VARIEGATION IN THE ZESTE EYE COLOR ALLELES AND ITS
BEARING ON GENE ACTION DURING THE DEVELOPMENT
OF THE EYE OF DROSOPHILA MELANOGASTER¹

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TWO of the four available zeste alleles show a variegation pattern of large red spots on a lighter colored background. The shape of these spots is identical to the shape of X-ray-induced mosaic spots that have been used earlier in an analysis of cell lineage and developmental physiology of the eye anlage (BECKER 1957a, b). In the present paper this identity will be used to analyse the variegation pattern of zeste eyes. This pattern will also be compared with the similar one of the eye deformations in Lobe alleles (see also BECKER 1957a, b). The data on the zeste alleles will then be used to add to the present knowledge of eye development. Finally, the particular type of gene action encountered in both the zeste and Lobe alleles will be discussed. The salt-and-pepper mottling that is also displayed by flies with the zeste alleles will be described but will not be analysed any further.

MATERIAL AND METHODS

The zeste locus lies in the X chromosome at 1.0. The two alleles *z* (zeste) and *z^a* (zeste-a) were found by M. GANS, the allele *z^m* (zeste-mottled) by M. M. GREEN. Stocks carrying these three alleles—*z*, *γ z^a* and *sc z^m* (*γ*, yellow; *sc*, scute)—were kindly provided by Dr. M. M. GREEN. The fourth allele arose spontaneously in one male of the *sc z^m* stock during the investigation. It was designated *z^l* (zeste-light), the stock accordingly *sc z^l*. Salivary gland chromosomes from a stock carrying *z* (GANS 1953) and from a stock carrying *z^m* were found to be normal.

Temperatures at which cultures were kept during the experiments will be given at appropriate places.

In X-ray experiments larvae of all stages were uniformly given the dose of 1250r. Two different machines had to be used; the data for one were: 100 kv, 6 ma, no filter, 200r/min, and for the other one were: 250 kv, 15 ma, 0.5 mm Al filter, 120r/min. This difference is, however, believed to be unimportant in these experiments.

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RESULTS

The zeste alleles

A thorough description and genetic analysis of the allele *z* has been given by M. GANS (1953). The pigmentation of the eyes is different in both sexes, and it also depends on the temperature under which the flies are raised. The characteristics of *z* and of the other three alleles are assembled in Figure 1. Cultures of stocks with all four alleles were raised at 14, 17, 25 and 30°C. Since the 14°C cultures showed the same features as the 17°C cultures, only to a slightly more extreme degree, they are summarized in the table as one group, "low". The same is true for the 30 and 25°C cultures respectively; they are summarized in group "high".

z: Females raised at high temperatures have lemon-colored eyes. When raised at low temperatures they show a salt-and-pepper type of variegation consisting of fine red spots on a lemon background. At 17°C there is a region of darker, more densely mottled ommatidia along the posterior margin of the eye. At 14°C almost the whole eye has this darker appearance, and only a narrow region at the anterior margin has the looser, lighter kind of mottling. In Figure 1 the appearance of an eye developed at 17°C is given. At first glance the eyes of males seem to be normal in cultures kept at all different temperatures. Closer inspection, however, especially under mineral oil, shows that in the eyes of males raised at high temperatures the pigmentation is incomplete. In these males the hexagonal pattern of red pigment—hexagonal because of the location of pigment cells on the borders between ommatidia—is interrupted at various places in the eye.

z^m: Females raised at high temperatures have lemon-colored eyes. When raised at low temperatures they are, especially at 14°C, slightly orange. They do not show any salt-and-pepper mottling, but have large consolidated spots instead. These spots are normal red or darker. The occurrence of these spots in any type of fly is represented in Figure 1 by one single, but typical, spot of this kind. Males brought up at high temperatures show the same type of large spots on eyes that

TEMPERATURE	<i>z^o</i>		<i>z</i>		<i>z^m</i>		<i>z^l</i>	
	♀	♂	♀	♂	♀	♂	♀	♂
LOW (14° and 17°C)
HIGH (25° and 30°C)

FIGURE 1.—Schematic representation of left eyes of the *zeste* alleles. Black = normal red; dotted = salt-and-pepper mottling; white = lemon; the black spot in *z^m* and *z^l* represents a c.l. spot.

otherwise have a lemon background with salt-and-pepper mottling. The eyes of low-temperature males have the same (almost normal) pigmentation as z males at high temperatures.

z^l : The eyes of these females are lemon color at all temperatures and only at 14°C show spots of the large type. Males raised at high temperatures show the characteristics of large spots most clearly, since the background color of the eyes is uniformly lemon without any other mottling. At low temperatures they look similar to z^m males raised at high temperatures, i.e., large red spots on eyes that otherwise have a lemon background with salt-and-pepper mottling.

z^a : This is a normal isoallele of the *zeste* locus, i.e., the eyes of both sexes are normal under all temperature conditions, and the *zeste* character shows only when z^a is in compound with any of the other three alleles.

The conclusions reached from the description of the four alleles are: In Figure 1 the four alleles are arranged according to increasing lightness from left to right. There is a consistent order from darker to lighter phenotypes from the upper to the lower part of the figure, i.e., as the culture temperature is increased. Males carrying any allele raised at a high temperature have a phenotype very similar to males of the next lighter allele raised at a low temperature. The difference in pigmentation due to temperature is not only a matter of the amount of pigment, but also, as was described in the z females, of its distribution over the eye. The pigment that caused the darker, more densely mottled area covers a greater area of the eye when the flies were raised at 14°C, compared to 17°C. The increase in pigmentation with decreasing temperatures proceeds obviously in a posterior-anterior direction. Two of the four alleles show, under appropriate temperature conditions, large uniformly red pigmented areas on a lemon background or on a lemon background with salt-and-pepper mottling.

The compounds

The eyes of compound females will give some further information about the characteristics of the alleles, especially about z^a .

The eyes of all flies raised at high temperatures are lemon. At low temperatures they show the following features: Whenever one of the alleles is z^a or z , the eyes show a salt-and-pepper mottling (the first five compounds in Figure 2). Whenever one of the alleles is z^m or z^l , the eyes show large uniformly pigmented spots typical for these two alleles (the last five compounds in Figure 2). Either these spots appear in addition to the salt-and-pepper mottling or they are the only type of variegation, as in z^m/z^l .

The indicated order of the alleles (as previously made) according to the darkness of pigmentation ($z^a \rightarrow z \rightarrow z^m \rightarrow z^l$) also holds true in their compounds. z^a/z is the darkest; z^m/z^l is the lightest. Among the z^a -containing compounds the order from dark to light is $z^a/z \rightarrow z^a/z^m \rightarrow z^a/z^l$. The darkness is obviously due to the distribution of pigment in the eye, which varies, as in z/z females, along a posterior-anterior gradient. z^a/z flies raised at 17°C show a pigment distribution similar to that described for z females raised at 14°C, i.e., most of the eye is densely mottled and dark, with only a small part along the anterior margin somewhat lighter. On

FIGURE 2.—Schematic representation of the left eyes of the zeste compounds. For further details see Figure 1.

the other hand, when z^a/z flies were raised at 14°C, the pigment-forming factor appears to have travelled anteriorly across the eye, leaving the posterior rim without red pigment: the eye is darkly mottled except for a small posterior middle portion. This condition is represented in Figure 2.

The compounds reveal one other property of the allele z^a ; that is with regard to the formation of the large uniformly pigmented spots. The compound z^a/z does not show this type of spot. In compounds of either one of these two alleles with z^m or z^l this spot type is found. It seems, therefore, that z^a is, like z , an allele that does not allow on its own the formation of large spots.

The origin of the large spot variegation in z^m and z^l

Figure 3 gives an idea of the variety of large spots that are formed in z^m and z^l . The pictures have been drawn from z^m males raised at 25°C. Figure 3 could also represent the spotting in z^m and z^l females raised at low temperatures. Because of their low spot incidence, however, eyes with large dark areas like those in Figure 3a will very seldom be found among these females. This matter will be discussed later in more detail.

As was mentioned earlier, these spots are identical in shape to mosaic spots, which can be produced by the irradiation of w/w^{co} larvae (w , white; w^{co} , white-coral). In that case, by X-ray induction of somatic crossing over in one presumptive eye cell, both its daughter cells were marked genetically; one of two sister cells became homozygous w/w , the other one w^{co}/w^{co} . Both of these cells keep dividing along with the rest of the eye anlage and finally yield a white and an adjacent white-coral spot in the otherwise intermediately colored w/w^{co} eye (see Figure 1 in BECKER 1957a). The size of these spots depends on the stage of the larvae at the time of irradiation; they are large when younger and small when older stages are irradiated. Figure 4 shows four examples of these "twin spots". In this case embryos and larvae with the constitution $sc\ z^m\ w^+/sc^+\ z^+\ w$ were X-rayed. Crossing over between the white locus and the kinetochore in the X chromosomes of an eye anlage cell yields a twin spot consisting of one lemon

FIGURE 3.—Left eyes of z^m males. Black = c.l. spots; dotted = lemon background with salt-and-pepper mottling.

FIGURE 4.—Twin spots induced by X-irradiation of younger (a) and older (b-d) first instar larvae of the constitution $sc\ z^m\ w^+/sc^+\ z^+\ w$. Black = normal red; dotted = lemon partner ($sc\ z^m\ w^+/sc\ z^m\ w^+$), white = white partner ($sc^+\ z^+\ w/sc^+\ z^+\ w$) of mosaic spot.

($sc\ z^m\ w^+/sc\ z^m\ w^+$) and one white ($sc^+\ z^+\ w/sc^+\ z^+\ w$) partner in an otherwise normally pigmented eye.

The shape and arrangement of large spots that were induced in the embryo and during the first larval instar showed a remarkable regularity, especially in the lower half of the eye. The spots in the upper half of the eye are not basically different but are not as regular in shape and position as the spots in the lower half. The reason for this difference is apparently that beginning with the end of the first larval instar, the growth centrum of the eye anlage is in the presumptive upper half of the eye, and growth in this center seems to be less regular than in parts of the anlage more distant from it. For this reason the analysis of the zeste alleles will be limited to the lower half of the eye.

It has been shown (BECKER 1957b) that the spots induced by treatment at the end of the first larval instar and earlier subdivided the lower half of the eye into roughly eight sectors. This was found when all these spots were drawn on the outlines of a single eye. A schematic representation of these results is given in Figure 5. Each one of the spots induced at the end of the first larval instar covers the area of one of the eight sectors, each one induced earlier covers the area of two or more of the sectors. The presumptive area for the lower half of the eye consists, therefore, at the end of the first larval instar, of about eight cells, and the presumptive total eye area consists of about 20 cells.

In the two alleles z^m and z^l the pattern of the variegation spots corresponds completely to the cell lineage pattern of X-ray-induced mosaic spots. It can be concluded, therefore, that those variegation spots are, like the mosaic spots, derivatives from single cells of the eye anlage. This means that in early development in z^m and z^l , some cells are determined to form lemon parts of the eye, and other neighboring cells are determined to form red parts of the eye. Whether or not this potential difference is realized depends on the temperature under which the flies are raised, as was discussed earlier. These large variegation spots will be referred to from now on as cell lineage or c.l. spots.

FIGURE 5.—Schematic representation of the shape and position of mosaic spots in the lower half of the eye, induced by irradiation at the end of the first larval instar. Earlier induced spots cover the area of two or more of the eight sectors.

Flies of the constitution z^m ; st/st or z^l ; st/st have almost white eyes with scarlet c.l. spots. The mutant scarlet (st) is known not to allow the formation of the brown pigment component. Apparently, therefore, the difference between the two cell types, i.e., those determined to form lemon and those determined to form red, is: some cells are able and some cells are not able—the appropriate temperature conditions provided—to form red pigment, the second of the two pigment components.

The stage at which cells are determined as to whether or not their descendants form red pigment should be given by the size of c.l. spots. The size of mosaic spots induced at different developmental stages and the size of c.l. spots have for this reason been compared.

The smallest c.l. spots of z^m and z^l eyes have the size equivalent to one of the sectors in Figure 5 (Figure 3b, c); all other spots have the size of two or more sectors. The smallest ones, then, are derived from cells determined at the end of the first larval instar. The larger ones could have been derived either from cells which were determined earlier or from a number of adjacent cells at the end of the first larval instar. To settle this question, 110 eyes of z^l males, each with a total red area of two sectors in the lower half of the eye, were rated as to whether they were adjacent or separate (compare Figure 3d–g). If in these cases the determination would have taken place at the “one sector stage”, i.e., would have affected any two cells out of eight at random, one would have to expect—under the given condition of eight sectors in a row—that in 25 percent of the cases the two sectors would be adjacent (Figure 1g) and that in 75 percent of the cases the two sectors would be separate (Figure 1d–f). Among the 110 eyes investigated, 33

(30 percent) showed adjacent sectors and 77 (70 percent) showed separate sectors. This indicates that the determination preferably takes place at only one stage during development and, furthermore, that this stage is the end of the first larval instar.

The quantitative treatment of variegation in z^m and z^l in which the number of sectors covered by c.l. spots are counted provides an opportunity to make further distinctions between the alleles and their compounds. First, however, it has to be shown that the c.l. spot incidence is independent of culture conditions.

It is known from the work of CHEN (1948) that the temperature effective period for the differences of pigmentation controlled by the allele z (as given in Figure 1) is in the pupal stage. The same is true for z^m and z^l . This was shown by transferring larvae at different developmental stages from 25°C to 17°C and *vice versa*. Whenever pupae developed at 17°C the eyes of the flies looked like the 17°C controls; whenever pupae developed at 25°C the eyes looked like the 25°C controls.

Next an attempt was made to find out whether the number of sectors covered by c.l. spots, i.e., the number of cells that are determined for red pigment formation, can be influenced by temperature. The temperature effective period would be expected before or during the time of determination, i.e., the end of the first larval instar, which is approximately 40–42 hours after egg deposition.

The experiment was done with z^m males. After preliminary tests had shown that no differences could be expected from comparing 17 and 25°C as experimental temperatures, z^m males were uniformly raised at 25°C and transferred at different stages either for six days into 13°C or for three hours into 36°C. The first four rows of Table 1 show the result of two different cold treatments and one heat treatment. Out of all sectors investigated, i.e., eight sectors per eye, the percentage covered by a c.l. spot was determined and is given in the rightmost column of the table. This is identical to the percentage of anlage cells determined to red pigment formation. The number after the transfer to 13°C of 20-hour old stages lies slightly above the control value; the number after transfer of 30-hour old stages lies slightly below it but is not significantly different from it. The flies whose larvae were transferred 30 hours after egg deposition for three hours to 36°C show also a value slightly but not significantly below the control. z^m and probably z^l have, therefore, no temperature effective period at the end of the first larval instar, but they both do have the same pupal temperature effective period as was found for z .

Notwithstanding this apparent independence of the c.l. spot incidence from the temperature during the larval stages, all flies whose spots were counted later were uniformly raised at 25°C until the end of their larval life. Then they were transferred into the temperature in which c.l. spots develop most clearly, and that is given in the third column of Table 1. The detailed data on the distribution of eyes with various proportions covered by c.l. spots are given in Table 2.

z^m females show c.l. spots only when they are raised at low temperature. Table 1 shows that only 2–3 percent of their anlage cells become determined for red pigment formation (rows 5 and 6).

TABLE 1

The percentage of sectors in the lower half of the eye covered by cell lineage spots, for different genotypes and under different culture conditions

No. of row	Genotype	Culture conditions	No. of eyes investigated*	Total no. of c.l. spot sectors	Percent sectors (or cells)†
1	$sc\ z^m/Y$	25°C	608	1,149	23.6
2	$sc\ z^m/Y$	{20h after egg deposition for 6 days 13°C}	185	368	24.9
3	$sc\ z^m/Y$	{30h after egg deposition for 6 days 13°C}	142	249	21.9
4	$sc\ z^m/Y$	{38h after egg deposition for 3 hours 36°C}	284	498	21.9
5	$sc\ z^m/sc\ z^m$	17°C	2,510	400	2.0
6	$sc\ z^m/sc\ z^m$	14°C	490	114	2.9
7	{ $(\gamma f: = \times)$ $sc\ z^l/Y$ }	25°C	1,813	1,558	10.8
8	{ $(\gamma f: = \times)$ $sc\ z^l/Y$ }	17°C	280	214	9.6
9	$sc\ z^l/Y$	25°C	534	541	12.7
10	$sc\ z^m/Y$	{both from $sc\ z^m/sc\ z^l$ mothers; 25°C}	912	1,663	22.9
11	$sc\ z^l/Y$		931	1,427	19.1
12	$sc\ z^l/sc\ z^l$	14°C	616	149	3.0
13	$\gamma\ z^a/z$	14 and 17°C	614	...	0.0
14	$\gamma\ z^a/sc\ z^m$	14°C	748	124	2.1
15	$\gamma\ z^a/sc\ z^l$	14°C	1,184	106	1.1
16	$z/sc\ z^m$	14°C	1,004	166	2.1
17	$z/sc\ z^l$	14°C	766	91	1.5
18	$sc\ z^m/sc\ z^l$	14°C	1,043	407	4.9
19	$sc\ z^l/0$	25°C	267	266	12.5

* Since each eye has eight sectors (see Figure 5), the total amount of sectors investigated is eight times the number of eyes investigated.

† Column 6 gives the percentage of sectors covered by c.l. spots. This percentage is at the same time the percentage of cells determined for red pigment formation.

The first z^l male found was crossed to double X females ($\gamma f: =$) and kept with them for several generations before a homozygous z^l stock was established. Rows 7 and 8 of Table 1 show that in the genetic environment of this stock ($\gamma f: = \times\ sc\ z^l$) the average number of cells determined for red pigment formation (10.8 and 9.6) lies well under the value of z^m males (23.6). Row 9, on the other hand, shows that the value went up in males of the homozygous z^l stock (12.7). In order to be able to compare the c.l. spot incidence in z^m and z^l males in an identical environment and with identical genetic background, the male offspring of

TABLE 2

The distribution of eyes with various proportions covered by cell lineage spots

No. of row*	Number of sectors affected (percent)								
	0	1	2	3	4	5	6	7	8
1	29.5	20.8	18.1	12.7	7.6	6.1	3.5	1.5	0.5
2	22.2	26.5	23.2	10.3	7.0	3.8	2.7	2.7	1.6
3	28.2	29.6	14.8	12.0	4.9	4.9	4.2	0.7	0.7
4	28.8	25.7	22.5	7.4	4.6	4.6	3.2	3.2	..
5	88.3	8.0	2.9	0.6	0.0	0.0	..	..	..
6	83.0	12.0	4.5	0.4	..	0.2	..	..	..
7	52.5	25.3	12.1	6.2	2.0	1.3	0.3	0.1	0.0
8	59.2	20.4	10.7	6.4	1.4	1.1	0.7	..	..
9	48.5	26.4	12.4	5.6	3.4	2.8	1.1	..	..
10	33.0	23.0	14.6	11.0	7.0	4.3	3.4	2.3	1.3
11	36.5	24.2	16.1	10.3	5.4	3.2	1.8	1.6	0.8
12	81.3	14.3	3.7	0.8	..	..	..	..	..
13	100.0	..	..	..	..	..	..	..	..
14	90.5	5.1	2.4	1.5	0.4	0.1	..	..	..
15	94.1	3.6	1.5	0.4	0.3	..	..	..	..
16	89.6	6.0	2.8	1.1	0.4	..	..	..	..
17	93.2	4.0	2.1	0.4	0.1	0.1	..	0.1	..
18	73.2	18.4	5.5	1.8	0.7	0.2	..	..	..
19	46.1	27.4	15.0	6.4	3.7	0.7	0.7	..	..

* Row numbers correspond to those given in Table 1.

z^m/z^l compound females were raised at 25°C and investigated. Rows 10 and 11, Table 1, give the result. The difference is small (22.9 vs. 19.1), but still highly significant, using a contingency chi-square test for the sum of the data: $\chi^2 = 29.0$; $P < 0.001$. The eyes of z^m males are darker than the ones of their z^l brothers, because of their salt-and-pepper mottling (see Figure 1). That this difference in background color of the eyes is not a source of error can be seen from rows 7 and 8, Table 1, where the lighter z^l males (row 7) show even a slightly higher spot incidence (10.8) than the darker ones (9.6; row 8).

The investigated z^l females (row 12, Table 1) have a different genetic background from the z^m females and, therefore, cannot be compared exactly with them. In any case, both are very similar, i.e., 2–3 percent, so that in z^l , as in z^m , the spot incidence in females is very much lower than in males.

On the basis of the difference between z^m and z^l males (rows 10 and 11, Table 1) it was justified to expect a smaller average number of spots in z^l than in z^m females. That this might still be the case, identical genetic background provided, is suggested by the data of the compounds. In compounds with both z^a and z , z^m shows a somewhat higher spot incidence than z^l : z^a/z^m , 2.1 percent; z^a/z^l , 1.1 percent (rows 14 and 15, Table 1) and z/z^m , 2.1 percent; z/z^l , 1.5 percent (rows 16 and 17, Table 1). z^a/z flies show, as stated earlier, no c.l. spots at all (row 13, Table 1), while z^m/z^l females show a surprising increase in spots compared to the homozygotes of either allele: z^m/z^m , 2.9 percent; z^l/z^l , 3.0 percent; z^m/z^l , 4.9

percent. Again, however, they should be coisogenic before further comparison is made.

As M. GANS (1953) has shown, the Y chromosome has no influence on the salt-and-pepper mottling of z females. X0 males with the allele z^l were investigated in order to find out whether the Y chromosome influences the number of c.l. spots. The males were derived from a cross of attached-X females without Y chromosome to $sc\ z^l$ males ($\underline{y} \times sc\ z^l$). The result, 12.5, is much like the value of z^l males from the homozygous stock (12.7; row 9, Table 1). The absence of the Y chromosome, therefore, does not seem to influence the incidence of c.l. spots.

The distribution of c.l. spots on the eye

Those presumptive eye cells in the anlagen that are the predecessors of the lower half of the eye grow more regularly than those which form the upper half of the eye, as was stated earlier. It was for this reason that the quantitative scoring of c.l. spots was limited to the lower half of the eye. Judging from the general appearance of the eyes, there is no reason to assume that the percentage of cells that become determined for red pigment formation is different in the presumptive upper half of the eye. The difficulty of scoring in the upper half, however, is a hindrance in getting definite evidence for this statement.

The distribution of c.l. spots over the lower half of the eye is shown in Table 3, upper row. Three hundred and sixteen sectors, each covered by one or part of one c.l. spot, are apparently equally distributed over all eight sector positions. This shows that the determination affects the cells of the anlage randomly, at least in the presumptive lower half of the eye.

This action of the alleles z^m and z^l on cells at the end of the first larval instar is interestingly comparable to the action of the Lobe alleles. The data from a newly arisen Lobe allele (BECKER 1957b) are summed up in the second row of Table 3. When the flies were raised at 18°C, 35 percent of the eyes were normal, whereas in the other 65 percent the eyes had gaps very similar in size, shape and position to X-ray-induced mosaic spots or c.l. spots of z^m and z^l (Figure 6). The analysis showed that this Lobe allele acted at the same stage as z^m and z^l , i.e., at the end of the first larval instar. The analysis of both, X-ray-induced mosaic spots and the Lobe deformities, indicated that at this stage in larval life the ommatidia field of the anlage is created. One of the properties that could be assigned to this field was the position of its center and its minimal diameter for the develop-

TABLE 3

The distribution of cell lineage spots in z^m and eye defects in Lobe over the different sectors of the lower half of the eye (in percent)

Allele	Sector								Total sectors covered by spot (z^m) or affected by defect (Lobe)
	I	II	III	IV	V	VI	VII	VIII	
z^m	13.3	12.3	11.7	13.3	11.1	11.7	15.8	11.7	316
Lobe	19.7	17.5	15.3	12.6	11.2	8.4	7.9	7.2	2,100

FIGURE 6.—Left eyes of the mutant *Lobe*, raised at 18°C (redrawn from BECKER 1957b).

ment of a normal eye. And the action of the investigated *Lobe* allele could be interpreted as a diminution of the field's diameter. The gradient that characterizes the distribution of 2100 registered defect sectors (Table 3, lower row) can be understood with the assumption that the field's center is laid down in that region of the anlage which forms the posterior central region of the eye. It should be pointed out that under these temperature conditions only the lower half of the eye is affected by the eye reduction.

One principal difference between the action of the *zeste* alleles z^m and z^l and the action of the *Lobe* allele is, then, that the determination of red pigment formation in the *zeste* alleles affects single anlage cells randomly, whereas the determination as to whether or not the descendants of a cell form ommatidia in *Lobe* eyes follows a gradient. Determination of cells in *Lobe* is apparently a part of the properties of a newly established, presumably centric ommatidia field. The action of both groups of alleles has, however, several common features. Both act at the same stage in larval life, and both determine at this stage the future fate of all the descendants of single cells. According to the size of the sectors and according to cell counts made of anlagen during the second larval instar (BECKER 1957b), about five cell divisions per cell take place between the time of determination at the end of the first larval instar and the time when the eye anlage consists of ommatidia stem cells in the third larval instar.

The gaps in the eyes of *Lobe* flies raised at 18°C do not distort the shape of the head. It is most likely, therefore, that all ommatidia stem cells keep growing at their normal rate. While ommatidia stem cells normally start with ommatidia formation by differential divisions, descendants of those anlage cells that failed to become determined for ommatidia formation do not start with differential division, but apparently divide into epithelial cells instead. These latter cells form later a normal kind of head cuticle that fills the gaps of the *Lobe* eyes.

The primary pigment cells of the ommatidia are the site where the red pigment is formed. They do not appear until four cell divisions after the beginning of ommatidia differentiation, i.e., about nine cell divisions after the determination for red pigment formation in z^m and z^l .

Thus, the conclusions that can be drawn so far are: In the *zeste* alleles z^m and z^l there are a certain percentage of eye anlage cells randomly determined as to whether or not their descendants, nine cell divisions later, will form red pigment. In *Lobe* the anlage cells are determined non randomly as to whether their de-

scendants start, after having undergone five cell divisions, with the ommatidia formation by differential divisions or divide into epithelial cells.

The action of the Lobe allele has to be understood, as was pointed out earlier, as diminution of the ommatidia field, i.e., as interference with a normal developmental process. The randomness of the z^m and z^l action, however, poses the question, whether the determination for red pigment formation at the end of the first larval instar is part of the normal development of the eye and whether, consequently, z^m and z^l interfere with such a process. It is possible to get at least a clue toward an answer. One can raise z/z^m or z/z^l heterozygotes (that develop normal eye color) until the second or third larval instar, and at this stage change single cells of the anlage into homozygous z^m/z^m or z^l/z^l cells. These changed cells, then, were heterozygous at the end of the first larval instar. If they had been determined at that time for normal red pigment formation, one would not expect to find any mosaic spots developing from these homozygous cells. If z^m/z^m or z^l/z^l cells induced as late as the third larval instar do form ommatidia with zeste phenotype, then there is apparently no determinative process concerning the eye pigmentation at the end of the first larval instar of normal flies.

To decide between these two alternatives, larvae heterozygous for z^m ($sc^+z^+/sc z^m$) were X-rayed. Their age was 65–75 hours after egg deposition, i.e., they were at the beginning of the third larval instar. The flies were checked for z^m/z^m mosaic spots. These spots were found in abundance. This means, then, that in normal cells no determination concerning the eye pigment takes place at the end of the first larval instar. In z^m , however, once the determination as to the type of pigmentation has been made, the cells keep the newly acquired property and hand it down to the daughter cells until the pigment becomes formed.

The zeste alleles interfere with the formation of the eye pigments. For the alleles z^m and z^l , in particular, the creation of the ommatidia field at the end of the first larval instar seems to be a critical point of interference. Once the action of z^m and z^l has been exerted, the cells hand down the new, abnormal property to their daughter cells. If a cell becomes homozygous for these alleles at any later stage of development, this genetic constitution still allows an interference with the normal process of pigment formation.

DISCUSSION

Up to this point the discussion has been driven as far as the observations and the experimental results warranted. In this section a comparison between our results and those of other workers will be made in an attempt to define the nature of the determination further.

The determination has always been referred to as a determination to form red pigment. All one can see, however, is that parts of the eye contain red pigment while others lack it. To be precise, one can only speak of the cells being determined to contain red pigment, as long as nothing exact is known about the properties by which cells of a c.l. spot differ from the other cells. The possible properties might differ in the capability to form pigment, to take up pigment into the

cells or to incorporate pigment into the pigment granules within the cells. Since pigment formation is one of the possibilities and almost nothing is known about the others, the zeste action will be discussed in connection with some known facts about red pigment formation.

The work of HADORN and others (see HADORN 1958) revealed the first case of a gene dependent enzyme in *Drosophila*, xanthine dehydrogenase. This enzyme was found to be present in normal larvae as early as the first larval instar and is for the most part produced in the Malpighian tubules and the fat body. It is missing in flies homozygous for the eye color mutant *rosy*. The enzyme is involved in the formation of isoxanthopterin, a precursor of the red pigment. In spite of the early presence of the enzyme, isoxanthopterin is produced only in the pupal stage. Thus the enzyme is formed very early in development, and either the enzyme itself or the pigment precursor is able to be transported to the site of their interaction. The production of the pigment precursor takes place right at the time when the eye becomes pigmented. If the absence of red pigment in zeste eyes would be due to the absence of this enzyme, one certainly could not expect any c.l. type of variegation.

There are, however, other mutants that interfere in a different way with the production of red pigment. In most of these cases the interfering mutant acts autonomously, i.e., in mosaics each cell develops a phenotype according to its own genotype. The reason for this could be that other enzymes or pigment precursors are incapable of diffusing or of being transferred from one cell to another. One would, however, be more inclined to assume that those parts of the reaction chain leading to red pigment, with which the autonomous mutants interfere, are taking place at a stage in development when transportation from normal cells to mutant cells is not possible any more.

Under such an assumption the determination in z^m and z^l eye anlagen at the end of the first larval instar does not seem to be of a kind that endows some cells with a fixed substance and not others. One would have to think of the determination rather in terms of endowment of the cells with different functional potentialities. These potentialities would have to be attached to certain cellular entities. It is at present not possible to say what cellular constituent might be the site of the determination.

In other lines of investigations one finds cases that offer themselves as models for an interpretation of the present findings. Thus, in their nuclear transplantation work in *Rana pipiens* KING and BRIGGS (1956) and BRIGGS and KING (1957) showed that stabilized changes of the capacity for differentiation take place in nuclei of developing endoderm. In another example, the localized swellings (puffs) in giant chromosomes of Dipterans are believed to be forms of differential activation of chromosome loci (BEERMANN 1959). In some of these cases rather stable changes take place in connection with the formation of a puff (PAVAN and BREUER 1955). In *Drosophila melanogaster* under experimental conditions, a puff has even been induced at a chromosome locus where puffing normally has not been observed (BECKER 1959). This puff appears in some cells of a gland and not in others.

Because of the close resemblance to the variegation in the zeste alleles mention must be made also of variegation due to chromosome rearrangements in which heterochromatin is involved. In eyes of *Drosophila virilis*, BAKER (1953) found a variegation pattern apparently identical to that of the c.l. spots of the zeste alleles z^m and z^l ; in his case the heterochromatic peach locus had been transposed into euchromatin. Many other effects of heterochromatin on variegation are reviewed by SCHULTZ (1939), LEWIS (1950) and HANNAH (1951). Of special interest in connection with the zeste variegation are more recent findings by SCHULTZ (1956). He found in a variegating white translocation that an irreversible differentiation of pigment in cells of Malpighian tubules occurs in the embryonic stage. In another case where the Stubble locus is transferred into heterochromatin, it could be shown that the action of heterochromatin on the variegation of the locus is involved with the nucleic acid metabolism. The extent of the heterochromatin-dependent variegation is even paralleled by the degree of heterochromatization of salivary chromosome bands carrying the respective genes (SCHULTZ 1941).

All the cases mentioned above have in common with the variegation phenomenon of z^m and z^l the phenomenon of stable changes in somatic cells; and in all those cases the site of the change was the nucleus. Similarly, the site of differential determination for red pigment formation in z^m and z^l could also be the nucleus.

SUMMARY

Of the four zeste alleles z , z^a , z^m and z^l , the two latter ones show a variegation pattern characteristic for cell lineage (c.l.) in both males and females, and also in compounds with the two former alleles. In flies raised in favorable temperatures, which are different for each allele and each sex, the c.l. spots are normal red or slightly darker than normal; they contain both the red and the brown pigment component. The background of the eye is lemon and salt-and-pepper mottled or only lemon; in this latter case the red pigment component is missing.

The size and distribution of the c.l. spots were compared with X-ray-induced mosaic spots, whose size depends on the larval age at the time of irradiation.

It can be concluded that the time of determination as to whether the descendants of a cell develop red pigment or not takes place at the end of the first larval instar. Of the approximately 20 cells of which the presumptive eye area consists at this time of development, any number can be determined to red pigment formation in any individual head anlage, on the average 20–25 percent in z^m males, 10–20 percent in z^l males, 2–3 percent in z^m/z^m and z^l/z^l females and none, 2.1 percent, 1.1 percent, 2.1 percent, 1.5 percent and 4.9 percent in z^a/z , z^a/z^m , z^a/z^l , z/z^m , z/z^l and z^m/z^l compounds respectively. The site of the red pigment formation, the primary pigment cells, does not appear until about nine cell divisions after the determination.

The action of the zeste alleles is discussed and compared with the action of the eye-deforming mutant Lobe.

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