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Temperature-Sensitive Mutations in *Drosophila melanogaster*, VII. A Mutation (*para^{ts}*) Causing Reversible Adult Paralysis

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ABSTRACT **A temperature-sensitive mutation, *para^{ts}*, in *Drosophila melanogaster* causes an immediate, but reversible, paralysis of adult flies when they are shifted from 22°C to 29°C. The mutation is a sex-linked recessive that maps 2.8 units to the left of *f*. Wild-type flies observed for 2-hr periods exhibited normal mobility at all temperatures between 22°C and 35°C. From 22°C to 25°C, *para^{ts}* flies were wild type in walking, climbing, and flying ability. At 1-degree intervals above 25°C, *para^{ts}* flies became increasingly debilitated; at 29°C, complete paralysis occurred. After flies were maintained for prolonged intervals at 29°C, some activity could be recovered at that temperature. Studies of the behavior of mosaics at 29°C revealed a requirement of the (+) allele in the head for mobility, and a thoracic component for proper leg movement. Normal electroretinograms were obtained at both 22°C and 30°C. The results suggest a temperature-sensitive defect in the nervous system.**

The genetic regulation of neural structure and function, and the relationship of such regulation to behavior, are of fundamental biological interest. The ready induction and recovery of mutations affecting behavior in Drosophila, and their genetic manipulation, is now being exploited in several laboratories (1–3). By selecting flies manifesting the phenotype of paralysis, we felt that mutations affecting nerves and/or muscles could be efficiently obtained. However, flies exhibiting such a mutant phenotype would not be expected to be viable; therefore, we searched for mutations that showed conditional paralysis that was temperature dependent (4). This report concerns the discovery and properties of such a mutation, paralytic-temperature-sensitive (*para^{ts}*), in *Drosophila melanogaster*.

METHODS AND MATERIALS

Screening

Newly emerged adult Oregon-R males were fed the mutagen ethyl methanesulfonate dissolved in a 1% sucrose solution (0.025 M) (5). 24 hr later, they were mated at 22°C to attached-X-bearing females in 125-ml bottles. Adult progeny were then placed in a heated plexiglass screening apparatus that allowed the ready separation of immobilized flies from those retaining normal movement (6). 1000–8000 adults (at a time) were placed in the box and left for $^1/_4$–2 hr before selection. All motionless flies were then returned to 22°C and any that regained mobility were mated (males to $\widehat{XX}$ females, females to Oregon-R males); all offspring of fertile individuals were tested for paralysis at 29°C. The specific properties and details of analysis of the temperature-sensitive paralytic mutant are discussed in the next section.

Abbreviation: ERG, electroretinogram.

RESULTS

Table 1 shows the results of the screening. The bulk of the immobilized flies recovered were dead or sterile at 22°C. However, out of an estimated 250,000 flies screened, one was detected (by R.W.) that carried a mutation causing a temperature-sensitive paralysis.

Genetic properties

The temperature-sensitive paralyzed fly was a male; all of its male progeny were paralyzed upon shifting to 29°C, whereas the females were not, thereby showing this mutation to be sex-linked. Females heterozygous for the mutation were not paralyzed, whereas homozygous females were. Consequently, the mutation was named paralytic-temperature-sensitive, *para^{ts}*. The mutation was mapped genetically by crossing *para^{ts}* males to females carrying the markers (7) (followed by their genetic positions): *y* (0.0), *cv* (13.7), *v* (33.0), *f* (56.7), and *car* (62.5) and test crossing the F_1 females at 22°C. The *para^{ts}* and (+) male progeny of the test cross were separated at 29°C, then scored for the visible markers. The mutation was readily located 2.8 units to the left of *f* (2993 males scored).

General biological properties

The effects of temperature on *para^{ts}* flies were studied in detail. At 22°C, the mutants exhibited normal walking and flying ability. When adult *para^{ts}* flies were shifted from 22°C to 29°C, complete paralysis was induced in less than 5 sec; when the paralyzed flies were shifted back to 22°C, mobility was recovered in less than 2 sec. Paralysis and recovery could be induced repeatedly in the same individuals with no apparent harm. Flies maintained at 29°C for several hours recovered normal mobility immediately upon shifting down to 22°C. While paralysis was initially complete at 29°C, a recovery of

TABLE 1. *Results of screening adult offspring of mutagenized flies for paralysis at 29°C*

Type of flies	Number
Total flies screened (estimated)	250,000
Immobilized at 29°C	293
Dead	200
Recovered mobility at 22°C	93
Fertile at 22°C	34
Temperature-sensitive paralytic mutations	1

some movement at this temperature could be seen. After 30 min at 29°C, the flies were able to right themselves and regained limited walking ability. After an hour or more, the flies were capable of climbing up the sides of the vials. However, it must be emphasized that these flies were visibly weak and never regained the strength and coordination that *para*^{ts} flies showed at 22°C. Flies that had regained mobility after 2 hr at 29°C immediately recovered wild-type behavior upon shifts to 22°C, and were paralyzed again upon being shifted back to 29°C. Preliminary tests suggest that *para*^{ts} flies that were left at 29°C for 12 hr, then were shifted down to 22°C for 5 min, and then were shifted back up to 29°C are not paralyzed. Thus, initial recovery of flies held at 29°C remains temperature labile, whereas long-term recovery at 29°C is not as temperature labile and may reflect a different basis for the two types of recovery.

In order to determine whether the *para*^{ts} mutation also affected larval mobility, two tests were performed. A direct test was made by placing 3rd instar larvae reared at 22°C into Drosophila Ringer's (8) solution at 29°C. The larvae continued to move normally for several hours and were totally unaffected by the increased temperature. A second method compared the relative fitness of *para*^{ts} individuals with wild-type flies at 29°C. Attached-X-bearing females (which were wild type with respect to *para*^{ts}) were crossed to *para*^{ts}/Y males for 24 hr at 22°C. The inseminated females were allowed to lay their eggs for 3 days in fresh bottles at 29°C. The F_1 progeny were left to develop at 29°C until adults began to emerge, whereupon 219 late pupae were collected and placed in fresh vials; half of these were shifted to 22°C. 12 hr later, the flies had emerged and the ratio of *para*^{ts}/Y males to wild-type females was scored. At 22°C, the ratio was 97 males to 122 females, showing that *para*^{ts} males had competed successfully with their wild-type sisters up to pupation at 29°C (females tend to hatch sooner than males). In the sample left at 29°C, a ratio of 83 males to 215 females was obtained. However, when the number and sex of adults found dead in the food and unhatched in their pupal cases was scored and included in the sex ratio, the ratio returned to 230:240 (male:-female). It was concluded that the *para*^{ts} mutation does not interfere with the development of the larval or early pupal stages and, therefore, is expressed only in differentiated adults. The fact that some males did emerge at 29°C could be accounted for by the earlier observation that *para*^{ts} adults recover some mobility after prolonged exposure to 29°C. The hatched males had obviously recovered sufficiently to crawl out of their pupal cases.

The effect of prolonged exposure of *para*^{ts} adults to different temperatures was then examined in order to determine the degree to which movement, coordination, and recovery of activity were affected over a wide temperature range. A dissecting microscope was mounted over two identical watertight chambers immersed in a temperature-controlled circulating-water bath. Adult *para*^{ts} and (+) flies (at least 40 of each) that had been raised at 22°C were placed in separate chambers and observed continuously for 2 hr at each temperature. Observations were made on different flies at 1-degree intervals between 22°C and 35°C. All movements, from flying to tarsal twitching of individual legs, were recorded; however, for analytical purposes, the behavior during recovery has been classified into six categories: complete paralysis, kicking, ability of flies to right themselves, walking, climbing, and flying.

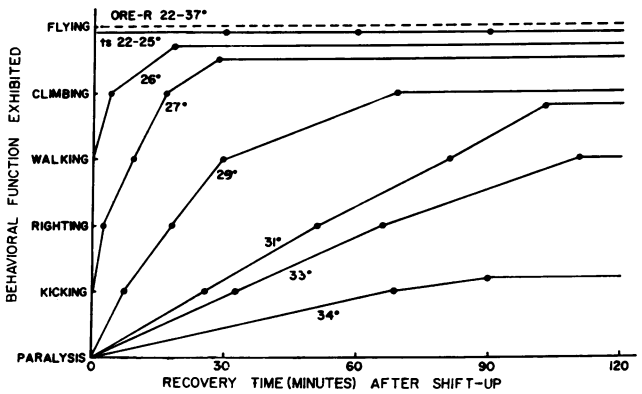

FIG. 1. Recovery of behavioral activity of *para*^{ts} flies at different temperatures during a 2-hr interval. Each point represents the time at which at least half of the flies observed exhibited the behavioral trait.

The results are presented in Fig. 1. The units separating the categories on the ordinate are arbitrary, so that the shapes of the curves are not significant. Oregon-R wild-type flies were not visibly affected over the entire range of temperatures from 22°C to 37°C. On the other hand, *para*^{ts} flies behaved normally only up to 25°C. They were visibly debilitated between 26°C and 28°C, and completely paralyzed at higher temperatures. With increasing temperatures, the phenotypic effects of the mutation became more severe and the length of time required for recovery was prolonged. For example, at 29°C, flies began to climb after 70 min, whereas at 31°C it took 105 min to regain the same ability. At 33°C, the flies showed only a weak capacity for walking toward the end of the observation period and, at 34°C, only 5% of the flies were able to even right themselves. It should be added that after 2 hr at any temperature up to 33°C, all *para*^{ts} flies shifted down to 22°C recovered. However, after 2 hr at 34°C, only 10% of the flies remained alive after a shift down to 22°C.

Since *para*^{ts} flies can be maintained at 33°C for up to 2 hr, we assumed that all vital organs still function in the paralyzed fly. Indeed, the heart, which can be easily seen through the dorsal wall of the abdomen, was observed to beat normally in paralyzed individuals. Similarly, slight movements of the abdomen, which might result from gas exchange through the spiracles, could be observed in *para*^{ts} flies at 29°C.

Tissue specificity

It was then asked whether the *para*^{ts} mutation functions autonomously, and whether any tissue specificity of the mutation could be determined. These questions could be answered by generating somatic mosaics of *para*^{ts} and (+) tissue. The ring chromosome, *In(1)w*^{vC}(7), is somatically unstable and is lost with a high frequency in mitotically dividing nuclei (9). By marking a normal rod X chromosome bearing *para*^{ts} with *y*(7) (a recessive mutation that produces yellow cuticle and bristles), the loss of the ring chromosome (which carried the y^+ and *para*$^{ts+}$ alleles) could be detected on the exterior chitin as yellow patches in a wild-type background (10). *In (1) w*^{vC}, + +/*In(1)dl-49, y w spl* females were crossed to *y para*^{ts}/-*sc*^{8}·Y, y^+ males at 22°C. Mosaics of *In(1)w*^{vC},+ +/*y para*^{ts} zygotes were recovered and the exact area of yellow tissue of each mosaic was recorded. Each mosaic was then placed in a

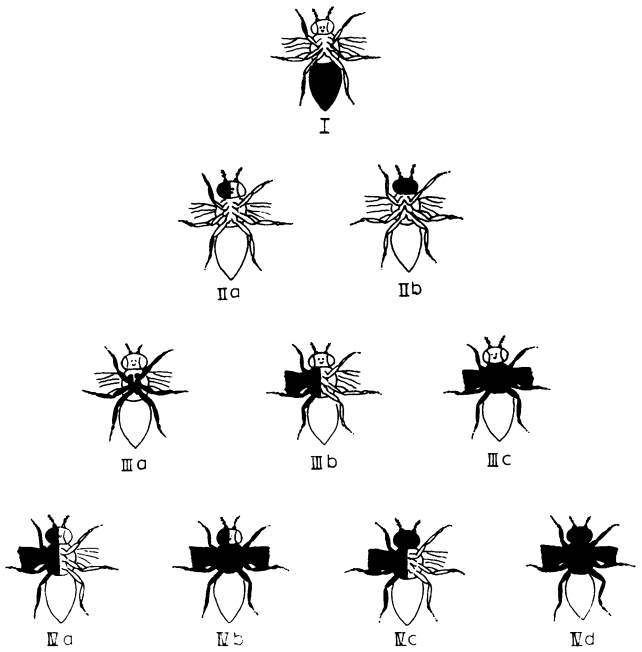

FIG. 2. Classification of mosaic females generated by somatic loss of $In(1)w^{vC}$ according to the ventral location of mutant tissue. The darkened area represents $y\ para^{ts}$ tissue in which the ring is lost.

coded vial by one person, and a different person noted the behavior of each fly under a dissecting microscope for 20 min at 29°C. Nonmosaic wild-type females were placed at random in coded vials as controls. After 150 mosaics had been scored, the data on the location of the mutant patches and the behavior at 29°C were compared.

The relation between specific behavioral patterns and the location of the patches was so clear-cut that the scorer of mosaicism could accurately predict the behavior of each fly at 29°C and, vice versa, the observer of behavior could predict the locations of the mutant tissue. A total of 300 mosaics was scored, with the amount of yellow tissue ranging from tiny patches involving one or two bristles to almost complete $y\ para^{ts}$/O males. A detailed analysis of the mosaic data will be published elsewhere. We have summarized the pertinent data by placing the mosaics into the following classes based on the location of yellow tissue: I: abdomen, II: head, III: thorax, and IV: head–thorax. Each class could be further subdivided into specific subgroups that differed in behavioral patterns (Fig. 2).

A description of the behavior of each class of flies indicated in Fig. 2 can be seen in Table 2. All class-I mosaics were wild type in behavior at 29°C. Flies of the reciprocal class, in which only the abdomen was wild type, were completely paralyzed. Complete-head mosaics (class II*b*) assumed a normal stance at 29°C but could not move, thus showing that $para^{ts+}$ head tissue is necessary for normal leg movement. Flies with mutant tissue in the legs only (class III*a*) could move, but the legs were stiff during movement. This class is actually a composite of 38 different mosaics, involving from one to five mutant legs. Of these, 19 had one mutant leg, 11 had mutant regions on two legs, 5 on three legs, 1 on four legs and 2 on five legs. Bilateral thoracic mosaics (class III*b*) moved their legs on the wild-type side, but the mutant legs were paralyzed in an extended position. Mosaics having completely mutant thoraxes

TABLE 2. *A correlation of the position of mutant patches and the pattern of paralysis of mosaic females at 29°C*

Class	Number of mosaics	Location of mutant patch	Behavioral characteristics at 29°C
I	6	Abdomen	wild type
II*a*	9	Bilateral head	walks, climbs in helical pattern
II*b*	5	Entire head	stands normally, cannot move
III*a*	38	Legs only	walks in stilted manner, legs stiff
III*b*	30	Bilateral thorax	mutant legs paralyzed, wild-type legs continue to move
III*c*	7	Entire thorax	legs paralyzed
IV*a*	17	Bilateral head and thorax	mutant legs paralyzed, wild-type legs continue to move
IV*b*	8	Bilateral head, entire thorax	completely paralyzed
IV*c*	26	Entire head, bilateral thorax	completely paralyzed
IV*d*	9	Entire head and thorax	completely paralyzed

(class III*c*) were paralyzed, as were the complete head and bilateral thoracic mosaics (class IV*c*). In addition, 18 flies that had mutant tissue only on the dorsal surface of the thorax were completely normal in mobility at 29°C. In another fly, the entire dorsal portion of the thorax and two legs were mutant, yet the four (+) legs moved normally. These data clearly demonstrate dual components for normal leg movement: wild-type head for motion, and wild-type thorax and legs for the posture and normal movement of each leg.

The use of mosaics to locate the regions governing normal mobility depends upon the detection of mutant tissue on the external surface of the fly. We have assumed that the external mutant patch is an indication that the underlying internal tissue is also mutant. Indeed, a good correspondence has been found in certain nonneural and some neural tissues (J. Merriam personal communication, and ref 11). Supporting evidence for this contention derives from our screening of 216 phenotypically nonmosaic $In(1)w^{vC}$, $+\ +/y\ para^{ts}$ females and over 200 phenotypic $y\ para^{ts}$/O males at 29°C. If, in fact, considerable internal mosaicism existed that was not indicated externally, we would have expected many of the externally nonmosaic females to exhibit aberrant behavior and some of the presumed X/O males to move at 29°C. This was not observed. All of the X/O males were completely paralyzed at 29°C. 12 females initially screened as nonmosaic were observed to walk abnormally at 29°C; upon reexamination, ten were found to be missing a leg, one had a mutant patch that had been overlooked, and only one appeared to be completely wild type.

When different classes of mosaics (classes II*b*, II*c*, IV*c*, and IV*d*) were compared with $para^{ts}$ files for 2 hr at 29°C, there were no detectable differences in the rates of recovery of coordinated movement in the mutant areas. This finding, in conjunction with the observation that class II*b*, II*c*, and IV*b* mosaics (Fig. 2) cannot move at 29°C, shows that paral-

ysis is not induced by the loss of a freely circulating factor necessary for movement. Moreover, the ability of class I and II*a* mosaics, as well as the wild-type side of classes III*b* and IV*a* mosaics, to move rules out the existence of a freely circulating inhibitor of movement produced by the *para*^{ts} mutation.

Visual and flight response

Flies having mutant tissue around one eye (class II*a*, Fig. 2) could walk at 29°C, but when they climbed vertically, they invariably followed a helical path and always kept the mutant eye up. Whether this behavior indicated blindness in the mutant eye could be tested easily by examining its optomotor response at 29°C. Wild-type flies invariably turn in the direction of moving stripes (12). When we painted both eyes and ocelli of the wild-type flies, they no longer showed a positive optomotor response. We then painted the wild-type eye and ocelli of bilateral head mosaics (class II*a*, Fig. 2). At 22°C, the flies showed a positive optomotor response, whereas the results at 29°C were ambiguous. On occasion, the flies did respond positively, but much less strongly and more slowly. We therefore measured the electrical response of the eyes of *para*^{ts} flies to light by recording the electroretinogram (ERG)(1). The ERG was obtained by Drs. Yoshiki Hotta and Seymour Benzer. A positive ERG was obtained at both 22°C and 30°C. This result indicated that the eyes of *para*^{ts} flies do transduce light into electrical responses at 30°C.

Finally, we tested the effect of *para*^{ts} on flying ability. The tips of toothpicks were glued perpendicularly to the center of the dorsal surface of the thorax. When the flies were lifted, they began to "fly" as soon as their feet left a solid surface. At 22°C, *para*^{ts} flies initiated flight immediately upon lifting, whereas at 29°C, flight was not initiated. Also, once it was initiated at 22°C, upon a shift to 29°C, flight continued for only about 5 sec. The response of bilateral thoracic mosaics was similar to *para*^{ts} flies, that is, wing movement on neither the mutant nor the wild-type sides could be initiated at 29°C.

DISCUSSION

The genetic and biological properties of the mutation, *para*^{ts}, point to a specific defect in the nervous system of adults that regulates both flight and walking. The rapidity with which paralysis and recovery can be induced by temperature shifts argues against a direct involvement of *de novo* macromolecular synthesis. Coordinated activity at 29°C, gained after a 2-hr exposure to 29°C, is eliminated by a 5-min exposure to 22°C (shift-down-and-up). This suggests that early recovery from restrictive temperatures does not involve processes different from those affected by an initial shift-up.

The lack of effect of *para*^{ts} abdomens on paralysis of flies having a wild-type thorax and head (class I) shows the absence of some freely circulating inhibitor of mobility that might be made in any part of the fly. Furthermore, the complete autonomy of each half of bilateral thoracic mosaics (class III*b*) in both paralysis and recovery time rules out a temperature-induced humoral inhibitor or temperature-resistant promoter of movement. On the assumption that the external phenotype demarcates the boundaries of internal nervous tissue of the same genotype, the mosaics demonstrate three components that govern normal movement: the head, the ventral thorax (including wings), and the legs. These regions correspond to the location of the cephalic and thoracic ganglia and the lateral elements of innervation.

The normal ERG obtained at 30°C indicates that light can be transduced by the eye into electrical responses and rules out any generalized physiological effect of temperatures on CO_2 concentrations. The mutant, Hyperkinetic-1, *Hk*^{1}, has a defect that causes rapid rhythmic-impulse generation in the motor areas of the thoracic ganglion (2). These impulses induce a characteristic leg twitching in flies lightly anesthetized with ether (2). The double mutant, *Hk*^{1} *para*^{ts}, exhibits leg shaking at 22°C that immediately stops upon shifting to 29°C (manuscript in preparation). This result suggests that the *para*^{ts} mutation causes a disorder that affects neural tissue. Recent studies on the time of appearance of activity of acetylcholine esterase and choline acetyltransferase during development (13) prompted a study of these enzymes in *para*^{ts} flies. Activity of both enzymes was not reduced at 30°C (manuscript in preparation). Of crucial interest is a determination of whether transmission within a neuron, between neurons, or across a neuromyal junction is affected by high temperatures. Such studies are currently under investigation by Dr. K. Ikeda.

The phenotype of temperature-dependent paralysis permitted the recovery of a hereditary behavioral defect. Selection schemes for paralytic larvae can also be readily constructed. Methods for large-scale mutagenesis and screening should make the search for such mutations feasible and promise to yield various mutations of considerable biological interest.

The search for temperature-sensitive paralytic mutations was stimulated by a discussion on the possibility of detecting muscle mutants 3 years ago with Dr. Ed Reich of the Rockefeller University.

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