TEMPERATURE-DEPENDENT EXPRESSION OF THE APERTOUS PHENOTYPE IN DROSOPHILA MELANOGASTER

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ABSTRACT

Mutations at the apertous (ap) locus in Drosophila melanogaster produce a variety of developmental defects, including several classes of wing abnormalities. We describe the wing phenotype produced by homozygotes and hemizygotes of three different temperature-sensitive apertous alleles grown at 16, 18, 20, 22, 25, and 29°C. We also describe the phenotype produced by each of these three alleles when heteroallelic with the non-temperature-sensitive ap' allele. Constant-temperature and temperature-shift experiments show that each of the heteroallelic genotypes can produce several of the previously described apertous phenotypes and that the length of the temperature-sensitive period for a given phenotype depends on the allelic combinations used to measure it. We suggest that the stage-specific requirements of the tissue for gene product, rather than the time of gene expression per se, determine the temperature-sensitive periods for apertous and other loci. The results support the hypothesis that the various wing phenotypes produced by apertous mutations are due to quantitative reductions in the activity of gene product and that failure to meet specific threshold requirements for gene product can lead to qualitatively different phenotypes.

CONDITIONAL mutations, the expression of which can be controlled by manipulating environmental factors, have proven useful in the study of developmental mechanisms (for review, see SUZUKI et al. 1976). Temperature-sensitive (ts) mutations are often used to determine the “time of gene action”; the appropriate manipulation of temperature conditions by upshift, downshift or heat-pulse experiments makes it possible to ascertain the temperature-sensitive period (tsp), which is interpreted as the time in development when the abnormal gene functions to produce a given phenotype (SUZUKI et al. 1976). In heat-sensitive mutations, the first downshift (from 29°C to 18°C) to produce the restrictive-temperature phenotype indicates the beginning of a temperature-sensitive period, whereas the first upshift culture that produces the permissive-temperature phenotype indicates the end of temperature sensitivity.

Several ts mutations affecting wing development in Drosophila melanogaster have been described (vestigial, ROBERTS 1918; Notch, SHELLENBARGER and

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Mohler 1975; I(3)c43H1, Martin, Martin and Shearn 1977; ecd1, Sliter and Bryant 1982). In addition, apterus mutations produce several different wing phenotypes in an allele-specific and temperature-dependent fashion (Wilson 1981; Stevens and Bryant 1985). The apterus wing phenotype includes five types of abnormality: blistering, deficiency, duplication, higher-order repetition and transformation of wing structures. We have proposed an explanation for the variety of wing phenotypes produced by different ap alleles (Stevens and Bryant 1985) in which quantitative reductions in the activity of the ap gene product produce qualitatively different phenotypes due to different threshold requirements of the ap+ function for critical events in wing disc development.

Given the assumption that exposure to the restrictive temperature (the temperature that produces the mutant phenotype) decreases the activity of the ap product and that heat pulses of different severity or different duration lead to varying degrees of reduction of gene product activity, it is possible to test the foregoing hypothesis. In this paper, we describe the effects of several temperature regimens on three ts ap alleles in hemizygous, homozygous and heteroallelic genotypes. In addition, we have investigated the interaction of the alleles with a non-ts allele and have compared the tsps of three different heteroallelic genotypes which produce wing margin repetitions.

MATERIALS AND METHODS

Stocks: We analyzed three ts mutations at the apterus (ap; 2-55.2) locus, ap49, ap54, and ap1080, which have been described previously (Wilson 1981; Stevens and Bryant 1985). All three alleles fall into a single class (class II; Stevens and Bryant 1985) as determined by genetic and phenotypic analysis. These alleles were kept balanced over the SM5 balancer chromosome (see Lindsley and Grell 1968); this made it possible to obtain more progeny than is possible with homozygous stocks and offered the additional advantage of providing control animals grown in the same culture bottles as the mutants. A non-ts class IV (Stevens and Bryant 1985) allele, ap7, was maintained as a homozygous stock, so that when these animals were crossed to flies heterozygous for the class II alleles, 50% of the resulting F1 progeny were heteroallelic for the two ap mutations. A chromosome carrying M(Z)SZ4 (Lindsley and Grell 1968), which is deficient for apterus, was used to produce animals hemizygous for various ap alleles.

Culture conditions: Animals were cultured in half-pint bottles, using a standard cornmeal, yeast, corn syrup and agar medium overlain with a thin layer of instant Drosophila medium (Carolina Biological). All temperatures listed are within ± 0.5°C. For the constant-temperature experiments, adults were allowed to lay eggs in the culture bottles at each temperature (16, 18, 20, 22, 25 and 29°C) for 3 days and were then removed. All eclosing adults were then scored, with a minimum of 50 per experiment. For temperature-shift experiments, eggs were collected over a 1-hour period, and the culture was then shifted to the designated temperature after a specific time interval and was left at the latter temperature until the adults eclosed. Eclosing adults were collected for 2 days, and the culture bottles were examined for any animals which had died as pupae or pharate adults. The wing phenotype was scored using a Wild dissecting microscope at 120X. Specimens from the constant-temperature experiments, as well as some of the animals from the shift experiments, were mounted between coverslips and scored under a Zeiss compound microscope at 125X.

Developmental times: Pupariation times were determined by counting pupae at 2-hour intervals, and eclosion times were determined by counting emerged adults at daily intervals.
Wing phenotypes of *apterous* homozygotes and hemizygotes when reared at constant temperature

<table>
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<tr>
<th>Temperature</th>
<th>Genotype</th>
<th>ap <em>78</em></th>
<th>ap <em>78</em></th>
<th>ap <em>49</em></th>
<th>ap <em>54</em></th>
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<td>B</td>
<td>B</td>
<td>S</td>
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<td>B</td>
<td>S</td>
<td>S</td>
<td>Nu</td>
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<td>Nu</td>
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<td>Nu</td>
<td>RT</td>
<td>Nu</td>
<td>RT</td>
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</table>

All animals in each sample (*N > 50 in each case*) showed the same phenotype: B = notched-blistered; No = notched; Nu = nubbin; RT = reduced thorax; S = strap.

### RESULTS

**Constant-temperature experiments—homozygotes:** All homozygous animals raised at any given temperature showed the same adult phenotype. The wing phenotype of *ap* *78* homozygotes grown at 15, 22, and 29° has been described by Wilson (1981). We have repeated these treatments with similar results (Table 1) and have also examined animals grown at 18, 20 and 25°. In our experiments, growth at 16° produced nearly normal wings with slight notching of the margins, similar to that described by Wilson (1981) at 15°. At 18°, the wing blade was about three-quarters the normal size, with slight blistering, and most of both the anterior and the posterior wing margin was missing (Figure 1A). Growth at 20° produced a slightly smaller blistered wing about one-half the normal size, totally lacking margin elements except for remnants of the double-bristle row at the tip of the wing. Growth at 22° produced a similar phenotype but with no margin whatsoever. Growth at 25° produced blistered strap wings (Figure 1B), and at 29° the wings were reduced to small nubbins lacking most of the wing-blade material (Figure 1C); the latter is the most common *apterous* wing phenotype produced by non-ts alleles.

Homozygotes of both *ap* *49* and *ap* *54* showed a similar range of phenotypes (Table 1), but these alleles were more severe than *ap* *78* homozygotes in that the more extreme phenotypes were produced at lower temperatures. For example, *ap* *49* and *ap* *54* homozygotes grown at 20° produced strap wings similar to those produced by *ap* *78* homozygotes at 25°, and *ap* *49* and *ap* *54* homozygotes grown at 25° showed a nubbin wing phenotype identical to that produced in *ap* *78* animals at 29°.

**Constant-temperature experiments—hemizygotes:** The phenotype produced by each hemizygous genotype at a given temperature was more severe than that produced by the corresponding homozygous genotype at the same temperature. Hemizygotes for *ap* *78* grown at 18° produced wings that were about one-half the normal length (Figure 1D); the phenotype resembled that of homozygotes grown at 22° (Table 1). Growth at 20° produced a normal notum with an intact scutellum, and small strap wings were present that varied
Wing phenotypes produced by homozygous and hemizygous *apterous* animals reared at different constant temperatures. Bar = 0.1 mm. A, *ap^78J/ap^78J* reared at 18°; B, *ap^78J/ap^78J* reared at 25°; C, *ap^78J/ap^78J* reared at 29°; D, *ap^78J/M(2)S2^* reared at 18°; E, *ap^78J/M(2)S2^* reared at 20°; F, *ap^78J/M(2)S2^* reared at 29°.

from one-eighth to one-quarter of wild-type wing length (Figure 1E). Animals grown at 22° had a notum with a scutellum, but were missing all scutellar bristles. Stevens and Bryant (1985) described the phenotype produced by hemizygous *apterous* genotypes, including the ts alleles, in animals grown at 25°. This phenotype consisted of a severely deficient notum, lacking all wing structures, the scutellum and most of the notal macrochaetes.

The phenotypes of *ap^49* and *ap^54* homozygotes were as follows: at 18° the scutellum was normal and the wings were one-quarter the normal length and strap-shaped; at 20° the scutellum was normal and the wings were reduced to
Frequency of wings of *apterous* heteroallelic combinations showing different phenotypes after rearing at constant temperature

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Temperature °</th>
<th>Normal</th>
<th>Notched</th>
<th>Notched-blistered</th>
<th>Margin repetitions</th>
<th>Strap</th>
<th>Nubbin</th>
<th>N (No. of wings)</th>
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* In each case of strap or nubbin wings, the other wing of the animal showed margin repetitions.

nubbins; at 22° the scutellar bristles were missing, but the scutellar cuticle was present.

All three hemizygous genotypes produced similar phenotypes at 29°: the thorax was extremely reduced, with either no macrochaetes at all or only a single anterior dorsocentral bristle. The scutellum and all wing tissue were missing (Figure 1F).

**Constant-temperature experiments—heterozygotes:** The results of the constant-temperature experiments for heteroallelic combinations are summarized in Table 2. Unlike homozygotes and hemizygotes, these genotypes often produced mixed phenotypes at a given temperature. Most *apterous* alleles interact with *ap⁵¢*, a non-ts allele, to produce repetitions of wing margin elements (STEVENS and BRYANT 1985). Heteroallelic combinations between *ap⁵¢* and each of the three ts alleles produced a wing phenotype that varied in a temperature-dependent manner; thus, the temperature sensitivity was maintained in the presence of the non-ts allele.

In heteroallelic *ap⁵¢'/ap⁵j* animals grown at 16, 18, 20, 22 and 25°, a mixture of normal, notched and notched-blistered wings was produced (Table 2; Figure 2A and B). The phenotype was slightly more severe at the higher temperatures in that fewer normal wings and a higher frequency of notched-blistered wings were observed. Growth at 29° produced wings with margin repetitions (Figure 2C) in 100% of these animals: 6 of 73 showed a strap wing
on one side, with margin repetitions on the other wing; and 3 of 73 showed a nubbin wing on one side, with margin repetitions on the other side.

Animals heteroallelic for \( ap^{g78}/ap' \) showed a similar progression of phenotypes to those of \( ap^{g78}/ap' \), but as in the case of the homozygotes, a given temperature treatment tended to produce a more severe phenotype than in \( ap^{g78}/ap' \). Again, lower temperatures (16, 18 and 22°) produced a mixture of normal, notched and notched-blistered wings, but even at 16° the frequency of normal wings was lower than that observed for \( ap^{g78}/ap' \) heterozygotes. In addition, margin repetitions were first observed at 25°, rather than at 29°, as was the case with \( ap^{g78}/ap' \). Of the animals raised at 29°, 100% showed margin repetitions: 2 of 45 had a strap wing on one side, and 2 of 45 had a nubbin wing on one side, in each case with margin repetitions on the other side. Similar results were obtained with \( ap^{54}/ap' \) animals, with a mixture of normal, notched and notch-blistered wings produced at lower temperatures. At 25°, the switch to the repetition phenotype was complete; at both 25 and 29°, 100% of the animals showed margin repetitions (Figure 2D), with 1 of 27 animals showing a nubbin wing on one side.

For \( ap^{59}/ap' \) and \( ap^{54}/ap' \) as well as \( ap'/SM5 \) controls, the mean eclosion times at 18° were in the range of 18.6–18.8 days (N = 74-264), whereas \( ap^{g78}/ap' \) showed a slight developmental delay, with a mean eclosion time of
19.6 days ($N = 201$). Mean pupariation times at 18 and 29° were 215 and 85 hr for cultures from $ap^{49}/SM5 \times ap^c$ ($N = 87$ and 44); 215 and 82 hr for cultures from $ap^{54}/SM5 \times ap^c$ ($N = 62$ and 52); and 224 and 86 hr for cultures from $ap^{78j}/ap^{78j} \times ap^c$ ($N = 83$ and 57). We conclude that $ap^{78j}/ap^c$ shows slight developmental delay compared to controls, whereas $ap^{49}/ap^c$ and $ap^{54}/ap^c$ develop at the normal rate.

**Temperature-shift experiments—heteroallelic combinations:** The phenotypes produced in temperature-shift experiments can be classified into two groups: the permissive-temperature phenotype, which includes normal, notched and notched-blistered wings similar to those described for 18° constant temperature, and the restrictive-temperature phenotype, which refers to the wing margin repetitions seen after constant 29° culture. With most shifts, the resulting populations could be placed into one or the other of these groups with no overlap; only $ap^{49}/ap^c$ and $ap^{54}/ap^c$ animals shifted from 18 to 29° after 5 days after egg laying (AEL) produced a mixture of animals with notched-blistered wings and margin repetitions (Figure 3).

In $ap^{78j}/ap^c$ animals grown at 18° for 4 days or less and then shifted to 29°, 100% of the animals showed wing margin repetitions. With shifts after 5 days AEL at 18°, an abrupt transition to the permissive-temperature phenotype occurred, and shifts at all subsequent times also produced the permissive-temperature phenotype of normal, notched and notched-blistered wings. No margin repetitions were observed in any animals shifted to 29° later than 4 days AEL.

Upshift experiments on $ap^{49}/ap^c$ and $ap^{54}/ap^c$ produced results similar to those obtained with $ap^{78j}/ap^c$, except that the transition from the restrictive-temperature phenotype to the permissive-temperature phenotype occurred slightly later. Animals shifted at day 5 AEL still showed margin repetitions at high frequency: 77% of $ap^{54}/ap^c$ and 92% of $ap^{49}/ap^c$ had repetitions. With shifts at day 6, 100% of the animals of both genotypes showed the permissive-temperature phenotype.

Downshift experiments (from 29° to 18°) showed similar results for all three genotypes. The permissive-temperature phenotype was observed in animals grown for 60 hr or less at 29°; longer culture at 29° resulted in the restrictive-temperature phenotype.

Both $ap^{49}/ap^c$ and $ap^{78j}/ap^c$ shifted from 18 to 29° late in development—after 15 days at 18°—produced a few animals (3% of $ap^{49}/ap^c$, 2% of $ap^{78j}/ap^c$) with the mitten phenotype, otherwise produced only by animals heterozygous for the non-ts allele, $ap^{3a}$ (STEVENS and BRYANT 1985). The remaining animals showed a mixture of notched and blistered wings.

**DISCUSSION**

We have used ts mutations of *apterous* to investigate the production of several classes of wing abnormalities. By decreasing the *ap* function at different times and to different degrees with the appropriate temperature conditions, we have been able to reproduce most of the known *apterous* phenotypes in each of two genotypes, $ap^{78j}/ap^c$ and $ap^{49}/ap^c$. This suggests that the pheno-
typic differences between *apterous* alleles may not be due to qualitative differences in the way the gene product is altered, but to quantitatively different effects on gene product activity which are translated into mutant phenotypes because of the existence of thresholds in the requirement of the tissue for the
active gene product. Different phenotypic categories appear to be characterized by different threshold levels (Stevens and Bryant 1985).

The *apterous* phenotypes range from nearly normal wings to severely deficient nubbin wings. Homozygotes for the least severe allele, $ap^{bt}$, show a mild blistering of the wing blade (Sedlak, Manzo and Stevens 1984; Stevens and Bryant 1985) and occasional transformations of posterior margin into anterior margin (Whittle 1979). A dominant allele, $ap^{xa}$, produces in the heterozygote a mitten-shaped wing lacking anterior wing margin elements, as well as the distal wing tip. Several recessive ts alleles and a second dominant allele, $ap^{id}$, produce strap wings in homozygotes. A heteroallelic combination of the two dominant alleles ($ap^{id}/ap^{xa}$) produces notal duplications accompanied by complete wing loss, and other heteroallelic combinations produce repetitions of wing margin elements and transformations of posterior margin elements to anterior margin structures. The most common phenotype, produced in homozygotes for 18 of the 24 $ap$ alleles we have studied (Stevens and Bryant 1985), consists of nubbin wings which lack most of the wing-blade material.

We have proposed an explanation of this plethora of wing phenotypes based on a phenotypic and genotypic analysis of 24 $ap$ mutations (Stevens and Bryant 1985). We suggest that the different alleles reduce the activity of the gene product to varying extents and that different alleles behave additively in most heteroallelic combinations. Each phenotype is assumed to be produced over a given range of $ap$ activity, and the total gene product activity for each genotype is simply the sum of the two allele-specific activities, with some exceptions where there appear to be nonadditive interactions between alleles in heteroallelic combinations. We suggest that quantitative reductions in the activity of the $ap$ gene product lead to qualitatively different wing phenotypes because of threshold requirements of the gene product for critical events in wing-disc development.

Our constant-temperature studies support the additive nature of this model and, in addition, suggest that the less-severe deficiency phenotypes can be altered in a quantitative as well as a qualitative manner. The ts *apterous* mutations produce phenotypes ranging from normal or nearly normal wings ($16^\circ$) to no wings ($29^\circ$). The phenotypes observed in homozygous and hemizygous animals grown at constant temperatures show quantitative changes in that increases in temperature are accompanied by decreasing amounts of wing tissue, as would be expected if the increased temperature reduced the activity of the gene product. The gradual shift from nearly normal wings to severely reduced thoraces may be due to a gradual reduction in $ap$ activity.

Some aspects of various $ap$ phenotypes respond discontinuously to changes in rearing conditions. There is, for example, no intermediate phenotype observed in $ap^{id}/ap^{xa}$ heterozygotes: these animals show either reduced wings (strap or nubbin, depending on the temperature) or a duplicated notum with total wing loss. We have never observed animals of this genotype with a normal thorax and no wing material, or a duplicated notum associated with strap or nubbin wings. The same generality applies to the reduced thorax phenotypes seen in hemizygotes. There is some evidence of a quantitative effect on the
amount of scutellar material and notal bristles, since \( ap^{ts78J} \) hemizygotes grown at 22° have scutellar cuticle but not scutellar bristles. However, the wing hinge elements appear in an all-or-none fashion. Thus, qualitatively different phenotypes occur in a single genotype and may be produced by a threshold mechanism.

We have used temperature-shift experiments to determine the tsp for the production of margin repetitions in three genotypes: \( ap^{49}/ap^c \), \( ap^{54}/ap^c \) and \( ap^{ts78J}/ap^c \). Our results suggest that the tsp for wing margin repetitions extends from the late second to the early third instar; this overlaps the tsp for wing deficiencies in \( ap^{78J}/ap^{78J} \) homozygotes, a period extending from the late second to the mid third instar (WILSON 1981). The transition time between the permissive-temperature and the restrictive-temperature phenotype in our experiments, however, is very abrupt compared to the tsp reported by WILSON (1981) and suggests a much shorter tsp ending early in the third instar. The tsp that we observe for margin repetitions corresponds exactly with the time that a heat pulse to \( I(1)ts-726 \) larvae produces leg triplications (GIRTON 1981), a phenotype that may be analogous to margin repetitions in the wing. GIRTON (1981) also found a very short tsp for leg triplications.

The tsp ends sooner in \( ap^{ts78J}/ap^c \) animals than in either \( ap^{49}/ap^c \) or \( ap^{54}/ap^c \) animals. This is not due to a difference in developmental times; in fact, the \( ap^{ts78J}/ap^c \) larvae develop slightly more slowly than do \( ap^{49}/ap^c \) or \( ap^{54}/ap^c \) animals. Although it is possible that the tsp reflects the time of gene expression

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**Figure 4.**—A model to explain how the measured tsp could reflect stage-specific requirement for \( ap \) gene product. The threshold requirement for \( ap \) gene product (solid line) is assumed to change during the development of the wing disc, increasing suddenly at 4 days and decreasing gradually from 4½ to 7 days. Dashed lines show levels of gene-product activity in wild type and in three heterozygotes subjected to the restrictive temperature. If gene-product activity level falls below threshold, the restrictive-temperature phenotype is produced. The temperature-sensitive period for \( ap^{49}/ap^c \) and \( ap^{54}/ap^c \) will be longer than that for \( ap^{78J}/ap^c \), because their activity levels fall below the threshold for a longer time.
and that allelic substitutions change the timing of ap gene expression, we find that our results are more easily interpreted by assuming that the tsp is determined by the stage-specific requirement of the tissue for ap gene product and that tsp differences between alleles are due to their differing severities. According to this model the threshold requirement for gene product activity, which is assumed to be the same for all genotypes, would change during development, as shown in Figure 4. The threshold would be low early in development so that lowering the activity at this time would not produce any developmental abnormalities in the disc. Just before the second to third-instar molt, the threshold requirement for gene product would suddenly increase, remain high during the beginning of the third instar and, then, slowly decline to a lower level. Although the various ap mutations presumably reduce gene product activity to different degrees, we assume that the degree of activity for any one genotype is constant throughout this part of development. Thus, the time interval during which an animal of any given genotype shows temperature sensitivity would depend both on the degree of reduction of gene product activity and on the changing requirements of the disc for gene product. For example, ap49/ ap and ap54/ap would show a longer tsp than ap678/ ap because the activity level produced by these two genotypes is lower and, therefore, is below the threshold level for a longer period of time than is the case with ap678/ ap.

Many different mutations which produce different tsps for different developmental defects show the same tsps for any one particular defect. For example, Notch (Shellenbarger and Mohler 1978) shibire (Poodry, Hall and Suzuki 1973) and ecd1 (T. Sliter, personal communication) all have similar third-instar tsps for eye scarring, small rough eyes, shortened tarsi and wing defects, as well as pupal tsps for extra and missing microchaetae. It is possible that all of these genes are under the same temporal regulation, but it is also possible that the stage-specific requirement of the tissue for these gene products, rather than the time of gene expression per se, determines the tsp.

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LITERATURE CITED


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