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Temperature-Sensitive Mutations in *Drosophila Melanogaster*, IV. A Mutation Affecting Eye Facet Arrangement in a Polarized Manner*,†

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Abstract. *Drosophila melanogaster* females heterozygous for the mutation N^{60g11} have wild type eyes when raised at 29°C but a disrupted arrangement of facets and extra bristles at 21°C. Shifts of cultures from one temperature to the other at different stages in development revealed that facet arrangement is altered by temperature during the third larval instar. The facet pattern is affected in a vertical wave that proceeds anteriorly from the posterior rim of the eye. The role of the Notch locus in development is briefly discussed.

Mutations with phenotypes that are expressed conditionally as a function of temperature have been useful tools in the analysis of development in such diverse organisms as *Drosophila*,[1] bacteriophages,[2] and slime molds.[3] The utility of such temperature-sensitive mutants in developmental studies results from the ability to manipulate the temperatures at specific time intervals. In *Drosophila*, shifting cultures from restrictive to permissive temperatures and vice versa, at successive stages in development, has delineated the period of temperature-sensitivity and revealed a number of different patterns.[4] The radiation-induced sex-linked mutant N^{60g11}, which maps at a site within the Notch locus of *D. melanogaster*, has been reported to have a dominant temperature-sensitive eye facet phenotype[5] and has also been found to have a temperature-sensitive wing effect. Shift experiments reported here have shown a temperature-sensitive period (TSP) during the third larval instar. During this interval, facet arrangement is determined in a vertical wave that proceeds from the posterior rim of the eye anteriorly.

Methods and Materials. All scoring was on w^a N^{60g11} *rb*/+ + + females[6] (which will henceforth be referred to as *N* females) obtained from the cross, w^a N^{60g11} *rb*/*Dp*-(N^+)·Y♂ × +/+ ♀. The cultures were maintained at either 21°C ± 1°C or 29°C ± 0.5°C.

In order to define the developmental stages present at the time of shifts, larval instars were distinguished according to the morphology of their mouthparts.[7] Eggs were collected in quarter-pint bottles within a 2-hr interval, from approximately 100 pairs per bottle. In the last shift experiments, the interval from the time of the shift to puparium formation was measured by scoring white prepupae. This is very precise since the puparium remains white for only a few minutes before darkening.[7] The TSP was determined by scoring phenotypes in cultures shifted from 21 to 29°C (shift-up) and from 29 to 21°C (shift-down) at successive intervals.

Eyes of adult *N* females were prepared for inspection with the scanning electron micro-

scope in the following manner. Flies were decapitated; the heads were placed in chloroform for three days and then allowed to air-dry for at least three more days. Omission of the treatment with chloroform caused many of the eyes to collapse upon desiccation. The dried heads were mounted on aluminum discs with household cement, coated with gold–palladium alloy in a vacuum evaporator, and photographed with a Cambridge Stereoscan scanning electron microscope. Eye tissue was scored as mutant when the ommatidia were not arrayed regularly in a closely-packed hexagonal lattice, and/or if the ommatidial bristles were duplicated or multiplicated.

In addition, the wings of the individuals whose heads were prepared for inspection were scored for the occurrence of nicked tips.[6] An *N* female was said to have a mutant wing phenotype if one or both wings were nicked.

Results. All *N* females raised at 21°C have a characteristic mutant eye phenotype of disrupted ommatidia and duplicated bristles over the posterior three-quarters to four-fifths of the eye (Fig. 1*b*), whereas the eyes of similar females are wild type when raised at 29°C (Fig. 1*a*). In addition, we noted that 3.5% (24/681) of the *N* females raised at 21°C expressed the Notch wing phenotype, whereas in the 29°C culture, 78.4% (530/676) had mutant wings. Thus, the mutant eye phenotype of N^{60g11} is expressed at low temperatures, whereas the mutant wing expression of the same allele is increased by high temperatures.

The results of the shift experiments are shown in Tables 1 and 2. It can be seen that the number of mutant eyes rises sharply in shift-ups between 120 and 144 hr when only third instar larvae are present. This is also the time interval during which the proportion of mutant wings drops drastically (Table 1). The time when shift-downs decrease the number of mutant eyes and increase the

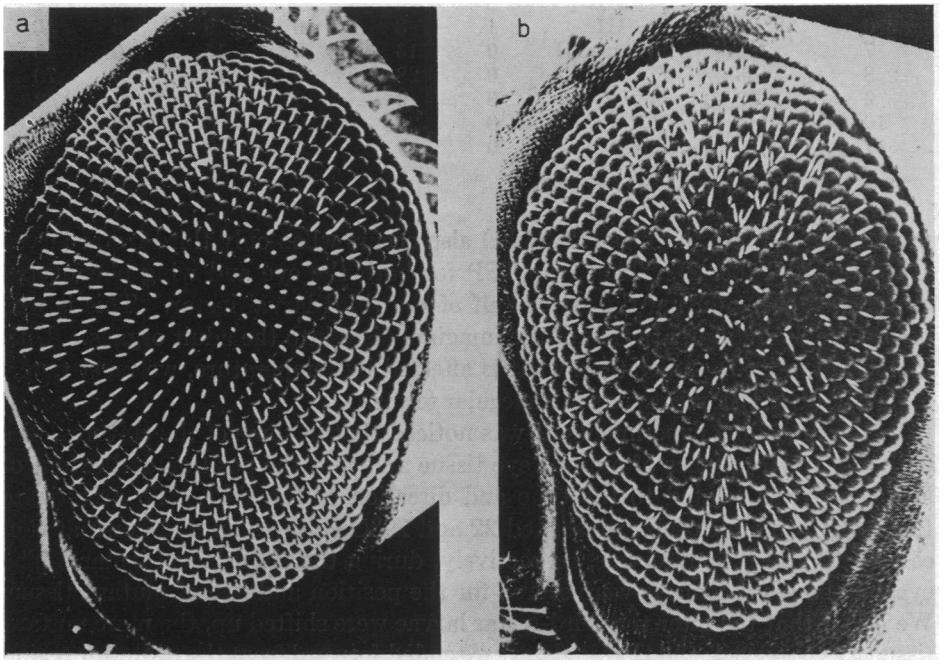

FIG. 1. Scanning electron micrograph of the eye of an $N^{60g11}/+$ female raised at (*a*) 29°C and (*b*) 21°C. Note that the anterior part of the eye is to the left.

TABLE 1. *Eye and wing phenotypes of $N^{60g11}/+$ adult females shifted from 21 to 29°C at different successive intervals.*

Culture no.	Time of shift-up: Culture age (hr)	Time of shift-up: Developmental stage	Number of flies in each phenotypic class: R N*	R⁺ N	R N⁺	R⁺ N⁺	% R	% N
1	24	I†	0	38	0	23	0	62
2	48	I, some II	0	28	0	27	0	51
3	72	II, some III	0	31	0	19	0	62
4	96	III	0	55	0	8	0	87
5	120	III	1	54	3	4	6	89
6	144	III, some P	9	0	54	0	100	14
7	168	P, some III	2	0	49	0	100	4
8	192	P	0	0	43	0	100	0
9	216	P	1	0	171	0	100	0.6
10	240	P	0	0	118	0	100	0
11	264	P	0	0	123	0	100	0
12	not shifted		1	0	180	0	100	0.6

* R = disrupted ommatidia, R^+ = wild type eyes, N = nicked wings, N^+ = wild type wings.
† I, II, III = 1st, 2nd, 3rd larval instar, respectively; P = prepupae & pupae.

TABLE 2. *Eye and wing phenotypes of $N^{60g11}/+$ adult females shifted from 29 to 21°C at different successive intervals.*

Culture no.	Time of shift-down: Culture age (hr)	Time of shift-down: Developmental stage	Number of flies in each phenotypic class: R N*	R⁺ N	R N⁺	R⁺ N⁺	% R	% N
1	24	I, some II*	0	0	53	0	100	0
2	36	II, some I	1	0	16	0	100	6
3	48	II	4	0	51	0	100	7
4	60	III	7	0	64	0	100	10
5	72	III	8	0	100	1	99	7
6	84	III	1	8	0	9	6	50
7	96	P, some III	0	13	0	15	0	46
8	108	P	0	28	0	12	0	70
9	132	P	0	9	0	8	0	52
10	144	P	0	20	0	8	0	71
11	not shifted		0	9	0	9	0	50

* See Table 1 for explanation of symbols.

proportion of mutant wings (72–84 hr) also occurs when only third-instar larvae are present (Table 2). Thus, the TSP for both the eye and wing phenotypes appears to occur during the latter half of the third larval instar. This is well before the final differentiation of the imaginal discs into the adult organs. Ultimately, in order to understand what is affected by temperature, we must determine what the TSP represents in molecular terms.

In the preceding experiments, it was noticed that in some of the cultures, flies had only small patches of mutant eye tissue and that the position and extent of this tissue was related to the time and direction of the shift. To investigate this pattern more closely, we shifted 22 and 29°C cultures up and down at successive 12 and 6 hr intervals, respectively, during the third larval instar. The eyes of the adult females were scored for the position and size of mutant tissue. We found that the later the third-instar larvae were shifted up, the more mutant tissue was observed, and that it always extended forward from the posterior region of the eye in a vertical strip. Similarly, the later the shift-down, the greater was the extent of non-mutant tissue in the same pattern. Since developmental

synchrony of the larvae was poor in this experiment, at any time of a shift, larvae in many different states in the third instar were present. The interval between a shift and the end of the third larval instar was determined precisely as follows. Several batches of eggs laid at 21°C were collected on petri plates containing *Drosophila* medium in successive 2- to 6-hr intervals over a 48-hr and a 72-hr period. All cultures recovered over the 72-hr period were incubated at 21°C until the first pupae were detected, at which time all of the cultures in this series were shifted up. Each culture collected in the 48-hr series was shifted to 29°C upon collection and the entire series was shifted down at the appearance of the first puparia. After the shifts, at both temperatures, white prepupae only were removed from the plates at defined times, placed in vials, and allowed to develop into adults. Thus, the interval between the time of the shift and puparium formation was accurately defined. The heads of all *N* females were inspected in the electron microscope and the wings inspected under a dissecting scope.

The results of this experiment can be seen in Fig. 2. The longer the interval between a shift-up and puparium formation (i.e., the earlier the shift-up during development), the less mutant eye tissue is found and the mutant area always begins at the posterior edge of the eye and extends forward (Fig. 2*a*). On the other hand, the longer the interval between a shift-down and puparium formation, the greater the amount of mutant tissue observed beginning at a point near, but not at, the anterior edge of the eye and extending posteriorly (Fig. 2*b*). Fig. 3 shows scanning electron micrographs of representative eyes that illustrate these patterns. It can be seen that the eye TSP occupies a similar time span (about 23 hr) at both 21°C and 29°C, even though development is accelerated at 29°C (the third instar occupies about 65 hr at 21°C and about 40 hr at 29°C).[8] Moreover, the eye TSP appears to occur earlier in the third instar at 29°C than at 21°C.

The incomplete penetrance of the wing mutant phenotype at 29°C, and the small number of flies scored, preempts a definitive delineation of the wing TSP. Nevertheless, the TSP for the wing phenotype appears to begin and end during the eye TSP (Fig. 2*b*, *a*). Experiments in which cultures are exposed to short pulses of high or low temperatures (shift-up-and-down and vice versa) at specific times may allow a more precise definition of the wing TSP relative to the eye TSP.

Discussion. The polarized progression of eye-facet arrangement suggests that a wave of determination of ommatidial organization originates in the portion of the eye disc destined to form the posterior edge of the adult eye and progresses anteriorly for at least four-fifths of the eye. The present observations and conclusions are very similar to those of Becker, who found[9] that a phenocopy of a "rough" eye could be induced by x-irradiation during the third instar and that the irregular arrangement migrated anteriorly across the eye in a vertical band with increasing larval age at the time of irradiation. Recently, Kuroda[10] reported that a vertical wave of ommatidial orientation proceeded forward from the posterior region of eye imaginal discs *in vitro*. He worked with mature third-instar larvae, which correspond to a later time in development than the beginning of the TSP noted in this report. Posterior-to-anterior progression of eye dif-

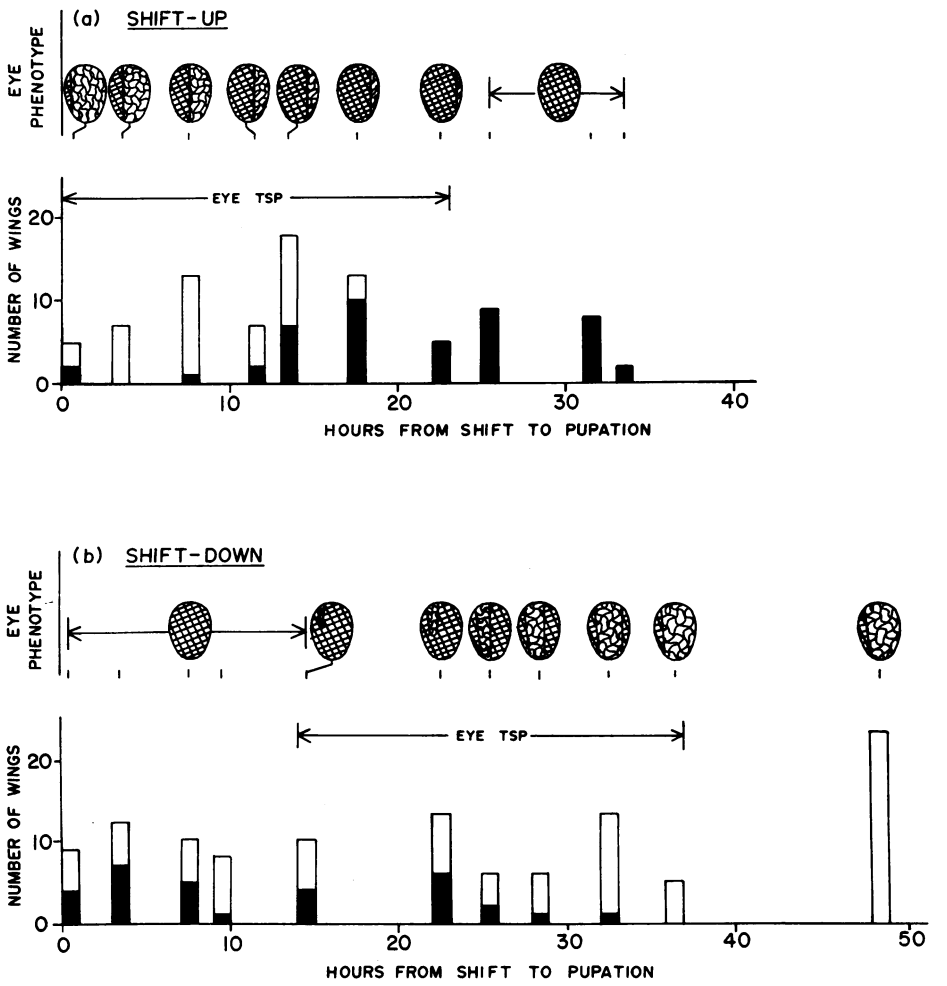

FIG. 2. The eye and wing phenotypes of $N^{60g11}/+$ females shifted from (*a*) 21–29°C, and (*b*) 29–21°C at different times prior to puparium formation. Note that the 3rd larvel instar stage lasts about 66 hr at 21°C and 40 hr at 29°C. ## wild type eye facet arrangement; ## mutant eye facet arrangement; □ wild type wings; ■ notched wings. Note that the anterior rim of the eye is on the left.

ferentiation has also been observed in other insects.[11] The observed eye TSP coincides roughly with the larval period during which a large increase in the number of ommatidial precursor cell-clusters is taking place[12] and it may be that N^{60g11} is defective in the formation of these clusters, a possibility that is presently under investigation.

Since it is known that Notch hemizygotes and homozygotes die in the egg stage,[13] the finding that the TSP for both the eye and wing phenotypes occurs during the third larval instar suggests that the Notch gene product is necessary for normal development at more than one stage during the life cycle of *Drosophila*. This corroborates the demonstration of tissue-specific temperature-sensitive periods for a gene known to act in several tissues.[14]

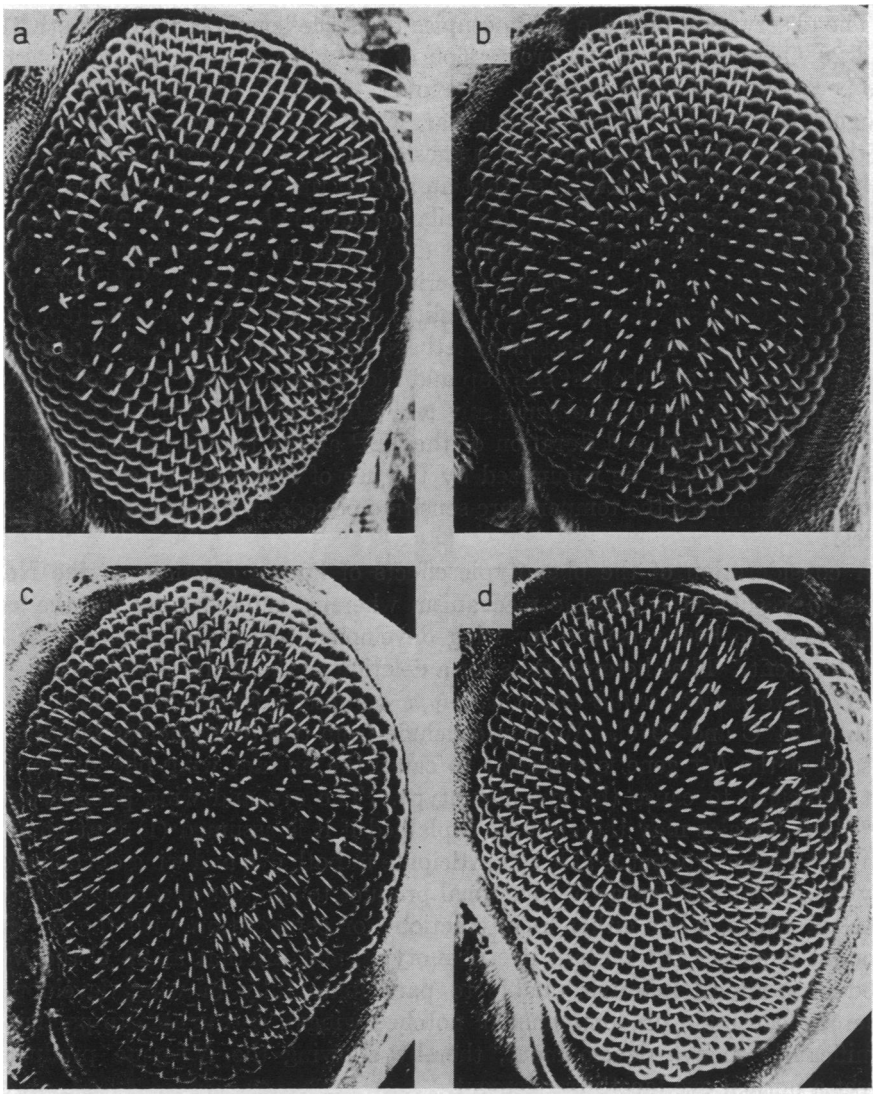

FIG. 3. Scanning electron micrographs of eye facets of $N^{60g11}/+$ females shifted down at (*a*) 14 hr and (*b*) 26 hr, and shifted up at (*c*) 7.5 hr and (*d*) 18.5 hr, prior to pupation. Note that the anterior part of the eye is to the left.

The function of the Notch gene in neurogenesis in the embryo[13] raises the possibility of neural involvement in differentiation of imaginal organs such as the eye and wing. It has been pointed out[12] that a neural connection between the brain and the developing eye disc is established long before ommatidial precursors can be recognized histologically and that each ommatidial precursor is innervated in the larval as well as the pupal stage. On the other hand, no neural connection is observed with the larval wing discs.[7] The question of a neural defect affecting eye development in N^{60g11} heterozygotes is presently being investigated.

The observation that the TSP occupies the same length of time at both 21°C and 29°C suggests that the progression of the wave of determination is not a temperature-dependent process. Moreover, at 29°C the eye TSP occupies roughly the first half of the third instar, while at 21°C it covers the last third of the third instar. This strongly suggests that some developmental events are only loosely related to others, so that an altered time or length relative to other events is not necessarily lethal. A similar conclusion has been reached in microorganisms.[15] This points to possible difficulties in relying exclusively upon reciprocal shift-up and shift-down experiments to establish the TSP of mutant characters (such as wing nicks or lethality) which are scored qualitatively. In experiments involving heat-sensitive lethals, for example, the TSP is defined as the interval between the last shift-up and the first shift-down in which lethality is observed.[4] This would obscure any real differences which might exist in the developmental stage and duration of the TSP at the two temperatures. This problem can perhaps be minimized by the use of reciprocal pulse-shift experiments to determine the temperature-sensitive periods at the respective temperatures.

A consideration of the phenotypic effects of known deletions of the Notch locus may suggest a possible mechanism whereby heterozygotes for the same allele may be heat-sensitive for wing development but cold-sensitive for eye development. Flies heterozygous for a deletion of the entire Notch locus, such as N^8, have *notched wings and a wild-type eye facet arrangement.*[6] This is true both at 21°C and 29°C. We can conclude from this that whereas halving the dosage of the N^+ gene is sufficient to cause the mutant wing phenotype, it is not sufficient to cause the eye phenotype. The eye and wing phenotypes of $N^{60g11}/+$ females may therefore be explained if it is assumed (1) that at 21°C, the N^{60g11} gene product is able to participate in both eye and wing development, having a sufficient dosage of functional product to produce predominantly non-notched wings, while either in interaction[16] or competition with the N^+-gene product it causes the mutant eye phenotype; and (2) that at 29°C the N^{60g11} product is almost inactive, unable to participate fully in wing development (thereby causing a predominance of notched wings), and unable to participate significantly in eye development, thereby allowing the N^+-gene product to direct a normal eye facet arrangement.

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Abbreviation: TSP, temperature-sensitive period.

Part III of this series is ref. 1*b*.

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