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Temperature-sensitive mutations in *Habrobracon**

By ROGER H. SMITH
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

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SUMMARY

Techniques for isolating and analysing temperature-sensitive mutations in *Habrobracon serinopae* are presented. The temperature-sensitive patterns and lethal phases are described for nine different mutant strains. Such mutants are well suited for the study of gene action during development.

INTRODUCTION

Temperature-sensitive lethal mutations in Habrobracon serinopae

A special class of conditional mutation, the temperature-sensitive lethal mutations, has been useful in the study of development and genetics in T4 phage and yeast (Epstein *et al.* 1963; Edgar & Lielausis, 1964; Hartwell, 1967). At the restrictive temperature, the organism either dies or further development is arrested; at permissive temperature, the organism survives and reproduces. The molecular basis for temperature-sensitive lethal mutations of micro-organisms is known (Edgar, Denhartz & Epstein, 1964). The majority are single base-pair substitutions of the missense type. At permissive temperatures the mutant protein is functional; under restrictive temperatures it is not.

Temperature-sensitive mutations have been induced in insects by mutagens and are now being used for various genetic and developmental studies: in *Drosophila* (Suzuki *et al.* 1967; Baillie, Suzuki & Tarasoff, 1968; Suzuki & Procunier, 1969; Tarasoff & Suzuki, 1970; Suzuki, 1970; Hotta & Benzer, 1970, 1972); *Habrobracon* (Smith, 1968, 1969); and *Musca* (McDonald, 1972). In addition, conditional lethals have been postulated to be useful in the control of insect populations (Smith & von Borstel, 1972).

In the present study, detailed methods for the induction and analyses of temperature-sensitive lethal mutations are described for *Habrobracon serinopae*, a parasitic wasp with a haploid–diploid genetic system.

MATERIALS AND METHODS

The genetic system

Females of *Habrobracon serinopae* are diploid ($2n = 20$) and the virgins produce haploid males parthenogenetically. Mated females produce both diploid and

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haploid offspring. Heterozygosity at the sex locus results in females, and either homozygosity or hemizygosity at this locus results in males (Whiting, 1933). When females are mated to haploid males which possess similar sex alleles

$$(x^a/x^b \times x^a \text{ or } x^b)$$

then both diploid males and females, x^a/x^a or x^b/x^b and x^a/x^b, respectively, are produced. In addition, mated females always produce some proportion of haploid males, x^a and x^b. When females are mated to males hemizygous for different sex alleles then, of course, only diploid females and haploid males result.

The female parasitizes the larvae of the European Flour Moth, *Anagasta kuhniella*. After stinging and paralysing the larvae she oviposits 40–50 eggs per day on its surface. The life-cycle from egg to adult of the wasp is 8 days at 28 °C and 6·5 days at 35 °C.

Induction and isolation of mutations

Fig. 1 presents the breeding scheme for isolating mutant strains from temperature-sensitive mutations in *H. serinopae*.

Haploid males of the wild-type strain (Hs^+) were starved for approximately 2 days and then fed a dilute honey solution containing either 0·01 M or 0·005 M ethyl methanesulphonate. Twenty hours later they were mated to untreated wild-type (Hs^+) virgin females. These mated females were placed individually into stender dishes containing *Anagasta* larvae and transferred to fresh larvae twice daily. Egg counts, hatchability, and adult survival were recorded to estimate the dominant lethality induced in sperm. Female progeny (F_1) were collected as virgins and set unmated to screen for temperature-sensitive mutations. The females oviposited overnight at 28 °C and the resulting haploid eggs were counted and allowed to develop to adulthood at 28 °C. The same females were then allowed to oviposit for 4 h at 28 °C, and the resulting haploid eggs were counted and allowed to develop to adulthood at 35 °C. There were three replicates at 28 °C and four at 35 °C for each F_1 female.

Adult survival of the F_2 progeny at both temperatures from each female was compared to determine whether or not any females were heterozygous for a temperature-sensitive lethal (*tsl*) mutation. If a female was heterozygous for a *tsl*, 50% of the haploid males died sometime during development at 35 °C but 90–100% of the offspring survived at 28 °C. The stages at death were recorded for all progeny from the F_1 females.

When the two dosages of EMS, 0·01 M and 0·005 M, were fed to the adult males, over 20% of the recessive lethal mutations were recorded as presumptive *tsl* mutations (Smith, 1973).

Each *tsl* mutation was taken through the breeding scheme diagrammed in steps 4–8 (Fig. 1). A few mutations caused the females to be sterile at both temperatures and therefore the mutants were maintained as heterozygotes or discarded. In some cases the lethal mutation was not detected in step 5 (Fig. 1) and therefore was considered to be lost by chance or to have been classed as a *tsl*-mutant in step 3 (Fig. 1) when it was actually a wild-type or a regular lethal. All the *tsl* mutant

stocks were given a *tsl* number (*tsl-1*, *tsl-2*, etc.). The breeding scheme was simplified when the mutant expressed lethality at the high temperature and a morphological mutant phenotype at the low temperature. In this case we used a more complex designation: *tsl-ble-1* for *tsl* (lethal at 35 °C), *ble* (black ears at 28 °C), and 1 (first allele at *ble* locus).

MUTAGEN

STEP

1 MALES ($1n$) × FEMALES ($2n$)

↓

2 F_1 VIRGIN FEMALES

↓

OVIPOSITION

↓

3 F_2 PARTHENOGENETIC HAPLOID MALE OFFSPRING

Genotype of F_1 mother	Expected survival of eggs to adults (%) 28 °C	35 °C	Interpretation of lethal mutation
(*a*) +/+	100	100	None
(*b*) +/1	50	50	Single
(*c*) +/*tsl*	100	50	Single temperature-sensitive

4 MALES FROM 3(*c*) × VIRGIN FEMALES (usually 12 crosses)

↓

VIRGIN FEMALES

5 TEST VIRGIN FEMALES FROM EACH CROSS

+/+ FEMALES → 100% SURVIVAL OF SONS AT 35 °C

tsl/+ FEMALES → 50% SURVIVAL OF SONS AT 35 °C

6 FATHER (*tsl*) × DAUGHTER (*tsl*/+)

↓

tsl/*tsl*, *tsl*/+ FEMALES

7 TEST INDIVIDUAL VIRGIN FEMALES

tsl/*tsl* ↓ Temperature (°C)	Survival of sons (%)	*tsl*/+ ↓ Temperature (°C)	Survival of sons (%)
28	100	28	100
35	0	35	50

8 SON (*tsl*) × MOTHER (*tsl*/*tsl*)

↓

HOMOZYGOUS *tsl* STOCK

Fig. 1. Scheme for isolating temperature-sensitive-lethal mutations (*tsl*) in *Habrobracon serinopae*. Percentage survival shown in tabular material has been simplified for clarity. Actual percentage survival for wasps without lethal mutations normally averages about 90%.

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Determination of the temperature-sensitive period

The temperature-sensitive period defines the time when the gene product of the mutant gene under study is necessary for survival to adulthood. This period can be determined by exchanging the developing wasps between the two temperatures (28 and 35 °C) at particular time intervals. By shifting the developing wasps from 35 to 28 °C at different time periods, the stage of development at which the gene product became necessary for survival to adulthood could be determined. When the wasps were shifted from 28 to 35 °C, the stage of development at which the gene product is no longer necessary was determined.

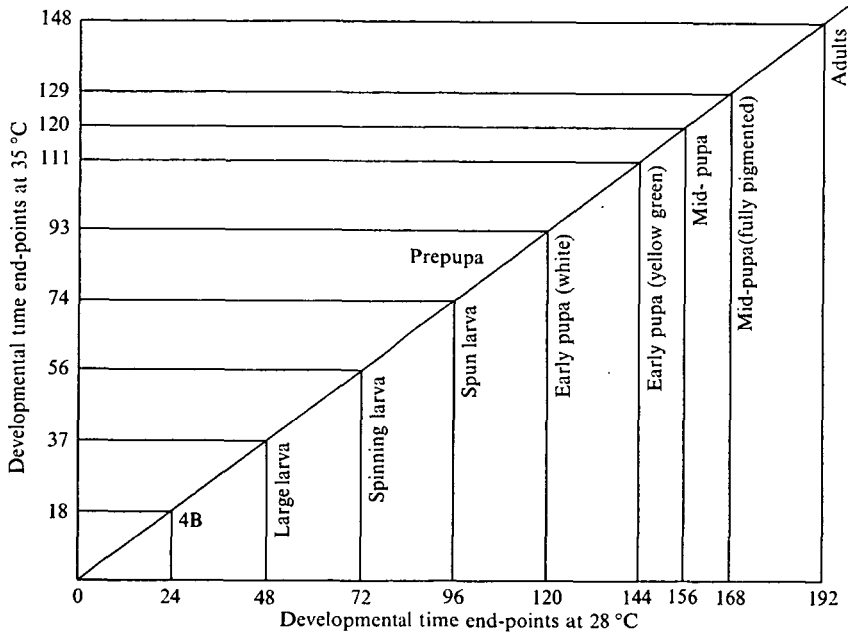

Fig. 2. Stages of development in *Habrobracon serinopae* haploid males at 28 °C plotted against the corresponding stages at 35 °C. This graph was used to determine the times when the wasps should be moved from 35 to 28 °C.

Virgin females were set for oviposition at 28 °C for a 3 h period. The eggs were counted and then were placed at 28 °C. The same females oviposited for another 3 h period and the eggs were placed at 35 °C. At 24 h intervals, the temperatures at which the developing haploid males (25–50) were maintained were changed from 28 to 35 °C, and from 35 to 28 °C. In later experiments, this procedure was modified so that wasps transferred from 35 to 28 °C were at the same stage of development as those transferred from 28 to 35 °C. The wasps developing at 35 °C reach adulthood 1·3 times faster than those reared at 28 °C. This difference in rate appeared to be fairly constant from egg to adult. Therefore we used the chart in Fig. 2 to determine the time of exchanges.

The end-points in this study were the developmental stages at death at 35 °C and the survival to adulthood for the various mutants. The stage or stages of development at death or arrest can be easily determined in *Habrobracon*. The egg chorion is transparent, and development can be observed by a dissecting microscope at × 40. The *Habrobracon* larvae are ectoparasites and are therefore easily observed on the immobilized host. The pupae also spin brittle cocoons which can easily be opened by a dissecting needle without damaging the pupae, or the pupae can be observed within the pupal cases when the cocoons are attached to the wall of a shell vial or the bottom of a stender dish.

Adult survival was scored after all adults had eclosed, 8–10 days after oviposition. At this time all inviable larvae and pupae were also counted and recorded.

RESULTS

As one might expect, and as other investigators have found (Suzuki *et al.* 1967), many mutants are 'leaky' and/or show pleiotropism. Although many *tsl* mutants are simple to maintain in the laboratory, others are not. A few, as mentioned below, had to be maintained in a heterozygous condition, and others were lost or intentionally discarded because of the problems in maintaining weak stocks. In the following paragraphs, *tsl* mutants are described on the basis of their temperature-sensitive patterns. All mutants described in this paper were found to be completely recessive.

tsl-5. This mutant is monophasic. Under the constant restrictive temperature of 35 °C all larvae died early, immediately after starting to feed. Under the constant permissive condition of 28 °C, or at room temperature (~ 22 °C), survival from egg to adult was the same as for the wild-type phenotype (~ 90 %).

The pattern of temperature sensitivity indicates that the gene product is necessary for survival from about the 36th h to about the 108th h of development. This is from about the time of hatching up to the prepupa stage (Fig. 3*a*).

When the animals were transferred from 28 to 35 °C between the 4th and 84th h of development, they died very soon after the shift (Table 1). When the shift was made on the 96th h, most of the males developed to a later stage and a small proportion survived to adulthood. The same pattern can be seen for the later transfers also, until after the 114th h when almost all survive.

As one can see, this is a very precise temperature-sensitive lethal mutation. For this reason, *tsl-5* can be used to select automatically for virgin females. Females homozygous for *tsl* are mated to wild-type males which possess different sex alleles. All progeny are exposed to 35 °C for a specified time period and then shifted back to 28 °C. Only females survive. In this way, several hundred virgins can be made available for various types of experiments with a minimum of effort.

tsl-1. The wasps that carry this particular mutant die between the midlarva to prepupa stages when reared at a constant 35 °C (Fig. 3*b*). By Hadorn's definition (1961), this mutant would be described as polyphasic. The shifts of individuals from 28 to 35 °C suggest that some gene product is made or is present after the

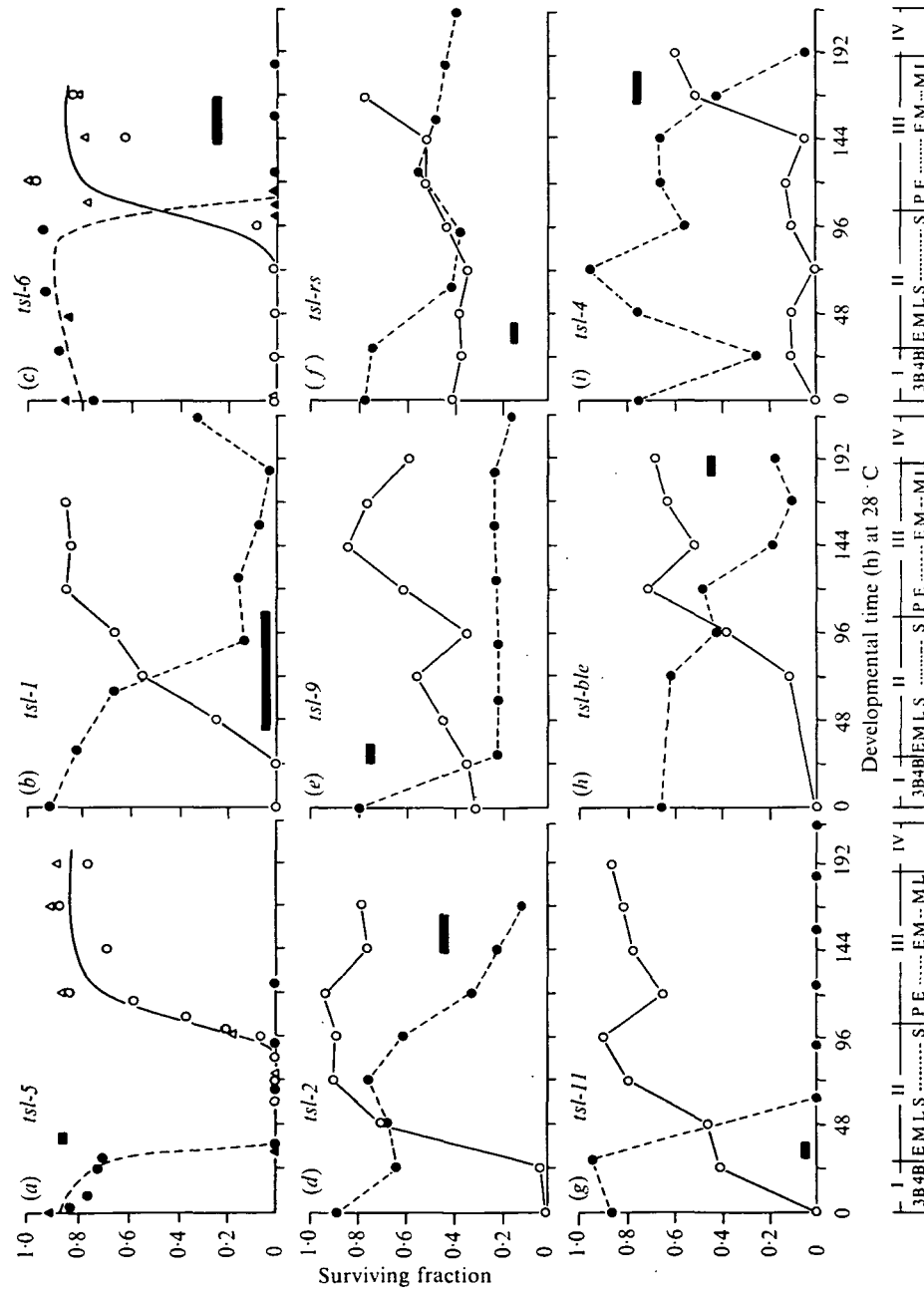

Fig. 3. Temperature-sensitive patterns for (*a*) *tsl-5*, (*b*) *tsl-1*, (*c*) *tsl-6*, (*d*) *tsl-2*, (*e*) *tsl-9*, (*f*) *tsl-rs*, (*g*) *tsl-11*, (*h*) *tsl-ble* and (*i*) *tsl-4*. The broken line in each case represents the shifts from 35 to 28 °C and the unbroken line represents the shifts from 28 to 35 °C. The circles and squares represent different experiments: ●, ■, 35–28 °C; ○, □, 28–35 °C. Roman numerals represent developmental stages: I, embryo; II, larva; III, pupa; IV, adult. The black rectangle represents the stage at death (lethal phase) at 35 °C.

critical phase, 24–72 h, because 5–30 % of the males survive to adulthood. The lethal phase roughly coincides with the pattern of temperature sensitivity.

tsl-6. The lethal phase for *tsl-6* occurs during the pupal period and the pattern of temperature sensitivity is relatively narrow, occurring about 24 h before the lethal phase (Fig. 3*c*). The gene product appears to be necessary for survival for only a few hours at the beginning of pupation.

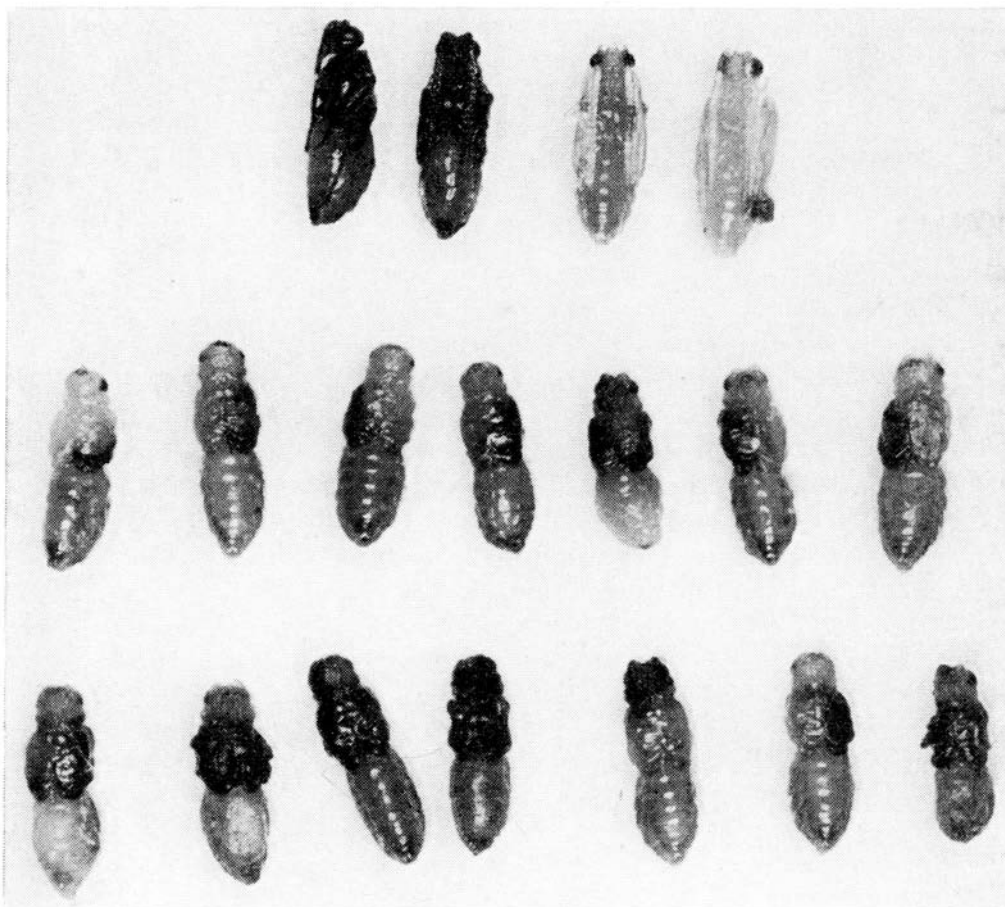

Fig. 4. The phenotypes of *tsl-6* individuals. The two pairs of individuals at the top are in middle and early pupal stages, respectively, and were reared at 28 °C. Development was normal and not arrested at these stages. Development was arrested in the individuals shown in the last two rows, and mosaic phenotypes are apparent.

Survival to adulthood and stage of development at 'death' were routinely scored on day 8 or day 9 for the individuals reared at 35 °C or shifted from 28 to 35 °C. We found that the wasps were not dead but that development was only arrested; when an individual was touched with a needle it responded. Also, over one-half the wasps arrested in development showed a mosaic morphology (Fig. 4).

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Table 1. *The number of dead males observed at the indicated developmental stages for a single test of* tsl-5

Time of transfer from 28 to 35 °C (h)	Stage when shifted from 28 to 35 °C	Developmental stages at death after the shift to 35 °C									Living adults
		Larvae					Pupae				
		Early	Middle	Late	Spinning	Spun	Prepupa	Early	Middle	Late	
4	Embryo	82	—	—	—	—	—	—	—	—	0
30	Very early larvae	87	—	—	—	—	—	—	—	—	0
60	Late	2	4	88	2	—	—	—	—	—	0
72	Late	—	—	—	110	—	—	—	—	—	0
84	Spinning	—	—	5	77	—	—	—	—	—	0
96	Spun	—	—	—	—	—	—	12	—	82	6
102	Spun	—	—	—	—	—	—	—	—	35	41
108	Spun-prepupa	—	—	—	—	—	—	—	—	22	33
114	Early pupa	—	—	—	—	—	—	—	2	—	89

As seen in the figure, most individuals exhibited morphological differences between their left and right sides, while others expressed differences between their heads and thoraxes, or thoraxes and abdomens. These differences, early and mid-pupa, were not correlated with light, temperature, or position within the dish. The expression seems to be under an intrinsic control. However, this mosaicism is not due to genetic mosaics in the classical sense because these are all haploid males from females homozygous for the *tsl-6* gene.

When the individuals reared at 35 °C for 7–9 days were placed at room temperature, pigment and appendages started to develop once again but never to an adult stage. The animals in the early pupa stage never initiated development again. The phenotypic pattern suggests that the mosaicism was predetermined very early in development, probably during very early cleavage. It is conceivable that during the first few cleavage divisions one of the *tsl-6* genes was 'conditioned' to respond differently, or not at all, to the restrictive temperature. For example, a control gene might have only partial control of the *tsl-6* gene – this control determined at cleavage. This gene makes a product during the prepupal stage but because the predetermined genes are now different the phenotype shows the mosaicism.

tsl-2. Death occurs during the pupal period in this mutant when reared at 35 °C (Fig. 3*d*). However, the gene product is not necessary for survival after the 48th h of development, as indicated by the shift from 28 to 35 °C. In contrast, the transfers from 35 to 28 °C at different time intervals suggest that the gene product was made and could be utilized throughout development from egg to adult, even though survival decreases after the early pupal stage. The 'plateau' in the 35 to 28 °C curve appears to be real because it was reproducible in four independent tests.

tsl-9. Many of the temperature-sensitive lethal mutations expressed pleiotropic effects. For example, haploid males which carried *tsl-9* died at the time of hatching at 35 °C but were viable and fertile when reared continuously at 28 °C (Fig. 3*e*). However, females homozygous for *tsl-9* were completely sterile at 35, 28 °C and room temperature ($\sim$ 22 °C). The ovaries of these females did not contain the normal array of developing trophocytes. Instead, each of the four ovarioles contained undifferentiated cells from the tip of the ovariole to the egg sac.

This mutant had to be maintained and tested as a heterozygote. The pattern of temperature sensitivity (Fig. 3*e*) was obtained from females heterozygous for the gene, and therefore adult survival did not drop to zero because one-half of the haploid males carried the wild-type allele of the *tsl-9* gene. As one can see, the gene product is necessary for survival in the embryonic period, and then becomes less essential throughout the larval period.

tsl-rs. This mutant was also pleiotropic. Haploid males which carried *tsl-rs* died in the early larval stage. At 28 °C these males made silk but did not make a functional cocoon. They spun a mat of silk on the bottom of the stender dishes and then pupated on top of the silk. The designation *tsl-rs* is for temperature-sensitive lethal and random spin. Females homozygous for this gene pupated but never became functional females. The wings and ovipositors were malformed at 28 °C and at room temperature, and at various relative humidities. This stock was

maintained in a heterozygous condition by selecting males which pupated outside of a cocoon (naked males), and then mating these males to wild-type (Hs^+) females. Over several generations the *tsl*-phenotype and random spin did not segregate, and therefore were assumed to be the same gene, or closely linked.

The pattern of temperature sensitivity indicates that the gene product of *tsl-rs* becomes necessary just before the lethal phase, and is necessary for survival up to the mid-to-late pupal stages (Fig. 3*f*).

tsl-11. The lethal phase for this mutant occurred immediately after hatching, and the time of gene action ranged from about the time of hatching to the late larval stage. When the males were changed from 28 to 35 °C at 24–48 h, death did not occur until they were early pupa (Fig. 3*g*).

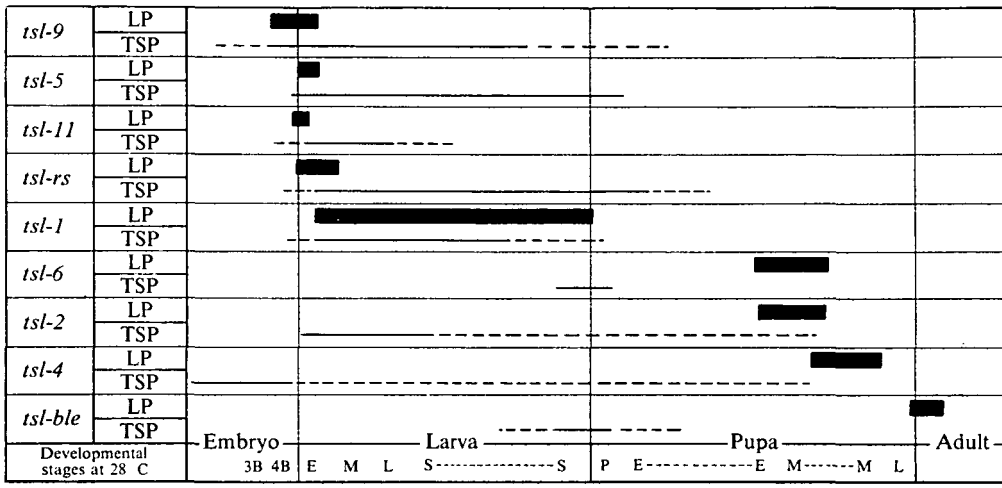

Fig. 5. A summary of the stages at death for each *tsl*-mutant from the earliest to the latest lethal phase and the deduced time of gene action. LP = lethal phase, TSP = temperature-sensitive pattern.

tsl-ble. This mutant, reared at 28 °C, possessed what we called 'black ears' phenotype because behind each eye there existed a transparent region that appeared as a black patch about the size of the insect's eye when viewed by the naked eye or a dissecting microscope at about × 10. At 35 °C most animals were complete adults (expanded wings and legs), but they died without eclosing. The temperature-critical period appeared to be between the 72nd and 120th h of development (Fig. 3*h*). Also, one can see that this particular mutant was 'weak' at 28 °C because, when compared to wild-type, survival was only 60–70 % from egg to adult.

tsl-4. This particular mutant is interesting because, for the animal to survive to adulthood, the gene product is necessary from the embryonic to the early pupal period (Fig. 3*i*). However, when reared at 35 °C, the wasps died as mid-to-late pupae. During the shifts from 35 to 28 °C, it can also be seen that it is a leaky

mutant, because up to 10% of the individuals survived to adulthood between the 24th and 144th h shifts.

Fig. 5 summarizes the lethal phase and temperature-sensitive pattern for each *tsl*-mutant. They are arranged from the earliest lethal phase to the latest lethal phase. Each mutant is unique when compared on the basis of these two parameters, independent of the obvious pleiotropic effects (*tsl-9*, *tsl-rs*, *tsl-ble*) and other unique morphological characteristics (*tsl-6*). The induction of temperature-sensitive mutations in *Habrobracon*, in addition to other insects, offers a method of obtaining, maintaining, and analysing mutations that are involved in the many developmental processes in higher organisms.

CONCLUSION

It is obvious from the work on *Drosophila* (Suzuki, 1970) that conditional mutations can be used to study many different aspects of developmental biology. With the evidence that temperature-sensitive mutations can be induced in the haploid–diploid genetic system of *Habrobracon serinopae*, an even wider range of developmental problems can be studied in a higher organism.

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REFERENCES

Baillie, D., Suzuki, D. T. & Tarasoff, M. (1968). Temperature-sensitive mutations in *Drosophila melanogaster*. II. Frequency among second chromosome recessive lethals induced by ethyl methanesulfonate. *Canadian Journal of Genetics and Cytology* **10**, 412–420.

Edgar, R. S., Denhartz, G. H. & Epstein, R. H. (1964). A comparative study of conditional lethal mutations of bacteriophage T4D. *Genetics* **49**, 635–648.

Edgar, R. W. & Lielausis, A. (1964). Temperature-sensitive mutants in bacteriophage T4D: their isolation and genetic characterization. *Genetics* **49**, 649–662.

Epstein, R. H., Bolle, A., Steinberg, C. M., Kallenberger, E., Boy De La Tour, E. Chavalley, R., Edgar, R. S., Susman, N., Denhardt, G. H. & Leilausis, A. (1963). Physiological studies of conditional mutations of bacteriophage T4D. *Cold Spring Harbor Symposia on Quantitative Biology* **28**, 375–392.

Hadorn, E. (1961). *Developmental Genetics and Lethal Factors*. New York: John Wiley.

Hartwell, L. (1967). Macromolecular synthesis in temperature-sensitive mutants of yeast. *Journal of Bacteriology* **93**, 1662–1670.

Hotta, Y. & Benzer, S. (1970). Genetic dissection of the Drosophila nervous system by means of mosaics. *Proceedings of the National Academy of Sciences, U.S.A.* **67**, 1156–1163.

Hotta, Y. & Benzer, S. (1972). Mapping of behavior in Drosophila mosaics. *Nature* **240**, 527–535.

McDonald, I. C. (1972). Temperature-sensitive mutations in the house fly: the characterization of heat-sensitive recessive lethal factors on autosome III. *Journal of Economic Entomology* **65**, 1364–1368.

Smith, R. H. (1968). Unstable temperature-sensitive lethal mutations in Habrobracon. *Genetics* **60**, 227.

Smith, R. H. (1969). Induction of mutations in Habrobracon sperm with mitomycin c. *Mutation Research* **7**, 231–234.

Smith, R. H. (1973). Induction of mutations in Habrobracon sperm with ethyl methanesulfonate. *Mutation Research* **21**, 17.

Smith, R. H. & von Borstel, R. C. (1972). Genetic control of insect populations. *Science* **178**, 1164–1174.

SUZUKI, D. T. (1970). Temperature-sensitive mutations in *Drosophila melanogaster*. *Science* **170**, 695–706.

SUZUKI, D. T., PITERNICK, L. K., HAYASHI, S., TARASOFF, M., BAILLIE, D. & ERASMUS, U. (1967). Temperature-sensitive mutations in *Drosophila melanogaster*. I. Relative frequencies among γ-ray and chemically induced sex-linked recessive lethals and semilethals. *Proceedings of the National Academy of Sciences, U.S.A.* **58**, 907–912.

SUZUKI, D. T. & PROCUNIER, D. (1969). Temperature-sensitive mutations in *Drosophila melanogaster*. III. Dominant lethals and semilethals on chromosome 2. *Proceedings of the National Academy of Sciences, U.S.A.* **62**, 369–376.

TARASOFF, M. & SUZUKI, D. T. (1970). Temperature-sensitive mutations in *Drosophila melanogaster*. VI. Temperature effects on development of sex-linked recessive lethals. *Developmental Biology* **23**, 492–509.

WHITING, P. W. (1933). Selective fertilization and sex determination in Hymenoptera. *Science* **78**, 537–538.