Pattern formation and differentiation center in the eye disc of *Drosophila*

YOSHIKO USHIODA

The ommatidial pattern formation and differentiation center during the development of the *Drosophila* compound eye is described using combined autoradiography and transplantation methods. The mitotic waves for photoreceptor formation produced from the posterior to the anterior of the eye disc during the third instar larvae. However, in the eye discs which removed the posterior edge does not occured such a mitotic waves. The fragments of the third instar eye discs implanted into adult abdomens with ecdysteroid, moulting hormone will differentiate to adult structures. Anterior or posterior fragments from the third instar discs were formed ommatidial structures. By contrast, the fragments from the early third instar discs, ommatidial formation were observed only posterior ones. However, dorsal and ventral half of the fragments in the early third instar discs were formed the ommatidium. These findings strongly suggest that the differentiation center exists at the two parts, that is dorsal and ventral part of the posterior region in the 60 hours eye disc and proceed from posterior to anterior region.

INTRODUCTION

The *Drosophila* eye disc is composed approximately 700 ommatidia arranged in a precise hexogonal array. Each ommatidium is complex light-sensitive organ composed of photoreceptor cells (22 cells included). The eye imaginal disc is the precurser of the adult eye and other head structures. It is attached anteriorly to the antennal disc and posteriorly to the brain by the optic stalk. The region of the undifferentiated eye disc that give rise to the adult eye consists of a monolayer of columnar cells in a hexogonal array.

The development of the eye disc proceeds through a series of inductive events which lead from an unpatterned epithelium to the highly organized adult com-

pound eye. The ommatidial assembly process starts in the eye imaginal disc of the third instar larvae as a wave of morphogenesis marked by a furrow in the epithelium advances from posterior to anterior. The development of the rhabdomeric pattern in the compound eye has been studied using various means (Ready et al, 1976 ; Campos-Ortega and Gateff, 1976 ; Campos-Ortega and Hofbauer, 1977 ; Fristrom and Fristrom, 1982 ; Kaji and Ushioda, 1983 ; Leboritz and Ready, 1986 ; Tomlinson and Ready, 1987 ; Ushioda, 1993). It was found from these experiments that the third instar larvae, a pattern first becomes evident as a clustering of cells at the posterior end of the eye disc.

To study the relation of cell division to formation of the pattern, has been used autoradiography. From these studies indicated that the mitotic waves for photoreceptor formation proceed from the posterior to anterior of the third instar larvae (Kaji and Ushioda, 1983 : Ushioda, 1993). In this report will be comformed that, a differentiation center for pattern formation exists or not in the eye disc during the larval development by means of transplantation experiments of the eye disc fragments.

Material and Methods

Individual of the *Oregon-R* strain of *Drosophila melanogaster* raised on standard medium at 25℃ were used as donors and hosts for the transplantation experiments.

Transplantation of eye disc fragments

Eye-antenna discs were dissected from larvae aged 60, 70, 72, 84 and 96 hours in a drop of sterile culture medium (Kaji and Ushioda, 1980). A tungsten wire with a sharpend edge was used to cut the eye discs. Discs were fragmented a head of the furrow, either paralled or obliquely to it, but far enough ahead to ensure that no furrow tissue had been included in the prospective transplant. Each disc fragment was transplanted with a glass micropipette to a sterile of culture medium. These fragments of the eye disc was implant to the adult abdomen. Thereafter, injection of ecdysteroid (moulting hormone) was repeated 2 days intervals into the host fly. The total amont of the hormone was 1.7 to 2. 0 μg / fly. After 10 days culture, metamorphosed implants was removed from the

host fly, and analysed of its imaginal cuticular structures (Fig. 1).

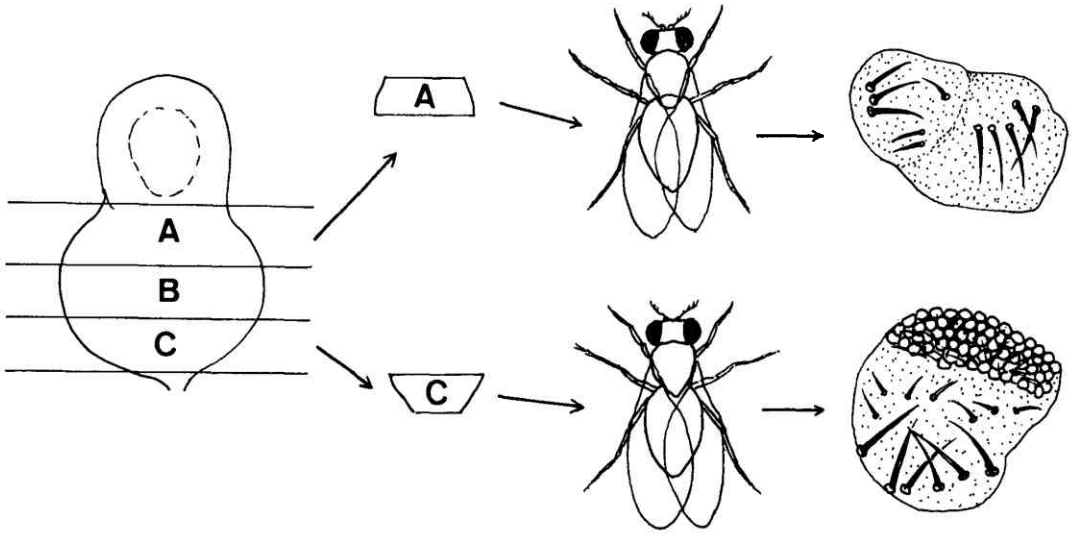


Fig. 1. Method for culturing imaginal eye disc fragments *in vivo*.
Schematic representation of experimental procedure. Eye disc fragment was transplanted into adult host hemocoel, after 10 days culture metamorphosed impiants was removed from the host fly and analysed its cuticular structure.

Autoradiographic method

The eye discs were labelled in 1 ml of culture medium containing 0.5 μl of ^{3}H--thymidine (specific activity 10 μCi / ml) for 40 min. After treatment with isotopes the materials were fixed by Squashed method (Ushioda, 1976, 1993) and were then stained with Giemsa solution.

RESULTS

The mitotic ability of eye discs implanted into adult hosts

The results of experiments were similar to that of the previous work (1993).

The eye disc from 70 hours old was dissected and implanted into adult fly. After 3 days culture the implant was removed from host fly, and exposed by ^{3}H--thymidine *in vitro*. Mitotic waves were detectable in the middle and anterior part of the eye disc. The middle wave produced to anterior region with the development and formed ommatidial clusters within the posterior region (Fig. 2. A). However, in the case of deeply and widely removed the part of the optic

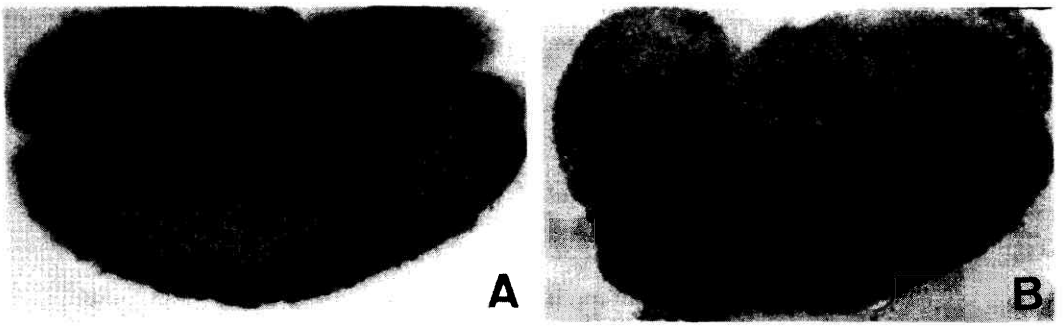


Fig. 2. Autoradiograph of two matured eye discs labelled by ^{3}H-thymidine.
70 hrs. eye discs (A and B) which had been cultured in adult abdomen for 3 days, and then pulse-labelled *in vitro* with ^{3}H-thymidine. A : control, B : removed the posterior region.

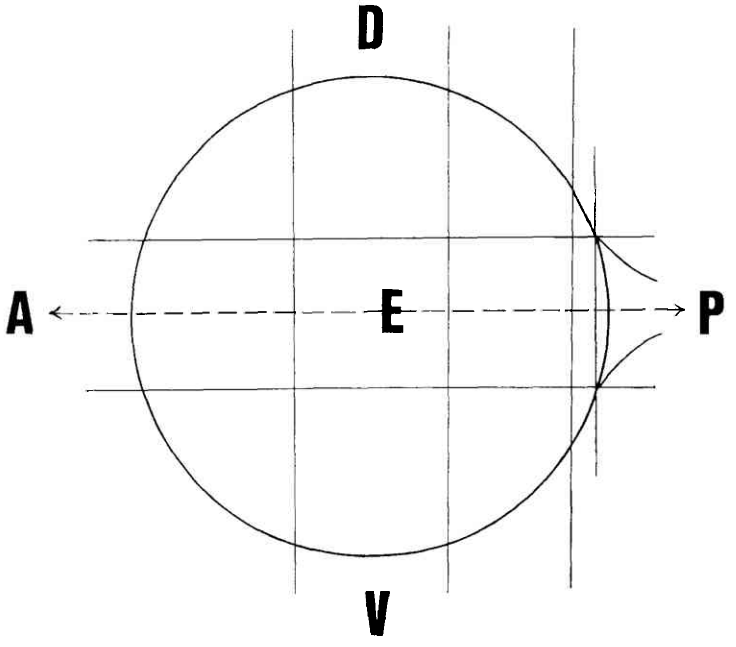
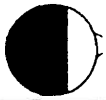
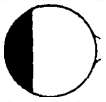
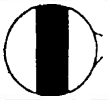


Fig. 3. Schematic drawings of eye disc.
A : anterior, P : posterior, D : dorsal, V : ventral, E : equater line.
The dotted line (A·····P), antero-posterior axis show the equater line. The eye stalk, at the posterior edge of eye disc connects to the brain lobe. The solid lines, showing the guide line for cutting.

stalk of 70 hours disc, incorporation could not observed (Fig. 2. B). These results indicated that the posterior portion of the eye disc is important field for the development of ommatidia.

Differentiating ability of the specific region in the eye discs

Based on these experiments, we attempted to clarify differentiating ability of the specific region in the eye discs during the 3rd instar larvae. Figure 3 is the schema of eye disc. The dotted line (A------P), antero-posterior axis show the equator line. The eye stalk, at the posterior edge of eye disc connects to the brain lobe. The solid lines, showing the guide line for cutting. We examined the imaginal differentiation of the fragments produced by transverse and longitudinal cuts.

	Age of eye disc	Facet formation	Drosopterin	Facetal bristle	Cuticle formation	Cuticle pigmentation	Bristle
	60	−	−	−	++	+	+
	72	±	−	−	++	++	++
	84	++	+	+	++	++	++
	96	++	++	++	++	++	++
	60	−	−	−	++	+	+
	72	−	−	−	++	++	++
	84	+	−	−	++	++	++
	96	++	+	+	++	++	++
	60	−	−	−	++	+	+
	72	±	−	−	++	++	++
	84	++	+	+	++	++	++
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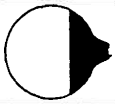	60	+	+	+	++	+	+
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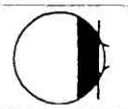	60	+	+	+	++	+	+
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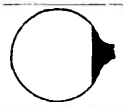	60	±	−	−	++	+	+
	72	++	+	+	++	++	++
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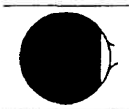
	60	±	−	−	++	+	+
	72	++	±	±	++	++	++
	84	++	++	++	++	++	++
	96	++	++	++	++	++	++

Table 1. Differentiation of the implanted eye disc fragments from the transverse cuts.
A shaded area indicate the implanted fragment.
Age of eye disc means the larval stage.
++ well developed, + developed, ± incomplete developed, − negative.

A. Differentiation of the implanted eye disc fragments from the transverse cuts

In the first series of experiments we cultured the eye disc fragments from the transverse cuts in the 60, 72, 84 and 94 hours ages. The results of experiments are illustrated in Table 1. The table shows that the particular structures of differentiating implants of different developmental stages.

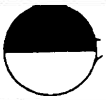
A shaded area in the eye disc indicate the implanted fragments. We examined the degree of the development in facet-formation, ommatidial pigmentation that is, drosopterin deposition, cuticle pigmentation and bristle formation. For example, the fragments which removed the posterior region of the eye disc from 60 hours old larvae, ommatidial formation, pigmentation and facetal-bristle formation were negative. The fragments from 70 hours age of discs show a similar pattern of differentiation except the facet formation. In this case, incomplete facet formation were observed. However, the fragments which removed from 84 or 96 hours eye discs are formed ommatidial structure. On the contrary, the fragments of posterior part of the eye disc could be observed the well-developed ommatidial structures in all of 60 to 96 hours eye discs.

B. Differentiation of the implanted eye disc fragments from the longitudinal cuts

In this series of experiments the eye disc fragments from the longitudinal cuts of different developmental stages were used (Table 2).

The fragments which removed the dorsal or ventral half of the 60 hours discs were formed ommatidial structures. By contrast, the fragment which include the equator line and posterior edge (narrow pieces), ommatidial formation were almost negative at the same stage. While, these fragments in the 84 hours discs were not difference in the ommatidial formation. In the case of the well-developed framents of the posterior region removed from 72 hours disc, differentiated ommatidia and their bristles were observed, but could not detected the ommatidial patterning, untanned thin cuticle, hairs and bristles were formed.

The above results of the both experiments are summerized schematically in Figure 4. As shown in Fig. 4, the differentiation of imaginal ommatidia derived from the fragment of 60 to 84 hours eye discs.

	Age of eye disc	Facet formation	Drosopterin	Facetal bristle	Cuticle formation	Cuticle pigmentation	Bristlc
	60	+	+	+	++	+	+
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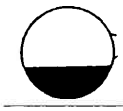
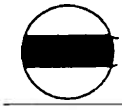
	96	++	++	++	++	++	++
	60	+	±	±	++	+	+
	72	++	+	+	++	++	++
	84	++	++	++	++	++	++
	96	++	++	++	++	++	++
	60	±	−	−	++	+	+
	72	+	+	+	++	++	++
	84	++	++	++	++	++	++
	96	++	++	++	++	++	++
	60	±	−	−	++	++	++
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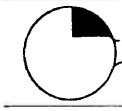
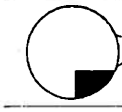
	84	++	++	++	++	++	++
	96	++	++	++	++	++	++
	60	+	±	±	++	++	+
	72	++	+	+	++	++	++
	84	++	++	++	++	++	++
	96	++	++	++	++	++	++
	60	+	±	±	++	++	+
	72	++	+	+	++	++	++
	84	++	++	++	++	++	++
	96	++	++	++	++	++	++

Table 2. Differentiation of the implanted eye disc fragments from the longitudinal cutts.

In the transverse series on the left hand, the fragments which does not contain the posterior region were not detected the ommatidial patterning in the 60 hours eye disc. By contrast, the fragment of posterior one's was well-formed ommatidial structure at the same age. Moreover, the posterior fragment which cut off the joint of optic stalk was also formed the ommatidial patterns. The fragment of posterior edge was low degree of ommatidia forming ability than in the case of posterior region which have a wide range. However, these fragments were formed ommatidial structure accompanyed by the progression of the development.

These results are interpreted that the differentiation center of the ommatidial formation exsists at the posterior region of the eye disc and proceed to the anterior region accompanyed by development.

In the longitudinal series on the right hand, the fragments of one-third of dorsal or ventral side were formed ommatidial structure at the 60 houres age. However, the fragments of the antero-posterior axis contained the equator line were less

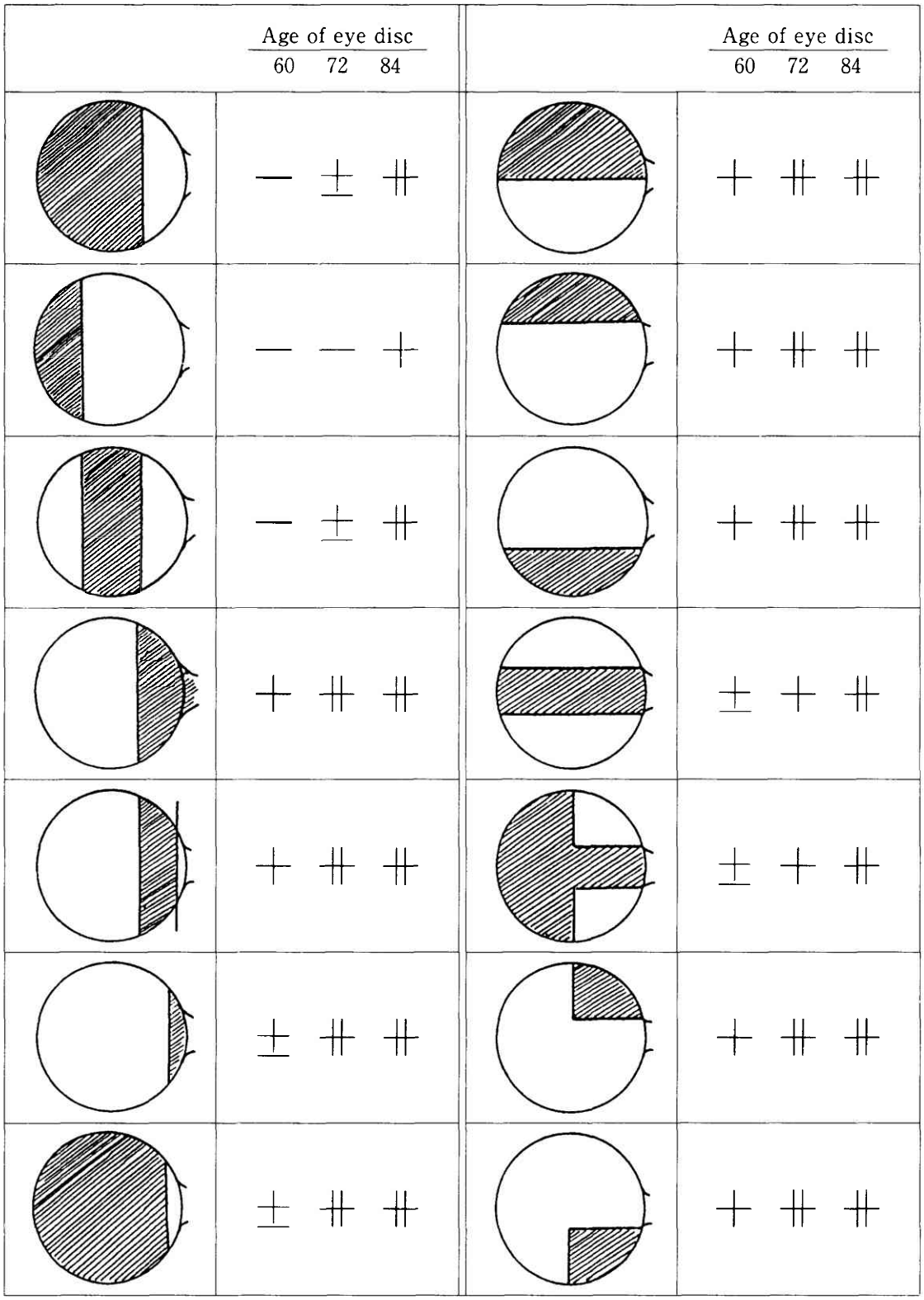


Fig. 4. Differentiation of immaginal ommatidia.

Showed the ability of ommatidial formation derived from the fragments of 60 to 84 hours eye disc.

ommatidia forming ability than the above experiments. Pieces from the upper half of dorso-posterial region and under half of ventro-posterial region of the 60 hours eye discs were formed ommatidia.

These resuls suggest that the differentiation center is already separated and exsits the dorsal and ventral part of the posterior region of the 60 hours eye disc.

Discussion

Pattern formation in the *Drosophila* compound eye begins at the posterior margin of the eye imaginal disc as undifferentiated cells assemble into ommatidial structures. During the third instar larvae, a pattern first becomes evident as clustering of cells at the posterior end of the disc by means of autoradiographic studies (Kaji and Ushioda, 1983; Ushioda, 1993). Similar results were observed with monoclonal antibodies (Lebovitz and Ready, 1982; Venkatesh, Zipursky and Benzer, 1985). In these studies, the fragments of the eye discs were cultured in larvae and removed from hosts before they pupated, Ommatidial clustars were visualized using a monoclonal antibody. In these implants, ommatidial formation progressed at it would have *in situ*; posterior region alway differentiated before more anterior areas.

The present expriments used implantation of the eye disc fragments into adult abdomen with moulting hormone. The implants was removed from the host fly after 10 days *in vivo* culture and investigated their structures. The results presented here indicated that the mechanism of pattern formation were also supported the author's previous studies. That is, anterior or posterior fragments from the third instar discs were formed ommatidial structures. By contrast, the fragments from the early third instar discs, ommatidial formation were observed only posterior ones. However, dorsal and ventral half of the fragments from the early third inster discs were formed the ommatidium.

Moreover, the present experiments demonstrated that the differentiation center for pattern formation exists or not in the eye disc using the implantation of the eye disc fragments in the adult flies.

It was suggested that the differentiation center in the developing eye of the mosquito, *Aedes aegypti* (White, 1963). He reported that a differentiation center exists at the posterior edge of the prospective eye region in the first instar of the

mosquito larvae.

It is concluded from the present experiments that direction of cell division is mirror image duplication to the equator line and ommatidial forming ability is not different between dorsal and ventral half of the eye discs. The differentiation center proceeded from posterior to anterior region along the direction of cell division.

Moreover so far as the present transplantation experiments concerned, it seems to be that the differentiation center exists at the two parts, that is, dorsal and ventral part of the posterior region (not edge) in the 60 hours eye disc.

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