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Temperature-sensitive paralytic mutations on the second and third chromosomes of *Drosophila melanogaster*

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SUMMARY

Seven temperature-sensitive paralytic mutants were recovered from 4544 lines of ethylmethanesulphonate (EMS) treated autosomes in *Drosophila melanogaster*. These mutants have been designated temperature-induced paralytic (*tip*). The *tip* mutations belong to six different genes; four of these, *tip-A*, *Tip-B*, *tip-C* and *tip-D* are on the second chromosome while *tip-E* and *tip-F* are on the third chromosome. This paper describes the paralysing behaviour and genetic localization of the *tip* mutants.

1. INTRODUCTION

Temperature-sensitive paralytic mutations of *Drosophila melanogaster* were first described by Suzuki and his coworkers (Suzuki, Griggliatti & Williamson, 1971; Griggliatti *et al.* 1973). The effect of these mutations on the nervous system has been described by a number of authors (Ikeda, Ozawa & Hagiwara, 1976; Siddiqi & Benzer, 1976; Wu *et al.* 1978; Singh & Siddiqi, 1981). Some of the paralysing mutations such as *shibire*^{ts} also cause developmental defects (Poodry, Hall & Suzuki, 1973). With few exceptions (Wu *et al.* 1978) studies on temperature-sensitive paralytic mutations of *Drosophila* have been focused on the X chromosome. The autosomes which represent about 80% of the *Drosophila* genome have remained relatively unexplored. We have isolated a set of temperature-sensitive paralytic mutations on the second and third chromosomes of *D. melanogaster*. In this paper we describe the behavioural characteristics and genetic localization of the autosomal paralytic mutations.

2. MATERIALS AND METHODS

(i) *Isolation of mutants*

Wild-type (Canton-Special) *Drosophila* males were fed 0·025 M ethylmethanesulphonate in 1% sucrose (Lewis & Bacher, 1968) for 24 h at 22 °C. Mutagenised males were mated according to the scheme described in Gethman (1974) to encourage

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elimination of lethals and to produce progeny, some of which were homozygous for the mutagenized second chromosome or third chromosome or both. The progeny were tested for paralysis at 38 °C. Flies that became paralysed within 5 min were recovered and bred to produce homozygous mutant stocks.

(ii) *Kinetics of paralysis*

Paralysis tests were performed as described by Siddiqi & Benzer (1976). Adult flies 2–3 days old were placed in a 20 × 150 mm thin walled glass tube. A glass tube wrapped with conducting aluminum foil was used as a stopper. Flies were confined to the lower 3 cm portion of the tube from the bottom and the lower 6–7 cm of the tube was immersed in a controlled temperature waterbath. Flies were

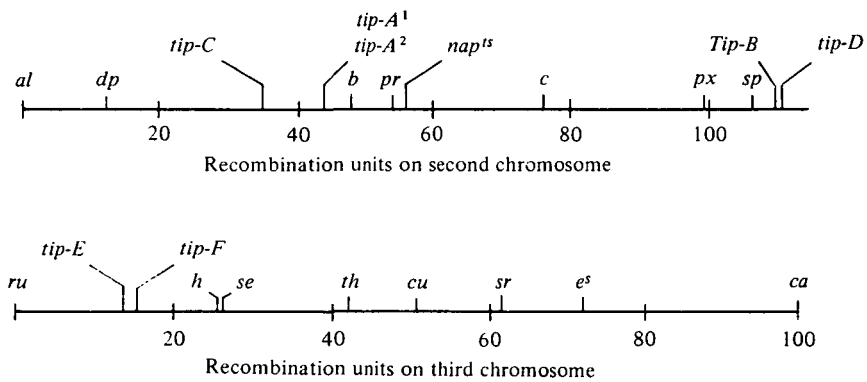

Fig. 1. Genetic map of temperature-sensitive paralytic mutations on the major autosomes. Mutant *nap*^{ts} was isolated in Benzer's laboratory at Caltech. All *tip* mutants were isolated at the Molecular Biology Laboratory, T.I.F.R., Bombay.

introduced into tubes previously equilibrated to the bath temperature. The number of flies that remained standing was recorded as a function of time. For determining the recovery kinetics, the flies were exposed to 38 °C for different intervals of time. The tube was then transferred to a waterbath at 23 °C. The time taken by each fly to regain a standing posture was noted as a function of the time period for which it was kept at elevated temperature.

The larvae were tested for paralysis in glass test tubes having inner sides coated with a thin and uniform layer of 1% agar. Paralysis of the larva was defined as a complete absence of observable body movements and recovery time was noted at the onset of body movements.

(iii) *Genetic localization*

tip mutations were placed in combination with recessive markers on their respective chromosomes and mapped by means of three-point tests. The mutations were localized on the second chromosome with respect to markers *aristaless*, *al* 0·0; *dumpy*, *dp* 13·0; *black*, *b* 48·5; purple, pr 57·5; *curved*, *c* 75·5; *plexus*, *px* 100·5; *speck*,

sp 107·0; and relative to third chromosome markers *roughoid*, *ru* 0·0; *hairy*, *h* 26·5; *thread*, *th* 42·5; *curled*, *cu* 51·0; *stripe*, *sr* 62·0; *ebony-sooty*, e^s 72·0; *claret*, *ca* 100·0. Complete descriptions of the markers and the balancer strains used in the experiments can be found in Lindsley & Grell (1968).

3. RESULTS

(i) *Dominance relationships*

tip/+ heterozygotes were tested for sensitivity to temperature. All except *Tip-B* were recessive. *Tip-B* was a homozygous lethal. (The only allele of *Tip-B* was recently lost due to low viability.)

Table 1. *Complementation test between various* tip *mutants*

((A) Mutants on second chromosome. (B) Mutants on third chromosome. +, No paralysis; 0, denotes paralysis.)

	A^1	A^2	C	D	
A^1	0	0	+	+	(A)
	A^2	0	+	+	(A)
		C	0	+	(A)
			D	0	(A)
		E	F		
	E	0	+		(B)
		F	0		

(ii) *Complementation studies*

Complementation tests were carried out to find out the allelic relationships between *tip* mutations. The results are summarized in Table 1. We conclude that *tip-A*, *tip-C* and *tip-D* are independent genes on the second chromosome. *tip-E* and *tip-F* are independent genes on the third chromosome. All *tip* genes except *tip-A* are represented by single alleles.

(*iii*) *Genetic localization*

Figure 1 shows the location of *tip* mutations on the second and third chromosomes. The mutation *tip-A* maps $4{\cdot}5 \pm 0{\cdot}2$ units to the left of *black* (*b*) at position 2-48·5. *Tip-B* and *tip-D* are located near the end of the right arm of the second chromosome. *Tip-B* is $2{\cdot}9 \pm 0{\cdot}2$ units to the right of *speck* (*sp*) at position 2-107·0 and *tip-D* is $10 \pm 0{\cdot}3$ units to the right of *plexus* (*px*) at position 2-100·0. *tip-C* maps $13{\cdot}2 \pm 0{\cdot}4$ units to the left of black (*b*) at position 2-48·5 (Table 2). The mutations *tip-E* and *tip-F* are on the third chromosome, *tip-E* at position $13{\cdot}5 \pm 0{\cdot}4$ and *tip-F* at position $15{\cdot}2 \pm 0{\cdot}4$. Both these mutants are located between *roughoid* (*ru*) 3-0·0, and *sepia* (*se*) 3-27·0 (Table 3).

The *tip-D* mutation, at position 2-109·5, mapped close to *Tip-B* at position

2-110·0. To determine whether *tip-D* and *Tip-B* were allelic, crosses were designed to recover *tip-D*$^+$–*Tip-B*$^+$ recombinants between these two mutations that had been placed in a trans configuration in females. Eleven wild type recombinants out of total 1263 flies were recovered. Thus *tip-D* and *Tip-B* were not allelic and were $1{\cdot}7 \pm 0{\cdot}1$ units apart assuming an equivalent number of reciprocal recombinants, i.e. the double mutants *tip-D–Tip-B* which escaped detection.

Table 2. *Three-factor test for mapping* tip-A^2, Tip-B *and* tip-C

	tip-A2					*Tip-B*					*tip-C*			
0	dp	ts	b	139	+	+	ts	779	0	dp	ts	b	437
	+	+	+	200	px	sp	+	847		+	+	+	437
I	dp	+	+	73	px	+	ts	45	I	dp	+	+	105
	+	ts	b	71	+	sp	+	67		+	ts	b	113
II	dp	ts	+	11	+	+	+	37	II	dp	ts	+	44
	+	+	b	9	px	sp	ts	10		+	+	b	63
I+II	dp	+	b	2	+	sp	ts	02	I+II	dp	+	b	22
	+	ts	+	4	px	+	+	03		+	ts	+	38
			Total	509			Total	1790				Total	1259

Table 3. *Three factor tests for mapping* tip-D, tip-E *and* tip-F

	tip-D				*tip-E*				*tip-F*			
0	c	px	ts	399	ru	ts	sc	318	ru	ts	h	165
	+	+	+	399	+	+	+	357	+	+	+	233
I	c	+	+	175	ru	+	+	53	ru	+	+	32
	+	px	ts	104	+	ts	sc	57	+	ts	h	29
II	c	px	+	62	ru	ts	+	62	ru	ts	+	18
	+	+	ts	45	+	+	sc	51	+	+	h	20
I+II	c	+	ts	9	ru	+	sc	6	ru	+	h	8
	+	px	+	3	+	ts	+	4	+	ts	+	8
			Total	1196			Total	908			Total	503

(iv) *Adult paralysis and recovery kinetics*

Mutant flies were tested for paralysis and recovery. The paralysis kinetics of *tip* mutants exposed to 38 °C is depicted in Fig. 2 and subsequent recovery from paralysis at 23 °C of these mutants is shown in Fig. 3. At 38 °C wild type (Canton-S) flies remain mobile for 30 min or more. The effect of temperature on the paralysis of *tip* mutants is illustrated in Fig. 4 and the effect of duration of exposure on their recovery in Fig. 5. The salient features of paralysis associated with each gene are described below.

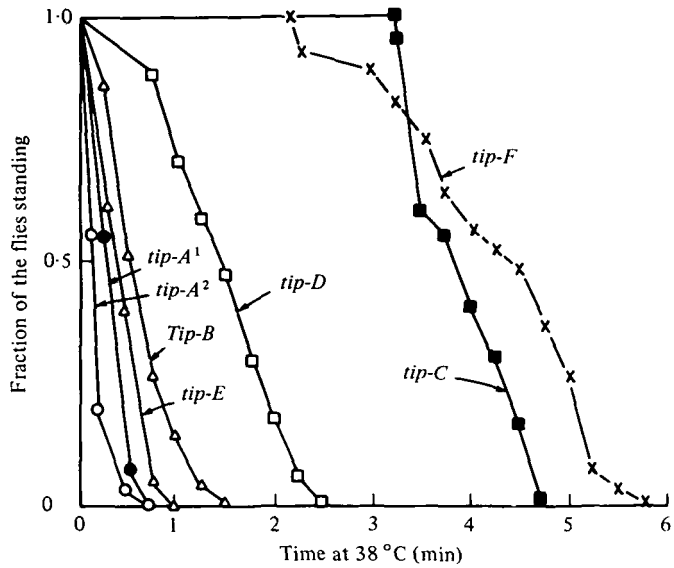

Fig. 2. Kinetics of paralysis. Flies exposed to 38 °C. Twenty flies were used.

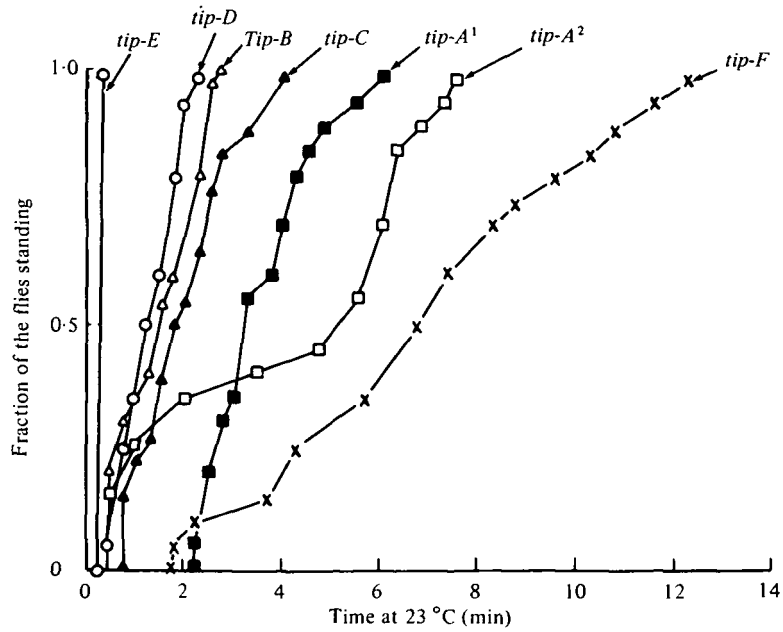

Fig. 3. Kinetics of recovery. Flies returned to 23° after complete paralysis. t_0 = time at which all the flies paralyse completely in each mutant strain.

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(*a*) *tip-A*

Mutants *tip-A*^{1} and *tip-A*^{2} become hyperactive after the initial temperature shock of 38 °C and exhibit uncoordinated walking movements before paralysis. The mutant flies begin to become paralysed within 15 s and all of them fall down in rapid succession within 1 min. *tip-A*^{1} and *tip-A*^{2} recover very slowly, taking more

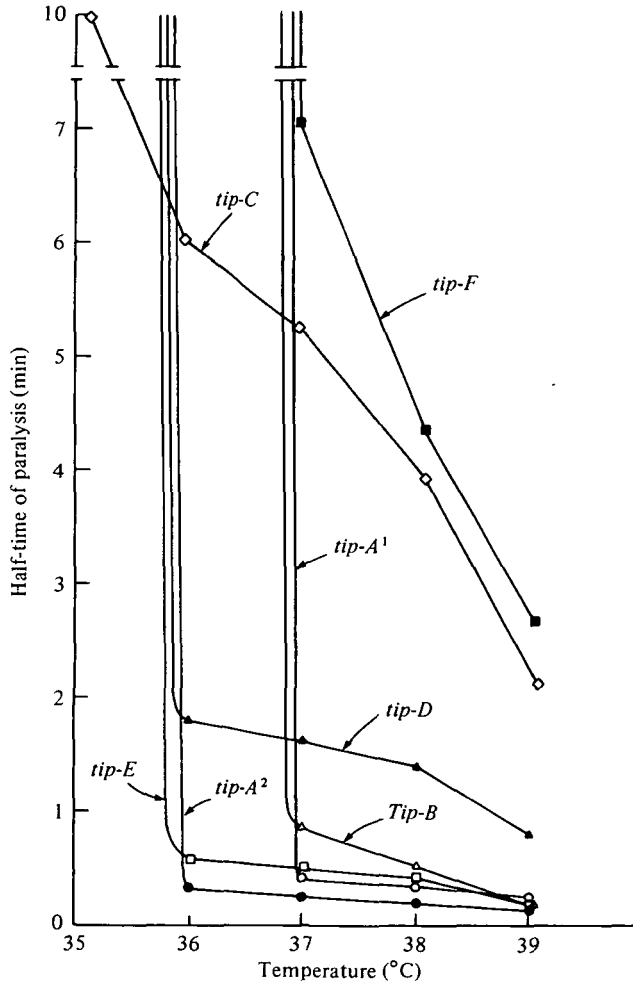

Fig. 4. Time required for paralysis of *tip* mutants *v.* temperature. Twenty flies were used to obtain each point.

than 6 min to recover after 1 min of previous exposure to 38 °C. Both these alleles show a sharp critical temperature of 37 °C and 36 °C respectively, below which no paralysis occurs. The recovery time for *tip-A*^{1} and *tip-A*^{2} increases rapidly with the previous exposure, becoming more than 15 min for an exposure of 4–6 min. Kinetic properties of *tip-A* are similar to *comatose* (*com*) in that the period of recovery from paralysis depends on the duration of previous exposure to heat.

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(*b*) *Tip-B*

Tip-B/+ flies were rapidly affected by an exposure to high temperature (38 °C). They jumped and fell down several times before paralysis, which occurred within 1·5 min. Recovery was also rapid, occurring within 2·5 mins. Mutant flies had a

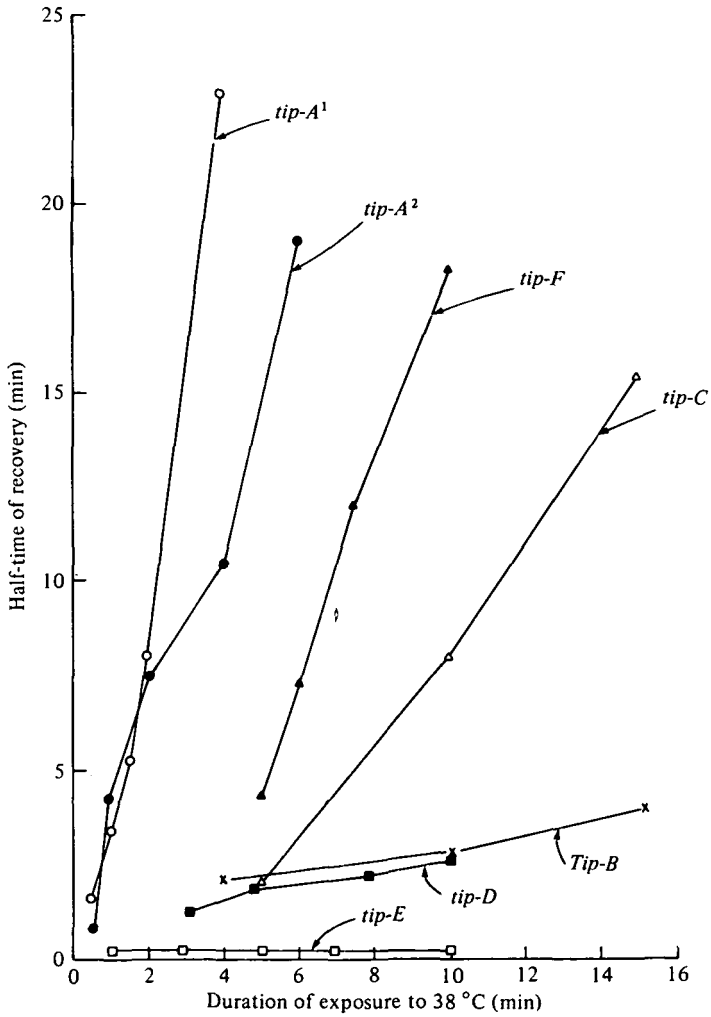

Fig. 5. Dependence of time required for recovery at 23 °C on the duration of previous exposure to 38 °C. Twenty flies were used to obtain each point.

sharply defined critical temperature of 37 °C for paralysis, and an increase in the temperature had little effect on the paralysis time. The recovery period was relatively independent of the duration of exposure.

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(*c*) *tip-C*

There is a lag of 3–4 min before *tip-C* flies are affected by 38 °C shock, followed by bouts of paralysis and recovery for 2–3 min. The flies fall on their back with a gentle tap on the vial. There is no sharply defined temperature critical for paralysis; such paralysis may occur at relatively low temperature after prolonged exposure. Recovery on return to 23 °C is slow, the time for recovery being proportional to the duration of exposure to elevated temperature.

(*d*) *tip-D*

Lesions in this gene cause a reversible paralysis within 45 s of exposure to 38 °C. Paralysis is complete within 2·5 min. *tip-D* flies become paralysed at a sharp critical temperature of 36 °C and prior to paralysis exhibit hyperactive and uncoordinated movements. The recovery is comparable to that described for the *Tip-B* mutation.

(*e*) *tip-E*

Members of this class show a very rapid paralysis at 38 °C. The flies appear at first stunned but soon fall on their sides without any accompanying locomotor defect. There is a sharply defined paralysis temperature of 36 °C, and the time taken for paralysis by half of the flies ($t_{\frac{1}{2}}$) is relatively independent of the paralysing temperature. *tip-E* flies recover very rapidly and recover within 10 s irrespective of the increasing periods of exposure to 38 °C.

(*f*) *tip-F*

The behaviour of *tip-F* at high temperature is comparable to that of *tip-C* and is more greatly affected by the magnitude and also by the duration of the temperature pulse.

In summary, mutation *tip-A* shows rapid paralysis above a critical temperature and slow recovery. The *Tip-B*, *tip-D* and *tip-E* mutants exhibit quick paralysis and quick recovery. The mutants *tip-C* and *tip-F* do not have sharply defined critical paralysing temperature. These mutants become paralysed slowly and also recover slowly.

(v) *Paralysis and recovery in larvae*

Early third-instar larvae were tested for paralysis. The normal (Canton-S) larvae remain active for at least 10 min at 39 °C. In only two of the *tip* mutations, *tip-D* and *tip-E*, did locomotor paralysis occur more rapidly than in wild type larvae; however, hypersensitivity to heat-induced paralysis of mutant larvae required higher temperature than was required for the paralysis of the same adults.

tip-D larvae become paralysed at 38 °C within 2 min and *tip-E* larvae at 39 °C within ½ min. The wild-type larvae remain active for more than 10 min at 39 °C. Paralysis and recovery kinetics of the larvae of these mutants is comparable to that of their adults. Other paralytic mutations on the X chromosome, namely shi^{ts}, $para^{ts}$ and comatose (*com*) also need higher temperature for larval paralysis than adults (Siddiqi & Benzer, 1976).

4. DISCUSSION

All but one (*tip-A*) of the six genes on the major autosomes of *D. melanogaster* that can mutate to cause temperature-sensitive paralysis are represented by single alleles. This suggests that there are many more related genes on these two chromosomes.

Siddiqi & Benzer (1976) described the behaviour of several X-linked temperature-sensitive paralytic mutants. Sex linked mutants $para^{ts}$, shi^{ts}, comatose (*com*), stn^{ts} and the autosomal mutant nap^{ts} (Wu *et al.* 1978) are well characterized in this respect. There is more than one allele for most of these genes. The *tip-A*, *tip-C* and *tip-F* mutants resemble comatose (*com*) in their kinetic properties in that the period of recovery depends on the extent of exposure to heat. *tip-E* on the other hand, is a rapidly recovering mutant allele like $para^{ts}$ and nap^{ts}. *tip-E* could possibly have absence of action potentials at high temperature as do $para^{ts}$ and nap^{ts}.

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