as well as choice between alternative available components will of necessity wait for the appropriate moment of system synthesis and then the different objectives will lead to different conclusions. A wide variety of system configurations will ultimately result.

References and Notes

1. R. Kompfner, *Science* **150**, 149 (1965).
2. S. E. Miller and L. C. Tillotson, *Appl. Optics* **5**, 1538 (1966); S. E. Miller, *Sci. Amer.* **214**, 19 (1966).
3. References are given to work all over the world, but owing to limitations of space in this type of article they are not complete. Furthermore, in view of my greater familiarity with work within Bell Telephone Laboratories, references to work done there are more numerous.
4. T. S. Chu and D. C. Hogg, *Bell Syst. Tech. J.* **47**, 723 (1968).

4a. G. Goubau and F. Schwering, *IRE Inst. Radio Eng. Trans. Antennas Propagation* **9**, 248 (1961).

5. G. D. Boyd and J. P. Gordon, *Bell Syst. Tech. J.* **40**, 489 (1961).
6. A. G. Fox and Tingye Li, *ibid.*, p. 453.
7. E. A. J. Marcatili, *ibid.* **46**, 149 (1967); *ibid.*, p. 1733.
8. D. Gloge, *Proc. IEEE*, in press.
9. J. Hirano and Y. Fukatsu, *ibid.* **52**, 1284 (1964); O. E. DeLange, *Bell Sys. Tech. J.* **44**, 283 (1965); J. R. Christian, G. Goubau, J. W. Mink, *IEEE Trans. Microwave Theory Tech.* MTT-15, 216 (1967).
10. O. E. DeLange and A. F. Dietrich, *Bell Syst. Tech. J.* **47**, 161 (1968); O. E. DeLange, *J. Appl. Optics*, in press.
11. D. Gloge, *Bell Syst. Tech. J.* **49**, 55 (1970); J. A. Arnaud and J. T. Ruscio, *J. Appl. Optics*, in press.
12. D. W. Berreman, *Bell System Tech. J.* **43**, 1469 (1964); D. Marcuse and S. E. Miller, *ibid.*, p. 1759; A. C. Beck, *ibid.*, p. 1815; E. A. J. Marcatili, *ibid.* **45**, 97 (1966).
13. W. H. Steier, *IEEE Trans. Microwave Theory Tech.* MTT-13, 740 (1965).
14. P. Kaiser, *Bell System Tech. J.* **49**, 137 (1970).
15. K. C. Kao and G. A. Hockham, *Proc. IEEE* **113**, 1151 (1966).
16. A. R. Tynes, A. D. Pearson, D. L. Bisbee, *J. Opt. Soc. Amer.*, in press.
17. T. Uchida, M. Furukawa, I. Kitano, K. Koizumi, M. Matsumura, *IEEE J. Quantum Electron.*, in press.
18. H. Kita and T. Uchida, "Light focusing glass fiber and rod," SPIE meeting, Dallas, Texas, 28 to 30 January 1970.
19. A. D. Pearson, W. G. French, E. G. Rawson, *Appl. Phys. Lett.* **15**, 76 (1969).
20. H. Kogelnik and Tingye Li, *Proc. IEEE* **54**, 1312 (1966).
21. S. E. Miller, *Bell Syst. Tech. J.* **48**, 2059 (1969).
22. C. G. B. Garrett, *Gas Lasers* (McGraw-Hill, New York, 1967).
23. O. E. DeLange, *J. Appl. Optics*, in press.
24. A. L. Bloom, *Gas Lasers* (Wiley, New York, 1968).
25. G. Burns and M. I. Nathan, *Proc. IEEE* **52**, 770 (1964); J. F. Butler, A. R. Calawa, R. J. Phelan, A. J. Strauss, R. H. Rediker, *Solid-State Commun.* **2**, 303 (1964).
26. A series of papers on the stripe-geometry junction laser appeared in *IEEE J. Quantum Electron.* **QE-4** (April 1968).
27. I. Hayashi, M. B. Panish, P. W. Foy, *IEEE J. Quantum Electron.* **QE-5**, 211 (1969); H. Kressel and H. Nelson, *RCA Rev.* **30**, 106 (1969); Zh. I. Alferov, V. M. Andreev, E. L. Portnoi, M. K. Trukan, *Fiz. Tekh. Poluprov.* **3**, 1328 (1969); M. B. Panish, I. Hayashi, S. Sumski, *Appl. Phys. Lett.*, in press.
28. L. E. Hargrove, R. L. Fork, M. A. Pollack, *Appl. Phys. Lett.* **5**, 4 (1964); P. W. Smith, *Proc. IEEE*, in preparation.
29. N. G. Basov, *IEEE J. Quantum Electron.* **QE-4**, 855 (1968); Bason and his co-owners did not use the stripe geometry, but did first use two contacts to establish self-pulsing.
30. T. P. Lee and R. Roldan, *ibid.*, in press.
31. I. P. Kaminow and E. H. Turner, *Proc. IEEE* **54**, 1374 (1966).
32. J. E. Geusic, H. J. Levinstein, S. Singh, R. G. Smith, L. G. vanUitert, *Appl. Phys. Lett.* **12**, 306 (1968); R. G. Smith, J. E. Geusic, H. J. Levinstein, J. J. Rubin, S. Singh, L. G. vanUitert, *ibid.*, p. 308.
33. F. S. Chen, *J. Appl. Phys.* **38**, 3418 (1967); A. Ashkin, G. D. Boyd, J. M. Dziedzic, R. G. Smith, A. A. Ballman, H. J. Levinstein, K. Nassau, *Appl. Phys. Lett.* **9**, 72 (1966).
34. E. G. Spencer, P. V. Lenzo, A. A. Ballman, *Proc. IEEE* **55**, 2074 (1967).
35. R. C. Miller and N. C. Witwer, *IEEE J. Quantum Electron.* **1**, 49 (1965).
36. M. V. Schneider, *Bell Syst. Tech. J.* **45**, 1611 (1966).
37. H. Melchior and W. T. Lynch, *IEEE Trans. Electron Devices* **ED-13**, 829 (1966); L. K. Anderson, M. DiDomenico, Jr., M. B. Fisher, in *Advances in Microwaves*, L. Young, Ed. (Academic Press, New York, 1970).
38. P. K. Tien, R. Ulrich, R. J. Martin, *Appl. Phys. Lett.* **14**, 291 (1969).
39. J. E. Goell and R. D. Standley, *Bell Syst. Tech. J.* **48**, 3445 (1969).
40. E. P. Ippen, *Appl. Phys. Lett.*, in press.
41. E. A. J. Marcatili, *Bell Syst. Tech. J.* **48**, 2071, 2103 (1969); J. E. Goell, *ibid.*, p. 2133.

Temperature-Sensitive Mutations in Drosophila melanogaster

Conditional lethality is a useful feature of mutations used in a variety of analyses in higher organisms.

David T. Suzuki

The explosive impact of bacteria and their viruses on the elucidation of many fundamental aspects of molecular biology has been extensively documented. The versatility of these organisms in the study of a variety of problems derives from the existence of an extensive array of conditional lethal mutations which survive under a "permissive" set of conditions but express a lethal phenotype in a "restrictive" environment. Thus, the recovery of auxotrophic mutations which can be supplemented for nutritional requirements, nonsense mutations which are genetically suppressible, and lethal mutations whose viability is temperature-dependent have been familiar tools for the resolution of various genetic and biochemical phenomena. The paucity of such mutants has greatly restricted the kind and scope of genetic studies that are possible in multicellular eucaryotic organisms. The apparent imminent resolution of the basic features of replication, mutation, coding, gene function, and recombination in microorganisms has aroused a new interest in the biological phenomena unique to multicellular organisms. Thus, problems of differentiation, behavior, chromosome structure and function, and genetic redundancy offer new horizons for molecular biologists. It is to be hoped that the development of methods for the recovery and analysis of conditional mutations, in *Drosophila*, which are sensitive to changes in temperature may provide a powerful technique in the analyses of various problems associated with multicellular organisms.

Background

At the outset, it may be instructive for the "non-Drosophilist" to note the life cycle of *Drosophila melanogaster* (Fig. 1). At 22°C, the entire cycle from egg to fertile adult takes 11 to 12 days; at 29°C, 7 to 8 days; and at 17°C, 22 to 28 days. For a developmental biologist, it is of great interest to note that the organism in essence goes through two different lives. The larva is differentiated to exploit a specific environment, yet must anticipate the development of completely different structural elements which constitute a very different animal, the adult. The

The author is professor of zoology at the University of British Columbia, Vancouver, B.C., Canada.

This article is adapted from a lecture presented in Madison, Wisconsin, in August 1969 at the Annual Meeting of the Genetics Society of America.

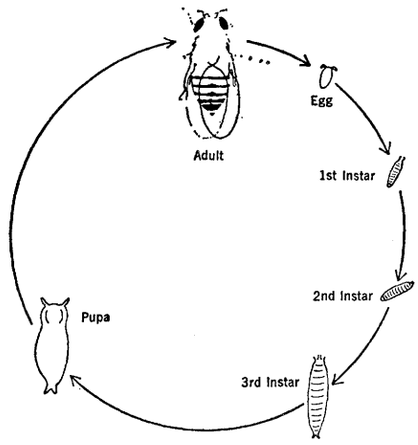

Fig. 1. Relative time scale for each developmental stage in the life cycle of *Drosophila melanogaster*.

genetic regulation of these two different functional forms poses a fascinating problem. *Drosophila*, the classic organism of choice for many geneticists, offers an extensive array of mutations and chromosome aberrations with which numerous problems can be studied. Some of the genetic markers mentioned below and their significant properties are summarized in Table 1.

Our work at the University of British Columbia on temperature-sensitive (ts) mutations resulted from a series of studies on the genetic and extrinsic regulation of crossing over. We were led to postulate that genes controlling many of the steps in cell division are located in proximal heterochromatin (*1*), an area of the chromosome known to be highly redundant (*2*). Thus, it was suggested that the apparent paucity of functional loci in this region could be the consequence of our inability to detect either recessive mutations which would be masked by their wild type duplicates or dominant mutations which would be lethal if cell division were affected. Therefore, in order to demonstrate the existence and function of redundant loci genetically, a method for the recovery of dominant mutations which are conditionally lethal was required, and temperature-sensitive lethality appeared to be more readily demonstrable than either auxotrophy or nonsense suppression. In fact, temperature sensitivity of mutations has long been known in a variety of multicellular organisms (*3*), including *Drosophila* (*4*). The molecular basis of temperature sensitivity was elucidated first in microorganisms. In T4 bacteriophage, ts lethals map extensively throughout the viral chromosome as point mutants of the missense type (*5, 5a*). Temperature sensitivity has been shown to be the consequence of a single amino acid substitution in a polypeptide (*6*), which alters the biological activity of the protein at different temperatures (*7*).

Before beginning a search for dominant temperature-sensitive mutations in *Drosophila*, we first set out to determine both the feasibility of the selection for sex-linked recessive ts lethals and the characterization of their genetic properties. In order to provide the greatest possible opportunity for detecting ts mutations, we exaggerated the temperature difference by using 17° and 29°C as the permissive and restrictive temperatures, respectively. Subsequent tests showed that over 90 percent of the ts lethals recovered in this manner were also sufficiently viable at 22°C to be scored as temperature-sensitive, and thereafter that was the temperature used as the permissive condition.

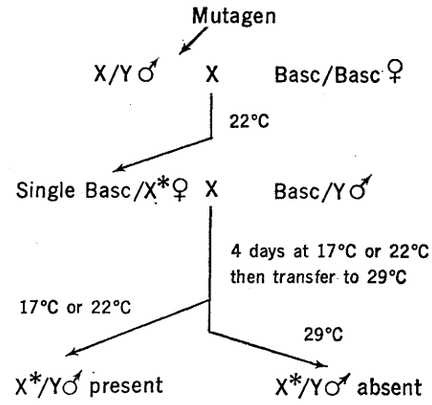

Fig. 2. Mating scheme for the detection of sex-linked recessive ts lethal mutations. The asterisk denotes the mutagenized X chromosome.

The protocol for detecting ts lethals is shown in Fig. 2. The survival of X*/Y males at low temperatures and their failure to survive at high temperatures indicates temperature sensitivity. In order to express in quantitative terms the relative viability of a mutation, the ratio of the number of F_2 X*/Y males to the number of *Basc*/X* females was used. Since Mendelian expectations predict a ratio close to 1.0, any depression in this value measures the reduced viability of the chromosome. It was arbitrarily decided to designate ts mutations as shown in Table 2.

With these criteria to define ts mutations, X chromosomes mutagenized by ethyl methanesulfonate (EMS), mitomycin C (MC), and γ-rays were tested for ts lethality (*8*). It was found that an estimated 11 to 12 percent of all EMS-induced sex-linked lethals are temperature-sensitive, whereas 3.0 to 3.5 percent of the lethals induced by MC and γ-rays are temperature-sensitive. In the case of autosomes, the same proportion of EMS-induced lethals is temperature-sensitive (*9*). These results, when correlated with the known

Table 1. Specific features of some of the chromosomes and mutations mentioned in the text.

Mutation	Chromosome	Properties of interest
	Y	Two-armed chromosome, factors for male fertility identified on the short (two) and long (five) arms.
Basc	X	Multiply inverted chromosome to suppress crossing over, marked by *B* (narrow eye) and w^a (orange eye color).
$In(1)w^{vC}$	X	Ring chromosome which is somatically unstable; its loss in X/X zygotes results in X/O patches.
Minute	X, 2, 3 and 4	A class of mutations with similar phenotypic properties of recessive lethality, prolonged development time, slender bristles, and enhancement of mitotic recombination.
DTS-lethal	2 and 3	Heterozygotes die at 29°C but survive at 22°C.
tra	3	Converts X/X zygotes into sterile, phenotypic males.
N^{60g11}	X	X-ray-induced; maps as a point within the Notch locus; recessive lethal; heat-sensitive nicked wing phenotype; cold-sensitive eye facet and bristle phenotype.
$para^{ts}$	X	Causes paralysis at 29°C, mobility at 22°C.
$l(1)E6^{ts}$	X	Temperature-sensitive lethal; levels of pteridines in eyes, testes, and Malpighian tubules are also temperature-sensitive.
$l(1)E7^{ts}$	X	Sexually dimorphic temperature-sensitive period, female temperature-sensitive period in egg and larvae; in males the temperature-sensitive period is in the pupal stage.
$l(1)E25^{ts}$	X	Mutant with repeated temperature-sensitive periods; the lethal phase is modified by time of upward temperature shift during the temperature-sensitive period.
$l(1)E34^{ts}$	X	Monophasic temperature-sensitive period in the pupal stage.

affinity of EMS to induce missense mutations (*10*), in contrast to the induction of chromosome rearrangements by MC (*11*) and γ-rays (*12*), suggest missense mutation as the most frequent basis for temperature sensitivity. This contention is further supported by failure to detect ts lethals among mutants induced by ICR-170, a mutagen assumed to cause frame shifts (*13*). Furthermore, we have recently observed that some ts lethals in *Drosophila* are very tolerant of a high salt diet (*14*), an observation resembling the observed resistance to osmotic shock of ts missense mutations in yeast (*15*). Probably the best test of the assumed missense basis of ts lethality will come from a study of EMS-induced mutants. In *Drosophila*, very few EMS-induced lethal mutations are associated with chromosome rearrangements or deletions (*16*), and, in striking contrast to x-ray-induced lethals (*17*), most lethals induced by EMS are single site point mutants (*18*). While it could be argued that temperature-sensitive lethality might result from single locus amorphs, this hardly seems an acceptable explanation for the bulk of mutants. The critical test will be a comparison of revertibility of nonconditional and ts lethals. These experiments are now in progress.

Table 2. Designations of ts mutations.

ts type	$(l^{ts}/Y\ ♂)/(Basc/l^{ts}\ ♀)$	
	29°C	22°C or 17°C
Lethal	0.0	>0.20
Semilethal	0.01–0.05	>0.30

The genetic distributions of 95 EMS-induced, 45 MC-induced, and 10 γ-ray-induced ts lethals on the X chromosome were determined in the following manner. Each mutant was first located within a "segment" delineated by the markers (*19*) followed by their genetic positions on the X chromosome, *y* (0.0), *cv* (13.7), *v* (33.0), *f* (56.7), and *car* (62.5). Any mutant falling between *y* and *cv* was then mapped to a "region" relative to w^a (1.5) and *rb* (7.5). Similarly, mutants falling between *cv* and *v* were mapped with *sn* (21.0) and *lz* (27.7), and those between *v* and *f* were mapped with *wy* (41.9) and *g* (44.4). Large regions were then subdivided into genetic "sections" on the basis of relative genetic size.

The distributions of 95 ts lethals and 163 non-ts lethals induced by EMS are shown in Fig. 3a. A "row × column" contingency test showed that the overall genetic distributions of the two types of mutants are not different. The distributions of 37 ts and 488 non-ts lethals induced by MC and of 10 γ-ray-induced ts lethals are shown in Fig. 3b. Again, the distributions are not statistically different. It should be noted that, whereas all EMS- and MC-induced ts lethals were readily localized to an interval, three out of the ten γ-ray-induced ts lethals were found to be associated with chromosome rearrangements; this suggests that radiation-induced ts lethals may be a heterogeneous group of mutations (*20*).

Sex-linked ts lethals, then, (i) are readily detectable in *Drosophila*; indeed, Wright (*21*) recovered three ts mutations at the lethal (*1*) myospheroid locus among 1500 EMS-treated X chromosomes; (ii) are more frequent among EMS-induced lethals than among MC-, γ-ray-, or ICR-170-induced lethals; (iii) map primarily as

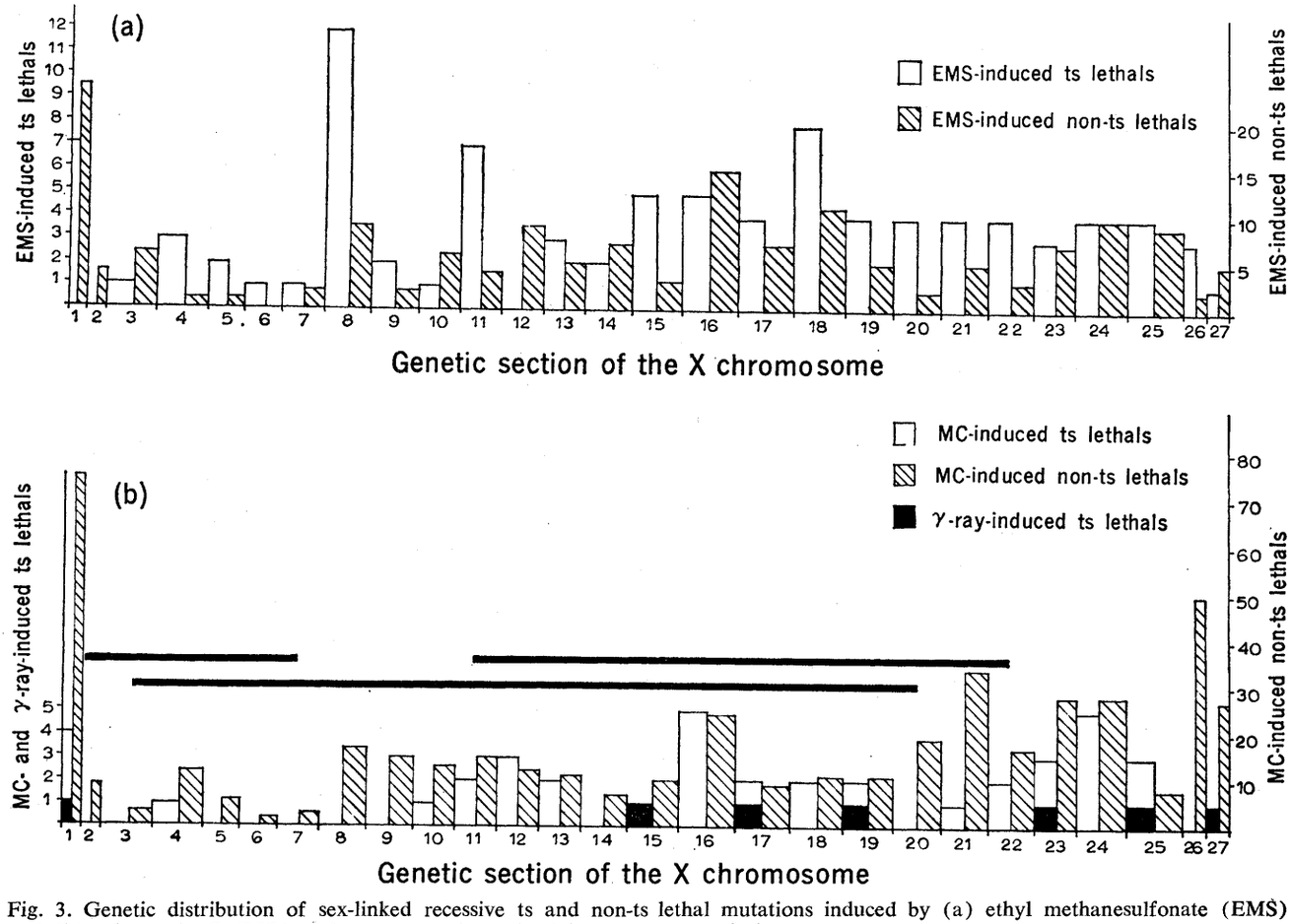

Fig. 3. Genetic distribution of sex-linked recessive ts and non-ts lethal mutations induced by (a) ethyl methanesulfonate (EMS) and (b) mitomycin C (MC) and γ-rays, as determined by crossover analysis.

point mutants within a genetic section, rather than as structural rearrangements; (iv) are located extensively throughout the X chromosome in a distribution similar to that of non-ts lethals; and (v) occur in the same proportion as among EMS-induced autosomal mutations. We conclude, therefore, that chemically induced ts lethals have genetic properties similar to those of microorganisms and that temperature sensitivity is not a property confined to a particular chromosome or limited to a few sites within a chromosome.

Biological Properties

The criterion used to define sex-linked ts lethal mutations is the survival of l^{ts}/Y males at the permissive temperature. It was observed that a number of these surviving males have an obvious visible phenotype. This characteristic of some of the ts mutations indicates that, while the mutation is sufficiently leaky to permit survival, it is not without biologically altered properties. Routinely, l^{ts}/Y males surviving at permissive temperatures were crossed to their heterozygous sibs. Of 83 stocks, males in 57 were fertile, and, of these, 53 yielded females homozygous for the ts lethal. Of the stocks with l^{ts}/l^{ts} females, 41 were fertile at permissive temperatures and, therefore, could be maintained as true-breeding stocks.

If hemi- and homozygous male and female adults which hatch at low temperature are shifted to an environment at 29°C, in most of the stocks the adults remain viable and fertile. Thus, in spite of the lethal genetic constitution, most flies that develop into adults at permissive temperatures are immune to temperature effects. Therefore, in these strains the incidence of temperature-dependent lethality must occur at a stage during development prior to eclosion. The fates of eggs deposited by such adults at high temperature vary with each mutant strain. In some cases the eggs never hatch; in others, larvae may emerge and proceed through development up to a specific stage, at which time death occurs. This period may be referred to as the "effective lethal phase" (LP) of the mutation (*22*) and represents the end result of continuous exposure to restrictive temperatures. The lethal phase, however, does not necessarily coincide with the actual temperature-sensitive period (TSP). That period can be established by a series of experiments in which cultures at low temperature are shifted to high temperatures ("shift-up") and vice versa ("shift-down") at different successive intervals after these cultures are started. An example is shown in Fig. 4, which is a study of a mutation having a lethal phase during the early pupal interval. It can be seen that the earliest shift-down which begins to decrease viability marks the initiation of the temperature-sensitive period (Fig. 4a). Similarly, the first shift-up which permits survival beyond the lethal phase defines the end of the temperature-sensitive period (Fig. 4b).

The temperature-sensitive period thus derived is inferred from two different experiments, shift-ups and shift-downs. In order to demonstrate that the interval so defined is a meaningful period in the developmental cycle of a mutant, continuous growth at high temperature except for the interval defined by the shifts should yield viable adults (Fig. 4c), and the reciprocal experiment should be lethal (Fig. 4d). This is, in fact, observed. For example, mutant $l(1)E7^{ts}$, in which the temperature-sensitive period in males was delineated in the early pupa, yields male survivors after a shift-down-and-up during the temperature-sensitive period (*23*). As might be expected with a biological system as complex as *Drosophila*, the demarcation of this period by shift studies is not clear-cut: shifts made around the temperature-sensitive period yield progressively more or fewer survivors, but the numbers fluctuate considerably (*23*). This can be seen in the results for $l(1)E34^{ts}$, a pupal lethal mutant (Fig. 5). In part, the scatter is due to the heterogeneity in development within each culture.

Another point of interest is the modification of the lethal period by genotypic or environmental changes, which has been noted for nonconditional lethals (*22, 24*). Thus, $l(1)E25^{ts}$, which was found to have a temperature-sensitive period during moulting from first to second larval instars and a lethal period during the second instar if a shift-up was made at 30 to 35 hours of the culture, survived to the third instar and pupal stages if the shift-up was made at 35 to 60 hours (*23*). Thus, although the temperature-sensitive period for lethality occupied a specific interval, the time of exposure to restrictive temperatures modified the lethal period. An example of genotypic modification of the lethal period is illustrated by the autosomal mutant *DTS-L2* (*25*). In the original stock, all mutants died as late-third-instar larvae

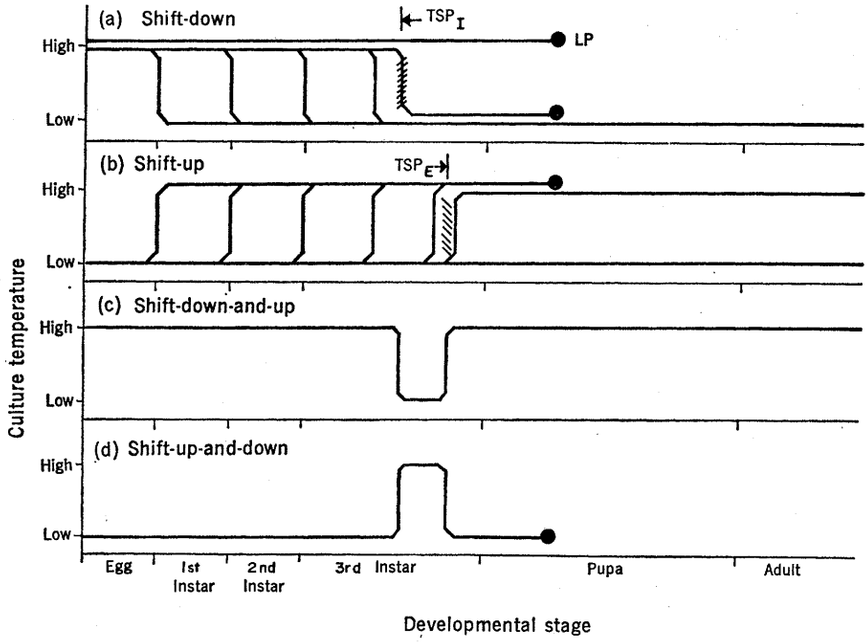

Fig. 4. Protocol for temperature-shift studies to determine the temperature-sensitive period (TSP) and the effective lethal phase (LP). (TSP_I) Initiation of the TSP; (TSP_E) end of the TSP. Cultures are shifted from 29° to 22°C, and vice versa, at different successive intervals, and survival or death is noted. The lethal phase is the time at which death occurs; the temperature-sensitive period is delineated by the earliest downward temperature shift ("shift-down") in which death is detectable and the earliest upward shift in which survival occurs.

or young pupae. Upon outcrossing to a balancer stock, the lethal period was shifted to the late pupal stage, and a further cross to another stock yielded a lethal period in the newly eclosed-adult stage (*25*).

We must ask, then, what is the molecular basis for the temperature-sensitive period? At present, our experiments have not been designed to answer this question. We have *assumed* that the primary effect of temperature is on the protein regulated by a given ts mutation. The temperature-sensitive period, we assume, corresponds to the interval during which the gene product controlled by the ts locus is biologically active—an assumption that makes no commitment as to the time of transcription or translation.

On the basis of preliminary temperature-shift studies on a number of ts lethals, several facts were established.

1) The bulk of ts mutations studied had a temperature-sensitive period or lethal phase at one of two intervals during development, either the embryonic egg stage or the interval from the late third larval instar through the pupal stage. These two developmental stages represent times of great morphogenetic activity and undoubtedly involve the activation of large numbers of developmental genes (*26*).

2) There is no predictable temporal correlation between the temperature-sensitive period and the lethal phase (*27*). Thus, in some mutants, the two coincide, while in others the temperature-sensitive period may be an interval preceding the lethal phase by from several hours to several days.

3) Among lethals located throughout the X chromosome there was no tendency for mutants at different positions to have temperature-sensitive periods around the same developmental stage (*27*). Four different ts lethals located within less than 1 map unit were found to have widely divergent temperature-sensitive periods (*23*), unlike closely linked phage cistrons, which are functionally as well as temporally related.

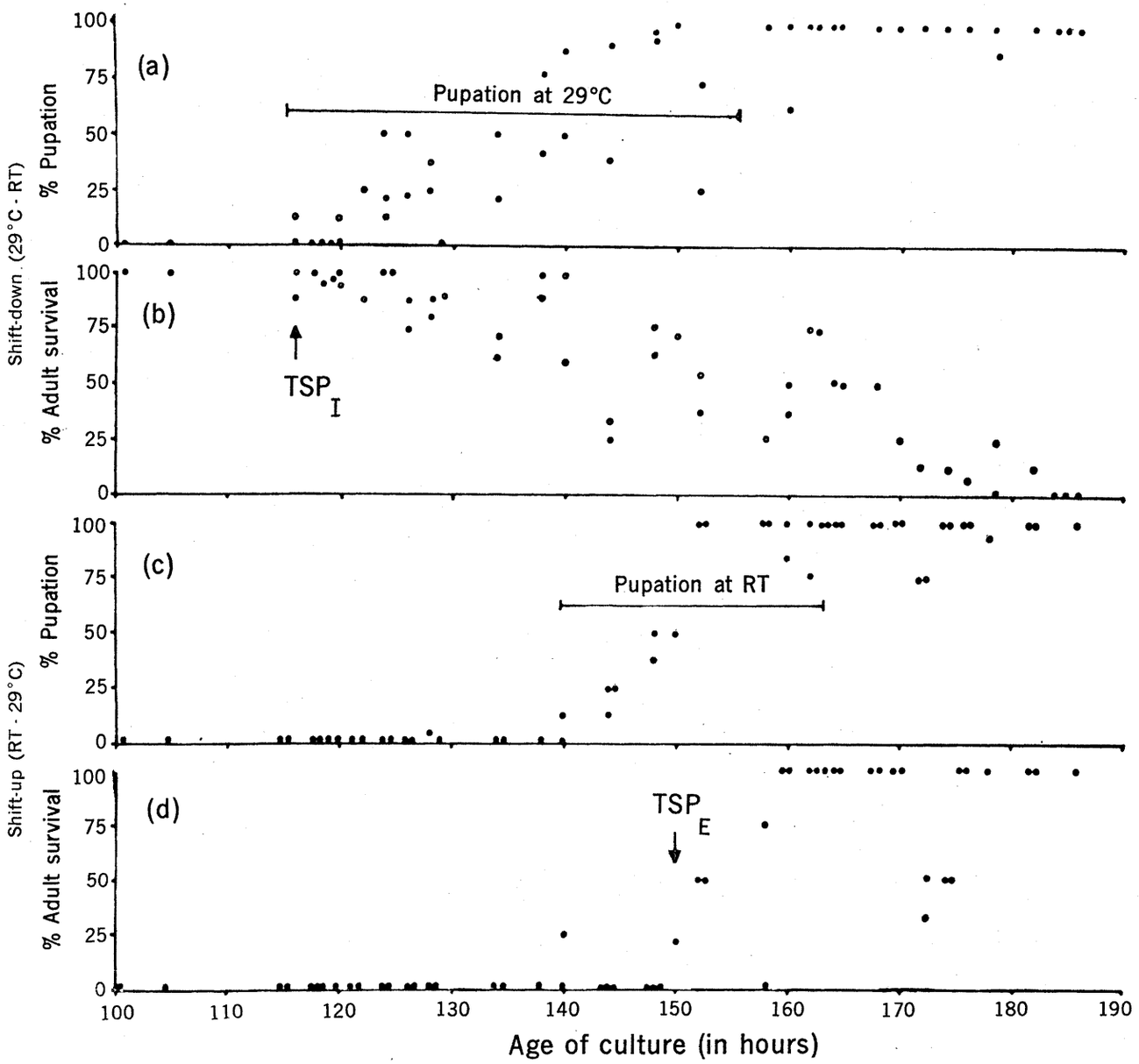

Fig. 5. Percentage of *E34* pupae present at the time of (a) a downward shift and (c) an upward shift and percentage of adult survivors after (b) a downward shift and (d) an upward shift. The arrows indicate the approximate times of the initiation (TSP_I) and end (TSP_E) of the temperature-sensitive period. *RT*, room temperature.

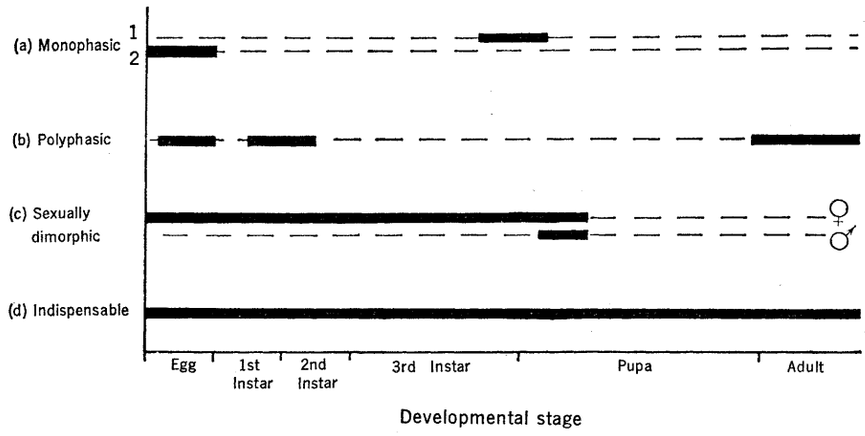

Fig. 6. Patterns of temperature sensitivity of different mutations as determined by temperature-shift experiments. Dark blocks denote the interval or intervals of the temperature-sensitive period.

4) In an analysis of whole embryonic mounts of 12 different "egg lethals," 11 were found to have a lethal phase long after gastrulation—6 after segmentation and 5 just prior to hatching—and one showed apparently arrested development prior to gastrulation (*28*).

In analyzing a number of stocks by the temperature-shift method, several different patterns of temperature-sensitive period emerged. The bulk of ts lethals were found to be monophasic with a single temperature-sensitive period occurring during the embryonic or pupal stages (Fig. 6a). Polyphasic mutants have also been detected in which several defined temperature-sensitive periods separated by non-temperature-sensitive intervals can be demonstrated (Fig. 6b). The polyphasic pattern may be interpreted in a number of ways: (i) it may indicate repetitive gene activation and inactivation; (ii) a locus may be activated and repressed in one tissue, then activated and repressed in a different tissue, and so on; or (iii) a gene product may be synthesized once and utilized at different successive times during development.

One mutant (Fig. 6c) exhibited a striking sexually dimorphic temperature-sensitive period. Males carrying the lethal gene had a single temperature-sensitive period of about 40 hours during the pupal stage. Females, on the other hand, were continuously sensitive to high temperature, from the egg stage to the pupal period. One might ask whether the difference in temperature-sensitive periods between the sexes is a consequence of gene dosage or of physiological differences. In order to determine this, l^{ts}/l^{ts} individuals homozygous for the autosomal recessive gene transformer (*tra*) (*19*), which converts X/X flies into phenotypic "males," were raised at 29°C for 24 hours, then at a lower temperature. It was found that l^{ts}/l^{ts};*tra*/*tra* "males" were killed, just as l^{ts}/l^{ts} females were. This result is not definitive, however, since the flies were grown at high temperature during early development at a time when the *tra* locus may not yet have been acting.

A fourth pattern of sensitivity is one which might be called "indispensable." At any stage of development, including the adult stage, a shift to the restrictive temperature results in an arrest in development, followed by death (Fig. 6d). Such a pattern may indicate continuous genetic activity of a locus or the formation of a thermolabile structural element that is necessary for viability at all times.

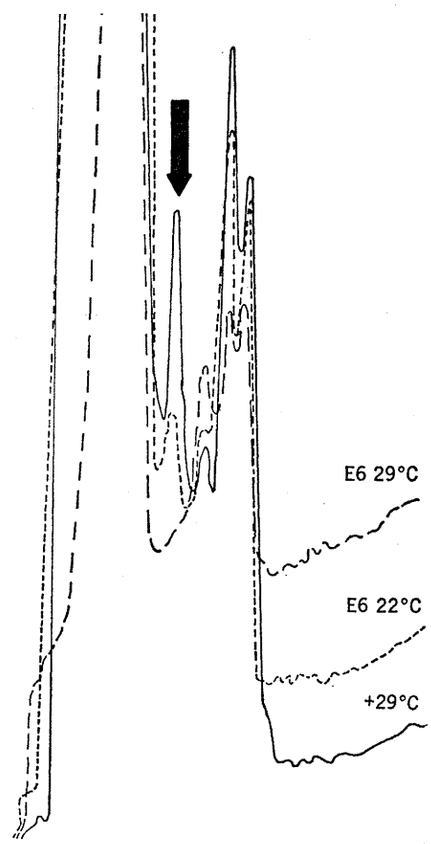

Fig. 7. Spectrofluorometric assay of thin-layer chromatograms of fluorescent pigments in the testes of *E6* and wild type stocks raised at different temperatures. The arrow indicates the compound measured to determine the temperature-sensitive period.

Detailed Analyses

Detailed analysis of ts mutants with special properties can yield insights into specific biological phenomena. Shift studies of an EMS-induced late pupal lethal (*E6*) showed a single temperature-sensitive period in the early part of pupation. The eyes of flies subjected to upward temperature shifts immediately *after* the temperature-sensitive period were found to be phenotypically mutant in color, whereas those of flies grown continuously at low temperature were wild type in color (*29*). The lethal factor in *E6* mapped genetically just to the left of *vermilion* (*v*, 33.0). In order to determine whether the ts lethal and eye color phenotypes were the consequence of mutation at a single locus or of a double mutation, scores were kept on eye and wing phenotypes of male offspring, raised at 29°C, and of females heterozygous for *E6* and *miniature* (*m*, 36.1). If, in fact, lethality and the eye phenotype result from two mutations, viable recombinants whose eyes are phenotypically mutant would be expected at 29°C. Of 3432 progeny, only wild-type crossovers were recovered, indicating that, if the eye color phenotype is, in fact, distinct from the site causing lethality, it is very tightly linked (*29*).

Pigmentation of *Drosophila* eyes results from a spectrum of fluorescent pigments some of which are also found in larval and adult Malpighian tubules and adult male testis sheaths; furthermore, the presence of several of the fluorescent compounds in the three tissues is affected by the same genes (*30*). Thin-layer chromatographic analysis of pigment distributions in testes (Fig. 7), Malpighian tubules, and eyes of *E6* flies exposed to a temperature of

29°C at different stages of development indicated altered levels of pteridines in all three tissues. Thus, it could be determined whether the temperature-sensitive period for mutant pigment synthesis by the *E6* allele occurs at the same time in all three tissues or whether the time of appearance of the pigment is, in fact, tissue-specific. By appropriate temperature-shift experiments and spectrofluorometric determination of the amount of pigment present, the temperature-sensitive period for the amount of pteridine was determined in each tissue (Fig. 8). It was found that a temporally distinct temperature-sensitive period for the mutant can be readily demonstrated in each tissue, although the separation of the periods for eye and testis was too short to be completely convincing (*29*). However, on the assumption that the tissue in which lethality is induced is different from the three organs chromatographed, at least three periods for *E6* could be clearly delineated. Although the data clearly suggest tissue specificity of the temperature-sensitive period, the possibility that pteridines are, in fact, accumulated rather than synthesized in the organs assayed complicates the interpretation. The dependence of phenotype upon the actual genotype of the cells analyzed was examined in the eyes, by means of the somatically unstable ring X chromosome, *In(1)w^{vC}* (*31*). Females genotypically *w^{vC}*,+/*E6* were grown at 29°C and inspected for the presence of patches of mutant eye color in a wild-type background. The ready detection of such patches, in which the unstable ring has been lost, shows that the pteridines are not accumulated from surrounding tissue (*29*). The incidental observation that phenotypically wild-type *E6* adult flies, when shifted to a temperature of 29°C and left for 4 weeks, lose no pigment indicates (on the assumption that pigment production remains autonomous) that eye pigments are extremely stable with respect to turnover.

The radiation-induced allele of the sex-linked Notch locus, *N^{60g11}*, has been reported to have a temperature-sensitive eye phenotype in heterozygous females (*32*). We observed that the facet arrangement in the eyes of *N^{60g11}*/+ females that had been raised continuously at 29°C was wild type (Fig. 9A), whereas facets in the posterior four-fifths of the eye were irregularly arranged (giving a rough appearance) when the flies had been grown at 22°C (Fig. 9B) (*33*). The temperature-sensitive period for the mutant eye phenotype was marked by the latest downward temperature shift and the earliest upward shift that produced the extreme mutant phenotype. This period was found to occur during the third larval instar stage. When the exact interval between the time of a temperature shift and pupation of a given individual was recorded, an interesting pattern emerged. The eyes of the flies subjected to an upward shift early in the temperature-sensitive period had a vertical strip of mutant tissue in the posterior portion of the eye (Fig. 9C), and the width of the mutant strip increased anteriorly with upward shifts at closer progressively later times in the temperature-sensitive period (Fig. 9D). The reciprocal downward shifts at progressively later times yielded a vertical strip of wild type facet arrangements in the posterior part of the eye; the margin of the strip advanced anteriorly with progressively later temperature shifts (Fig. 9E). These results are summarized in Fig. 10. Thus, the locus apparently regulates facet arrangement in a polarized fashion that begins in the posterior part of the eye and proceeds forward.

A number of points merit attention. The temperature-sensitive period occurs during a time when the prospective facet cells are found in undifferentiated imaginal disks prior to eversion. The pattern of facet control is unrelated to the clonal derivation of the facet cells (*34*). It may be relevant to look at the pattern of innervation of the imaginal disk at different temperatures and developmental times, since the regulation of nerve synthesis is defective in *N*/*N* and *N*/Y individuals (*35*). It is of interest to note that, while the mutant eye phenotype is expressed at 22°C, the nicked wing margins characteristic of *N*/+ heterozygotes are present in most females at 29°C but absent in all but a few at 22°C. A number of molecular interpretations can be constructed within the context of Welshons' (*36*) model explaining pleiotropy of the Notch locus.

The maximum usefulness of ts mutations in developmental problems will be realized when genetic, cytological, and biochemical techniques can be used to define the primary biological defect of such lesions. Ideally, then, ts mutations in loci known to act at a specific time in specific cells would be most useful. The Y chromosomal loci offer an opportunity for such study. The demonstration of a number of different sites necessary for male fertility (*37*), the mutability to sterility of such loci by EMS (*38*), the formation of lampbrush-like functional structures by the Y chromosome in primary spermatocytes (*39*), and the localization of Y chromosome–specific messenger RNA to testes (*40*) all indicate an ideal system for the molecular analysis of temperature sensitivity.

Since most wild-type flies raised continuously at 29°C are sterile, a temperature-resistant strain was derived from an Amherst wild-type stock. Y chromosomes of this stock were mutagenized with EMS, and eight mutagenized Y chromosomes that caused males to be sterile when the flies were raised at 28°C but allowed males to be fertile when the flies were raised at 22°C

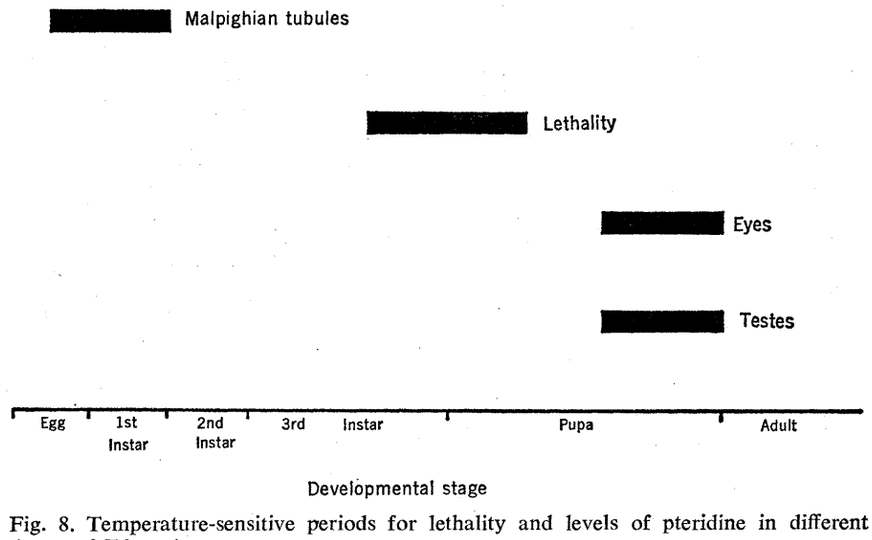

Fig. 8. Temperature-sensitive periods for lethality and levels of pteridine in different tissues of *E6* stock.

were recovered (*41*). These eight mutagenized chromosomes were combined with various Y chromosome fragments and deletions and tested for complementation at 28°C. All eight mutations were localized in the long arm of the Y chromosome and fell into at least four different complementation groups. The cell stages in the mutant testis affected by high temperature could be determined by exposing fertile males reared at 22°C to a 29°C heat shock for 48 hours. If males were then exhaustively mated at daily intervals to deplete the sperm supply, a decrease in fertility could be seen. While the patterns of sterility varied after a heat shock, sterility was generally significant by the fourth day after heat shock and lasted for 3 to 4 days (Fig. 11). It has been calculated that primary spermatocytes require at least 4 days to proceed through spermatogenesis to yield mature sperm (*42*), and it is thus suggested that the temperature-sensitive period of this class of mutants occurs in primary spermatocytes. Exposure to ts-mutation-bearing males to pulses of high temperature at different developmental stages provided good evidence that primary spermatocytes and perhaps later stages are temperature-sensitive (*41*). Electron microscopic examination by B. Kiefer of the testes of ts-mutation-bearing males raised at 29°C reveals no cytological abnormalities which are not seen in various Y chromosome deletions (*43*). The abnormalities observed included loss of paired fibrils in the axial filament, nonassociation of the axial filament with the mitochondrial element, and nonmotile sperm. The possible application of cytological and biochemical methods of analysis to Y chromosome–linked mutations that cause temperature-sensitive sterility promises to provide a fruitful area for further study.

Two other classes of temperature-sensitive mutations are worthy of mention. A number of stocks having the ts lethal mutation are seen, when examined at restrictive temperatures, to manifest a temperature-sensitive phenotype of melanotic pseudotumor formation prior to death. The size and location of the tumors vary from stock to stock, and it cannot be stated whether tumor formation is the primary cause of death or a secondary response to the mutant environment. A mutant stock which is viable at 29°C but is temperature-sensitive for melanotic pseudotumor formation has also been recovered (*44*).

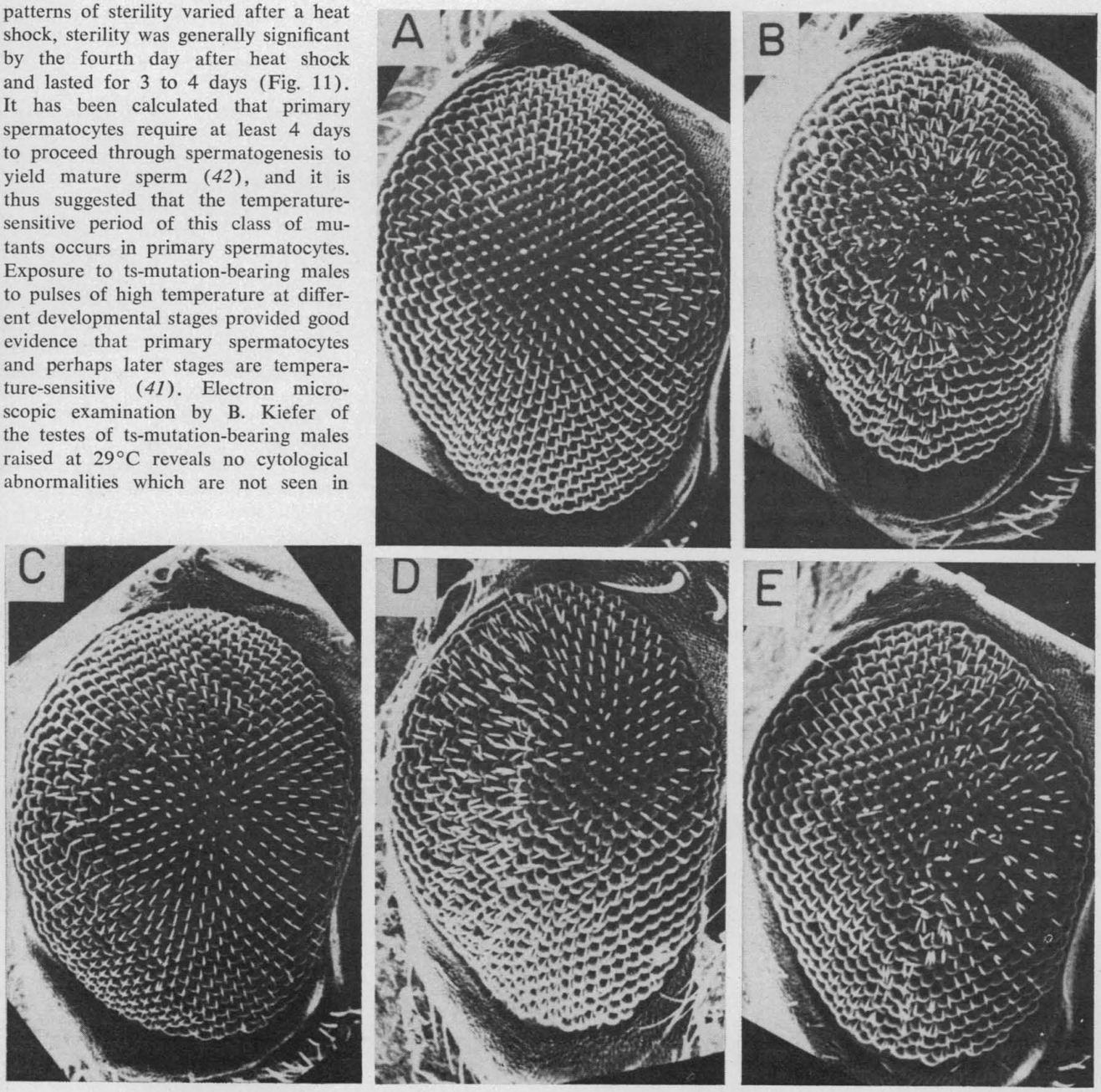

Fig. 9. Scanning electron micrograph of eyes of $N^{60g11}/+$ females (A) at 29°C and (B) at 22°C; when temperature was raised (C) early and (D) late in the temperature-sensitive period; and (E) when temperature was lowered late in the temperature-sensitive period. The anterior rim of the eye is at the right.

In a search for mutations affecting either muscle (*45*) or nervous tissue, adults have been screened for a reversible temperature-sensitive paralysis. Recently, a sex-linked recessive mutation, paralytic-temperature-sensitive (*para*^{ts}), was recovered (*46*). Paralysis of the mutants occurs at 29°C, in adults only, and is complete within 5 seconds of a shift from 22° to 29°C. The flies survive in a state of paralysis for at least 2 hours at 29°C. They recover mobility immediately upon being shifted from 29° to 22°C, and paralysis can be induced and removed repeatedly with the same individuals. This class of mutations promises to yield an exciting approach to muscle and nerve study.

While the potential of temperature-sensitive mutations for studying development remains to be fully exploited through biochemical and cytological analysis, some points of importance have already emerged. The usefulness of temperature sensitivity as a means of probing specific aspects of development is indicated by the sterility mutations on the Y chromosome and by the paralytic mutation. The mutant N^{60g11} has revealed a temporal effect on the pattern of eye facet organization not previously described; moreover, heat sensitivity of the wing phenotype and cold sensitivity of the eye phenotype of N^{60g11} provide an interesting puzzle. The tissue specificity of genetic activity of the *l(1)E6* locus points to an important problem in the regulation of activation. The disparate temperature-sensitive–period patterns of closely linked loci reemphasize the absence of any major chromosomal arrangement into groups activated sequentially or coordinately.

Dominant Temperature-Sensitive Lethals

The characterization of the general properties of recessive ts mutations and the demonstration of their potential as tools for a variety of analyses encouraged us to turn to the question of whether dominant ts (DTS) lethal mutations could be recovered. The results shown in Table 3 were obtained (*47*).

The reason for the disparity in the frequency of recovery of DTS lethals on the second and third chromosomes became apparent when the second-chromosome mutants were mapped genetically (*48*). Of 15 mutants mapped, 11 were localized to within 2.0 units between the mutations *dumpy* and *clot*. The functional relatedness of the mutations in the cluster was indicated by the appearance of recessive lethality at 22°C when the mutations in the cluster were combined with certain mutants in trans heterozygotes. Thus far, 28 mutations have been assigned to the cluster on the basis of the complementation patterns, and a complementation map similar to those determined in yeast has been constructed (Fig. 12). While circular complementation maps have been established for a number of loci (*49*), the genetic significance of these operational constructs has yet to be determined (*50*). Moreover, we have to determine the genetic size of the cluster and the relationship of the "locus" to a cistron. Fine-structure mapping of the alleles in the cluster should be readily accomplished in trans heterozygotes, since only recombinants between DTS alleles will survive at 29°C. The functional relatedness of mutants in the cluster was further indicated by a temperature-sensitive period 18 to 24 hours after egg deposition for all alleles tested (*48*). All of the second-chromosome DTS lethals were developmental lethals.

Each of the third-chromosome DTS lethals maps as a point within a crossover region (*25*). They are distributed extensively along the chromosome and all have a temperature-sensitive period and effective lethal phase during de-

Table 3. Data on recovery of DTS lethal mutations.

Chromosome tested	No. of chromosomes tested	No. of DTS lethals
X	1,440	0
2	17,000	48
3	26,000	8

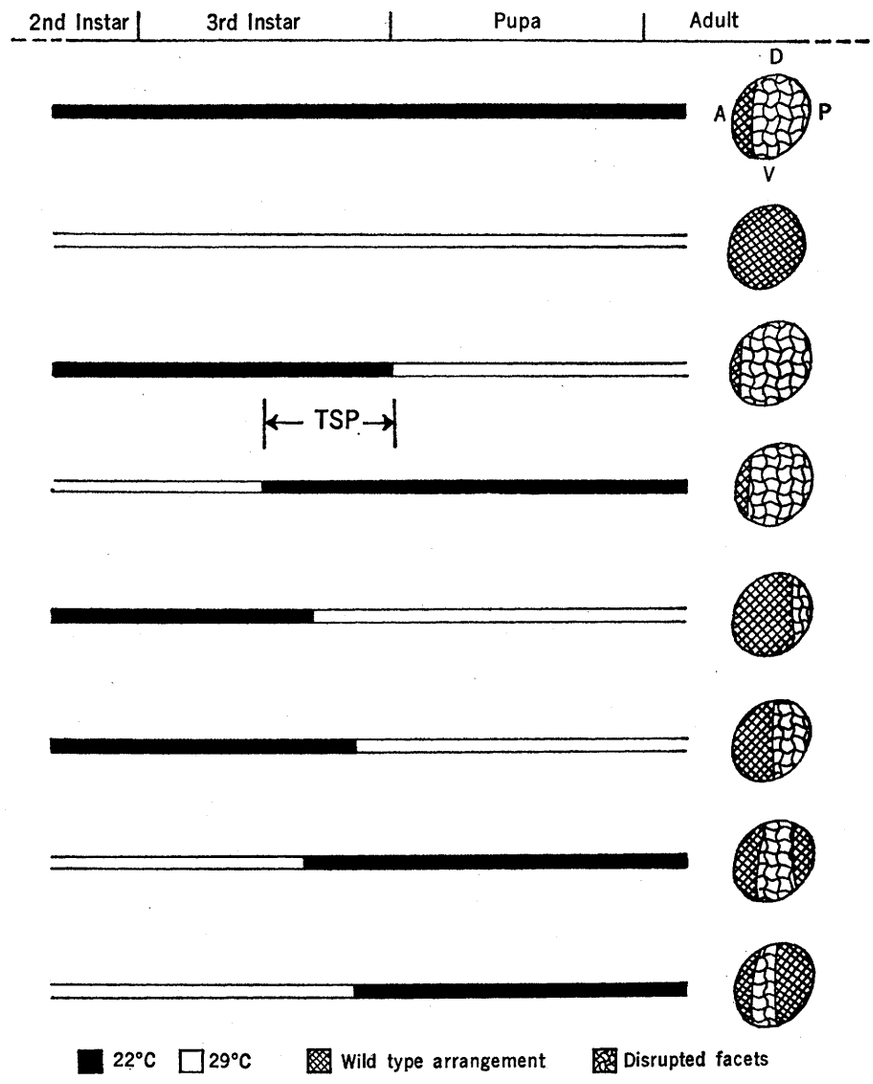

Fig. 10. Facet arrangement in eyes of $N^{60g11}/+$ females in temperature-shift experiments. (*A*) Anterior; (*P*) posterior; (*D*) dorsal; (*V*) ventral.

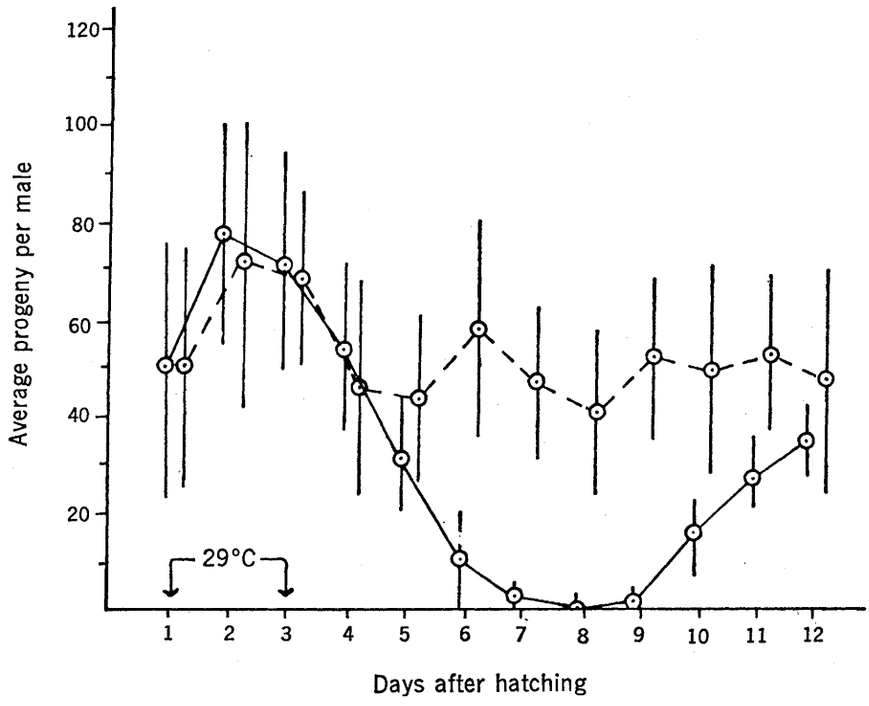

Fig. 11. Average daily fertility per male after a 48-hour temperature shock (29°C) administered 1 day after eclosion. (Dashed line) Amherst temperature-resistant stock, Am^{tr}; (solid line) ts mutant Y stock, $A145^{ts}$.

velopment. Two of the mutants are worthy of note. By all criteria that could be applied (bristle phenotype, developmental time, recessive lethality at 22°C, mitotic "recombinagenesis," interaction with *Dl*), they are *Minute* mutations. K. C. Atwood (see *19*) has suggested that *Minutes* represent mutations at sites for transfer RNA (tRNA) synthesis. Our ts mutants in *Drosophila* may provide a means for testing Atwood's suggestion. It should be stressed that the detection of ts *Minute* mutations does not automatically discount the tRNA-*Minute* relationship, since ts tRNA mutants are known in *Escherichia coli* (*51*).

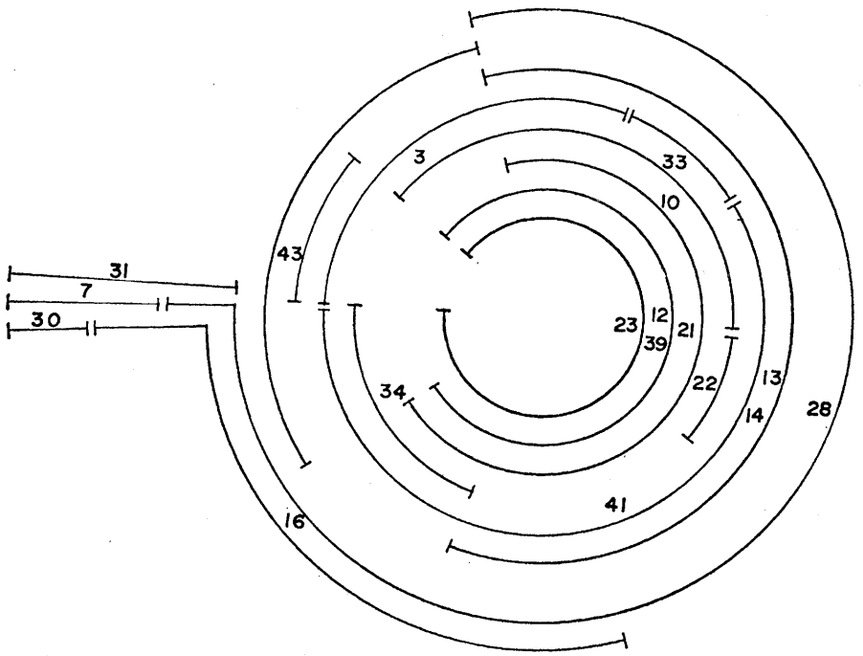

Fig. 12. Complementation map, at room temperature, of functionally related DTS lethal mutations clustered in one region of chromosome 2. Complementation was indicated by viability of DTS-a/DTS-b heterozygotes at 22°C.

The mechanism of dominant temperature sensitivity may be the consequence of several possible lesions: incorporation of defective polypeptides in either a structural element (*52*) or a polymeric enzyme (*53*); a defect in a regulatory protein (*54*); or an absolute requirement for more than one gene dose of product. The use of DTS lethals may be the only means of detecting and making genetic studies of highly redundant loci. The potential of DTS lethals as a means of making genetic studies of organisms not readily amenable to extensive genetic manipulation is tremendous. Recently, Chu (*55*) reported the recovery of lethal mutations in human cell cultures which are probably DTS lethals. Temperature-sensitive mutations which may be DTS lethals have also been reported in L cells (*56*).

Cold-Sensitive Lethals

Although Edgar and Lielausis (*5a*) found that ts lethals which die at high temperatures but survive at lower temperatures (heat-sensitive lethals) do not map randomly in T4 phage, their sample nevertheless was distributed extensively around the chromosome. In contrast, lethals which die at low temperatures but survive at high temperatures (cold-sensitive lethals) are strikingly clustered in a small number of cistrons (*57*). Whereas heat-sensitive lethals are defective with respect to a wide range of functions (*58*), cold-sensitive mutations involve changes primarily in regions of proteins concerned in regulatory functions (*59*). The remarkable property of self-assembly of ribosomal proteins is highly dependent upon temperature, an observation which led Nomura to the brilliant decision to screen cold-sensitive lethals of *Escherichia coli* for defects in ribosomal proteins (*60*). It was found that a significant proportion of cold-sensitive lethal mutations in this organism do indeed encode defective ribosomal proteins, and, moreover, that such cold-sensitive defects are dominant.

On the basis of these observations in microorganisms, we decided to examine cold-sensitivity as a possible means of selecting ribosomal mutants. Recessive cold-sensitive mutations had been de-

tected by chance earlier (*9*) and were recovered in a specific screen in 0.8 percent of 3583 X chromosomes tested (*61*). Since it was highly unlikely that loci regulating ribosomal proteins would be exclusively or primarily sex-linked, autosomes were screened for dominant cold-sensitive lethals. Among 5046 second chromosomes tested, 22 dominant cold-sensitive lethals have been recovered. This is in striking contrast to the failure to detect any dominant cold-sensitive lethals among 3200 mutagenized third chromosomes. Preliminary tests for ribosomal defects are in progress, and it is hoped that this screening technique will yield ribosomal mutants amenable to analysis of both structure-function relationships and biosynthetic control mechanisms of the organelle.

Conclusions and Prognosis

The findings reported here strongly suggest that, given a proper screening protocol, temperature sensitivity is a property that can be readily detected for a wide spectrum of loci throughout the genome. Thus, the detection of ts alleles at specific loci, recessive and dominant heat- and cold-sensitive lethals on the X chromosomes and autosomes, Y chromosome sterility mutations, and a ts paralytic provides an extensive array of mutants for genetic contrivance to facilitate certain analyses or to probe biological phenomena.

The general properties of the ts mutations studied in *Drosophila* resemble the known missense basis for temperature sensitivity in microorganisms. Thus, ts alleles are most frequent (11 to 12 percent) among mutants induced by the alkylating agent EMS, less common among MC- or γ-ray-induced lethals (3.0 to 3.5 percent), and not detected among ICR-170 mutations. All of the chemically induced ts lethals map readily within a genetic interval as points. Preliminary analysis of osmotic sensitivity suggests that DTS lethals are resistant to high salt concentrations.

The usefulness of ts mutations for analysis of a number of different kinds of problems in cell biology has been suggested, and the use of ts mutations amenable to genetic, cytological, and biochemical techniques of analysis should give great insights into the molecular biology of eucaryotic organisms. The restrictions in the extent to which the primary effect of a ts lesion may be determined depend upon the mutants chosen for study; the most profitable approach involves the detection of ts lethals affecting a known process or definable product amenable to analysis. The feasibility of using DTS mutations to study problems in organisms, such as cells in culture, for which there are no genetic methods of analysis is now being recognized in other laboratories.

In addition, conditional lethal genes can be used as a means for increasing genetic resolution, as has been done in the case of microorganisms. Thus, rare single or multiple recombinants between closely linked ts lethals can be readily detected at the restrictive temperature. Similarly, rare reversions or suppressors of ts lethals may be easily recovered as the sole survivors at the restrictive temperature. Further characterization of the specific developmental effects of different ts mutants (for example, DTS lethals or ts Y chromosome steriles), when coupled with the elegant genetic contrivances possible in *Drosophila*, allow the construction of special selective procedures for the recovery of specific classes of mutations.

Finally, the survival and fertility of adult insects carrying ts mutations which cause death during immature stages suggests a method for pest control. Adult organisms bearing dominant heat- or cold-sensitive lethals could act as vectors for the spread of mutations which would ensure death of offspring at restrictive temperatures normally encountered daily or seasonally. In fact, several laboratories have begun work toward such a means of biological control (*62*).

References and Notes

1. D. T. Suzuki, in "Oregon State University Genetics Lecture Series," R. Bogart, Ed. (Oregon State Univ. Press, Corvallis, in press).
2. A. A. Prokofyeva-Belgovskaya, *Genetics* **22**, 94 (19[illegible] F. M. Ritossa and S. Spiegelman, *Proc. Nat. Acad. Sci. U.S.* **53**, 737 (1965); H. D. Berendes and H. G. Keyl, *Genetics* **57**, 1 (1967).
3. For *Habrobracon*, see P. W. Whiting, *Genetics* **19**, 268 (1934); for rabbits, see R. Danneel, *Ergeb. Biol.* **18**, 55 (1941).
4. For a review of the very early work, see E. C. Driver, *J. Exp. Zool.* **59**, 1 (1931).
5. R. S. Edgar, G. H. Denhardt, R. H. Epstein, *Genetics* **49**, 635 (1964).
5a. R. S. Edgar and I. Lielausis, *ibid.*, p. 649.
6. H. G. Wittmann, B. Wittmann-Liebold, J. Jauregui-Adell, *Z. Naturforsch.* **20b**, 1224 (1965); B. Wittmann-Liebold, J. Jauregui-Adell, H. G. Wittmann, *ibid.*, p. 1235.
7. H. Jockusch, *Biochem. Biophys. Res. Commun.* **24**, 577 (1966).
→ D. T. Suzuki, L. K. Piternick, S. Hayashi, M. Tarasoff, D. Baillie, U. Erasmus, *Proc. Nat. Acad. Sci. U.S.* **57**, 907 (1967).
9. D. Baillie, D. T. Suzuki, M. Tarasoff, *Can. J. Genet. Cytol.* **10**, 412 (1968).
10. D. R. Krieg, *Genetics* **48**, 561 (1963).
11. M. W. Shaw and M. M. Cohen, *ibid.* **51**, 181 (1965).
12. D. E. Lea, *Actions of Radiations on Living Cells* (Cambridge Univ. Press, New York, 1955).
13. E. A. Carlson, R. Sederoff, M. Cogan, *Genetics* **55**, 295 (1967).
14. E. Tasaka, unpublished.
15. D. C. Hawthorne and J. Friis, *Genetics* **50**, 829 (1964).
16. J. K. Lim and L. A. Snyder, *Mutat. Res.* **6**, 129 (1968).
17. E. Lifschytz and R. Falk, *ibid.*, p. 235.
18. ———, *ibid.* **8**, 147 (1969).
19. For a complete description of all mutations and chromosome aberrations used, see D. L. Lindsley and E. H. Grell, "Genetic Variations of *Drosophila melanogaster*," *Carnegie Inst. Wash. Publ. 627* (1968).
20. D. T. Suzuki, L. K. Piternick, J. Healy, in preparation.
21. T. R. F. Wright, *Proc. Int. Congr. Genet., 12th* (1968), vol. 1, p. 141.
22. E. Hadorn, *Developmental Genetics and Lethal Factors* (Wiley, New York, 1961).
23. M. Tarasoff and D. T. Suzuki, *Develop. Biol.*, in press.
24. E. Hadorn and P. S. Chen, *Arch. Julius Klaus-Stift.* **27**, 147 (1952).
25. J. Holden and D. T. Suzuki, *Genetics* **60**, 188 (1968); J. Holden, *Can. J. Genet. Cytol.* **12**, 384 (1970); ——— and D. T. Suzuki, in preparation.
26. E. Hadorn, *Symp. Soc. Exp. Biol.* **2**, 177 (1948); *Advan. Genet.* **4**, 53 (1951).
27. D. T. Suzuki and P. Duck, *Genetics* **56**, 592 (1967).
28. J. Lucchesi, unpublished.
29. T. Grigliatti, *Can. J. Genet. Cytol.* **12**, 382 (1970); ——— and D. T. Suzuki, *Proc. Nat. Acad. Sci. U.S.*, in press.
→ E. Hadorn and H. K. Mitchell, *Proc. Nat. Acad. Sci. U.S.* **37**, 650 (1951).
31. C. W. Hinton, *Genetics* **40**, 951 (1955).
32. W. J. Welshons and E. S. von Halle, *ibid.* **47**, 743 (1962).
33. G. G. Foster, *Can. J. Genet. Cytol.* **12**, 381 (1970); ——— and D. T. Suzuki, *Proc. Nat. Acad. Sci. U.S.*, in press.
34. H. J. Becker, *Z. Indukt. Abstammungs-Vererb.* **88**, 333 (19[illegible] → W. K. Baker, *Amer. Zool.* **3**, 57 (1963).
35. D. F. Poulson, *Proc. Int. Congr. Genet., 12th* (1968), vol. 1, p. 143.
36. W. J. Welshons, *Science* **150**, 1122 (1965).
37. G. E. Brosseau, *Genetics* **45**, 257 (1960).
38. J. H. Williamson, *ibid.* **60**, 238 (1968).
39. O. Hess and G. F. Meyer, *Advan. Genet.* **14**, 171 (1968).
40. W. Hennig, *J. Mol. Biol.* **38**, 227 (1968).
41. T. Sanders and B. Ayles, *Can. J. Genet. Cytol.* **12**, 394 (1970); B. Ayles, T. Sanders, D. T. Suzuki, B. I. Kiefer, in preparation.
42. P. T. Ives, *Genetics* **48**, 981 (1963).
43. B. I. Kiefer, *ibid.* **54**, 1441 (1966); personal communication concerning the ts mutants.
44. W. Willis and D. T. Suzuki, unpublished.
45. Suggested in a conversation with E. Reich of Rockefeller University.
46. R. Williamson, T. Grigliatti, D. T. Suzuki, *Can. J. Genet. Cytol.* **12**, 400 (1970); T. Grigliatti, R. Williamson, D. T. Suzuki, *Genetics* **64**, 527 (1970).
47. D. T. Suzuki, D. Procunier, J. Cameron, J. Holden, *Proc. Int. Congr. Genet. 12th* (1968), vol. 1, p. 144.
→ D. T. Suzuki and D. Procunier, *Proc. Nat. Acad. Sci. U.S.* **62**, 369 (1969).
49. For a review, see J. R. S. Fincham, *Genetic Complementation* (Benjamin, New York, 1966).
50. A. M. Kapuler and H. Bernstein, *J. Mol. Biol.* **6**, 443 (1963); F. H. C. Crick and L. Orgel, *ibid.* **8**, 161 (1964).
51. J. D. Smith, personal communication; E. Galluci, G. Pachetti, S. Zangrossi, *Mol. Gen. Genet.* **106**, 362 (1970).
52. H. Bernstein and K. M. Fisher, *Genetics* **58**, 307 (1968).
53. A. Garen and S. Garen, *J. Mol. Biol.* **7**, 13 (1963).

54. J. R. Sadler and A. Novick, *ibid.* **12**, 305 (1965).
55. E. H. Y. Chu, as reported at the Annual Meeting of the Genetics Society of America, Madison, Wisconsin (1969).
→ L. H. Thompson, R. Mankovitz, R. N. Baker, J. E. Till, L. Siminovitch, G. F. Whitmore, *Proc. Nat. Acad. Sci. U.S.* **66**, 377 (1970).
57. P. D. Scotti, *Mutat. Res.* **6**, (1968).
58. R. H. Epstein, A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, A. Lielausis, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 375 (1963).
→ G. A. O'Donovan and J. L. Ingraham, *Proc. Nat. Acad. Sci. U.S.* **54**, 451 (1965); S. Condon and J. L. Ingraham, *J. Bacteriol.* **94**, 1970 (1967).
→ C. Guthrie, H. Nashimoto, M. Nomura, *Proc. Nat. Acad. Sci. U.S.* **63**, 384 (1969); P.-C. Tai, D. P. Kessler, J. Ingraham, *J. Bacteriol.* **97**, 1298 (1969).
61. R. Rosenbluth, H. Mayoh, D. Ezell, W. Willis, M. G. Somero, *Can. J. Genet. Cytol.* **12**, 394 (1970).
62. M. J. Whitten, personal communication; C. F. Wehrhahn, personal communication; R. H. Smith, personal communication.
63. The research discussed has been supported by the National Research Council of Canada (grant A-1764), the Atomic Energy Commission of the United States [contract AT (45-1)-1924], and the National Cancer Institute of Canada (grant 6051). The studies reported here were initiated by the energetic enthusiasm and efforts of Dr. Leonie K. Piternick and have been extended by a succession of people who invested a period of their lives in this work: Burton Ayles, Barbara Bahner, David Baillie, Paul Baumann, Jill Cameron, Diana Combes, Peter Duck, Udo Erasmus, Dean Ezell, Geoffrey Foster, Donna Gibson, Thomas Grigliatti, Susi Hayashi, Jeanette Holden, Richard Kiyomoto, Helen Mayoh, Rachel Pratt, Douglas Procunier, Raja Rosenbluth, Thomas Sanders, Michael Schewe, Lorne Shaw, Meredith Gould Somero, Elaine Tasaka, Mary Tarasoff, Rodney Williamson, William Willis, and Katherine Yang. Credit for any work of worth belongs to these individuals. I also thank Dr. John Lucchesi, Dr. Barry Keifer, and Mrs. Shirley Macaulay, who contributed their unique expertise to a part of these investigations.

Environmental Protection in the City of New York

Urban pollution control presents problems of great technical, legal, and political complexity.

Merril Eisenbud

The City of New York, by reason of its size, its geographic position in the midst of the world's most densely populated region, and decades of neglect, has been beset acutely with environmental problems. As has been generally true at all levels of government, a comprehensive approach to environmental protection had been handicapped in the past by traditional organizational separation of responsibilities, with inadequate coordination among the organizational units. To provide a unified approach, Mayor John V. Lindsay created the Environmental Protection Administration (EPA) in March 1968 to consolidate former administratively separate functions concerned with environmental hygiene. With its formation, EPA became responsible for street sanitation, water supply, water pollution control, air pollution, and noise abatement. It is an organization of more than 20,000 employees, with an annual operating budget of about $275 million, and a construction program of more than $2 billion during the next 5 years.

This article will deal with some of the pitfalls and successes of the program during its first 2 years of existence. Although no two communities are alike in all respects, the pollution problems of all cities do have many characteristics in common, and one generalization that can surely be made is that problems of urban pollution control present aspects of enormous legal, technical, sociological, and political complexities. No substantial progress can be made without huge expenditures of money and many years of sustained effort.

Air Pollution Control

The present active program of air pollution control began in the mid-1960's in response to widespread public interest. In 1965 Councilman Robert Low and the then mayoral candidate Lindsay began campaigns to strengthen the local laws governing air pollution control. A series of hearings before the City Council developed the first comprehensive report (*1*) of the problems of air pollution control in New York City, and early in 1966 a second report was published by a mayoral task force chaired by Norman Cousins (*2*). These two reports laid the groundwork for the energetic program developed by Commissioner Austin N. Heller, who headed the Department of Air Resources (*3*) from the late spring of 1966 until February 1970.

A new air pollution control law (Local Law 14) was passed by the City Council early in 1966 and mandated certain basic requirements among which were the following. (i) The sulfur content of all fuels burned in New York City would be limited to 1 percent by the 1969 to 1970 heating season. (ii) No incinerators could be installed in newly constructed buildings. (iii) All existing apartment house incinerators were to be shut down or upgraded according to a specified timetable. (iv) Emission controls were to be installed as soon as possible on all municipal incinerators. (v) All open burning of leaves, refuse, and building demolition materials would be banned within city limits.

The overall emissions of sulfur dioxide to the city's atmosphere were reduced by 56 percent by the end of 1969. This has been reflected by progressive reductions in the hourly peak concentration of SO_2 (Fig. 1). The annual maximum hourly concentration, which was 2.2 parts per million (ppm) in 1965, was reduced to 0.8 ppm by 1969, and further improvement has been observed in the early months of 1970.

Dust and soot are the most annoying form of air pollution in many cities. The sources of the particulate emissions in New York City are shown in Table 1, which indicates that space heating, municipal incineration, apartment house

The author is professor of environmental medicine at New York University Medical Center and has recently completed a 2-year leave from New York University to serve as the first administrator of the Environmental Protection Administration, City of New York.