*XXI. THE DEVELOPMENT OF THE EYE AND OPTIC TRACT IN DROSOPHILA MELANOGASTER AND ITS 'EYELESS' MUTANT.**

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The mutant of the fruit fly, *Drosophila melanogaster,* known as 'eyeless' has been the object of much interest since its discovery both because of its genetic behavior and because of changes in the embryological development which makes possible profound modifications of the head regions of the fly. Evidently in the structural changes involved in this mutation are to be seen modifications not only of an adult condition, but functional disturbances of the whole development processes so far as they concern the eye and optic tract and, secondarily, the other head structures. This interest in eyeless suggested the importance of an embroyological study of the development of the eye and optic tract in both normal and eyeless flies. The purpose was to compare their genesis in the normal and the eyeless stock, to determine the time of appearance and behavior of the mutation. Two methods of approach to the problem were worked out: (a) chrono-

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logical development of the optic apparatus through larval, pupal and adult stages and its comparison in the two sets of flies; (b) the use of graphs to compare rates of growth of the various life stages and organs in the flies.

That a study of the development of the eye in both normal and eyeless Drosophila might throw some light on the genetic question was suggested to the writer by Dr. A. Richards. His interest and helpful criticism have been the most valuable factor in the work. The writer is also indebted to Dr. A. O. Weese for aid with graphic work relating to growth and size relations; and to Mrs. Richards for the loan of notes and lists of literature.

Observations on the structure and development of the insect head have been made over a period of very many years, for insect anatomy proved a fascinating field of work for many of the earlier students of microscopy. The contributions to the modern knowledge of the development of the eye and head parts have been made by Hickson (1885). Villones (1886, 1887, 1891), Patton (1887, 1888), and Wheeler, (1893). Berger, 1878, described the optic tract and he identified the optic nerve. Weismann, 1864, has given an account of the external appearance of the developing eyes and optic tract. Recently a few embryological studies have been made on the eyes and associated parts of the nervous system in Drosophila. O. A. Johannsen, 1924, published a study of the structural variations of the ommatidia in various Drosophila mutants such as glazed, spineless-glass, and wax, together with some eye color mutants. A microscopic study of "The Eye and Optic Tract in Normal and 'Eyeless' Drosophila" by Mildred Hoge Richards and Esther Y. Furrow (1922-1925), forms the histological basis for the present work. According to these investigators, the terminal anastomosis or postretina lies directly behind the basal plate separated by a layer of granular cells from the external medullary mass. Embedded within the cortex of the more distal part of the optic lobe are two cap-shaped bodies, closely applied to each other and containing radially arranged fibres, the middle medullary mass. Behind it stand, with their long axes at right angles to it, three inner medullary masses, comprising an anterior, a middle and a posterior capsule. The middle capsule is also known as the inner chiasma, the outer chiasma lying between the outer and middle masses.

Krafka (1920) has determined the time of ommatidial differentiation in Drosophila, and established its identity with the 'critical period' in larval life. The temperature of the medium at this period determines, in 'bar-eyed' flies, the number of ommatidit ,to be present in the adult eye.

An account of the early development of the eye of the bee has been given by Nelson, 1915, in his "Embryology of the Honey-Bee." Observation on the Development.

The larvae of the normal flies, when they emerge from the egg, show no segmentation of the ventral nerve cord, the nervous system being, on the other hand, more strongly concentrated than in the adult stage. The two supraoesophageal ganglia and large, subspherical masses are attached by thick, short crurae to a thick-based cone, the anlagen of the ventral cord and the suboesophageal ganglia. Its whole length is about one-third of the body on the first day; in the full-grown larva it is about one-sixth of the body length.

The optic discs, which are the rudiments of the ommatidial layer, do not appear on the surface of the supraoesophageal ganglia (or so-called 'hemispheres') until the larva is around fifty hours old. It is at first a thin, transparent plate of undifferentiated cells, but rapidly thickens and becomes opaque. It is attached to the hemispheres by a thin, hollow stalk at its posterior border. Growth takes place principally in the anterior borders, which spread around the front of the hemispheres, folding and curling the edges of the disc. Simultaneously with this sudden growth, the ommatidial elements become visible, presenting a characteristic arrangement of large, polygonal cells in its outer surface.

As the optic disc develops, the spherical 'hemispheres,' as they are commonly named, although the term is misleading, begin to show a medial constriction, which rapidly deepens and enters into the substance of the hemisphere. Finally the ganglion presents two distinct lobes, an inner, still spherical 'corpus centrale' and an outer, cap-shaped optic lobe. Within the optic lobe is a concentration of medullary fibers, grouped into two large bundles, the inner and middle medullary masses. The outer medullary mass is formed in a peculiar manner, in the pupal stage of the fly.

Soon after the establishment of the pupal state, a striking dorsal flexure of the supraoesophageal and certain other ganglia appears, shifting the hitherto anterior surfaces of the hemispheres to face dorsally, and the faces that were posterior are pressed down upon adjoining end of the upper portion of the ventral cord. This brings the optic disc to face laterally and dorsally, as do the compound eyes of the adult fly. These discs now begin to expand, losing their folds, and moving out toward the outer surface of the head, finally coming to rest just beneath the new cuticle. The optic stalk, also, has become funnel-form, the large end applied to the inner face of the disc, the smaller to the optic lobe.

On the fifth day (first day nymph) the ommatidial rudiments lie in a cuboidal 'cell' of tissue, with the cuticle forming the corneal facets over them. On the sixth day, they begin to lengthen into their characteristic spindle shape. The dark rhabdomeres can be seen extending down through the opening in the surrounding cells, continuing as nerve fibers toward the optic lobe. These are well developed on the seventh day. But on the seventh day there is still another organ in the optic stalk; an irregular layer of cells making their appearance just below the basilar membrane of the ommatidia, sending fibers back to the optic lobe. From present evidence, it appears that these are cortex cells, which have migrated out through the stalk to this position. Just below the cells, thickly laced fibers are gathered into radial bundles, constituting the outer medullary mass, the last organ of the optic tract to be laid down.

So far as the actual mechanism of development is concerned, there is no difference between that of the normal eye and the small eye which is usually present in the 'eyeless' stock. The optic plates are nearly always smaller in the larvae and pupae of the latter, and in a few individuals at least one plate seemed to be missing. This state of affairs is similar to the variable sizes of the eyes in adult eyeless.

Two questions arise concerning this reduction: i. e., is this reduction constantly present, or is there a normal early development, and later a check? It is evident that the decision here is of genetical importance. If the second is the usual mode of development in eyeless, then the variation in the amount of reduction in the adult must be due to some external influence, which checks the growth in varying degrees according to its strength. This is probably the case in bar-eyed mutations. If on the contrary, the first mentioned is the mode, the variable sizes of the adult eye are due to the modifying action of many other factors present in the chromosomes, which are so interrelated and numerous that they breed out in a large number of combinations.

In sections of the larval stages of eyeless flies, it was easily seen that the optic discs were reduced, but measurement of these was impracticable. In the pupal stages, the ommatidia could be counted in cross sections of the plate, with some advantage. The following table gives the maximum number of ommatidial rudiments in sections of pupal and adult heads:

	Normal	'Eyeless'
6 days old	34	23
7 days old	36	26
8 days old	33	24
Adults	31	23

The constancy of the results is striking. It may indicate that there is a limiting number of the ommatidia in 'eyeless' development.

Observations of Growth and Size Relations.

Graphs were first made of the growth of the body-length in terms of time, and also of the calculated body-volumes, with time. The curves produced were the typical sigmoid growth curves. However, body-size proved somewhat variable with the age, so the measurements of the optic discs were plotted against the diameter of the hemispheres, and body length, as being more accurate.

The first graphs were made of the relation of the optic-lobe portion of the hemisphere with the diameter of the optic plate associated with it. A fairly clear curve could be sketched from the plotting positions of these organs in normal flies, but in the mutant form, the amount of eyelessness varied so widely that no definite curve could be drawn from the scattered points. It has been shown (Richards and Furrow, '25) that the size of the optic lobe varies with the amount of reduction of the eye-disc in mature animals.

To rule out this modification of lobe-size by the disc reduction the disc diameters were again plotted, against body length, for both sets of flies. A much clearer curve appeared, the optic disc showing a fairly constant relation to the body length in both normal and eyeless animals, though the proportions are different in the two. It is important to note that the angle made by each curve with the x-axis was approximately forty degrees, indicating the *same rate of growth of the disc in both cases.* This was the conclusion sought for.

Other points add weight to the evidence. The curve cuts the y-axis, when extended, at a higher point in the case of the 'eyeless' graph, indicating that the reduction was present from the first appearance of the disc on the external surface of the brain. The earliest larva ('eyeless') in which the optic disc could be identified in a dissection was already four millimeters long, whereas discs were found in normal larvae of two and one-half millimeters. This may however, be due to the extremely small size of the early discs in eyeless larvae, preventing their recognition.

Summary and Conclusions.

1. There is no structural difference in the development of the eye and optic tract in an eyeless form fr mohtat of a normal Drosophila.

2. The growth curve of flies of the eyeless stock is essentially similar to the normal stock.

3. There is, in the normal larva and pupa, a definite relation between the growth of the optic lobe portion of the hemisphere and of the optic discs; both increasing at a constantly porportional rate.

4. In the eyeless stock, this relation is less definite, but neither, as a rule, reach quite the same size in eyeless adults as they do in the normal flies.

5. The *rate* of growth of the optic discs in both types of flies is approximately equal, but the growth in eyeless is begun later, and from a smaller anlage, than in the normals.

It can then be concluded, in regard to development in eyeless forms, that:

1. The optic discs of eyeless larvae and pupae show varying degrees of reduction when the standard is taken as the size in a normal larva of the same length.

2. The reduction in size of the eye-plate in eyeless is present from the first.

3. It is not the size of the facets which affects, but their number.

It may be safely concluded that the effect of the gene which produces eyelessness is a deep-seated one, producing an actual change in the body structure; it is not due to a weakening of the developmental processes of the eye which renders it susceptible to modifying factors external to itself. The mutation acts as a fundamental alteration in the organization and development of the animal.

Bibliography

Berger, E., 1878, "Untersuchungen über den Bau des Gehirns und der Retina der Arthropoden," Arb. d. Zool. Inst. Wein. u. Triest. Bd. I; 173.

Hickson, S. G., 1885, "The Eye and Optic Tract in Insects." Quart. Journ. Micr. Sci., 25; 243.

Johannsen, O. A., 1924, "Eye-structure in Normal and Mutant Drosophilas." Journ. Morph. 39.

Krafka, J. Jr., 1924, "Development of the Compound Eye of Drosophila melanogaster and its Bar-eyed Mutant." Biol. Bull. 47: 143.

Nelson, J. A., 1915, "The Embryology of the Honey-Bee." Princeton University Press.

Patten, W., 1887, "Eyes of Molluscs and Arthropods." Journ. Morph. I, No. 1.

Patten, W., 1888, "Studies of the Eyes of Arthropods II., Eyes of Asilius." Journ. Morph. II.

Richards, Mildred Hoge, and Esther Y. Furrow, 1922, "A Preliminary Report on the Optic Tract of Eyeless Flies." Proc. Okla. Acad. Sci. 2.

Richards, Mildred Hoge, and Esther Y. Furrow, 1925, "The Eye and Optic Tract in Normal and 'Eyeless' Drosophila." Biol. Bull. 39.

Viallanes, E., 1886, "Le cerveaux du Criquet (de la Guepe)." Ann. d. Sci. Nat. ser. 7., tom. 12.

Viallanes, E., 1887, "Etudes histologiques et organologique slr les centres nerveaux et les organs des sens des animaux articles." 4th Mem Ann. Sci. Nat. Zool., tom. 2; 5.

Viallanes, E., 1891, "Sur quelques points de l'histoire du developpment embryonnaire de la mante religieuse." Ann Sci. Nat., 7, II, 283.

Weismann, A., 1364, "Die nachkommen Entwicklung der Musciden nach Beabachtungen, an Musca vomitoria und Sarcophaga Garnoria." Zeit f. wsis Zool., Bd. 14, 187-328.

Wheeler, W. M., 1893, "A Contribution to Insect Embryology." Jour. Morph. 8, 1-160.