Studies on the female-sterile phenotype of \( I(1)su(f)^{ts76a} \), a temperature-sensitive allele of the suppressor of forked mutation in *Drosophila melanogaster*

**BY THOMAS G. WILSON**

*From the Department of Biological Sciences, Northwestern University, Evanston, Illinois*

**SUMMARY**

A new allele of the suppressor of forked \([su(f)]\) mutation in *Drosophila melanogaster* has been found and designated \( I(1)su(f)^{ts76a} \). It is temperature-sensitive for suppression of forked \((f)\) and has additional temperature-sensitive phenotypes of lethality, female sterility, and abnormal bristle formation at 29 °C. It closely resembles two other conditional alleles of \( su(f) \), \( I(1)su(f)^{lsG7g} \) and \( I(1)ts726 \). Female sterility at 29 °C is characterized by both disorganized egg chambers in the ovarioles and also chorioc-deficient oocytes. Both of these abnormalities may be the result of premature follicle cell death. The observations on \( I(1)su(f)^{ts76a} \) are consistent with the proposal that the similar allele, \( I(1)ts726 \), is a cell-lethal mutation specifically affecting mitotically active cells.

**INTRODUCTION**

Numerous loci have been described for *Drosophila melanogaster* which express phenotypes of lethality (see Lindsley & Grell, 1968) or female sterility (King, 1970). Analysis of these loci is considerably easier when temperature-sensitive (ts) alleles are found (Suzuki, 1970), allowing one to determine sensitive periods and resulting phenotypes simply by shifting animals to the restrictive temperature. In a screen for ts juvenile hormone null mutants, I selected for phenotypes expected of a juvenile hormone deficient mutant, ts larval-lethality and non-vitellogenic female sterility (Vogt, 1943; Postlethwait & Weiser, 1973). I recovered one larval-lethal, female-sterile mutant which proved to be an allele of suppressor of forked \([su(f)]\). (Subsequent characterization of this allele demonstrated ample vitellogenesis and thus the unlikelihood of a juvenile hormone deficiency.) Two other labs have reported similar \( su(f) \) alleles (Dudick, Wright & Brothers, 1974; Russell, 1974). Both of these mutants possess an array of ts phenotypes, including suppression of the forked \((f)\) mutation, female
sterility and lethality. In addition, Russell's mutant, \textit{I(l)ts726}, shows abnormal pattern formation in leg and eye-antennal imaginal disc tissue when larvae are subjected to a restrictive temperature pulse, and bristle deficiencies when pupae are shifted to the higher temperature. It has been postulated that this phenotype results from imaginal disc cell death, and \textit{I(l)ts726} has proven useful in examining pattern formation in \textit{Drosophila} (Russell, 1974; Clark & Russell, 1977; Russell, Girton & Morgan, 1977).

The ts suppression and lethality phenotypes of this new allele, \textit{I(l)su(f)}\textsuperscript{ts76a}, are very similar to those described for the other ts alleles and will be only mentioned in this paper. The female-sterility phenotype will be described in detail. The results from these studies support the hypothesis of Russell (1974) that \textit{I(l)ts726} is a cell-death mutant specific for cells undergoing mitosis.

\textbf{MATERIALS AND METHODS}

\textit{Stocks}

The Oregon-R wild-type stock has been maintained as an inbred line at Northwestern University for 20 years. The \textit{FM7, y\textsuperscript{31dV} B} balancer stock (see Lindsley & Grell, 1968, for stock description) was obtained from the Oak Ridge collection. All other stocks were obtained from the Bowling Green collection. The \textit{y, cv, v} and \textit{f} genes in the \textit{y cv v f ts76a} chromosome were derived from a \textit{y cv v f car} chromosome from that stock. The \textit{I(l)su(f)}\textsuperscript{ts76a} stock used for allele complementation tests also contained \textit{I(l)mys\textsuperscript{ts2}}. In this report the genotypes of heterozygotes have the chromosome of maternal origin written first.

\textit{Mutagenesis}

Only \textit{X} chromosomes were screened in this work because of the ease of stock manipulations relative to screening autosomes. Male Oregon-R flies were fed ethyl methane sulphonate by the procedure of Lewis & Bacher (1968) and mated with attached-X females of the constitution \textit{C(1)DX, y f/Y}. \textit{F\textsubscript{1}} progeny were raised at 23 °C and males singly mated to \textit{C(1)DX} females. A sample culture of \textit{F\textsubscript{2}} progeny from each single-pair mating was raised at 29 °C to test for pre-adult lethality, indicated by the absence of \textit{F\textsubscript{2}} adult males. Homozygous females were constructed via an \textit{FM7} balancer chromosome from each culture exhibiting a pre-adult-lethality phenotype. Such females were tested for ts sterility by shifting to 29 °C at eclosion and monitoring fertility over a period of several days.

\textit{Staging for temperature shifts}

Temperature-shift experiments were carried out as described by Suzuki (1970). For the larval temperature-shift experiments larvae were isolated from 0–1 h after hatching at 24°(±1 °C) and either placed at 29°(±1 °C) (shift-down
Female-sterile phenotype of \(1(1)\text{su}(f)^{ts76a}\) in Drosophila

Fig. 1. Scanning electron micrographs of tergites of (a) \(ts76a/^+\) and (b) \(ts76a/ts76a\) shifted to 29 °C at pupariation for 40 h. \(\times 200.\)

experiments) or maintained at 24 °C (shift-up experiments) for various lengths of time before shifting to the lower or higher temperature. Similar shifts were made for pupae of different ages after they were restaged at the white puparial stage (Bodenstein, 1950).

Miscellaneous

Fecundity was determined from daily egg collections from females at 24 °C placed 20 per shell vial together with 20 Oregon-R males. Fertilized eggs were determined to be those turning brown within 3 days after oviposition (Wright, 1973); unfertilized eggs remain white during this time. Ovary transplants were done using the methods outlined by Ursprung (1967). Decisions regarding ovary autonomy were made after examination of at least 12 transplants for each experimental condition. Feulgen-stained whole mounts (King, Rubinson & Smith, 1956) of ovaries were prepared for examination. Flies were raised on a standard agar–cornmeal–yeast diet.

RESULTS

Mutagenesis and mapping

Several ts larval-lethal female-sterile mutants were picked up in the mutagenesis screen. Only one is described in this paper and was designated \(TSI\) in the preliminary work.

\(TSI\) was mapped by recombination by crossing \(TSI\) males to \(y\) \(cv\) \(vf\) \(car\) females. \(F_1\) females were mated to Oregon-R males, and the \(F_2\) generation was raised at 29 °C. Since preliminary tests had shown \(TSI\) hemizygotes to be consistently 100% larval-lethal at 29 °C, all \(F_2\) males carrying the \(TSI\) gene were eliminated. A preliminary examination of 316 surviving \(F_2\) males located \(TSI\) 3 map units
Fig. 2. Fecundity of ts76a females after shifting to 29 °C (solid arrow) and after returning to 24 °C (dashed arrow). The top curve represents the percentage hatch of morphologically normal eggs laid by these females. Data from 80 females, 20 per shell vial. Error bars designate ± 1 standard error.

to the right of car. This map position of 65–66 places TSI near su(f), given as 65.9 (Lindsley & Grell, 1968). Since alleles of su(f) have been described which show lethality and female sterility (Dudick et al. 1974; Russell, 1974), the possibility that TSI is an allele of su(f) presented itself. Allelism to su(f) was tested by crossing TSI with an allele of su(f), I(l)su(f)tas76g, which also exhibits conditional lethality and female sterility (Dudick et al. 1974). The TSI/I(l)su(f) tsp was demonstrated to exhibit lethal and female sterile phenotypes indistinguishable from TSI homozygotes. The final proof that TSI is an allele of su(f) rests on its ability to suppress f. TSI was found to suppress f in a ts manner analogous to that described for I(l)su(f)tas76g (Dudick et al. 1974). It was concluded that TSI is allelic to su(f) and will be redesignated I(l)su(f)tas76g and ts76a in this paper.

Pre-adult temperature sensitivity

The temperature-sensitive periods for lethality during development of ts76a were determined by temperature shift experiments. A period of time extending from second larval instar until 12 h after pupariation delineates the ts period.
Female-sterile phenotype of 1(1)su(f)^ts76a in Drosophila

Dudick et al. (1974) and Russell (1974) found a similar sensitive period in development for their mutants.

Pupae of ts76a shifted to 29 °C within 5 h after pupariation usually failed to survive eclosion, but those that did survive showed a striking bristle-deficiency phenotype (Fig. 1). The absence of bristles is more pronounced on abdominal tergites than on the head and thorax. Russell et al. (1977) also noted defective bristle formation when I(1)ts726 mid-third instar larvae were shifted to 29 °C.

Female sterility

ts76a females maintained at 24 °C exhibited excellent fertility and fecundity. When shifted to 29 °C, however, females ceased to oviposit within a 4- to 5-day period (Fig. 2). During this period, different types of eggs were laid. For the first two days, the eggs appeared morphologically normal, although egg hatch decreased after the first day (Fig. 2). Since 70–80% of the unhatched eggs were fertilized, the decrease in egg hatch was not due to decreasing fertilization. Eggs laid between two and four days at 29 °C were of two types: (1) morphologically normal eggs of decreasing hatch in proportion to time of exposure of females to 29 °C; and (2) abnormal eggs, which lacked a chorion, were smaller than normal and misshapen, and never hatched. After 4 days at 29 °C, 90% of eggs laid were abnormal; after 5 to 6 days, oviposition ceased. When ts76a females at 29 °C
were returned to 24 °C, they began laying fertile eggs after 4 days (Fig. 2). The first few eggs laid were abnormal, but subsequent eggs were morphologically normal.

Appearance of ovaries

Ovaries of ts76a females were examined as Feulgen-stained whole mounts. Whole mounts of ovaries from females maintained at 24 °C were morphologically indistinguishable from those of Oregon-R females (see King, 1970, for a description of oogenesis in *Drosophila melanogaster*). However, ovaries from ts76a females shifted to 29 °C within 4 h after eclosion and maintained at that temperature for 5 days were very different (Fig. 3). Usually, one or two stage-14 (mature) oocytes were present. Also, several fully vitellogenic (resembling stage-13) oocytes were present in each ovary; these oocytes resembled the abnormal eggs laid after several days at 29 °C and probably were laid as abnormal eggs when females were returned to 25 °C. No stage-8 to -11 oocytes (partially vitellogenic) were seen, suggesting that vitellogenic uptake was not actively occurring after 5 days at 29 °C, although partially vitellogenic oocytes have been observed after 3 days at 29 °C. No distinct egg chambers were present; rather, the vitellaria appeared to be packed haphazardly with cells resembling nurse cells. Follicle cells were absent, although debris suggestive of dead follicle cells surrounded late-stage oocytes.

Ovary transplants

To determine if ts76a ovaries were abnormal at 29 °C due to some environmental factor (non-autonomous development) or to a factor within the ovary (autonomous development), ovary transplants from ts76a into Oregon-R hosts were performed. ts76a females were raised at 24 °C and ovaries were extirpated 0–4 h after eclosion and transplanted into either of two types of Oregon-R hosts: 2–4 h post-eclosion females or females which had been shifted to 29 °C at eclosion and maintained at 29 °C for 5 days. All hosts were placed at 29 °C 1 h after surgery, and host and donor ovaries were examined after 5 days at 29 °C as Feulgen-stained whole mounts. Host ovaries showed normal development, the ovaries morphologically indistinguishable from Oregon-R unoperated control females. Implanted ts76a ovaries appeared similar to those of unoperated ts76a females shifted to 29 °C 5 days before examination. Therefore, both host and implant ovaries developed autonomously. Likewise, when ovaries were dissected from 0–2 h post-eclosion Oregon-R females, and transplanted into ts76a females which had been shifted to 29 °C at 30 h after pupariation and maintained at that temperature for 5 days, both donor and host ovaries showed autonomous development at 29 °C 5 days after implantation.

Male sterility

In order to distinguish between the possibility that ts76a affects oocyte development only or affects gamete development in both sexes, it was important to
Fig. 4. Fertility of males after shifting to 29 °C (solid arrow) and returning to 24 °C (dashed arrow), O, Oregon-R; ●, y cv v f ts76a/y + Y; □, ts76a. Each curve was constructed from data from 25 males, one per shell vial with two Oregon-R virgin females. Females were replaced daily and observed for progeny over a 10-day period at 24 °C. A male was scored as fertile on a particular day if progeny were produced from one of the females.

determine if ts76a males become sterile at 29 °C. This experiment was complicated by the observation that Oregon-R males became sterile after 7 days at 29 °C, although the effect was reversible (Fig. 4). When ts76a males were shifted to 29 °C, they rapidly became irreversibly sterilized (Fig. 4). This effect could be due to the ts76a mutation or to another EMS-induced mutation on the X chromosome affecting male sterility. To distinguish between these possibilities ts76a males were tested whose X chromosome had been largely replaced by a y cv v f chromosome through recombination, although an undetermined amount of the original X chromosome between f (56.7 map position) and ts76a (65.9 map position) remained. These y cv v f ts76a males proved to be considerably more fertile at 29 °C than ts76a males (Fig. 4), presumably due to the elimination of a mutation affecting male fertility at 29 °C. Thus the results suggest that ts76a depresses male fertility but does not render males completely sterile at 29 °C. Russell (1974) reported that 29 °C has no adverse effect on male fertility of 1(1)ts726,
alleles, but also to the fact that they are highly penetrant and produce such striking phenotypes. All three alleles have similar phenotypic characteristics.

Cell death was found in imaginal disc and brain tissue of \( I(l)ts726 \) by Russell (1974), and he suggests that it is a cell-death mutant. Subsequently, a histological examination of heat-shocked \( I(l)ts726 \) larvae by Clark & Russell (1977) demonstrated lysosomal activity indicative of cell death in imaginal disc cells. The molecular basis of the cell death may be defective ribosomal protein, suggested as the \( su(f) \) gene product (Finnerty et al. 1973; Wright, 1973; Dudick et al. 1974 (but see Lambertson, 1976; Vaslet and Berger, 1976).

Russell noted that cell death in \( I(l)ts726 \) is somewhat specific, affecting cells which are actively undergoing mitosis. In addition to imaginal disc cells, affected cell types include brain cells and abdominal histoblasts (Russell, 1974). With the finding that follicle cells are sensitive to 29 °C, the present work is in agreement with Russell’s results, since follicle cells undergo mitosis during oocyte development (see King, 1970). The female sterility of \( ts76a \) may be due entirely to premature follicle-cell death. If follicle cells surrounding egg chambers are responsible for shaping these chambers, then the absence of viable follicle cells would explain the amorphic arrangement of nurse cells and oocytes in the ovarioles of females shifted to 29 °C. Since follicle cells are responsible for deposition of the chorion (King & Koch, 1963; Quattropani & Anderson, 1969; Petri, Wyman & Kafatos 1976), premature death would account for the development of oocytes lacking chorions in females which have been shifted to 29 °C for several days. At least 25 h at 29 °C is necessary to either kill follicle cells or impair their functioning. This is evidenced by the observation that \( ts76a \) females shifted to 29 °C at eclosion contain one or two stage-14 oocytes (having chorionic appendages) when examined 5 days later, as do ovaries transplanted from newly eclosed \( ts76a \) females to Oregon-R hosts which are then shifted to 29 °C. Approximately 30–35 h are required for newly eclosed wild-type females to form stage-14 oocytes at 25 °C (calculated from data of Handler & Postlethwait, 1978 and David & Merle, 1968), and probably less time (perhaps 25–30 h) is required for stage-14 oocyte maturation in \( ts76a \) females at 29 °C. Therefore, follicle cells of \( ts76a \) females at 29 °C are functional for at least this time period.

Bristle and socket formation is a product of two specialized epidermal cells, the trichogen and the tormogen, respectively (Lees & Waddington, 1942). The final differentiative divisions producing these cells occur during a period of from 14½ to 36 h after pupariation, based on the sensitivity of the dividing cells to X-ray (Poodry, 1975). These cell types are sensitive to 29 °C in \( ts76a \), since both bristle and socket structures are absent on abdomens of adults shifted to 29 °C shortly after pupariation. The radiosensitivity period determined by Poodry for the abdominal bristles allows an estimation of the time required for the effect of the restrictive temperature on \( ts76a \) pupae. Since the radiosensitive period of abdominal bristles is 24 h after pupariation (Poodry, 1975), and since the ts period for abdominal bristle formation is 0–5 h after pupariation of \( ts76a \),
Female-sterile phenotype of 1(1)su(f)ts76a in Drosophila 255

a minimum of 20 h at 29 °C is required to suppress abdominal bristle and socket formation. Of course, this calculation assumes that the effect of ts76a is on the final cell division and not on bristle and socket formation.

Mitosis in Drosophila embryos is a very active process, yet none of the ts su(f) alleles shows a sensitive embryonic period. This may be due to the presence of sufficient su(f)+ gene product deposited in the oocyte to satisfy any mitotic requirements for this product during embryogenesis. The decreasing egg hatch of morphologically normal, fertilized eggs laid by ts76a females placed at 29 °C (Fig. 2) possibly reflects insufficient su(f)+ product placed in the oocytes to satisfy mitotic requirements during embryogenesis.

Other cell types undergoing mitosis are the stem cells of the reproductive tissues. The fact that female sterility is reversible (Fig. 2) and male sterility is partially reversible (Fig. 4) suggests that stem cells may not be killed by the restrictive temperature. The basis for this sensitivity difference between stem cells and other dividing cell types to ts76a remains to be elucidated.

Russell's laboratory has made extensive use of 1(1)ts726 for studying various aspects of pattern formation. With the finding that ts76a affects follicle cell viability, perhaps this mutant will be useful for investigating follicle cell functions during oocyte maturation. One fruitful approach might be an examination of ovaries of ts76a/+ females shifted to 29 °C after inducing ts76a follicle cell clones by mitotic recombination.

The author would like to thank Dr Lawrence I. Gilbert for his support, and Drs Robert C. King and Jack Girton for their helpful comments during this work.

Research supported by National Institutes of Health National Research Service Award ES 05050–01 to T. G. Wilson, and National Institutes of Health Grant AM 02818 and National Science Foundation Grant PCM 76-03620 to L. I. Gilbert.

REFERENCES


(Received 4 May 1979, revised 10 September 1979)