[5-9] However, relatively little has been done concerning their behavior. Whiting's excellent study on gynandromorphs of the parasitic wasp, *Habrobracon*,[10] in which males and females display distinct reactions, showed the head to be the controlling structure for sexual behavior. Similar results have been reported for the mosquito, *Aedes aegypti*.[11]

A *Drosophila* gynandromorph may be formed by the loss of one of the two X-chromosomes of a female egg during the first mitotic division in embryonic development. Fig. 1 illustrates the subsequent events leading to a mosaic adult

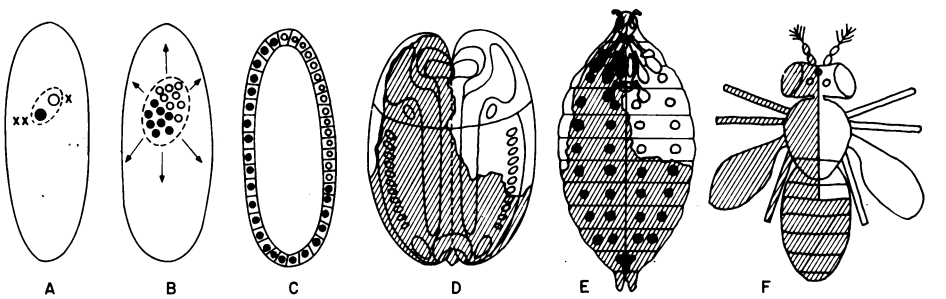


FIG. 1. Schematic diagram illustrating formation of a gynandromorph. (*A*). Starting with a female (XX) egg, one of the X-chromosomes is lost during the first nuclear division. This produces one nucleus that contains only a single X, and develops into male tissues, while the XX nucleus develops into female tissues. The orientation of the first mitotic spindle is (in *Drosophila*) random,[6] so these nuclei produce, as they divide, descendent nuclei that are variously oriented in different embryos. (*B*). The nuclei divide in a syncytium without cell walls. After about a dozen divisions, the nuclei migrate to the surface of the egg, and (*C*) cell membranes are laid down to form a blastoderm,[21] the female nuclei forming some areas, male nuclei forming others. (*D*) The blastoderm is sketched as if cut along the dorsal side and folded open like a book, showing a fate map of the regions destined to become the various larval organs (redrawn from Poulson[22]). In the example shown, the shaded portion will develop into female parts, the unshaded into male parts, eventually hatching as a composite larva. (*E*). A mature larva split dorsally and opened flat, indicating the various imaginal discs which are destined to develop into adult surface structures after metamorphosis (redrawn from Parks[6]). The adult (*F*) is a composite formed from some female and some male discs.

fly. To make flies that are composite for, say, a recessive behavioral deficit, the mutant gene is placed, by recombination, onto one of the X-chromosomes, along with other recessive markers that affect phenotypes readily visible in the adult, e.g., white eyes, yellow body color, and forked bristles. In the female body parts, all these mutant genes will not be expressed, since they will be dominated by their normal counterparts in the second X-chromosome; in the male parts, the mutant genes will be uncovered. Examination of the fly for the visible markers thus will indicate the parts of the body in which the behavioral mutation should be expressed. The following experiments apply this technique to a series of mutants selected for deficits in phototactic behavior.

Materials and Methods. (*a*) ***D. melanogaster* strains:** Nonphototactic mutants were isolated from the Canton-Standard (C-S) normal strain by induced muta-

genesis and countercurrent distribution.[12] The detailed properties of the mutants, complementation tests, and genetic mapping will be described elsewhere. All are located on the X-chromosome and have defects detectable by the electroretinogram (ERG).

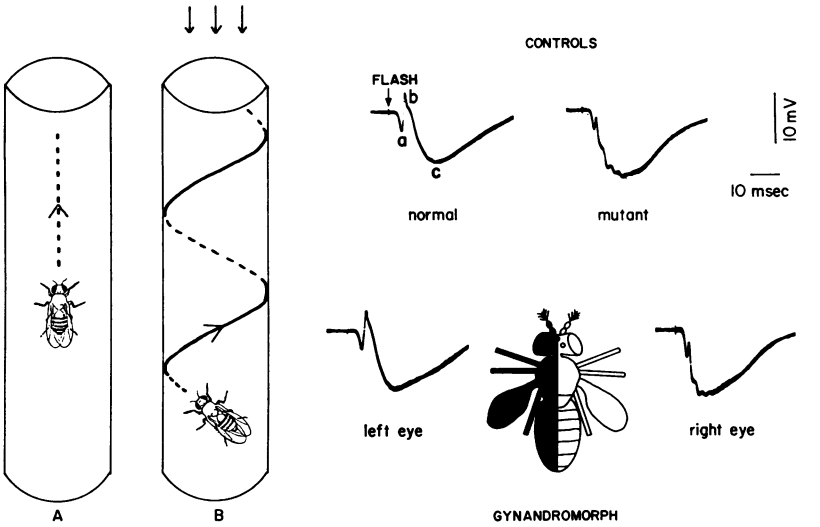
(*b*) **Production of gynandromorphs:** *Method I:* In the strain $In(1)w^{vC}$ (inversion in first (X) chromosome, white-variegated eyes, Catcheside), which will be abbreviated as w^{vC}, the ring-shaped X-chromosome is frequently lost from one daughter nucleus at the first mitotic division of a heterozygous egg. This property is not simply due to the ring shape of the chromosome, since rod variants are obtainable that still retain the property.[13] The eyes of this strain have variegated pigmentation which is dominant to the *w* (white eyes) gene. Males carrying the X-linked recessive mutation (*x*) to be studied, plus marker genes, such as *y* (yellow body) and *w* (white eyes and ocelli) were mated to virgin females heterozygous for w^{vC} to generate $w^{vC}/y,w,x$ females. Approximately 10% of the female progeny showed mosaicism evident on inspection under the dissecting microscope. These arose from female embryos in which elimination of the ring chromosome produced male parts of the constitution $y,w,x/O$. Since the genes *y*, *w*, and *x* are recessive, the female parts had wild phenotype, except for variegation of eye color. The male parts showed the *y* and *w* characters, thereby indicating uncovering of the *x* gene in those parts. Other marker genes[14] used in some cases were sn^3 (singed bristles) and f^{36a} (forked bristles).

Method II: In eggs laid by a mother homozygous for the gene ca^{nd} (claret eye, nondisjunction-inducing) on the third chromosome, elimination of any of the four maternal chromosomes often occurs in the initial nuclear division.[15] Loss of one of the second or third chromosomes is lethal, but this is not true for the X-chromosome, so that gynandromorphs containing XX and XO tissues are produced. Elimination of one of the pair of tiny fourth chromosomes is recognizable by the presence of minute bristles, a prominent trident pattern on the thorax, and abnormality in the L5 wing vein. In these experiments, gynandromorphs in which this haplo-4 property was detected were excluded. Since haplo-4 individuals show a normal ERG, any undetected mosaicism for haplo-4 should not have affected the results. *Method II* is less efficient than *Method I*, because of the loss of many progeny, but it has the advantage of being useful with dominant X-linked mutations, since the X-chromosome bearing the dominant gene can be eliminated.

(*c*) **Scoring of gynandromorphs:** Each gynandromorph was examined for the presence of marker phenotypes over the entire body and the results recorded on a form sheet with prepared outlines. The eye color markers *w* and w^{vC} and the body surface markers, such as *y*, f^{36a} or sn^6, being visible over most of the body, permitted determination of the division line between male and female parts.

(*d*) **Electroretinograms:** The technique described by Hotta and Benzer[16] was used, employing a strobe flash lamp 30 cm from the front of the fly and saline-moistened wick electrodes placed on each eye. The ERGs of both eyes were recorded simultaneously; the common reference electrode was a capillary inserted within the body cavity. For these measurements, mosaics in which an entire eye was not of a single genotype were not used.

Results. (*a*) **Behavior of mosaic flies:** The positive phototactic response in *Drosophila* is an example of tropotaxis,[17] i.e., the fly moves toward the light in such a way as to equalize the light intensities falling on the two eyes. Flies of the normal C-S strain are also negatively geotactic. When placed in a vertical tube in darkness, a fly climbs straight up, using gravity as a cue (Fig. 2*A*). If light shines from the top, a normal fly still climbs straight up, the proper direction for phototaxis being consistent with gravity. If this experiment is now done with a gynandromorph having one eye of normal genotype, the other eye of mutant type tan^1, which has deficient visual function, the result in darkness is the same as for a normal fly, but when the light is turned on, the fly climbs in a



(*Left*) FIG. 2. Behavior of mosaic fly with one visually defective eye. (*A*) In darkness. The fly, being negatively geotactic, climbs straight up. (*B*). With light shining from above, the fly turns its defective eye toward the light and climbs a helical path. Control flies, with two good eyes, climb straight up both in light and in darkness.

(*Right*) FIG. 3. Electroretinogram (ERG) of normal, mutant, and mosaic flies. Stimulus is a 20-microsecond strobe flash of white light. *Upper left:* Normal ERG, recorded from a w^{vC} fly. *Upper right:* Abnormal ERG, recorded from a *w*, *tan*^{1}, f^{36a} fly. *Below:* Schematic drawing shows a gynandromorph in which the shaded left half is XX female (w^{vC}), and the right half is XO male, in which the *tan*^{1} gene is uncovered, as indicated by the markers *w* and f^{36a}. The male eye gives a typical *tan*^{1} ERG, while the ERG of the female eye is normal.

helical path (Fig. 2*B*), always turning the bad eye toward the light in a futile attempt to balance light sensations on the two sides. Thus, if the right eye is abnormal, the fly traces a right-handed helix; if the left eye is abnormal, it traces a left-handed helix. The behavior is similar to that observed long ago by Garrey in the robber fly, after blackening one eye[18], and similar observations have been reported in many cases.[4,17] Ten *tan*^{1} bilateral gynandromorphs, made by *Method I*, were tested, five having the mutant eye on the right side, five on the left. In every case, the result was as in Fig. 2, the sign of the helix being the same as the bad eye side. Control experiments were done, under the same conditions, using gynandromorphs in which no *tan*^{1} mutation was present, i.e., with only the marker genes *w* and f^{36a}, to test for any asymmetry due to male or female parts or the difference in pigmentation of w^{vC} (female) versus *w* (male) eyes. Of 14 flies tested, 12 climbed straight up, both in darkness and in light, just as normal flies do. (The remaining two flies climbed poorly in darkness and gave erratic results on repeated trials in light.) These results indicate that the *tan*^{1} visual deficit of the mutant is indeed expressed in the behavior of composite flies; parabiosis with a normal half fly does not remove the deficit.

(*b*) **Electroretinograms of mosaic *tan*^{1} flies:** The ERG of *Drosophila*, in response to a short flash of light, shows two main components.[16] The first is a receptor potential which apparently arises from depolarization of the photoreceptor cells and is recorded as a corneal negative wave; this triggers a neural

discharge which is seen as a positive spike. In the nonphototactic mutant *tan*[1], the negative wave is normal, but the positive spike is not.[16] Thus, in a fly that is mosaic for this character, the ERG can be tested separately in each eye. A typical result obtained from a bilaterally mosaic fly made by *Method I* is illustrated in Fig. 3. The ERG obtained from the female eye, in which the *tan*[1] gene is masked by its normal allele, is the same as for a normal fly. The ERG recorded from the male eye, in which the recessive *tan*[1] mutation is uncovered, is defective in the same way as in a non-mosaic *tan*[1] fly. Thus, the presence of a normal half-body, in parabiosis with the mutant half, does not compensate for the ERG defect in the *tan*[1] eye.

The same result is obtained regardless of the amount of normal female tissue present, even when the mutant eye is associated with an otherwise entirely normal body. Fig. 4 shows the complete results for 44 flies mosaic for the *tan*[1] mutation; the normal body parts are shaded. Group A, in which both sides of the head were of normal genotype, always gave normal ERGs from both eyes, even though all the rest of the body was mutant. In group C, where both sides of the head were mutant, both eyes always gave defective ERGs, even when the remainder of the body was normal. Group B shows flies whose heads were split into normal and mutant portions, arranged roughly in order of increasing amount of normal tissue. In every case, the mutant eye gave a defective ERG, the normal eye a normal one. This mutation is therefore clearly autonomous, residing within or close to the eye.

(c) Results with ERG mutants of various cistrons: A series of nonphototactic mutants have been isolated that show detectable ERG abnormalities. By genetic mapping and complementation tests, these have been assigned to five distinct functional genes (cistrons) on the X-chromosome. The properties and genetics of these mutants will be described in detail elsewhere; only the experiments with gynandromorphs are given here. Other workers have also isolated abnormal-ERG mutants, some of which probably belong to these groups.[19]

(i) Receptor potential I group: Mutants of this group are characterized by near-absence of even a receptor potential in the ERG, indicating lack of response of the photoreceptor cells to light. Six independently-arising mutants of this group have been isolated. They fail to complement each other, i.e., a female heterozygote, containing one mutation on one X-chromosome and another mutation of the same group on the other X-chromosome, has the mutant phenotype; the mutations are hence regarded as affecting the same cistron. Three of the mutants (JM11, EE5, and KO50) have been made into mosaics, mainly by *Method I*. A total of sixty gynandromorphs were tested, including 38 having one mutant and one normal eye. Every eye behaved autonomously. KO50 is a special member of this group in that it is temperature-dependent, both with respect to ERG and phototactic behavior: at high temperature (28°C) it shows no phototaxis and no receptor potential, while at 18°C it is almost normal in both respects. A fly with one KO50 eye and one normal eye gives one defective ERG and one normal ERG at high temperature, but at low temperature the KO50 eye gives a near-normal ERG. Thus, the good eye in a gynandromorph can be used as an internal control in experiments on the mutant eye.

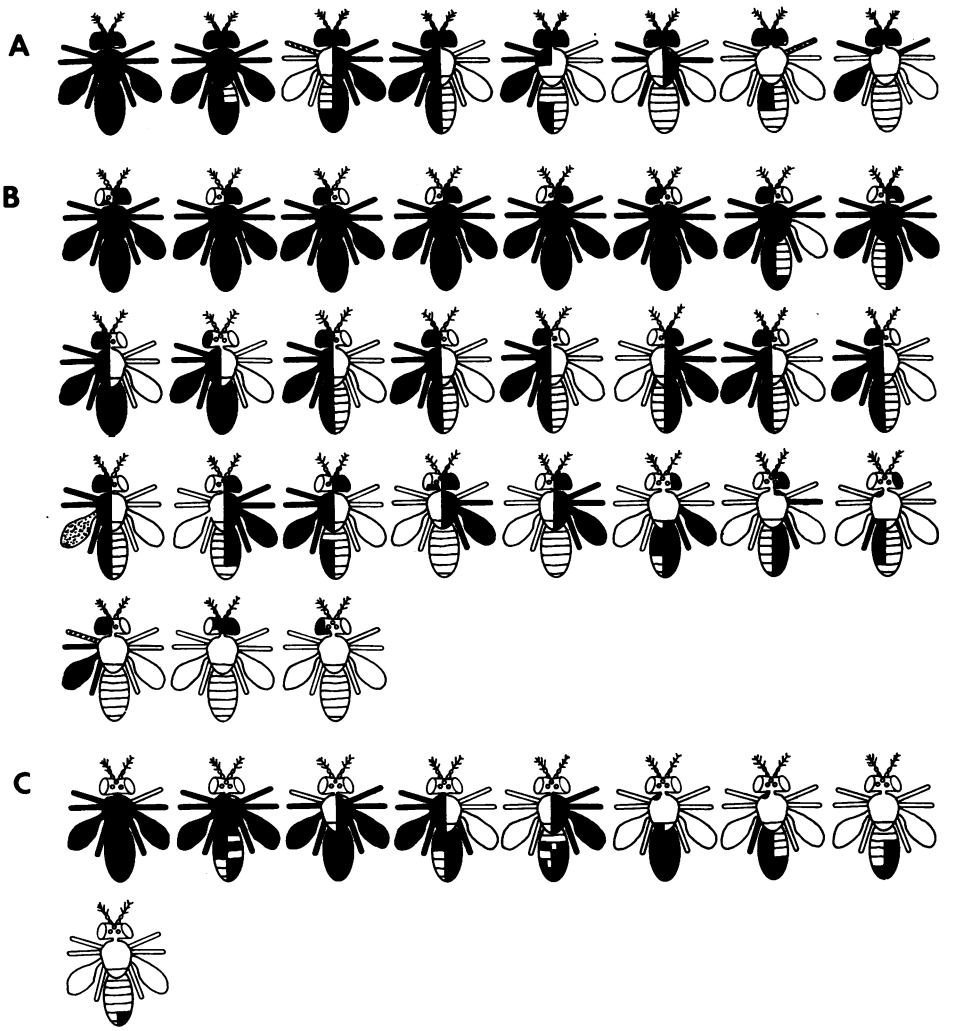


FIG. 4. Autonomy of the *tan*^{1} visual defect in 44 gynandromorphs. Female parts shaded; male parts, in which the *tan*^{1} mutation is uncovered, are unshaded. *Group A:* Both eyes female. These flies gave normal ERGs from both eyes. *Group B:* Split heads with eye female, the other eye *tan*^{1} male. These flies all produced a normal ERG from the female side and a *tan*^{1} ERG from the male side. *Group C:* Flies with heads totally male. These flies produced *tan*^{1} ERGs from both eyes.

(*ii*) *Positive spike I cistron:* Fifteen independently-arising mutants have been obtained which show properties similar to *tan*^{1} (see ERG in Fig. 3) and which fail to complement *tan*^{1} (or each other) in heterozygotes. Among them, *tan*^{1} was chosen for the test by the mosaic technique; 24 gynandromorphs were made by *Method I* and 20 by *Method II*. As illustrated in Fig. 4, the *tan*^{1} defect was in every case autonomous.

(*iii*) *Positive spike II cistron:* The two mutants of this group show the same ERG abnormality as *tan*^{1}, but do not have the associated light body color.

Moreover, they complement mutants of the *tan*[1] group and map at a different region of the X-chromosome. One of the mutants (BS18) was tested in five gynandromorphs (made by *Method I*) that had one normal and one mutant eye. All gave autonomous results.

(*iv*) *Receptor degeneration I cistron:* Five noncomplementing mutants are known in this group, in which the rhabdomeres of the receptor cells degenerate with age, and the ERG is characteristically small and delayed. One of these mutants (PC47) was tested in 34 gynandromorphs (2 made by *Method I*, 32 by *Method II*) of which 15 had one normal and one mutant eye. Autonomy was observed in every case. Degeneration of the rhabdomeres in male eyes was also confirmed in each case by observation of the pseudopupil which, in the normal eye, has a precise shape due to the optical properties of the photoreceptor-cell rhabdomeres;[20] in the mutants the pseudopupil presents a scrambled aspect. Another mutant of the group (BS12) gave similar results in five gynandromorphs (*Method I*) having one normal and one mutant eye. A third mutant (KO14) gave the same result in one bilateral gynandromorph tested.

(*v*) *Receptor degeneration II cistron:* This group contains six mutants that do not complement each other, but do complement mutants of the group (*iv*), although both groups present the same histological picture of rhabdomere degeneration. A total of 31 gynandromorphs (3 made by *Method I*, 28 by *Method II*), of which 18 had one mutant and one normal eye, were tested for one of these mutants (KO45). The eye was in all cases autonomous in respect to both ERG and degeneration.

(*d*) Mosaicism within a single compound eye: In the ERG measurements described above, gynandromorphs in which the dividing line cut across a compound eye were not used, because the ERG, as measured, is a mass potential to which many facets of the eye contribute. More precise localization could be done by intracellular recording, which is more difficult. With degenerative mutants, it is possible to observe the effect of the mutation on the individual receptor cells by anatomical examination. The male tissue can also be marked by *w*, which prevents formation of the screening pigment, thus identifying the sex of the pigment cells that separate the ommatidia. In six such mosaic eyes studied histologically, representing both of the degenerative cistrons, the boundary line between pigmented and nonpigmented areas coincided closely with that which separated degenerated receptor cells from normal ones, demonstrating a high degree of autonomy of the degenerative effect.

Discussion. For the mutants described here, the effect of the mutation is autonomous within the eye or very closely associated tissue. For the purpose of dissecting the nervous system with mosaics to locate functional sites, that is a desirable feature. Since these mutants were chosen for ERG abnormalities, it is not surprising to find their primary defects associated with the eye. Other kinds of behavioral mutations might be expected to affect other sensory systems or more centrally located nervous system structures. Ikeda and Kaplan[23], in recent studies of gynandromorphs for the *Hk* (hyperkinetic) mutation which causes shaking of the legs when the fly is etherized, find that the effect is closely linked to the individual legs, and presumably the associated thoracic

ganglion regions. In our studies on mutants with abnormal circadian rhythm (Konopka, Hotta, and Benzer, unpublished experiments), the results with gynandromorphs indicate that the rhythm-determining mechanism resides in the head of the fly.

With only surface markers, such as body color or bristles, the internal distribution of male and female tissues cannot be identified. A mutant eye does not guarantee that the associated optic ganglia are also of mutant type. To pursue the gynandromorph method further into the central nervous system will require means to distinguish internal male and female cells. This might be accomplished by a chromosome staining technique analogous to the distinction of male from female human cells; Lewis and Hodgetts (personal communication) have had promising results with this method. Other possible methods include the use of a mutation affecting some cellular enzyme or antigen that can be detected histochemically, or a mutation causing a nutritional requirement that could be used to label the male cells by autoradiography. Such cellular markers should provide powerful techniques for tracing the details of cell lineage during development, as well as genetic dissection of the functioning nervous system.

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Abbreviation: ERG, electroretinogram.

[1] Sturtevant, A. H., *Proc. Soc. Exp. Biol. Med.*, **17**, 70 (1920).
[2] Beadle, G. W., and B. Ephrussi, *Genetics*, **21**, 225 (1936).
[3] Butenandt, A., W. Weidel, and E. Becker, *Naturwissenschaften*, **28**, 63 (1940).
[4] Morgan, T. H., and C. B. Bridges, *The origin of gynandromorphs* (Washington: Carnegie Inst. Publ. 278, pp. 1–122, 1919).
[5] Sturtevant, A. H., *Z. Wiss. Zool.*, **135**, 323 (1929).
[6] Parks, H. B., *Ann. Entomol. Soc. Amer.*, **29**, 350 (1936).
[7] Patterson, J. T., and W. Stone, *Univ. Tex. Publ.*, **3825**, 1 (1938).
[8] Garcia-Bellido, A., and J. R. Merriam, *J. Exp. Zool.*, **170**, 61 (1969).
[9] Stern, C., *Genetic Mosaics and Other Essays*, (Boston: Harvard Univ. Press, 1968).
[10] Whiting, P. W., *J. Comp. Psychol.* **14**, 345 (1932).
[11] Rai, K. S., and G. B. Craig, Jr., *Proc. Int. Congr. Genet. 9th*, **1**, 171 (1963).
[12] Benzer, S., *Proc. Nat. Acad. Sci. USA*, **58**, 1112 (1967).
[13] Hinton, C. W., *Genetics*, **40**, 951 (1955); **42**, 55 (1957).
[14] Lindsley, D. L., and E. H. Grell, *Genetic variations of Drosophila melanogaster* (Washington: Carnegie Inst. Publ., 627, 1967).
[15] Sturtevant, A. H., *Z. Wiss. Zool.* **135**, 323 (1929); Lewis, E. B., and W. Gencarella, *Genetics*, **37**, 600 (1952).
[16] Hotta, Y., and S. Benzer, *Nature*, **222**, 354 (1969).
[17] Fraenkel, G. S., and D. L. Gunn, *The orientation of animals* (Oxford: Oxford Univ. Press, 1940).
[18] Garrey, W. E., *J. Gen. Physiol.*, **1**, 101 (1918).
[19] Pak, W. L., J. Grossfield, and K. S. Arnold, *Nature*, **227**, 518 (1970). Heisenberg, M., and K. Götz, personal communication.
[20] Kirschfeld, K., *Exp. Brain Res.*, **3**, 248 (1967).
[21] Sonnenblick, B. P., in *Biology of Drosophila*, ed. M. Demerec (New York: Hafner Publ. Co., 1965), p. 88 ff.
[22] Poulson, D. F., in *Biology of Drosophila*, ed. M. Demerec (New York: Hafner Publ. Co., 1965), p. 246.
[23] Ikeda, K., and W. Kaplan, *Proc. Nat. Acad. Sci. USA*, **67**, *this issue* (1970).