

THE USE OF MOSAICS IN THE STUDY OF THE DEVELOPMENTAL EFFECTS OF GENES

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One of the central problems of biology is that of differentiation—how does an egg develop into a complex many-celled organism? This is, of course, the traditional major problem of embryology; but it also appears in genetics in the form of the question, "How do genes produce their effects?" One of the most productive methods of studying the question in its embryological aspects is that of grafting, as elaborated by LEWIS, HARRISON, SPEMANN and others associated with them. The use of the grafting technique is also possible in studying the question from the geneticist's point of view, and much valuable information is available. The method has, however, certain limitations, some of which can be avoided by the use of spontaneously occurring mosaics, such as gynandromorphs and somatic mutants.

There are two questions that one may hope to study through the use of such mosaics, namely in what parts of the body and at what times of development are specific genes effective? The latter (the question of time of action) may also be studied by methods involving spontaneous changes in the course of development, as in GOLDSCHMIDT'S experiments, or induced changes such as those described by LILLIE and JUHN; the former question (as to the place at which genes act) may also be studied by means of grafting, constriction, excision, centrifuging, or other treatment.

If a *Drosophila* egg starts development as a female, with two X chromosomes, it was shown by MORGAN and BRIDGES that one of these X's is occasionally eliminated from a cleavage nucleus. The cells descended from such a nucleus are male in character, and there results a gynandromorph. It often happens in experimental cultures that the male parts of such a gynandromorph have sex-linked recessive genes that are suppressed by their dominant allelomorphs in the female parts of the specimen. MORGAN and BRIDGES showed that, in general, the sex and the sex-linked characters in such gynandromorphs are completely autonomous in development. That is to say, each part develops according to its own constitution, producing the same end-result as though it had formed part of an entire individual of that same constitution. This remains the rule in *Drosophila* gynandromorphs; but a few exceptions have appeared, and these may be described briefly.

I have shown that the sex-linked dominant gene for Bar eye is an exception to the general rule. Bar acts to reduce the area of the eye, and the number of facets removed is the most convenient index of its action. Study of mosaics for Bar shows that the area occupied by facets in the normal

eye may be divided into three regions with respect to the activity of the Bar gene. In the periphery of this area Bar removes facets not only from tissue that is genetically Bar but also from small not-Bar regions; in the central area Bar never removes facets under any condition; in the intermediate zone Bar removes facets unless not-Bar tissue is present, in which case facets develop in regions that are Bar in constitution and that lie close to not-Bar regions. I have interpreted these results as demonstrating a mutual effect of Bar and not-Bar areas on each other. It must be admitted, however, that it remains possible that we are dealing with a time relation, and that the zones differ only in the relative time at which it is determined in development that facets are or are not to be developed. Bar mosaics have been studied by the use of the Minute-n of BRIDGES, which causes eliminations late in development; if a series of early cleavage mosaics for Bar could be studied the question could be settled. Unfortunately there is no convenient method of obtaining such cleavage mosaics in quantity in *Drosophila melanogaster*, and Bar is known only in this species.

I have recently studied several of the allelomorphs of the sex-linked recessive scute in mosaics, and have found that scute is also an exception to the general rule of autonomous development. Here again most of the mosaics studied are due to eliminations occurring late in development. The results show that scute, which has as its effect the removal of specific bristles, does not remove these bristles as frequently when they lie in small patches that are scute in constitution as it does in flies that are wholly scute. Here also there is a possibility that we are dealing with a time relation, though my own interpretation of the scute series of allelomorphs leads me to suspect that the smallness of the areas scute in constitution is responsible. In this case cleavage mosaics are again needed; a few have been found, and these strongly indicate that scute shows autonomous development in large areas that result from eliminations occurring early in development.

It was observed several years ago by several investigators that yellow body-color present in the form of small spots on wild-type flies is usually not as distinctly yellow as the same area in wholly yellow flies. It has been uncertain whether this incompleteness of the yellow effect was a real one or was due to the presence of underlying not-yellow tissues partly visible through the yellow area, or possibly to some reaction in the eye or nervous system of the observer. Both of these possibilities are rendered unlikely by the recent observation that such small spots of silver body color are regularly perfectly distinct and possess sharper outlines than do yellow spots—though silver itself is a color similar to yellow but less different from wild-

type. Yellow, like scute, must be classed as a gene whose effects are weakened in small spots but not in large areas of mosaics.

The first exception found to the rule of autonomous development in *Drosophila* was the sex-linked recessive eye color, vermilion. It has long been known that gynandromorphs with one or both eyes genetically vermilion may have eyes wild-type, vermilion, or intermediate in color. The results from mosaics due to elimination late in development were not particularly helpful here; but vermilion has recently appeared in *Drosophila simulans*, where there exists a method of obtaining early cleavage mosaics in large numbers. A series of such specimens has now been studied, and definite conclusions are available. In such mosaics, as the experiment was carried out, ovaries are always not-vermilion in constitution and testes are always vermilion in constitution. Of the specimens having eye-tissue vermilion in constitution and large enough in area to classify certainly for color, 51 of the 53 flies that had two ovaries were wild-type in eye color; all 20 of those with two well-developed testes were vermilion or intermediate in eye color; those with poorly developed testes, or with one ovary and one testis, fell into any one of the three color classes.

These results mean that the vermilion color in such mosaics is suppressed by something produced by the wild-type ovary. Three separate elements in the system may be distinguished: (1) constitution of the facet area itself; (2) constitution of the gonad; (3) constitution of some other region, or perhaps merely presence or absence of not-vermilion tissue in the fly. The third element is necessary to account for the differences between specimens with like gonads. Tabulation of such specimens indicates that this third element does not lie in the abdomen; but no particular region of the head or thorax appears to be more effective than another. One may surmise that the effective element is some internal organ not very closely related (in terms of cell lineage) to the surface areas.

These experiments were carried out by mating claret ♀ × vermilion garnet forked ♂. The garnet and forked served to identify the male regions in the 180 gynandromorphs observed. Two controls were also made: claret ♀ × garnet forked ♂ made it certain that garnet is completely autonomous in development; vermilion claret ♀ × vermilion garnet forked ♂ gave 86 wholly vermilion gynandromorphs with vermilion garnet areas, from which it is clear that poorly developed gonads alone do not interfere with the development of vermilion eye color.

The relation between eye pigment and gonad constitution described above is especially interesting in connection with the results of DOBZHANSKY, who

studied *D. simulans* gynandromorphs in which the male parts were genetically white. The eye color in such mosaics showed autonomous development, as has long been known. White-eyed males also have transparent vasa efferentia and testicular envelopes, those of wild-type males being bright yellow at emergence; DOBZHANSKY showed that this yellow color does not show autonomous development. A testis or a vas efferens attached to a female (that is, under the conditions of the experiment, wild-type) duct or ovary will be yellow at emergence even though it be white in constitution. Furthermore, even if the entire genital apparatus be white and male in constitution, color may still develop, though much more slowly, if wild-type tissue is present elsewhere in the fly.

DOBZHANSKY and I have recently studied this slower development of color in somewhat more detail. It appears that, under the conditions stated, if both eyes of the mosaic are red (in some cases with very small white areas) the testes develop some color about 4 days after emergence; if both eyes are white or with a small spot of red, color does not appear in the testes until 9 to 10 days after emergence. If the eyes have both white and red present in considerable areas, the result is more variable, but color usually develops in the testes 5 to 8 days after emergence. One may conclude that there is a reciprocal relation here; in vermilion the gonads affect the eyes, in white the eyes affect the gonads.

It is clear that in most cases there is a chain of reactions between the direct activity of a gene and the end-product that the geneticist deals with as a character. One may surmise that any valid generalizations about these reactions are more likely to concern the initial links than the terminal ones. However, it is the terminal ones that are usually more open to experimental attack, since the only index to the effectiveness of a given experimental technique is the condition of the end-product. Looked at from this point of view, the type of experiment that I have described may be considered as a beginning in the analysis of certain chains of reactions into their individual links.