GENETIC ANALYSIS OF TWO ALLELIC TEMPERATURE-SENSITIVE MUTANTS OF *DROSOPHILA MELANOGASTER* BOTH OF WHICH ARE ZYGOTIC AND MATERNAL-EFFECT LETHALS*

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ABSTRACT

After fertilization, the development of a zygote depends upon both gene products synthesized by its maternal parent and gene products synthesized by the zygote itself. To analyze genetically the relative contributions of these two sources of gene products, several laboratories have been isolating two classes of mutants of *Drosophila melanogaster*: maternal-effect lethals and zygotic lethals. This report concerns the analysis of two temperature-sensitive mutants, *OX736hs* and *PC025hs*, which were isolated as alleles of a small-disc mutant, *l(3)1902*. These alleles are not only zygotic lethals, but also maternal-effect lethals. They have temperature-sensitive periods during larval life and during oogenesis. Mutant larvae exposed continuously to restrictive temperature have small discs. One- or two-day exposures to the restrictive temperature administered during the third larval instar lead to a homeotic transformation of the midlegs and hindlegs to the pattern characteristic of the forelegs. Mutant females exposed to the restrictive temperature during oogenesis produce eggs that can develop until gastrulation, but do not hatch. ——The existence of these mutants, and one that was recently described by another group, implies that there may be a class of genes, heretofore unrecognized, whose products are synthesized during oogenesis, are essential for embryogenesis and are also synthesized during larval stages within imaginal disc cells.

THERE exists substantial evidence, in eukaryotes, that much of early development depends upon components in the egg cytoplasm that are under maternal gene control (reviewed by DAVIDSON 1976). To identify those genes in Drosophila that are involved in such maternal effects on development, several laboratories have isolated maternal-effect lethal mutants (reviewed by KING and MOHLER 1975). These mutants are a subset of female-sterile mutants defined by the property that homozygous mutant females produce eggs that can be fertilized and begin development but die before eclosion (RICE and GAREN 1975).

The genes identified by these mutations act only during oogenesis. If there are additional genes whose products are synthesized during oogenesis and have a specific maternal effect, but are also essential later in the development of the zygote, then such genes will not be represented among mutants isolated as

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maternal-effect lethals. This is a consequence of the method required to isolate female-sterile mutants (Figure 1). In cross III of the generalized protocol shown in Figure 1, zygotic lethal mutations do not produce homozygotes that can be tested in cross IV for female sterility. Any of these lethal mutations of genes that are also required during oogenesis will not be recognized as such. There are at least two ways to avoid this failure to identify an entire class of genes. One way is to transplant ovaries from zygotic lethal larvae into normal hosts and test for development of progeny derived from these mutant ovaries. Another way is to screen temperature-sensitive zygotic lethals raised to the adult stage at permissive temperatures for female sterility at restrictive temperatures. ARKING (1975)

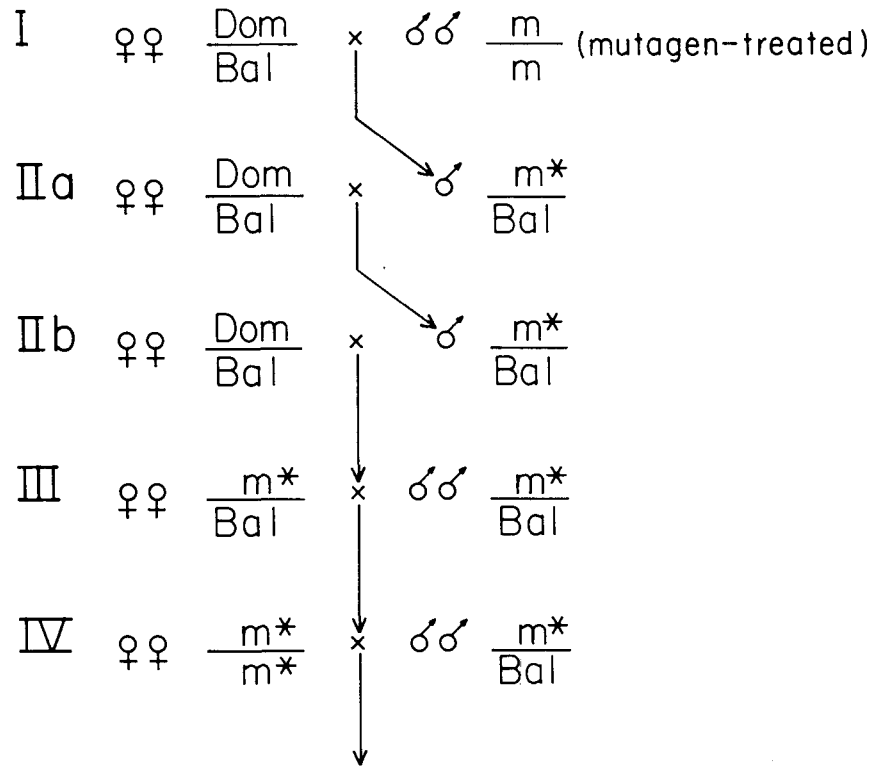

FIGURE 1.—Generalized scheme for isolating female-sterile mutations. Cross I: Mutagen-treated males homozygous for a recessive marker (*m*) are mated to females heterozygous for a dominant marker (*Dom*) and a balancer chromosome (*Bal*). Cross IIa: Male progeny of the first cross that are heterozygous for the balancer are mated individually with females from the balancer stock (The asterisk indicates mutagenized chromosomes). Cross IIb: To prevent the recovery of mutational mosaics, only one male from each of the IIa crosses is used to mate again with females from the balancer stock. Cross III: Males and females heterozygous for the balancer and an isogenic mutagenized chromosome are crossed to each other. If a lethal mutation is present on the marked chromosome, then no adult progeny, homozygous for the marker, will result from this cross. Cross IV: If no lethal is present, then female homozygotes can be tested for fertility by mating to heterozygous males. The absence of progeny from such a cross indicates the presence of a recessive female-sterile mutation.

found that 15 of 20 *X*-chromosome temperature-sensitive cell lethal mutants tested in this way are, indeed, also female sterile. None of these, however, is an apparent maternal-effect lethal (KING and MOHLER 1975). FAUSTO-STERLING, WEINER and DIGAN (1977) have recently described the first temperature-sensitive zygotic lethal that is also a maternal-effect lethal.

The mutants that are the subject of this study were isolated as part of a project, which is described in the preceding report (SHEARN *et al.* 1978) to isolate alleles of five small-disc mutants. The small-disc mutants are a heterogeneous group sharing the properties that their imaginal discs are smaller than normal discs and do not differentiate into characteristic adult structures when injected into metamorphosing larval hosts (SHEARN and GAREN 1974). In some of these mutants, the primary defect is in the imaginal disc cells themselves; in others the primary defect is in nonimaginal cells, which leads to secondary imaginal disc defects; in the remaining mutants the imaginal disc phenotype might be the product of both primary and secondary defects. The mutants described here are alleles of *l(3)1902*, which is in the first category; *l(3)1902* has an autonomous imaginal disc defect and no apparent larval defect (SHEARN *et al.* 1978).

We originally had two purposes for isolating temperature-sensitive alleles of small-disc mutants: (1) to determine temperature-sensitive periods (TSP), and (2) to provide a pure source of homozygous mutant larvae that could be used for biochemical studies. We fulfill the first objective in this study; biochemical studies are in progress.

Once isolated, these temperature-sensitive alleles provided an opportunity to test whether the *l(3)1902*$^+$ gene product is essential during oogenesis. We present evidence that these temperature-sensitive alleles are indeed maternal-effect lethals with a temperature-sensitive period during oogenesis. Although many zygotic mutants of Drosophila are also female-sterile (KING and MOHLER 1975), these two alleles and the *X*-chromosome mutation isolated by FAUSTO-STERLING, WEINER and DIGAN (1977) are the only ones that are both zygotic and maternal-effect lethals.

MATERIALS AND METHODS

Stocks: The heat-sensitive mutations *l(3)1902*^{OX736hs} (abbreviated OX736hs) and *l(3)1902*^{PC025hs} (abbreviated *PC025hs*) were induced by ethyl methanesulfonate treatment of male flies carrying third chromosomes marked with red Malpighian tubules (*red*, 3–53.6). They were identified by their failure to complement *l(3)1902* (3–30.9 ± 0.7) using the scheme described by SHEARN *et al.* (1978). All of the alleles of *l(3)1902* considered in this report are marked with *red*, including *l(3)1902* itself, which is also marked with multiple wing hairs (mwh, 3–0.0) and ebony (*e*, 3–70.7). They are all maintained as balanced lethal stocks using *TM3* that is marked with Stubble (*Sb*, 3–58.2), *e*, and Serrate (*Ser*, 3–92.5). For a description of the markers and balancers used, see LINDSLEY and GRELL (1968). All stocks and crosses were maintained in shell vials on a medium of cornmeal, molasses, yeast and agar at 20° unless stated otherwise.

Incubation at 29°: The restrictive temperature for *OX736hs* and *PC025hs* is 29°. In our experience, ordinary refrigerated incubators are not adequate for raising flies at this temperature because it is too close to the upper limit tolerated by nonmutant *Drosophila melanogaster*. We had instead modified several water-baths to serve as 29° incubators. A sheet of Plexiglass

was drilled with holes slightly larger than the diameter of our vials and firmly attached to the top of each water bath. The baths were filled with a 0.13% aqueous solution of zephiran chloride to a level just below the Plexiglass sheet. Tight-fitting rubber rings were placed near the top of each vial to allow them to be suspended in the water without slipping through the holes. Rubber stoppers were then placed in each of the unused holes. The water bath cover was placed over the Plexiglass sheet to maintain a high relative humidity in the vials.

Complementation: Females (3–5) heterozygous for *OX736hs* or *PC025hs* were mated to males (3–5) heterozygous for each of the other *l(3)1902* alleles. After several days at 20°, each set of parents was transferred to 29°. The relative viability of hybrid progeny was calculated by dividing the number of hybrids by one-half the number of heterozygotes. Statistical analysis of the data was performed using the *G* test of contingency.

Temperature shifts to determine the zygotic TSP: For shifts from permissive to restrictive temperature, eggs were collected for 8–16 hours at 20° from the cross of females heterozygous for *l(3)1902* by males heterozygous for *OX736hs*. After incubation for one to twelve days at 20°, cultures were shifted to 29°. The shifts from restrictive to permissive temperature were analogous.

Restrictive temperature exposures during the larval period: Eggs were collected for two to four hours at 20° from the heterozygous stock, *OX736hs/TM3*. After a varying number of days at 20°, cultures were exposed to the restrictive temperature (29°) for one to five days and then returned to a permissive temperature (20°). Eclosed flies were recovered only after one- or two-day exposures. These flies were examined under a dissecting microscope for morphological abnormalities.

Larval ovary transplantation: Ovaries dissected from third-instar mutant (*mwh l(3)1902 red e*) or control (*red*) female larvae were injected into female larvae homozygous for the heat-sensitive, female-sterile mutation, $fs(3)L8^{ts}$, using a procedure similar to that for injecting imaginal discs (URSPRUNG 1967). The $fs(3)L8^{ts}$ mutant was kindly supplied to us by JOHN POSTLETHWAIT. Host females that develop at 29° without transplanted ovaries do not lay eggs. Hosts [$fs(3)L8^{ts}$] that eclosed after transplantation of a donor ovary were mated at 29° to males with the genotype *DTS-2 Gl/TM3, red e Ser*. [DTS-2 (3–33.4) is a dominant heat-sensitive mutant isolated by HOLDEN and SUZUKI (1973); Glued (*Gl*, 3–41.4).] Progeny derived from transplanted ovaries are recognizable by their *red* phenotype. No progeny derived from the host's ovaries were ever recovered at 29°.

Temperature shifts to determine the maternal-effect TSP: For shifts from permissive to restrictive temperature, *OX736hs* homozygotes were raised at 20° until they began to lay eggs and then were transferred to 29° for further egg laying. For shifts from restrictive to permissive temperature, *OX736hs* homozygotes were raised at 20° until puparium formation (to avoid the zygotic TSP), transferred to 29° until they began to lay eggs and then shifted back to 20° for further egg laying. After these shifts, eggs were collected twice a day for two to four hours at the shifted temperatures; eggs collected from females at either temperature were incubated at 20°C and examined for further development.

RESULTS

Zygotic lethality: We have isolated 29 alleles of *l(3)1902* from 39,694 mutagenized chromosomes. Two of these, (*OX736hs* and *PC025hs*) are temperature sensitive. Based on the data of BAILLIE, SUZUKI and TARASOFF (1968) this number of temperature-sensitive mutants is not significantly different from the expected number according to the *G* test ($P > 0.9$). Most of the experiments reported here involve one of these two temperature-sensitive alleles, *OX736hs*, which is homozygous viable and fertile at 20°, a permissive temperature. At 29°, however, mutant homozygotes die after puparium formation. Imaginal discs dissected from mature, third-instar larvae of *OX736hs* homozygotes are signifi-

TABLE 1

Sizes of imaginal discs from mutant and normal mature third-instar larvae

Genotype	Area of wing discs* ($mm^2 \times 10^3$)	
mwh red e	179 ± 7 (4)	
		$t = 11$†; $P < 0.01$
OX736hs	67 ± 20 (8)	
		$t = 8.9$†; $P < 0.01$
l(3)1902	15 ± 4 (16)	

* Product of length and width (± standard deviation) measured with ocular micrometer (SHEARN *et al.* 1978); number of discs measured is in parentheses.

† The significance of the difference between the means was calculated according to the *t* test.

cantly smaller than normal discs, but significantly larger than discs from the reference mutant, *l(3)1902* (Table 1).

Complementation with 1(3)1902 *alleles:* Both temperature-sensitive alleles were tested for complementation with all of the other alleles at both permissive and restrictive temperatures. At 29°, the restrictive temperature, no hybrids eclosed from any of the crosses. However, at 20°, the permissive temperature, three different patterns of results were obtained (Table 2). Ten of the alleles (Group I) complement both *OX736hs* and *PC025hs* fully, like *l(3)1902* does. Eleven of the alleles (Group II) do not complement either *OX736hs* or *PC025hs* to the same extent as does *l(3)1902*. Seven of the nine alleles in Group III (*MK436*, *OR030*, *OA001*, *MZ1007*, *NV931*, *OT502*, and *NH006*) complement *OX736hs* to a significantly different extent than they do *PC025hs*. The lack of complementation of some pairs at 20° might be considered negative complementation.

Zygotic temperature-sensitive period: The approximate TSP of *OX736hs* and *PC025hs* was measured by a series of reciprocal temperature shifts. The results for both mutants were similar; only the results with *OX736hs* are presented in Figure 2. Males heterozygous for *OX736hs* were mated to females heterozygous for *l(3)1902*. This cross produces three relevant genotypic classes: mutant hybrids and two kinds of mutant heterozygotes. The viability of the two kinds of heterozygotes is indistinguishable in crosses maintained continuously at 20° ($\chi^2 = 1.7$; $P > 0.10$) or at 29° ($\chi^2 = 1.2$; $P > 0.10$). Therefore, after any temperature shift, the relative viability of the mutant hybrids may be calculated by dividing the number of hybrids by one-half the number of heterozygotes. Figure 2 shows that the TSP begins prior to hatching and does not end until the beginning of pupation. Thus, it extends throughout the developmental period during which imaginal disc cells proliferate in nonmutant individuals.

Since the TSP covers about half of the life cycle, we exposed the progeny of the *OX736hs/TM3* heterozygous stock to 29° for one to five days during develop-

TABLE 2

Complementation of heat-sensitive alleles with all other mutant alleles of l(3)1902 *at 20°, a permissive temperature*

Group*	Allele	*OX736hs* Number of hybrids	*OX736hs* Relative viability (%)	*PC025hs* Number of hybrids	*PC025hs* Relative viability (%)
I	*l(3)1902*	114	99	64	>100
	NY721	81	>100	62	>100
	PI1517	25	>100	23	>100
	PK911	23	>100	25	>100
	PQ1129	36	>100	12	96
	OW105	53	91	84	>100
	OS628	64	84	67	>100
	OU511	72	82	57	>100
	MM130	55	72	68	>100
	PB901	31	72	26	>100
II	*MR127*	50	63	54	79
	MY939	62	67	48	74
	OD522	33	54	48	76
	NW522	2	2.5	6	24
	OZ440	0	<2.2	0	<6.3
	OQ626	0	<1.3	0	<4.1
	OZ1340	0	<1.6	0	<1.8
	OP813	0	<1.8	0	<1.3
	MM634	0	<1.6	0	<1.3
	NU808	0	<1.3	0	<1.4
	MM701	0	<1.3	0	<1.3
III	*OX736hs*	213	100	69	66
	PC025hs	69	66	165	>100
	MK436	41	39	81	95
	OR030	20	30	44	>100
	OA001	77	30	27	74
	MZ1007	36	37	63	64
	NV931	14	19	54	67
	OT502	7	9.8	40	65
	NH006	3	8.1	53	>100

* Each allele in groups I and II shows no significant difference in its complementation with either heat-sensitive allele. The complementation of alleles in group I with the two heat-sensitive alleles is not significantly different from the complementation of the reference mutation, *l(3)1902*, with either of these two alleles. The complementation of the alleles in group II is significantly different from the complementation of *l(3)1902*. Each allele in group III does show a significant difference in its complementation with the two heat-sensitive alleles.

ment to determine the shortest exposure to restrictive temperature that causes lethality. We found that one-day exposures during the third larval instar cause a significant reduction in viability (Table 3) and delay eclosion. However, individuals that do not eclose survive until the pharate adult stage. Leaky or hypomorphic alleles of *l(3)1902*, such as *NH006*, also allow development until the pharate adult stage (SHEARN *et al.* 1978).

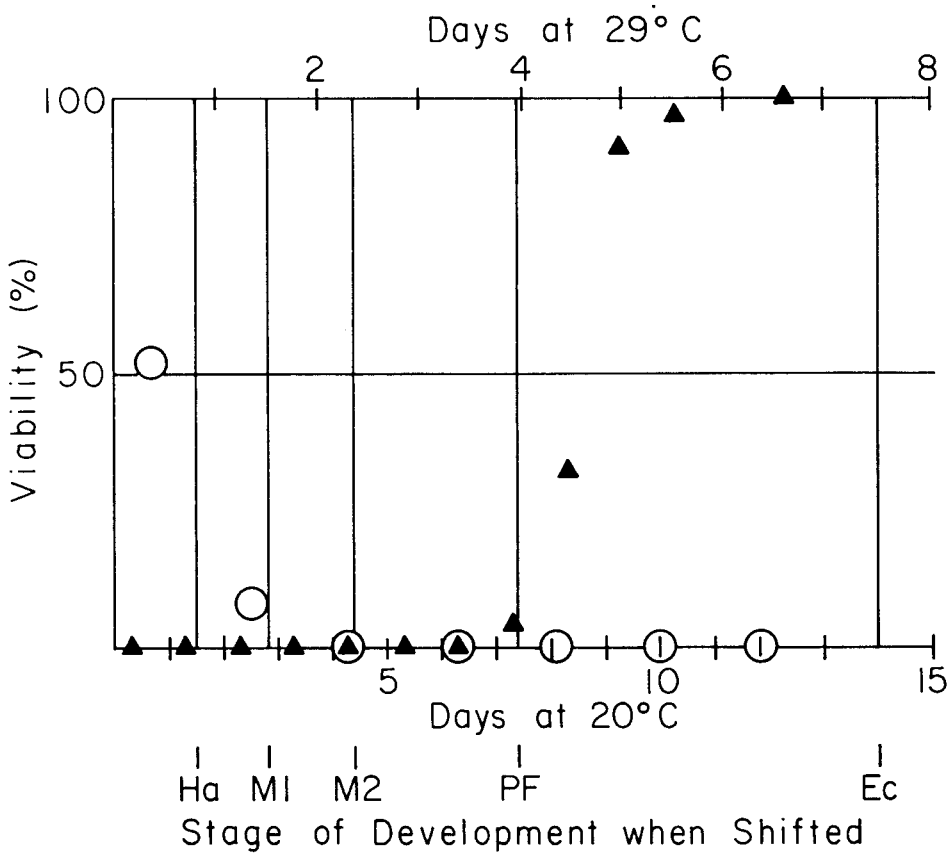

FIGURE 2.—Effect of temperature shifts on viability of *OX736hs* homozygotes. Progeny from the heterozygous stock were shifted at the indicated times either from permissive to restrictive temperature (▲) or from restrictive to permissive temperature (○). The viability was calculated as the number of eclosed homozygotes divided by one-half the number of eclosed heterozygotes. Abbreviations: Ha, hatching; M1, first larval molt; M2 second larval molt; PF, puparium formation; Ec, eclosion.

Homeotic transformation after exposure to restrictive temperature: All of the progeny that eclose after a one-day exposure from days five to six or after two-day exposures from days three to five or days four to six manifest a homeotic transformation of the midlegs and hindlegs to forelegs (Figure 3). This transformation resembles the phenotype of the recessive mutant, extra sex combs (*esc*, 2–54.9), and the dominant mutants *Polycomb* (*Pc*, 3–41), Multiple sex combs (*Msc*, 3–48) and Extra sex combs (*Scx*, 3–48), except that the expressivity in such *OX736hs* homozygotes is greater and the penetrance is 100%. Detailed examination of these transformed legs reveals that they retain aspects of their own pattern, while incorporating aspects of the foreleg pattern. For example, transformed midlegs and hindlegs have transverse rows of bristles on the tibia and first tarsal segment and sex combs characteristic of male forelegs.

TABLE 3

Effect on OX736hs *viability of one-day exposures to restrictive temperature at varying stages of development*

Day of exposure	Number of homozygotes	Relative viability (%)*
0–1	235	82.6
1–2	322	96.6
2–3	188	113
3–4	183	106
4–5	111	71.4†
5–6	98	70.3†
6–7	229	71.3‡
7–8	50	71.9
8–9	100	113
20° continuous	213	99.8
29° continuous	0	<0.162

* Compared to control grown continuously at 20°.
† Significantly different from control according to G test ($P<0.05$).
‡ Significantly different from control according to G test ($P<0.01$).

The midlegs retain their characteristic apical bristles and spurs on the tibia; the hindlegs retain their characteristic transverse rows of bristles on the first and second tarsal segments. As a result, the first tarsal segment of the transformed hindleg has two sets of transverse rows, a posterior set of 10 transverse rows and a ventral set of three to five transverse rows of bristles.

Ovary transplantation: We observed the maternal effect of mutations at the *l(3)1902* locus both by ovary transplantations and by use of temperature-sensitive alleles. Ovaries from homozygous *l(3)1902* larvae were transplanted into larvae homozygous for an unrelated female-sterile mutation, *fs(3)L8^{ts}*. Host females that eclosed were mated to genetically marked males. For a control, normal ovaries from genetically marked larvae were transplanted into female-sterile hosts (Table 4). None of the eggs derived from the 91 females that received mutant ovaries hatched. By contrast, 23 of the females that received normal ovaries produced progeny derived from the donor ovaries.

Temperature shifts during oogenesis: To test *OX736hs* for a maternal effect, homozygous larvae raised at 20° were shifted to 29° beginning at puparium formation. This avoided the zygotic TSP and allowed oogenesis to proceed at a restrictive temperature. Eggs collected for two to four hours from such heat-treated females were transferred to 20° to allow further development. Such eggs develop normally through gastrulation, but die before hatching. Presumably, they die because of a defect in larval organogenesis. To examine this point in detail we plan a histological study of defective embryos.

During the egg-collection period, the embryos were briefly exposed to 29°. We do not believe that this exposure was responsible for the lethality of these embryos because more than 50% of homozygous embryos, derived from heterozygous parents raised at 29°, are viable after exposure to 29° from 0–24 hours

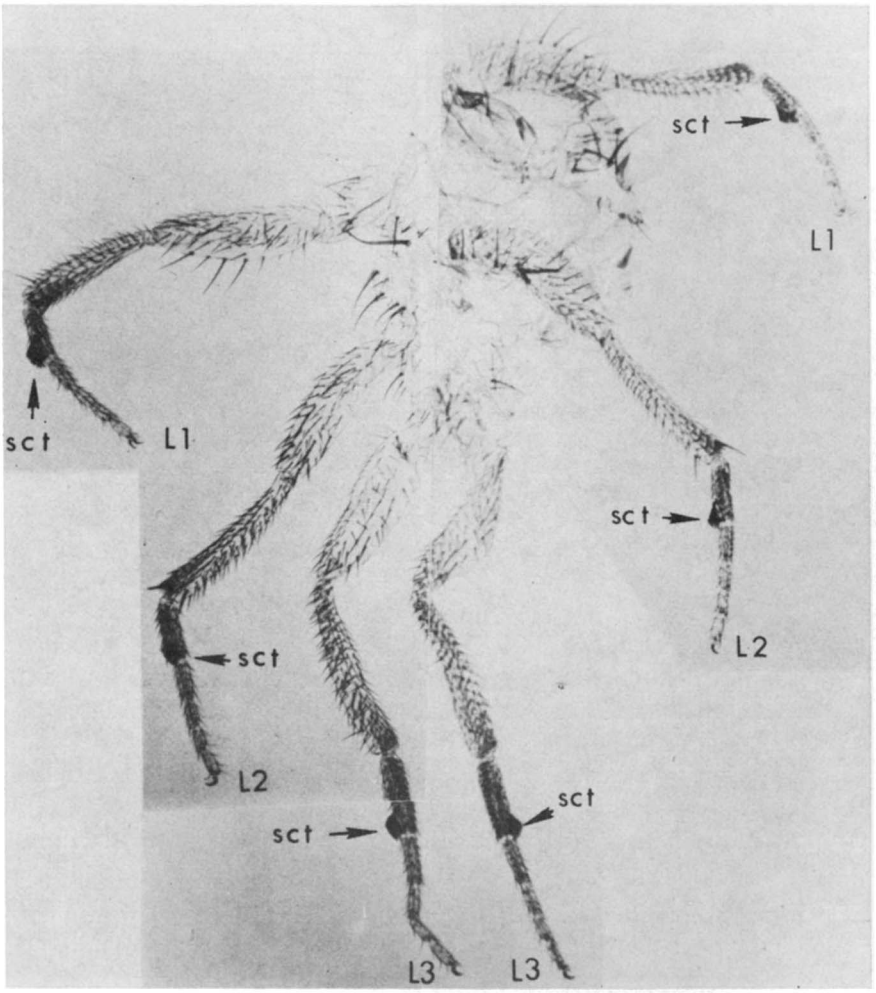

FIGURE 3.—Ventral thorax of *OX736hs* homozygote exposed to 29° for 48 hr from the fourth to the sixth day of development after oviposition. This figure is a composite of three photographs of a single specimen. Although sex combs (sct) are present on all six legs, the midlegs (L2) can be distinguished from the forelegs (L1) by the presence of an apical bristle on the tibia. The hindlegs (L3) can be distinguished by the presence of transverse rows of bristles on the second tarsal segment. Magnification is 40×.

TABLE 4

Transplantation of larval ovaries into female-sterile, fs(3)L8^{ts}, *host larvae*

Donor genotype	Number of hosts injected	Number of hosts recovered	Number producing progeny	Genotype of gametes
red	179	63	23	*red*
mwh l(3)1902 red e	150	91	0*	—

* The results are significantly different from the control ($G = 31.7$; $P << 0.01$).

of development (Figure 2). To show that the maternal effect depends on the genotype of the embryo's mother, but not on the embryo's own genotype, heat-treated homozygous *OX736hs* females were mated to nonmutant males. In this case all of the embryos were heterozygous; nevertheless, they died before hatching.

It is unlikely that the maternal effect could be due to a second mutation, since both *l(3)1902* and *OX736hs*, which are of independent origin, express such an effect. Nevertheless, we examined this possibility further by testing hybrids of *OX736hs* with three different lethal alleles of *l(3)1902* for the maternal effect. All three hybrids express the maternal effect. Therefore, we conclude that a single temperature-sensitive mutation is responsible for the zygotic lethality and maternal-effect lethality of *OX736hs*.

Temperature-sensitive period: Beginning on the first day after a shift from 29° to 20°, some of the eggs collected from *OX736hs* females will develop into morphologically normal progeny. Beginning on the first day after a shift from 20° to 29°, the proportion of eggs collected from *OX736hs* females that develop into adult progeny decreases.

DISCUSSION

Complementation: Both *OX736hs* and *PC025hs* were recognized as heat-sensitive mutants because hybrids of either of them with *l(3)1902* were viable at 20°, but lethal at 29°. Although hybrids of these temperature-sensitive alleles with all other mutant alleles of *l(3)1902* are also lethal at 29°, they are not all viable at 20° (Table 2). It is unlikely that the explanation for these results at 20° depends on quantitative differences in the residual amount of *l(3)1902*$^+$ activity associated with the lethal alleles, because that would not be consistent with the phenotypic differences among the alleles. For example, *l(3)1902* expresses the most extreme phenotype, and hybrids of it with *OX736hs* have 99% viability, while *NH006* expresses the least extreme phenotype (presumably because it is the "leakiest" allele) and hybrids of it with *OX736hs* have a relative viability of only 8.1%. A more consistent interpretation postulates that the active *l(3)1902*$^+$ gene product is a multimeric protein with identical subunits. Most of the alleles, according to this interpretation, are missense mutations leading to a reduction or elimination of multimer activity. Hybrid multimers with subunits from one of the heat-sensitive alleles and from one of the lethal alleles may be active or inactive depending on the specific interaction between the subunits. This interpretation, if correct, should allow us to identify which of the alleles are missense mutations. A corollary of this interpretation is that *OX736hs* and *PC025hs* subunits have different amino acid substitutions because their patterns of complementation are not identical (Table 2).

Homeotic transformation of the leg: We have now found homeotic defects in temperature-sensitive mutations of two different genes, neither of which was initially identified as a "homeotic" gene (this study and MARTIN, MARTIN and SHEARN 1977). The distinction between homeotic genes and other genes may be arbitrary rather than of fundamental significance. This leads us to suspect that

many of the nonlethal homeotic mutations may be leaky alleles of genes with a less specialized function than the control of imaginal disc determination. We believe that *l(3)1902*$^+$ functions primarily in disc proliferation, not in leg disc determination. However, exposures to restrictive temperature during the third instar interfere with the proliferation of *OX736hs* leg discs in such a way that these discs respond by manifesting a particular homeotic transformation, which can also be caused by mutations in at least three or four other genes. According to this view, the normal function of *l(3)1902*$^+$ is not to prevent the midlegs and hindlegs from forming the foreleg pattern. This view, that homeotic transformations originate from causes like those that stimulate transdetermination, has been expressed earlier by OUWENEEL (1969, 1970).

TSP of maternal effect: It is not yet possible to define precisely the stage or stages of oogenesis that are heat sensitive in *OX736hs* homozygous females, because the length of time required for each of the stages during normal oogenesis has been described only for development at 25° (KING 1970), and because we do not know the effect of temperature shifts on the rate of normal oogenesis.

Maternal-effect lethality: Our analysis of *l(3)1902* (SHEARN *et al.* 1978) led us to conclude that the *l(3)1902*$^+$ gene product is essential only for imaginal disc development and that this mutation causes the null-activity phenotype. However, transplantation of *l(3)1902* ovaries and analysis of temperature-sensitive alleles now leads us to conclude that the *l(3)1902*$^+$ gene product is also essential for normal embryogenesis. (We do not yet know whether or not this product normally functions directly in the embryo.) The fact that *l(3)1902* homozygotes derived from heterozygous parents are viable until puparium formation, while heterozygotes derived from heat-treated *OX736hs* homozygotes do not hatch, can be explained by postulating that during normal development the embryonic requirement is met by the *l(3)1902*$^+$ gene product synthesized during oogenesis and that the imaginal disc requirement is met by synthesis of the product in the imaginal disc cells themselves. Since homozygous *l(3)1902* embryos can be produced only by heterozygous (and therefore nonmutant) mothers, they hatch normally. This interpretation of the late zygotic lethality of *l(3)1902* is analogous to that for the anucleolate mutant of *Xenopus laevis* (BROWN and GURDON 1964). The anucleolate mutation is a deletion of ribosomal RNA genes. Homozygous progeny from heterozygous parents survive until the tailbud stage, even though they are incapable of synthesizing ribosomal RNA. The need for this gene product at earlier developmental stages is apparently met by the supply placed in the oocyte by the maternal parent. The embryo dies when this maternally derived supply becomes inadequate.

Number and specificity of maternal-effect lethals: The results of culturing cells *in vivo* from dissociated embryo halves (CHAN and GEHRING 1971) and of ligating embryos (SCHUBIGER 1976) both demonstrate that at the blastoderm stage, when cells are first formed, the process of determination has already begun. However, prior to the blastoderm stage, embryos synthesize relatively little RNA (ZALOKAR 1976; MCKNIGHT and MILLER 1976). Thus many of the gene products involved in the determination process at blastoderm formation

might be derived from genes that are transcribed during oogenesis. To identify such genes, maternal-effect lethals have been isolated and their developmental defects have been described (BAKKEN 1973; RICE and GAREN 1975; GANS, AUDIT and MASSON 1975; ZALOKAR, AUDIT and ERK 1975; MOHLER 1977). However, the range of defects observed is much smaller than was expected (RICE 1973; ZALOKAR, AUDIT and ERK 1975). Moreover, we estimate that there are only 163 ± 77 genes in the entire Drosophila genome that could give rise to maternal-effect lethal mutations of the type isolated by these groups. Our number is based on the estimate of GANS, AUDIT and MASSON (1975) that there are 142 ± 67 genes on the *X* chromosome that can mutate to yield a female-sterile mutation, that 23% of these (12/52) may be maternal-effect lethals (ZALOKAR, AUDIT and ERK 1975), and that the *X* chromosome comprises about 20% of the genome. This estimate assumes that such genes are randomly distributed throughout the genome.

Other genes with maternal effects: The mutations analyzed in this study, plus the one described by FAUSTO-STERLING, WEINER and DIGAN (1977), may represent an entire class of genes not previously recognized: namely, those coding for products synthesized during oogenesis for use during embryogenesis, and again later in development for use during imaginal disc development. If so, the absence of these genes from the previous estimate could account for its low number. Moreover, the maternal-effect phenotypes caused by mutations in such hypothetical genes might show a greater range than found among those maternal-effect lethals already studied. Finally, the existence of such genes would be consistent with recent results concerning the sequence complexity of RNA during sea urchin development. GALAU *et al.* (1976) have shown that the complexity of oocyte RNA is much greater than that of polysomal RNA extracted from embryos of several different stages. Moreover, all of the sequences detected, for example, in gastrulae are included among those detected in oocytes. Those results, if they are relevant to Drosophila, predict the existence of genes like *l(3)1902*$^{+}$, which are transcribed during oogenesis and also transcribed at one or more stages during zygotic development.

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