*STUDIES ON THE MORPHOGENESIS OF MOSAIC COMPOUND EYE IN THE SILKWORM MOTH**

By

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I. INTRODUCTION

The colour mosaic patterns hitherto being found frequently in the compound eye of the silkworm moth appear in the anteroposterior position of the eye without exception. This fact was primarily found by TAJIMA (1942). He tried to perform the mosaic pattern in the eye by using a strain which has the mosaic gene, and produced a lot of mosaic eggs composed of black (normal) and red cells in the serosa. Majority of the larvae from these eggs grew to the adults which show various types of the mosaic eye. All these mosaic patterns generally took anteroposterior stripes. Similar tendencies had been inspected on the structure of abnormal compound eyes of the moths treated by X-ray and radium radiations when in egg stage (ARUGA, 1936).

On the origin of the mosaic eye, TAJIMA presupposed that there might be the definite direction in the development of the optic disc. This proposition must be assured by the morphogenetic investigation along the course of division of the imaginal disc.

UMBACH (on *Ephestia*, 1934) and WOLSKY (on *Bombyx*, 1949) have been interpreted that the differentiation of ommatidia proceeds to take the definite pattern contradicting the simultaneous development of the whole eye disc and proposed that it begins at a certain limited area located in the posterodorsal region.

The previous study on the development of the Bombyx moth eye (KOYAMA & TANAKA, 1954, 1956) principally agreed with the above interpretation, but a question why all the mosaic stripes appear anteroposteriorly has still been remained to be resolved.

The present article mainly treated the axial problem of the development of the compound eye using mosaic eye individuals to clear up the question.

Before going further the author wishes to express to his hearty gratitude to Professor Dr. N. YAGI, who gave kind advices through the investigation and read the original manuscript. Thanks are also due to Dr. Y. TAJIMA who

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sent valuable materials for the study and Mr. T. TAKIZAWA who assisted the experimental operation.

II. EXAMPLES OF THE MOSAIC EYE FOUND IN THE SILKWORM MOTHS

From 1954 to 1956 the author reared worms of the hybrid* between *re*/+ gene component and *re*/*re* one, and got fifteen mosaic eye individuals. Detection of the mosaic pattern is able in the eye of 7~8th day pupa (Photo 1·2·3), because the difference between black and red area of the eye is very conspicuous, becoming indistinguishable in the older age. In Fig. 1 mosaic

Fig. 1 Various types of pupal mosaic eyes which were obtained by the author.

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* It was produced by Dr. TAJIMA. Minority of its eggs had colour mosaic patterns and generally resulted the eye colour mosaic.

eyes of pupae are schematically shown.

As the figures indicate mosaic eyes exhibit various features in the shape of pattern, taking anteroposterior direction in all examples. They, further, show that the mosaic pattern is different between the right and the left eyes; several moths show mosaicism only in one side (Fig. 1, 2~8) and an example (Fig. 1, 1) takes entirely different colouration.

III. PARTIAL REMOVAL OF THE OPTIC ANLAGE AND DEVELOPMENT OF THE COMPOUND EYE

It has generally been considered that the formative origin of the imaginal eye lies in the hypodermal layer beneath the limited area of lateral ocelli which have no direct correlation in formation of compound eye according to their fate. The ocelli diminish histolytically after retardation towards the periopticon. (Koyama & Tanaka, 1954).

In consequence of the above phenomena the author carried out the operation of partial removal of the optic disc of the matured larva and traced the development of the part by the imaginal stage.

Each group of five individuals of the hybrid between *Hakuba* and *Tenryu* (normal black eye) was used for this purpose. The experiments are described in the following respective topic. After operations the worms were protected under room condition (temperature, 22~25°C; R. H., 60~70%) and observed on change of the external structure.

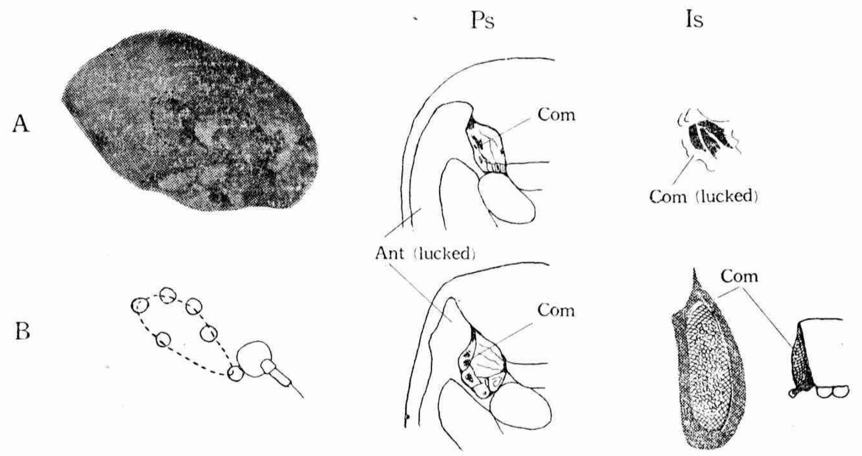


Fig. 2 Expt. 1 and the result
Circled with dotted line; Removed part, Com; Compound eye, Ant; Antenna, Ps; Pupal stage, Is; Imaginal stage.
The same explanations are used in the succeeding figures.

1. Removal of the Plate Including All Lateral Ocelli

In two cases of operations, A case resulted the loss of compound eye in papae and adults, leaving only chitinous development at the eye seat (Fig. 2, A). This fact means complete removal of the optic disc. The antenna was heavily destructed or could not be differentiated at all. On the other hand the eye of much reduced size or abnormal one developed sometimes in B case (Fig. 2, B).

The developed eye usually took slender shape, being circled with a thick ring of blackish chitin. The so called non-facetted area which had been pointed out by UMBACH (1934) in *Ephestia* and by WOLSKY (1949) in *Bombyx* was not recognized but large facets arranged irregularly. Thus in B case developmental potency seems to be still remained.

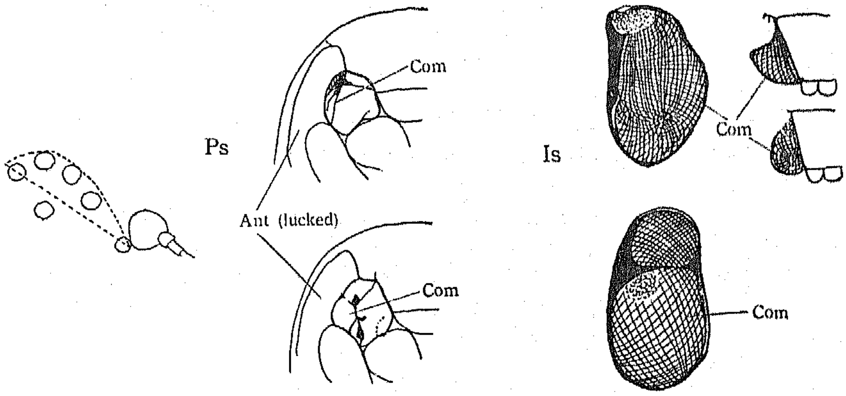


Fig. 3 Expt. 2 and the result

2. Removal of the Anterodorsal Half of the Disc

Fig. 3 shows the results of the removal effect of the optic disc cut in half. The operated region could be detected near at dorsal part of the disc. The antenna was often destructed. The same region is considered to be closely related to the development of the antenna as seen in the case of Expt. 1.

The developed eye from the abovementioned treatment reduced into abnormal shape. In majority of the eye the large facets were seen at the dorsal side (Fig. 3, Is–upper figure) and the rest part was divided into two regions by the suture anteroposteriorly (Fig. 3, Is–under figure). The latter fact suggests the existence of characteristic axis in development of the optic disc.

3. Removal of the Posteroventral Half of the Disc

In this case the opposite side contrasting to the above operation was treated. The pupal and imaginal eyes became smaller when compared to the case of Expt. 2 (Fig. 4). By this operation no individuals lost the antenna. The operation is certainly more effective to the growth process than that of Expt. 2.

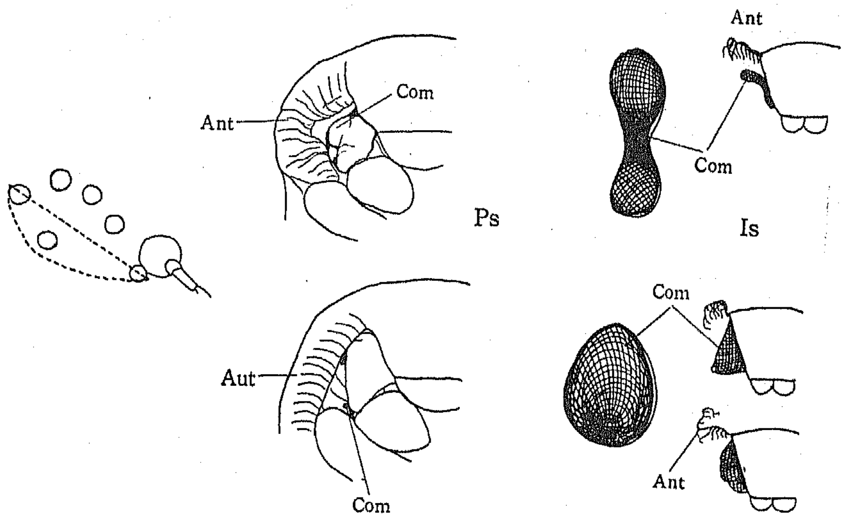


Fig. 4 Expt. 3 and the result

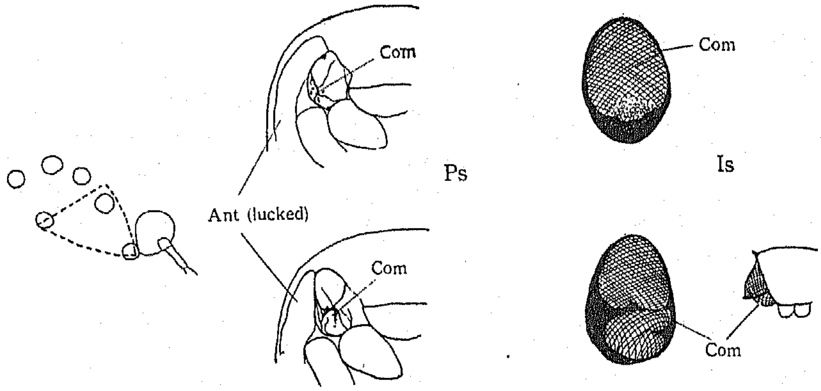


Fig. 5 Expt. 4 and the result

4. Removal of the Anteroventral Half of the Disc

In this experiment the region containing the anterior three ocelli was removed. Generally the ventral part of the compound eye and the antenna

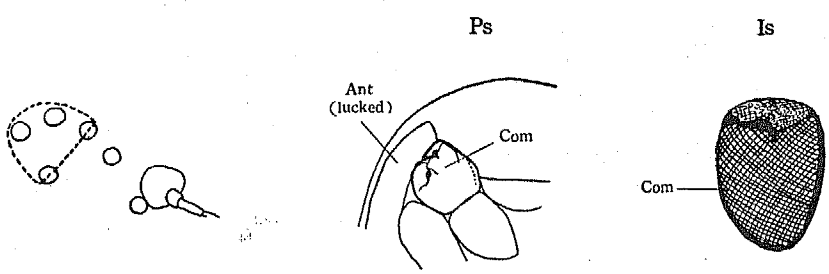


Fig. 6 Expt. 5 and the result

were broken in the resulting pupae and moths. The deep suture (Fig. 5, under figure) could also be seen in some individuals.

5. **Removal of the Posterodorsal Half of the Disc**

In this case the opposite side to the former operation was removed. Almost of compound eyes from this experiment lucked the dorsal region (Fig. 6.) Expt. 4 and 5 contribute to decision of the correlation between the optic disc and the dorsal position or the ventral one of the imaginal eye.

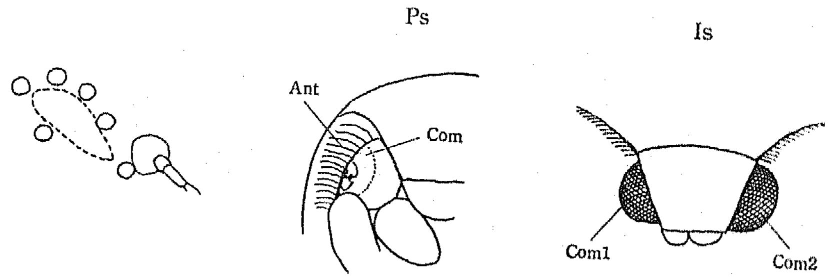


Fig. 7 Expt. 6 and the result

6. **Removal of the Central Part of the Disc**

In this experiment the central area of the optic disc surrounded by the lateral ocelli was removed. The resulting imaginal eye (Com1) was reduced to smaller size than the normal one (Com2), the size being approximately 1.4 mm in length and 1.2 mm in width, showing no significant difference in structure as compared with the normal one. The fact involves that the optic anlage expands over the central region, where the differentiation potency is still remained.

7. **Consideration**

From abovementioned observations the following assumptions will be summarized.

1. The optic anlage occupies considerably wider area than that circled with all ocelli. It has been proved by Expt. 1 and 6.
2. The posterodorsal and the anteroventral parts of the optic disc correspond to the upper and the under parts of the imaginal eye, respectively (Expt. 4 and 5)
3. The removal of the anterodorsal and the opposite halfs of the optic disc is the most influencial on the formation of compound eye, so the starting center of differentiation exists nearly along the border line between the above halfs.
4. The differentiation center can not be limited as a point or a small area by the results of these experiments.
5. The suture observed in the operated eye shows the existence of a definite direction in regard to the developmental process.

IV. STIPPLING TREATMENT FOR THE OPTIC DISC AND DEVELOPMENT OF THE COMPOUND EYE

Proceeding experiments were the removal of considerable wide area of the optic anlage. In this experiment the author tried to know the developmental mechanism by another way, in which various positions of the optic disc were burned by stippling.

Materials used were matured larvae of the same hybrid as in Chapt. III.

A. EXPERIMENTS FOR THE OPTIC ANLAGE IN THE LARVAL STAGE

1. Stippling for the Most Anterior Ocellus

The most anterior ocellus near the base of the antenna was burned down (Fig. 8). The pupa and the moth resulted have an abnormally reduced eye, antenna and maxilla, the last one being frequently missed (Fig. 8, Ps and Is–under figure). The imaginal eye is virtually affected by the operation at the under side (Photo 6).

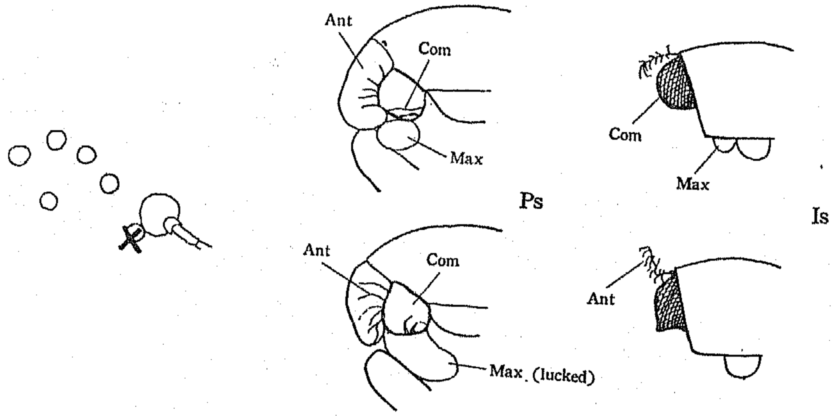


Fig. 8 Expt. 1 and the result
Crossed mark; stippling position, Max; Maxilla
The same explanations are used in the succeeding figures.

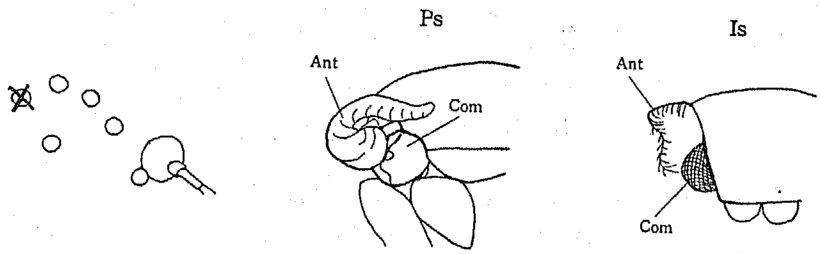


Fig. 9 Expt. 2 and the result

2. Stippling for the Most Posterior Ocellus

This was the experiment of stippling for the opposite side to Expt. 1. The eyes of resulting pupae were destroyed at the posterodorsal region. The form of the antenna was also modified. The fact indicates that the antennal anlage has very much wide expansion. Only the under side of the future compound eye differentiates as illustrated in Fig. 9, Is.

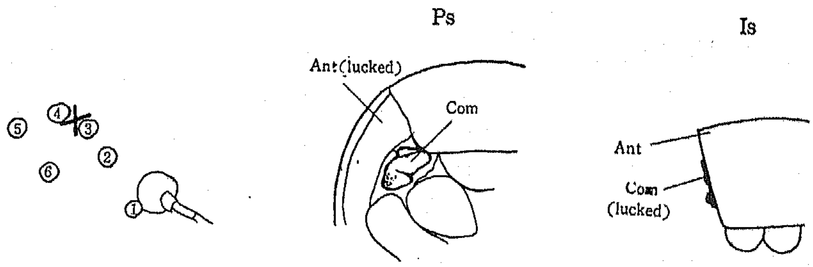


Fig. 10 Expt. 3 and the result

3. Stippling for the Position between Ocellus No. 3 and No. 4

In this operation the two ocelli would be burned (Fig. 10, left figure). In its result the coming eye and antenna were remarkably reduced and sometimes unable to develop at all.

Notwithstanding the treatment was similar to the case of Removing Expt. (Chapt. III—2), the effect was more striking than the expectation. The consideration on the cause of development will be given in Chapt. VI.

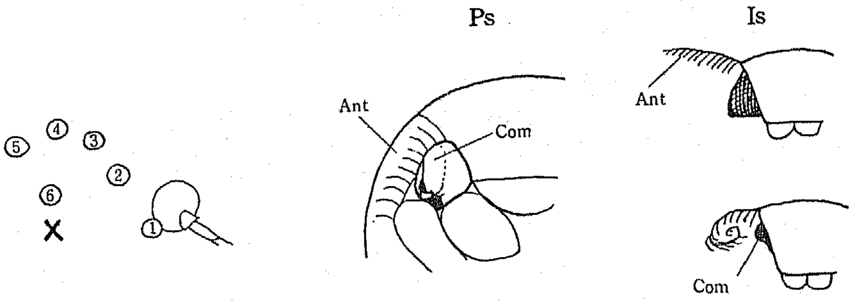


Fig. 11 Expt. 4 and the result

4. Stippling for the Position just under Ocellus No. 6

In this case the majority of resulting eyes took a half form as missed the ventral side (Fig. 11, Is–upper figure), minority of the case having trace of the imaginal eye at the dorsal region (Fig. 11, Is–under figure).

5. Stippling for the Central Position encircled by the Ocelli

In this treatment almost similar results to Expt. 3 were obtained (Fig. 12). No individual, however, lucked the antenna though it took an abnormal form.

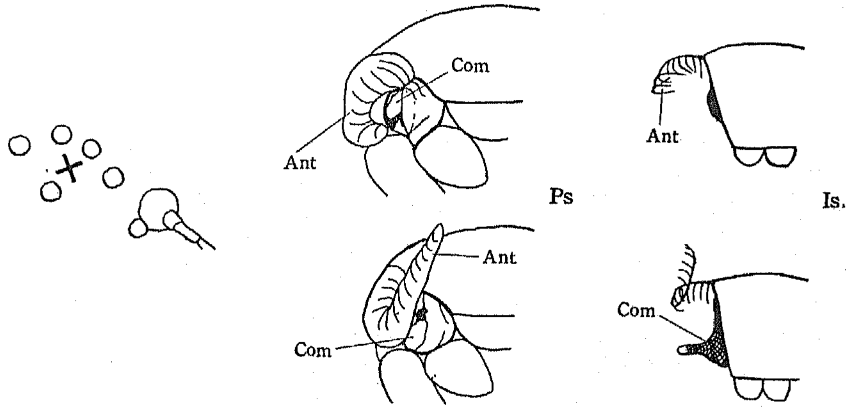


Fig. 12 Expt. 5 and the result

6. Stippling for the Position between Ocellus No. 1 and No. 6

All of individuals operated had the stippled trace at the under margin of pupal eye and reduced eyes in the imaginal stage. The antenna was somewhat affected by the heat but not the maxilla (Fig. 13).

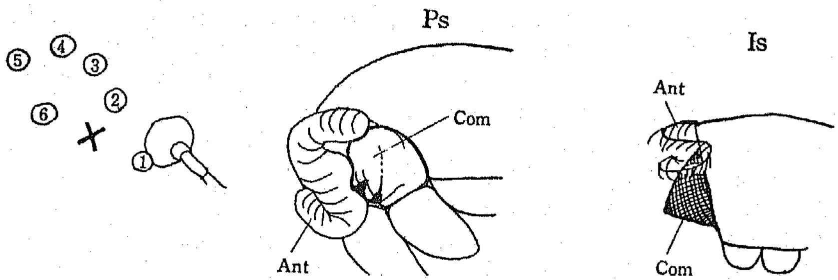


Fig. 13 Expt. 6 and the result

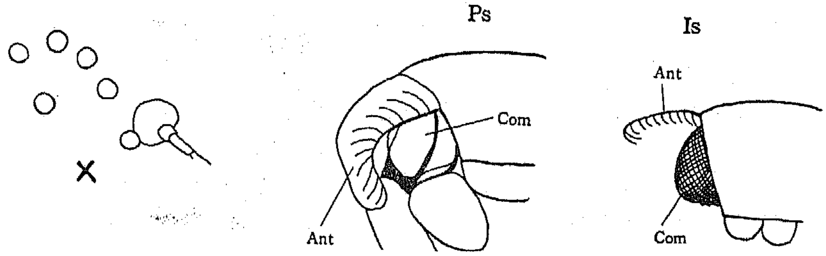


Fig. 14 Expt. 7 and the result

7. Stippling for the Most Under Position of the Disc

In this experiment the imaginal eye was reduced into about a half of the normal size taking a roundish form, though the trace was remained at the ventral position of the compound eye (Fig. 14). The antenna was deformed.

B. EXPERIMENTS FOR THE RUDIMENTARY EYE IN THE PUPAL STAGE

The previous experiments were all concerned to the optic disc in the larval stage. In this case the author carried out similar operations on the pupal eye, of which he could determine the differentiaition center, and got a knowledge of facet formation of the resulted eyes in the adult stage. All the results are shown with summarized expression in Fig. 15.

Experiment a: In this case the effect was not so conspicuous. The eye in a–3 collum showed one broken portion at the anterior side, taking almost normal form. The same results were seen in subsequent experiments. The facetless region was not formed though the undifferentiated chitinous part was sometimes detected around the eye (Fig. 15, a–3, photo 7).

Experiment b: "Differentiation center" in the pupal eye disc was operated in this case (Fig. 15–b). The compound eye is hardly constructed until b–2 (Photo 8), only being formed abnomally after b–3 (Photo 9) and a horizontal line is formed in it. At the posterior margin the facetless area is formed (Photo. 10).

Experiment c: The same treatment had been done by WOLSKY (1949) though the author's experiment was not always conformed. For example, the resulted eye in sample operated at "24 hrs after pupation" (Fig. 15, c–6, Photo 11) differentiated sufficiently in his work, while in the author's case it could not completely be reestablished. According to this fact the causation will depend upon the degree of stippling.

Experiment d: Treated eyes in Expt. b to d developed always as the form divided anteroposteriorly by a chitinous furrow. In this operation the facetless area extends at the posterior margin (Fig. 15, d, Photo 12).

Experiment e and f: These cases as well as Expt. a did not bring so eminent effect upon the eye, which developed almost completely, being harmed limitedly at the dorsal or at the ventral side (Fig. 15, e・f, Photo 5・13・14).

C. CONCLUSION

From the experiments described above the following conclusions should be deduced.

1. The burning effect on the rudimental eye of the pupa in Expt. a to d, in which the central portion of "differentiation suture" was stippled, was harder for the eye development than Expt. e and f at the ventral or the dorsal part of the eye. It seems to show that the most potential part underlies near at the central region of the suture.

2. When either zone to the differentiation suture was treated, the effect

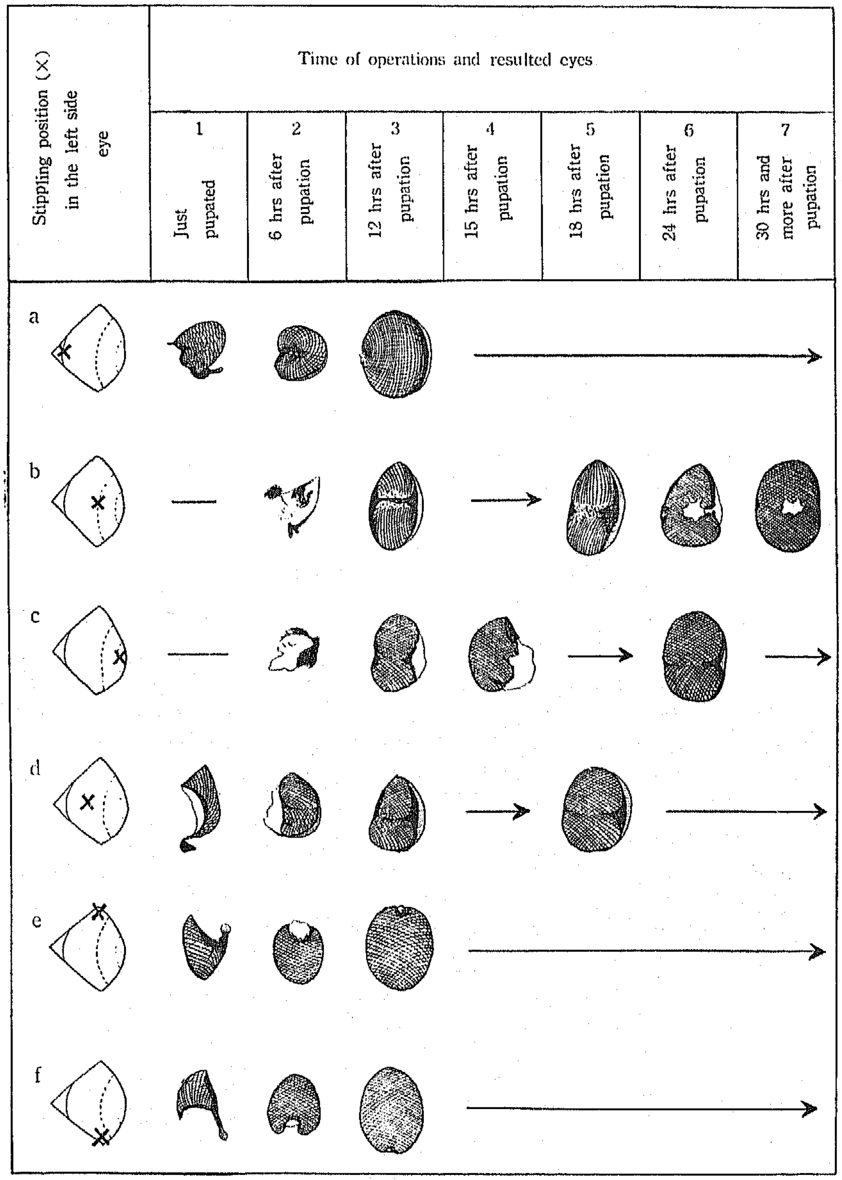


Fig. 15 Stippling treatment for the pupal eye and summary of the results

was lesser in the anterior zone (Expt. a and d) than in the posterior one (Expt. c). This will mean that the development proceeds mainly towards the posterior direction.

3. The imaginal eye could not be completed at all in Expt. b-2 and c-2. The potentiality of facet differentiation is considered to be related to the degree of invagination which is indicated by the suture on the surface of the eye. Accordingly, in the stippling of this part antecedent to the differentiation, there occurs no formative growth (see also KOYAMA & TANAKA, 1956).

4. The dorsal and the opposite side of the pupal disc are undoubtedly concerned to be the formative origin of the upper and the under part of the imaginal eye, respectively (Expt. e and f). From these experiments the author inclines to assume that the differentiation potency is mainteined towards the dorsal (Fig. 15, e-1) or the ventral side (Fig. 15, f-1).

V. RELATIONSHIP OF OCELLAR MOSAICS AND MOSAIC PATTERNS OF THE IMAGINAL EYE

KOYAMA & TANAKA (1954) pointed out that the lateral ocelli have no direct relation to the formation of compound eye and retreats into the inner part

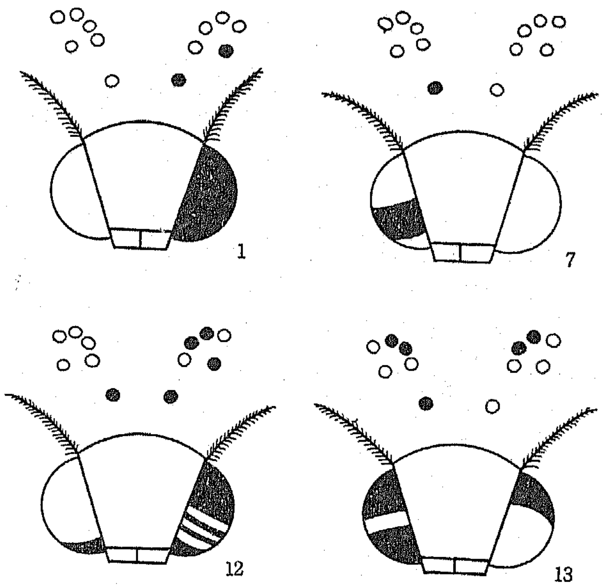


Fig. 16 Correlation between the mosaicism of lateral ocelli and the mosaic pattern of the imaginal eyes. Numbers indicated in each figure correspond to those in Fig. 1.

White and black show red and black colouration in eyes, respectively.

of eye. Mosaic eyes of the imago however have a certain relation to the remnant of the pigmental part of mosaic ocellus which is seen between the periopticon and the epiopticon (Photo 4). This fact will be proved by the existence of correlation between the mosaic ocellus together with its surrounding hypodermal cells and the mosaic pattern of the compound eye.

For the purpose to certain the former presumption, four specimens which have mosaic ocellus were brought to the morphological study. The results are shown in Fig. 16.

It is clearly evidenced that the situation of the mosaic ocellus has close coincidence with that of the stripe in compound eye. It may be understood by seeing the persective correlation of these parts. The case of the left figure in Fig. 16-7 is only an exceptionable example, in which a black band appeared near at the medial part not at the lowest one (compare with the left figure of Fig. 16—12). But the fact will be understood in such assumption that any mosaic pattern has been contained at far upper region of the lowest ocellus, for the mosaic factor is not always provided with the hypodermis just arround the mosaic ocellus.

None the less the observation obviously shows that the differentiation center is not situated at a point but expands quite widely towards the vertical direction of the optic disc in the larval stage.

VI. DISSCUSSION AND CONCLUSION

As Wolsky (1956) noted it will be of accessible fact that the anlage of compound eye starts its development in the embryonic stage and persists its growth throughout the larval one as a distinct imaginal disc, differentiating to a certain extent before pupation. In *Notonecta*, *Drosophila*, and Odonata, one type is seen that several cells grouped in the larval eye disc make the growth center, from which the cellular division proceeds towards the periphery (Lüdtke, 1940; Bodenstein, 1953; Ando, 1957). Another type in which the development starts anteroposteriorly from a differentiation suture in the optic disc, is recognized in a Lepidopterous eye (Wolsky, 1949: Koyama & Tanaka, 1956), the suture being formed at dorsoventral situation by 10 hours before pupation when lateral ocelli sink into the inner part of eye (Koyama & Tanaka, 1954). In the latter case, however, the range of differentiation potency had been left to be decided strictly in the eye disc.

According to several experiments in the present investigation in addition to above researches, it is sufficiently reliable that the differentiation center is situated at the vertical suture between the anterior zone taking smooth surface and the posterior one with granulous surface in the pupal optic disc. In this time the problem must be subjected to solve where the potential center is set in the larval eye disc. Certainly the center lies neither in single point nor in a small limited area and exists in the vertical line which runs through medial zone being surrounded with whole ocelli (Chapt. III). Further the dorsal region and the ventral one of the optic disc correspond apparently to the upper and the under fo the imaginal eye, respectively (Chapt. III—4・5, Chapt. IV–A, 1・2). Either experiment of Chapt. III–1 or –2, in which a line through Ocellus No. 1 to No. 5 was cut off, proved to effect on the dorsal or the ventral side in majority cases, showing the latter being more affected than the former. It means that much more potential part is contained in the former case. So the differentiation center is assumed to be performed at the same situation as a thick line (Dc) in Fig. 17, more or less crossing with the line through Ocellus No. 1 to No. 5 (straight dotted line in Fig. 17, A). Along this process the imaginal eye can be formed, even if the region encircled with whole ocelli was removed as in the case of Chapt. III–6.

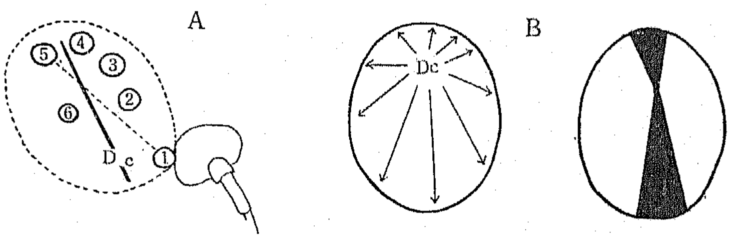


Fig. 17 A; Situation of the differentiation center (Dc) B; General interpretation on the differentiation in Lepidopterous eye (left figure). Arrows show streams of differentiation. If a certain mosaic factor exists in Dc, the resulting eye might have such mosaic pattern as the right figure.

It will be note worthy that adequate differentiation is not established so far as the ocellar retardation in metamorphosis has entirely performed; the growth potentiality seems to be insufficient for furnishing the development until the vertical suture has completely been composed, for instance in an abnormally developed eye the remnant of ocelli is by no means observed between the periopticon and the epiopticon, staying at the distal region of the periopticon (see also KOYAMA, 1953). That the stippling treatments (Chapt. IV) were certainly more influencial than the removal operations on the same part (Chapt. III), will depend, in the former, upon the inadequate ocellar retreatment which is disturbed by the burned tissue.

From above considerations the mosaic pattern is believed to be caused by the characteristic of cellular division in the optic disc. When a certain mosaic pattern exists in any position of the larval disc, it will become the mosaic potency in the differentiation center as metamorphosis goes on. If so, the horizontal mosaicism of colour pattern will reasonably be brought because the eye growth proceeds definitely towards the anteroposterior direction (Fig. 18). The general interpretation as to the differentiation center in Lepidopterous eye developments would wholly be admitted, the dorsoventral mosaic pattern should probably be formed by the radiative differentiation (Fig. 17, B).

Conclusionaly it will be said that TAJIMA's presumption introduced in Chapt. I was quite suitable.

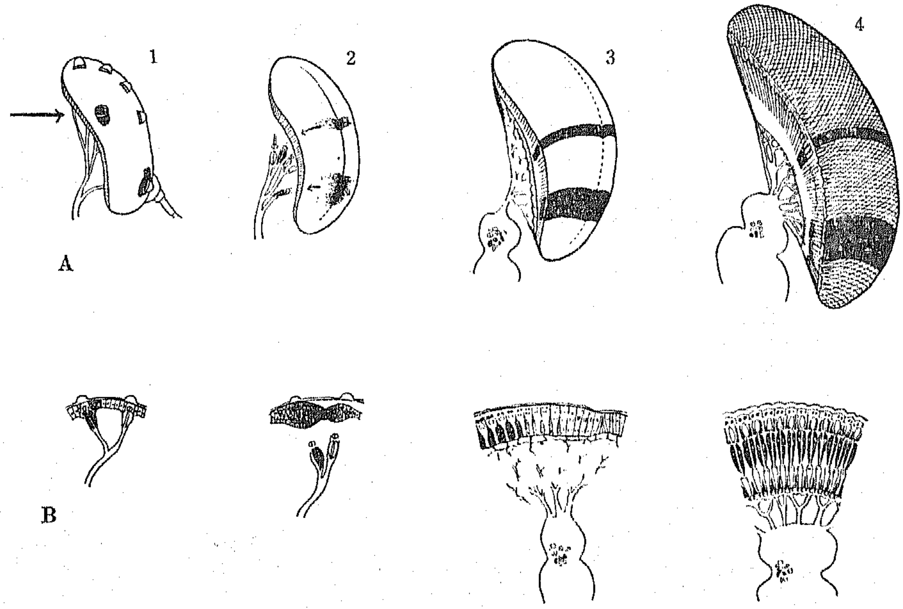


Fig. 18 Diagrammatic explanation on the process of differentiation of the mosaic eye.
A. General aspect
1. Larval optic disc. Black spots show the position of mosaic pattern.
2. Primary differentiation period (from just pupation to 3rd day after pupation)
3. 6th day after pupation
4. 9th day after pupation
B. Changes of optic hypodermis corresponding to each stage in A figures. Each figure is shown by longitudinal section at the position of an arrow in A-1 figure.

VII. SUMMARY

The mosaic pattern of the compound eye frequently found in the silkworm moth is arranged anteroposteriorly without exception. This fact suggests that the characteristic division takes place in the hypodermal cell of the optic disc. In the present article morphogenesis of the compound eye has fundamentally been studied with special reference to the situation and limit of the differentiation center, and the direction of the cellular division.

The results are summarized as in the followings.

1. The optic disc extends over the ovoidal zone containing the whole lateral ocelli (the dotted zone of the left figure in Fig. 2-A and Fig. 17, a).

2. The differentiation potency is set in the dorsoventral suture formed in the disc center as the morphogenesis from larva to pupa goes on. From the suture the cellular division proceeds mainly anteroposteriorly (Fig. 18).

3. If a certain mosaic pattern exists in any part of the disc, the pattern occupies a position of the suture at the ocellar retardation and reasonably the anteroposterior pattern of mosaic colour is performed in the imaginal eye as illustrated in Fig. 18.

4. The general interpretation, in which a small area of the disc is concerned to the differentiation center, on the morphogenesis of a Lepidopterous eye, can not be said to be adquate enough, taking the present study into consideration.

Literatures Cited

ANDO. H. : Sci. Rep. Tokyo Kyoiku Daigaku, Sect. B, 8 (128) : 174–216 (1957)

ARUGA, H. : J. Jap. Appl. Zool. 8 (6) 290–298 (1936)

BODENSTEIN, D. : Insect Physiology, New York (1953)

KOYAMA, N. : Res. Rep. Fac. Text. & Seric., Shinshu Univ. 3: 44–47 (1953)

——— : J. Jap. Seric., 25 (3) : 244–245 (1956)

——— & S. TANAKA : Res. Rep. Fac. Text. & Seric., Shinshu Univ., 4 : 50–55 (1954)

——— · ——— : J. Fac. Text. & Seric., Shinshu Univ., Ser. A, 6 : 1–14 (1956)

LÜDTKE, H. : Z. Morph. Ökol. Tiere, 37 : 1 (1940)

TAJIMA, Y. : J. Jap. Genet., 18 (6) : 305–308 (1942)

UMBACH, W. : Z. Morph. Ökol. Tiere, 28 : 561 (1934)

WOLSKY, A. : Expt. Cell. Research, Suppl. 1 : 549–554 (1949)

——— : Trans. New York Acad. Sci. Ser. 2, 18 (7) : 592–596 (1956)

Explanation of Photographs

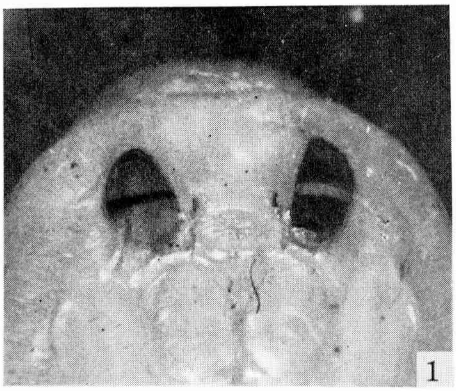
PLATE I

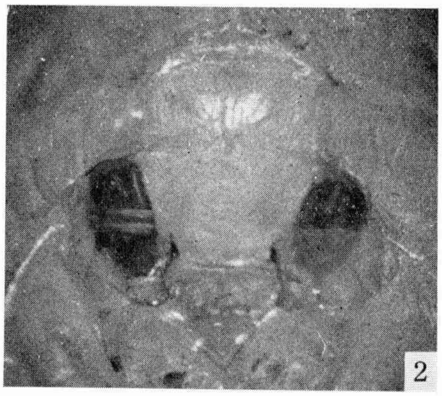
1. Mosaic pattern in the pupal eye, which is shown in No. 11 of Fig. 1.
2. Ditto, in No. 14 of Fig. 1.
3. Longitudinal section of the mosaic eye in the pupal stage.
4. Colour mosaicism in the remnant of ocelli.
5. The eye resulted by the stippling operation (Chapt. IV, B-Expt. e : Fig. 15, e-1). The dorsal portion of the pupal disc was burned.
6. Ditto (Chapt. IV, A-1 : Fig. 8). The most anterior ocellus was stippled.

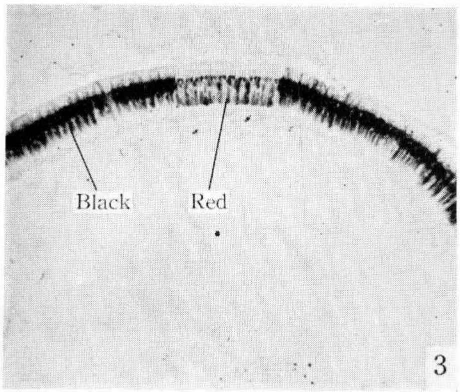
PLATE II

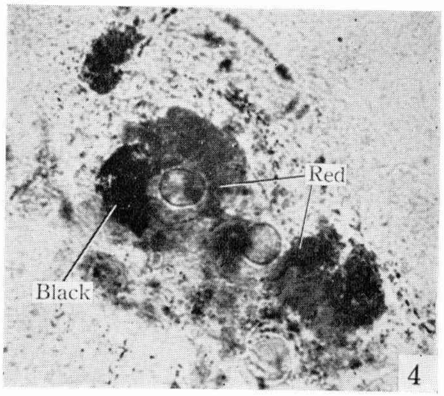
7. The eye resulted by the stippling treatment (Chapt. IV, B-Expt. a ; Fig. 15, a-3). The posterior part (left side) is seen being destroyed.
8. Ditto (Chapt. IV, B-Expt. b; Fig. 15, b-2). No facet is formed.
9. Ditto, Fig. 15, 6-3.
10. Ditto, Fig. 15, b-5. In the periphery the facetless part is seen.
11. Ditto (Chapt. IV, B-Expt. c : Fig. 15, c-6).
12. Ditto (Chapt. IV, B-Expt. d : Fig. 15, d-3). The facetless margin is recognizable.
13. Ditto (Chapt. IV, B-Expt. e : Fig. 15, e-3).
14. Ditto (Chapt. IV, B-Expt. f : Fig. 15, f-3).

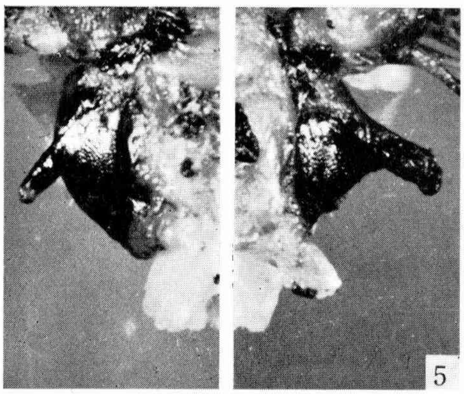
PLATE I

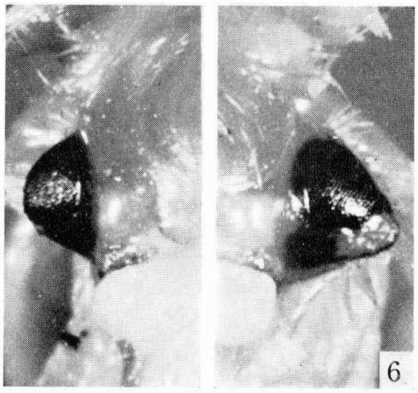


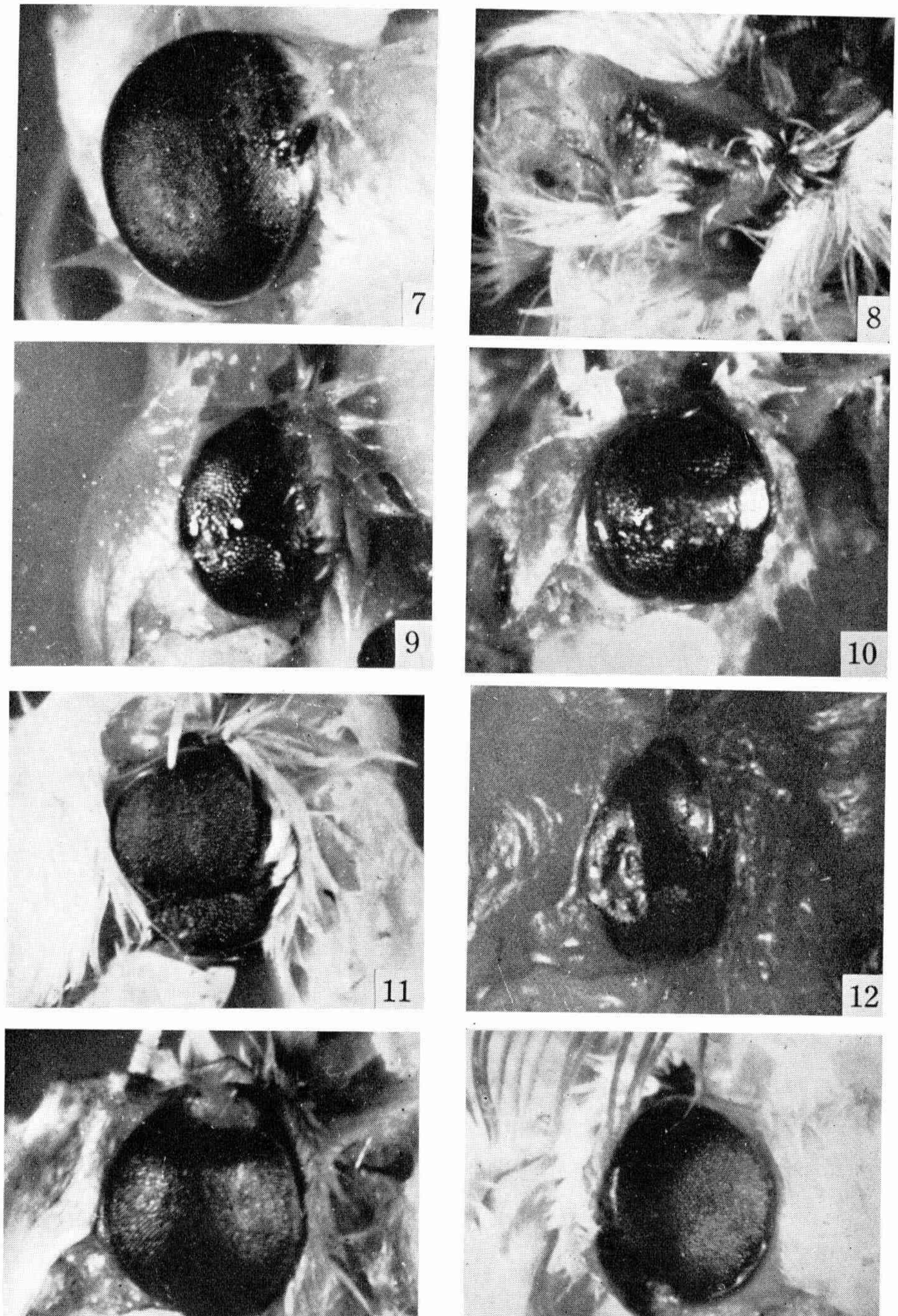








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