P Element-Induced, X-Linked Lethal Mutations Causing

Melanotic Tumors in Drosophila melanogaster

by

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CORRIGENDUM

The cytological map position of air8 as displayed in
Figure 3B (page 40) should be extended to include 7C9
(ie. 7C4-6; 7C9)

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS/DESCRIPTIONS

A	Adults
AM	Anterior midgut
air	Aberrant immune response mutation
Binsn	X-chromosome balancer
BH (Bh)	Brain hemispheres
DV	Dorsal vessel
EL	Early larvae, 1st and 2nd instar
EP	Early pupae
Eye/an	Eye-antennal imaginal disc
FM7	X-chromosome balancer
Gar	Garland cells
Gc	Gastric caeca
ID	Imaginal discs
In	Intestine
LG	Lymph gland lobes
LL	Late larvae, 3rd instar
M cytotype	Permissive cellular environment for P elements
M strain	Drosophila strain devoid of intact P elements
P	Proventriculus
PC	Pericardial cells
P cytotype	Restrictive cellular environment for P elements
Psp	Pseudopupae, combination of larval and pupal characteristics
P strain	Drosophila strain containing intact P elements
RG	Ring gland (corpus allatum, prothoracic gland, corpus cardiacum)
Tum^1	Tumorous-lethal, X-linked melanotic tumor mutation
$tu(1)Sz^{ts}$	Tumor of Suzuki, X-linked melanotic tumor mutation
tu-W	Tumorous-W, autosomal melanotic tumor mutation
VG (Vg)	Ventral ganglion
w^1	White-1, X-linked marker mutation
y^1	Yellow-1, X-linked marker mutation

LITERATURE SURVEY

P Element Mutagenesis

The ability to understand the role of a particular gene
or its product in the development and/or maintenance of an
individual is dependent on clearly defining all the
functions and interactions of that gene. One experimental
approach is to perturb or abolish the function of a gene
and examine what effect this action has on the ability of
that individual to complete proper development. Recently,
a technique known as P element mutagenesis was developed
for use in the genetically well-characterized organism,
Drosophila melanogaster. The mutagenesis technique
utilizes the transposable genetic elements called P
elements as mutators. This mutagenesis technique was
inspired by the syndrome of correlated genetic
alterations/defects including high mutation rates that is
known as P-M hybrid dysgenesis (ENGELS, 1983). Hybrid
dysgenesis results when Drosophila males obtained from
natural populations (P strains) are mated with laboratory-
reared (M strains) females (ENGELS, 1983). P strains
contain numerous copies of the genetic element P, dispersed
among their chromosomes. In addition, P strains are
characterized by a restrictive cellular environment known
as P cytotype that is capable of repressing P element

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transposition (BINGHAM, KIDWELL and RUBIN, 1982; RUBIN, KIDWELL and BINGHAM, 1982). Pure M strain chromosomes are devoid of P elements and these strains possess a permissive cellular environment termed M cytotype (ENGELS, 1983). P elements become activated through dysgenic crosses (P strain males X M strain females) which place P element-containing chromosomes in the permissive cellular environment (M cytotype) of the M strain egg (ENGELS, 1983; SIMMONS and BUCHOLZ, 1985). The active state is characterized by transposition events in the hybrid germline that include insertions, excisions, deletions and various chromosome rearrangements mediated by the mobilized P elements. The newly-induced germline mutations are transmitted to the progeny of the dysgenic flies. The insertion of a P element into a new chromosome location can perturb or abolish the function of a gene and in the process give rise to a mutant product (LEVIS, O'HARE and RUBIN, 1984).

The transposition of P elements can occur by either a "replicative" or "conservative" mode (SIMMONS and KARESS, 1985). In the "replicative" mode which involves the transfer of sequence information, a replica of the resident element is made and inserted at a new site while the template element is retained at its original chromosome site. The alternative "conservative" mode would involve transfering the P element to a new chromosome location with

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the loss of the original element from its chromosome site. The total number of endogenous elements should not increase as a result of transposition events that are operating under conservative regulatory mechanisms. P elements have been demonstrated to rapidly spread through M cytotype populations supporting the replicative theory of transposition (KIDWELL, NOVY and FEELEY, 1981; KIDWELL and KIYASU, 1984).

P elements can exist in two general forms, complete or intact elements and incomplete or defective elements. The intact P element is 2907 base pairs (bp) with 31 bp terminal inverted repeats flanking the element (O'HARE and RUBIN, 1983). There are four open reading frames and collectively they form a protein product known as "transposase" that is required in trans to catalyze transposition events (KARESS and RUBIN, 1984; LASKI, RIO and RUBIN, 1986; RIO, LASKI and RUBIN, 1986). Defective elements usually contain variable internal deletions of the complete element but retain the terminal inverted repeats. These deletions prevent the element from making a functional transposase product but the element is still capable of transposing if supplied with functional transposase from another source (O'HARE and RUBIN, 1983; RUBIN and SPRADLING, 1982; SPRADLING and RUBIN, 1982). Pseudo-M strains exist which contain only defective P elements among their chromosomes. These pseudo-M strains

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are more refractory to transposition events under dysgenic conditions (KIDWELL, 1985). These observations support the theory that defective elements are capable of making a product that regulates the transposition of intact elements (ENGELS, 1986). A positive feedback regulator model originally proposed by O'HARE and RUBIN (1983) suggested that P elements are capable of supplying regulator molecules which suppress transposition and stimulate their own activity. In accordance with this type of model, the permissive cellular environment or M cytotype must lack functional regulator molecules, whereas P cytotype should contain functional regulator molecules.

In assessing whether P elements serve as effective mutators, a number of different P element features must be considered. P element insertions have some site specificity that may be regulated at the nucleotide level or some higher order of chromatin structure (O'HARE and RUBIN, 1983). Various researchers have reported "hotspots" (SIMMONS, et al. 1984) or "coldspots" (SIMMONS and LIM, 1980) for P element insertions under dysgenic conditions, suggesting that this type of mutagenesis would not be feasible for saturation experiments. Some obvious advantages of P element mutagenesis are that transposition is easily controlled with an appropriate mating regime, and newly-induced mutations can be stabilized by maintaining them in a strong P cytotype (ENGELS, 1983). The use of

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dysgenically-induced mutations facilitates the cloning of these genes by a technique known as "transposon tagging" (BINGHAM, LEVIS and RUBIN, 1981; BINGHAM, KIDWELL and RUBIN, 1982). P element-induced mutations are usually stabilized by maintaining the mutation in a P cytotype, thus preventing further transpositon events. The stabilization of these mutations also retains a P element "tag" or marker inserted at the gene of interest. P element probes are available (i.e. pπ25.1, O'HARE and RUBIN, 1983) and can be used to probe for homologous, recombinant clones containing the gene of interest from cloned libraries made using mutant stock DNA.

X-chromosome lethal mutations induced by P element transposition in males can occur at rates as high as 2.5% (ZUSMAN, 1985). As previously mentioned, dysgenic conditions cause a variety of different mutational events and this can be applied to studies concerned with examining how variability in the expression of a gene/product affects the mutant phenotype. In addition, P element-induced mutations can be reverted under dysgenic conditions at very high frequencies (i.e. 2.6-6.5%, VOELKER et al. 1984), with occasional imprecise excisions that create small deletions in the flanking DNA (DANIELS et al. 1985; VOELKER et al. 1984). All of the aforementioned features need to be considered when interpreting the characteristics exhibited by mutants induced by P elements.

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The Cellular Defense System of Drosophila

Drosophila possess an "open type" circulatory system
containing a single tubular dorsal vessel which spans the
larvae from the third thoracic segment through to the last
abdominal segment (RIZKI, 1978). The dorsal vessel is
divided anteriorly into the aorta (anterior to the brain-
ring gland complex), which lacks ostia, and posteriorly
into the heart (abdominal segments 4-5 through 8)(RIZKI,
1978). The lymph glands are the haematopoietic organs of
Drosophila and contain three precursor cell types: the
prohaemocytes, the proplasmatocytes and the procrystal
cells (GATEFF, 1984). The prohaemocytes differentiate into
proplasmatocytes which give rise to the group of haemocytes
that is normally released into the haemolymph late in the
third instar period (GATEFF, 1984). The cellular defense
responses in Drosophila are mediated by the haemocytes,
which can distinguish between "self" and "non-self" in
their phagocytic and encapsulation responses (CHADWICK,
1975; NAPPI, 1975, 1977). Encapsulation is defined as the
formation by haemocytes of a cellular envelope around
foreign objects (GOTZ, 1986). The three different
reactions classified as encapsulation responses are: 1)
nodule formation, involving haemocytes and small

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particulate material, 2) capsule formation (haemocytic

encapsulation), involving haemocytes and foreign objects

that exceed the size of haemocytes and 3) humoral

encapsulation, involving the formation of melanotic crusts

without the participation of haemocytes (GOTZ, 1986).

Haemocytes are not involved in the transport of gases to

and from internal tissues and are analagous to mammalian

leukocytes (NAPPI, 1975). A brief description of the terms

used to distinguish morphologically or functionally between

the haemocytes of Drosophila is presented in Table 1

(RIZKI, 1978; NAPPI, 1975, 1977).

The ability of an insect to respond when invaded by

foreign material requires that active immune-surveillance

(or -recognition) mechanisms must be operating in the

insect haemocoel. These mechanisms are capable of

triggering both cellular and humoral responses but the

controlling elements and many of the details remain

unresolved. The development of melanotic tumors typically

begins with the process of cellular encapsulation. The

encapsulation of foreign material that is too large to be

phagocytosed involves the aggregation, adhesion and

flattening of one haemocyte type (lamellocyte) in a

coordinated manner (NAPPI, 1975). Haemocytic encapsulation

has also been noted to be stimulated by large masses of

particulate matter (RIZKI, 1978). The rapid transformation

of rounded haemocytes (plasmatocytes) into long, spindle-

TABLE 1

Drosophila Haemocytes

A. <u>Plasmatocytes and variant forms</u>:
<u>(podocyte and lamellocyte)</u>

-constitutes the main bulk of haemocyte population
-phagocytic cells with adhesive cell surface properties
-7 to 12 micron diameter
-displays mitotic activity

<u>Podocytes</u>

-morphological variant of the plasmatocyte
-has filamentous or membranous extensions of the
 cytoplasmic surface
-a massive plasmatocyte-podocyte transformation usually
 occurs during late larval life
-may represent the transient intermediate in the
 differentiation of plasmatocytes to lamellocytes

<u>Lamellocytes</u>

-flattened, disc-like cells that form the walls of
 immune capsules
-30 to 60 micron diameter; thickness: 0.2 micron
-reported to initially appear at 67 h of development
 (late third instar)

B. <u>Crystal Cells</u>:

-large, non-phagocytic cells containing non-membrane
 bound paracrystalline inclusions
-cell cytoplasm contains many electron-dense, amorphous
 bodies
-represent 5-10% of larval haemocyte population
 (all stages)
-sole source of haemolymph phenoloxidases
-paracrystalline structure contains both phenoloxidase
 and tyrosine phenolic substrates
-comparable to coagulocytes, spherule cells, oenocytoids
 and cystocytes of other invertebrates

shaped lamellocytes and the lysis of other haemocytes
(crystal cells) occurs during capsule formation (RIZKI,
1978). Flattened lamellocytes surround the foreign
material and continue to enwrap the structure until a
multi-layered capsule is formed (RIZKI and RIZKI, 1986).
Capsule formation is then followed by the intra- and
extra-cellular deposition of the pigment melanin late in
the third instar stage of development. In some dipterans,
melanotic capsules are formed around foreign material
without the participation of haemocytes (i.e. haemolymph
lacking haemocytes will melanize) (GOTZ, 1986; JONES, 1975;
NAPPI, 1975; RIZKI, RIZKI and BELLOTI, 1985; VEY and GOTZ,
1986). This humoral encapsulation event (also called
melanosis) usually coincides with low haemocyte counts and
generates "protective" polyphenol-protein complexes (NAPPI
and CARTON, 1986).

Melanins are formed by the oxidation and polymerization
of phenols such as tyrosine and dopa using phenoloxidase
enzymes (NAPPI, 1975). Tyrosine and phenoloxidase have
both been localized to the paracrystalline inclusion bodies
of the crystal cell (NAPPI, 1975; RIZKI, RIZKI and GRELL,
1980; RIZKI, RIZKI and BELLOTI, 1985). The mechanism(s)
that regulates crystal cell deposition of melanin is
unclear, but there is evidence that supports the
involvement of ecdysteroid hormones (RIZKI, 1978; NAPPI,
1977). It has been demonstrated using the Drosophila

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mutant Black cells (Bc) that amelanotic immune capsules can be formed (RIZKI and RIZKI, 1986). This supports a multi-step encapsulation process in which haemocytic capsule formation is clearly separated from the reactions that form and deposit melanin. In addition, the activation of the precursor prophenoloxidase into its biologically-active form leads to the generation of melanin, parasite destruction, coagulation and opsonization in some invertebrates (GOTZ and VEY, 1986; ROWLEY et al. 1986; SODERHALL and SMITH, 1986; VEY and GOTZ, 1986). This may be due to the exocytotic release of crystal cell components that trigger other humoral and cellular responses (ROWLEY et al. 1986). Although invertebrates lack immunoglobulins and complement they are capable of recognizing and responding to foreignness through an integrated system of agglutinin-like molecules on haemocyte surfaces and humoral factors generated by the prophenoloxidase cascade.

Melanotic Tumor Mutants of Drosophila

The presence of melanotic tumors in Drosophila has been described by many investigators (NAPPI, 1977; GATEFF, 1978a; RIZKI, 1978; SPARROW, 1978; SILVERS and HANRATTY, 1984). Although some of the mutants have been commonly studied by these investigators, there seems to be little

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consensus as to the underlying cause(s) of the tumorous phenotype. There is general agreement about the involvement of haemocytes in the encapsulation event, which is then followed by melanin deposition. The specific details, of these two processes in each of the different tumorous mutants are difficult to discern due to significant investigator bias. Melanotic tumors are observed as single or multiple masses either free-floating in the haemocoele or attached to internal tissues (SPARROW, 1978). These melanotic masses appear only infrequently in wild type strains but are consistently found in the tumorous strains. The melanotic tumors generally appear during the third larval instar period in the tumorous strains, and some strains have tumors which persist to the adult stage (SPARROW, 1978).

The melanotic tumor phenotype is usually ascribed to a recessive mutation in one of a number of major genes which, when mutated, give the tumorous phenotype (SPARROW, 1978). There are many minor modifying genes which have the ability to alter the frequency of tumors (SPARROW, 1978). Incomplete penetrance and variable expressivity are phenotypic features associated with the known melanotic tumor mutants, presumably due to the modifier gene influence of the background genotype (SPARROW, 1978).

Studies using the melanotic tumor mutants of Drosophila have revealed two general classes of mutants based on the

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type of abnormality that initiates the defense response.
These two classes are represented by: (Class 1) mutants in
which abnormal tissue or cell surfaces trigger a specific
autoimmune response and (Class 2) mutants with invasive
neoplasms that non-specifically encapsulate tissues or
cells. One mutant tu-W, has been extensively studied by
Rizki and co-workers and is a typical example of Class 1
melanotic tumor mutants (RIZKI and RIZKI, 1974; RIZKI,
1957; RIZKI, 1978; RIZKI and RIZKI, 1979). Melanotic
tumors in tu-W are formed in response to abnormalities that
develop in the basement membrane of caudal adipose cells
(RIZKI and RIZKI, 1986). It was hypothesized that the
degeneration of the basement membrane exposes (or creates)
molecules which trigger autoimmune responses (RIZKI and
RIZKI, 1986).

In the tumorous-lethal (Tum[1]) mutant, neoplastic lymph
glands (haematopoietic organs) give rise to an
overproduction of precociously-transformed lamellocytes
which non-specifically encapsulate "self" tissues (HANRATTY
and RYERSE, 1981; NAPPI, KMIECIK and SILVERS, 1984; SILVERS
and HANRATTY, 1984). The Tum[1] mutation exhibits a dominant
precocious-transformation of haemocytes and a temperature-
sensitive, recessive lethality and dominant enhancement of
tumorigenesis (HANRATTY and RYERSE, 1981; NAPPI and CARTON,
1986; NAPPI, KMIECIK and SILVERS, 1984; SILVERS and
HANRATTY, 1984). The Tum[1] mutation is representative of

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the Class 2 type of tumor mutant, in which neoplastic
blood-forming organs cause an overproduction of haemocytes
that non-specifically encapsulate "self" tissue. It was
also demonstrated in this mutant that during infection by
live parasites the differentiation of plasmatocytes to
lamellocytes was inhibited and the host became more
susceptible to the lethal effects of parasitization (NAPPI
and CARTON, 1986; NAPPI, KMIECIK and SILVERS, 1984). The
inhibition was presumably due to the release of an immuno-
suppressive agent that prevented encapsulation and
melanization events from occurring in the parasitized host.
This demonstrated the important role of cellular
encapsulation reactions in the prevention of successful
parasitic infections in $\underline{Tum}^1$ larvae.

The $\underline{tu-Sz}^{ts}$ mutant also exhibits a dominant precocious-
transformation of plasmatocytes to lamellocytes and a
temperature-sensitive enhancement of melanotic tumor
formation (RIZKI and RIZKI, 1980a; RIZKI and RIZKI; 1980;
RIZKI and RIZKI, 1986). At the restrictive temperature
$(27^{o}C)$, the encapsulation responses are specific for the
abnormal caudal fat body cells of the mutant larvae (RIZKI
and RIZKI, 1986). Thus in this mutant, abnormal cellular
components are responsible for initiating the reactions
leading to melanotic tumor formation. A partial list of
some characteristics of the known X-linked melanotic tumor
mutants is presented in Table 2.

TABLE 2
X-Linked, Melanotic Tumor or Immune-Defective Mutants

Map Position--Mutant Name (Symbolic name, synonyms)
    Phenotype; References

0.3 / 1F1; 2A2--deep orange-lethal (dor[1], l(1)7)
    -males lethal at late 3rd to early pupal stage, abnormal
    gut, melanotic tumors most prevalent at 25°C, abnormal
    melanotic anal organ, haemocytes aggregate and melanize
    in caudal region.[a,b]

1.0 / 3A3--lethal(1)melanomalike (l(1)ml)
    -3rd instar lethal, internal melanotic masses.[c]

20.4--small tumoroid (stu)
    -viable, small adult, variable small pseudotumors.[c]

21.3--lethal(1)8 (l(1)8)
    -3rd instar lethal, extended 3rd instar (10 days),
    testes and lymph glands degenerate, imaginal discs
    maintain developmental capabilities, abnormal protein
    metabolism, high free amino acids and peptides.[c]

29.0--focal melanosis (me)
    -late pupal to early adult lethal, tibia/femur junction
    becomes melanized.[c]

29.0--pigmy (pig)
    -viable, small melanotic adult.[c]

34.3 / 10A10-11--tumor of Suzuki[ts] (tu(1)Sz[ts])
    -ts @ 26°C (tumors), melanotic tumors occur in
    heterozygous females at 26°C, precocious lamellocytes at
    both permissive and restrictive temperatures (dominant),
    abnormal caudal fat body.[d]

34.5--Tumorous-lethal (Tum[1])
    -3rd instar to early pupal temperature-sensitive,
    lethal, hypertrophy/hyperplasia of lymph glands, lymph
    glands detach from dorsal vessel, gastric caeca absent
    or reduced, internal melanotic masses, temperature-
    sensitive at 29°C, overproduction of circulating
    haemocytes at both permissive and restrictive
    temperatures.[e,f]

39.0--lethal(1)malignant blood neoplasm (l(1)mbn)
    -invasive, transplantable neoplasm, enlarged lymph
    glands, extensive melanization of larvae including
    caudal fat body and gut, precocious plasmatocyte-
    lamellocyte transformations occur.[a,g]

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41.0--tumor-53 (tu-53)
    -viable, low frequency of small melanotic tumors in
    adults, abnormal/delayed egg hatching, blistered/nicked
    wings in adults, knotted or shortened wing veins.[c]

58.7--tumorous (tms)
    -viable, adults slightly small and some with diffuse
    tumors.[c]

64.1--melanized (mel)
    -viable, dark adult body color, curled wing tips, dull
    red eye color.[c]

64.5--tumorous head in chromosome 1 (tuh-1)
    -weakly viable, has some maternal effect, asymmetrical
    growths in head region, asymmetrical eye-antennal disc
    observed in 32 hr. larvae.[c]

lethal(1)malignant (l(1)m)--not localized.
    -late 3rd to early pupal lethal, lymph gland cells
    destroy imaginal buds, posterior fat body and testes,
    tumor cells melanize after tissue damage has
    occurred.[a,c]

lethal(1)no differentiation (l(1)nd)--not localized.
    -prepupal to pupal lethal, imaginal buds fail to
    differentiate, imaginal disc mesoderm proliferates
    abnormally.[c]

lethal(1)no imaginal buds (l(1)nib)--not localized.
    -3rd instar lethal, small or absent imaginal buds,
    stomach epithelium excessively proliferates eventually
    occluding the gut, gut proliferations melanize.[c]

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a = SPARROW (1978), b = RIZKI (1978), c = LINDSLEY and
GRELL (1968), d = RIZKI and RIZKI (1980), e = NAPPI,
KMIECIK and SILVERS (1984), f = HANRATTY and RYERSE
'1981), g = GATEFF (1978b).

LITERATURE CITED

BINGHAM, P. M., R. LEVIS and G. M. RUBIN, 1981 Cloning of
    DNA sequences from the white locus of Drosophila
    melanogaster by a novel and general method. Cell 25:
    693-704.

BINGHAM, P. M., M. G. KIDWELL and G. M. RUBIN, 1982 The
    molecular basis of P-M hybrid dysgenesis: the role of
    the P element, a P strain-specific transposon family.
    Cell 29: 995-1004.

CHADWICK, J. S., 1975 Hemolymph Changes with Infection or
    Induced Immunity in Insects and Ticks, pp 241-271. In:
    Invertebrate Immunity, Mechanisms of Invertebrate
    Vector-Parasite Relations. Edited by K. Maramorosch & R.
    E. Shoppe. Academic Press, New York.

DANIELS, S. B., M. M^{C}CARRON, C. LOVE and A. CHOVNICK, 1985
    Dysgenesis-induced instability of rosy locus
    transformation in Drosophila melanogaster: analysis of
    excision events and the selective recovery of control
    element deletions. Genetics 109: 95-117.

ENGELS, W. R., 1983 The P family of transposable elements
    in Drosophila. Annu. Rev. Genet. 17: 315-344.

ENGELS, W. R., 1986 On the evolution and population
    genetics of hybrid-dysgenesis-causing transposable
    elements in Drosophila. Phil. Trans. R. Soc. Lond. B.
    312: 205-215.

16

GATEFF, E., 1978a Malignant and Benign Neoplasms of
   Drosophila melanogaster, pp 182-261. In: The Genetics
   and Biology of Drosophila, vol. 2B. Edited by M.
   Ashburner & T. R. F. Wright. Academic Press, New York.

GATEFF, E., 1978b The Genetics and Epigenetics of
   Neoplasms in Drosophila. Biol. Rev. 53: 123-168.

GATEFF, E., 1984 Comparative Ultrastructure of Wild-Type
   and Tumorous Cells of Drosophila, pp 559-578. In:
   Insect Ultrastructure, vol. 2. Edited by R. C. King & H.
   Akai. Plenum Publishers.

GOTZ, P., 1986 Encapsulation in Arthropods, pp 153-170.
   In: Immunity in Invertebrates. Edited by M. Brehelin.
   Springer-Verlag, Berlin.

GOTZ, P. and A. VEY, 1986 Humoral Encapsulation in
   Insects, pp 407-429. In: Hemocytic and Humoral Immunity
   in Arthropods. Edited by A. P. Gupta. John Wiley & Sons,
   New York.

HANRATTY, W. P. and J. S. RYERSE, 1981 A Genetic Melanotic
   Neoplasm of Drosophila melanogaster. Dev. Biol. 83:
   238-249.

JONES, J. C., 1975 Forms and Functions of Insect
   Hemocytes, pp 119-128. In: Invertebrate Immunity,
   Mechanisms of Invertebrate Vector-Parasite Relations.
   Edited by K. Maramorosch & R. E. Shoppe. Academic Press,
   New York.

KIDWELL, M. G., 1985 Hybrid dysgenesis in Drosophila

melanogaster: nature and inheritance of P element
regulation. Genetics 111: 337-350.

KIDWELL, M. G. and P. K. KIYASU, 1984 Hybrid dysgenesis in
Drosophila melanogaster: the evolution of mixed P and M
populations maintained at high temperature. Genet. Res.
44: 251-259.

KIDWELL, M. G., J. B. NOVY and S. M. FEELEY, 1981 Rapid
unidirectional change of hybrid dysgenesis potential in
Drosophila. J. Hered. 72: 32-38.

KARESS, R. E. and G. M. RUBIN, 1984 Analysis of P
Transposable Element Functions in Drosophila. Cell 38:
135-146.

LASKI, F. A., D. C. RIO and G. M. RUBIN, 1986 Tissue
specificity of Drosophila P element transposition is
regulated at the level of mRNA splicing. Cell 44: 7-19.

LEVIS, R., K. O'HARE and G. M. RUBIN, 1984 Effects of
transposable element insertions on RNA encoded by the
white gene of Drosophila. Cell 38: 471-481.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic Variations
of Drosophila melanogaster. Carnegie Inst. Washington
Publ. 627.

NAPPI, A. J., 1975 Parasite Encapsulation in Insects, pp
293-326. In: Invertebrate Immunity, Mechanisms of
Invertebrate Vector-Parasite Relations. Edited by K.
Maramorosch & R. E. Shoppe. Academic Press, New York.

NAPPI, A. J., 1977 Comparative Ultrastructural Studies of

Cellular Immune Reactions and Tumorigenesis in
Drosophila, pp 155-188. In: Comparative Pathobiology,
Vol. 3: Invertebrate Immune Responses. Edited by L. A.
Bulla Jr. & T. C. Cheng. Plenum Press, New York.

NAPPI, A. J. and Y. CARTON, 1986 Cellular Immune Responses
and Their Genetic Aspects in Drosophila, pp 171-187. In:
Immunity in Invertebrates. Edited by M. Brehelin.
Springer-Verlag, Berlin.

NAPPI, A. J., J. KMIECIK and M. SILVERS, 1984 Cellular
Competence of a Drosophila Mutant with Neoplastic
Hematopoietic Organs. J. Invert. Pathol. 44: 220-227.

O'HARE, K. and G. M. RUBIN, 1983 Structures of P
transposable elements and their sites of insertion and
excision in the Drosophila melanogaster genome. Cell 34:
25-35.

RIO, D. C., F. A. LASKI and G. M. RUBIN, 1986
Identification and immunochemical analysis of
biologically active Drosophila P element transposase.
Cell 44: 21-32.

RIZKI, T. M., 1957 Tumor formation in relation to
metamorphosis in Drosophila melanogaster. J. Morphol.
100: 459-472.

RIZKI, T. M., 1978 The Circulatory System and Associated
Cells and Tissues, pp 398-452. In: The Genetics and
Biology of Drosophila, vol. 2B. Edited by M. Ashburner
& T. R. F. Wright. Academic Press, New York.

RIZKI, R. M. and T. M. RIZKI, 1974 Basement membrane abnormalities in melanotic tumor formation. Experientia 30: 543-546.

RIZKI, R. M. and T. M. RIZKI, 1979 Cell interactions in the differentiation of a melanotic tumor in Drosophila. Differentiation 12: 167-178.

RIZKI, R. M. and T. M. RIZKI, 1980 Hemocyte responses to implanted tissues in Drosophila melanogaster larvae. Wilhelm Roux's Arch. Dev. Biol. 189: 207-213.

RIZKI, T. M. and R. M. RIZKI, 1980a Developmental Analysis of a Temperature-Sensitive Melanotic Tumor Mutant in Drosophila melanogaster. Wilhelm Roux's Arch. Dev. Biol. 189: 197-206.

RIZKI, T. M. and R. M. RIZKI, 1986 Surface Changes on Hemocytes during Encapsulation in Drosophila melanogaster Meigen, pp 157-190. In: Hemocytic and Humoral Immunity in Arthropods. Edited by A. P. Gupta. John Wiley & Sons, New York.

RIZKI, T. M., R. M. RIZKI and R. A. BELLOTI, 1985 Genetics of a Drosophila phenoloxidase. Mol. Gen. Genet. 201: 7-13.

RIZKI, T. M., R. M. RIZKI and E. H. GRELL, 1980 A Mutant Affecting the Crystal Cells in Drosophila melanogaster. Wilhelm Roux's Arch. Dev. Biol. 188: 91-99.

ROWLEY, A. F., N. A. RATCLIFFE, C. M. LEONARD, E. H. RICHARDS and L. RENWRANTZ, 1986 Humoral Recognition

Factors in Insects, with Particular Reference to
Agglutinins and the Prophenoloxidase System, pp 381-406.
In: Hemocytic and Humoral Immunity in Arthropods.
Edited by A. P. Gupta. John Wiley & Sons, New York.

RUBIN, G. M., M. G. KIDWELL and P. M. BINGHAM, 1982 The
molecular basis of P-M hybrid dysgenesis: the nature of
induced mutation. Cell 29: 987-994.

RUBIN, G. M., and A. C. SPRADLING, 1982 Genetic
transformation of Drosophila with transposable element
vectors. Science 218: 348-353.

SILVERS, M. and W. P. HANRATTY, 1984 Alterations in the
Production of Hemocytes Due to a Neoplastic Mutation of
Drosophila melanogaster. J. Invert. Pathol. 44: 324-328.

SIMMONS, M. J. and L. M. BUCHOLZ, 1985 Transposase
titration in Drosophila melanogaster: A model of
cytotype in the P-M system of hybrid dysgenesis. Proc.
Nat. Acad. Sci. USA. 82: 8119-8123.

SIMMONS, M. J. and R. E. KARESS, 1985 Molecular and
population biology of hybrid dysgenesis. Drosophila
Inform. Serv. 61: 2-7.

SIMMONS, M. J., and J. K. LIM, 1980 Site specificity of
mutations arising in dysgenic hybrids of Drosophila
melanogaster. Proc. Nat. Acad. Sci. USA. 77: 6042-6046.

SIMMONS, M. J., J. D. RAYMOND, T. P. CULBERT and T. R.
LAVERTY, 1984 Analysis of dysgenesis-induced lethal
mutation of the X chromosome of a Q strain of Drosophila

melanogaster. Genetics 107: 49-63.

SODERHALL, K. and V. J. SMITH, 1986 The Prophenoloxidase
    System: The Biochemistry of Its Activation and Role in
    Arthropod Cellular Immunity with Special Reference to
    Crustaceans, pp 208-223. In: Immunity in Invertebrates.
    Edited by M. Brehelin. Springer-Verlag, Berlin.

SPARROW, J. C., 1978 Melanotic Tumours, pp 277-313. In:
    The Genetics and Biology of Drosophila, vol. 2B. Edited
    by M. Ashburner & T. R. F. Wright. Academic Press, New
    York.

SPRADLING, A. C. and G. M. RUBIN, 1982 Transposition of
    cloned P elements into Drosophila germ line chromosomes.
    Science 218: 341-347.

VEY, A. and P. GOTZ, 1986 Antifungal Cellular Defense
    Mechanisms in Insects, pp 89-115. In: Hemocytic and
    Humoral Immunity in Arthropods. Edited by A. P. Gupta.
    John Wiley & Sons, New York.

VOELKER, R. A., A. L. GREENLEAF, H. GYURKOVICS, B. B.
    WISELY, S. HUANG and L. L. SEARLES, 1984 Frequent
    imprecise excision among reversions of a P-element-
    caused lethal mutation in Drosophila. Genetics 107:
    279-294.

ZUSMAN, S., D. COULTER and J. P. GERGEN, 1985 Lethal
    mutations induced in the proximal X-chromosome of
    Drosophila melanogaster using P-M hybrid dysgenesis.
    Drosophila Inform. Serv. 61: 217-218.

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OBJECTIVES

At the start of this project, a large, P element-mediated mutagenesis experiment was ongoing whose purpose was to isolate new mutations causing altered growth patterns. Approximately 500-1000, X-linked hybrid dysgenesis-induced lethals had been isolated and classified according to their lethal stage. I selected 65 mutations that caused lethality during the late third instar or early pupal stage of development. A more extensive analysis of the lethal syndrome associated with each of these 65 mutants revealed 15 mutants with a heritable melanotic tumor phenotype.

The melanotic tumor mutants were chosen for study because 1) they had an interesting phenotype that was not documented very well in the literature and 2) lymph gland neoplasia had been demonstrated in one X-linked, lethal melanotic tumor mutant. It was expected that these studies would generate information to aid our understanding of the genetic basis of melanotic tumor formation in Drosophila melanogaster. In addition, we hoped that this investigation would determine whether any aberrant growth was caused by the air mutations.

T. K. Johnson, K. R. Hummels and M. Meili found additional dysgenesis-induced mutations with a melanotic tumor phenotype and some of these were included in the

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air collection. A. L. Johnson has mapped five
additional dysgenesisinduced lethals that exhibited
either a melanotic tumor or hypertrophied lymph gland
phenotype. These mutants were not allelic to any of the
air mutants characterized here, suggesting the existence
of additional unidentified X-linked melanotic loci.

INTRODUCTION

The formation of melanotic tumors in <u>Drosophila</u> larvae has
been thought to arise as a normal, heritable response to
some form of abnormal development (SPARROW, 1978). In third
instar larvae of melanotic tumor strains, cellular or
haemocytic capsules are formed around aberrant larval
tissue, and then melanin is deposited within and around the
capsule (RIZKI and RIZKI, 1986). This type of cellular
defense response also will occur normally if foreign objects
invade the larval haemocoele, such as during parasitization
or wounding events (GOTZ, 1986). The melanotic tumor
phenotype typically shows a recessive mode of inheritance
with incomplete penetrance and variable expressivity,
apparently due to numerous modifier genes present in the
genome (SPARROW, 1978).

We propose that melanotic tumor mutants can be divided
into two general classes based on the type of abnormality
which initiates the defense response. These two classes are
represented by: (1) mutants in which abnormal tissue or
cell surfaces trigger a specific autoimmune response (RIZKI
and RIZKI, 1974a; RIZKI and RIZKI, 1980a) and (2) mutants
with invasive neoplasms that non-specifically encapsulate
tissues/cells (ie. lymph gland neoplasia, HANRATTY and
RYERSE, 1981). Although a large number of non-allelic loci
can be mutated to give a melanotic tumor phenotype, the

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genetic basis of immune recognition and the regulation of the defense responses are still poorly understood. In a P elementmediated mutagenesis experiment, we have isolated 20 lethal mutations exhibiting a melanotic tumor phenotype. Experiments were conducted to determine the number of distinct X-chromosome loci represented in this collection of mutants. The lethal syndromes were characterized in an effort to identify specific developmental anomalies that might be associated with melanotic tumor formation in each mutant.

MATERIALS AND METHODS

Culture Conditions: All fly strains were maintained on standard cornmeal, molasses, yeast and agar _Drosophila_ medium in vials or half-pint milk bottles. The X-chromosome duplications and deficiencies used in this study are listed in Table 3. Descriptions of other mutations and chromosomes can be found in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1986, 1987). Fly stocks were obtained from the following sources: California Institute of Technology Stock Center; Mid-America _Drosophila_ Stock Center, Bowling Green State University; Drs. P. J. Bryant, A. T. C. Carpenter, W. R. Engels, B. W. Geer, J. L. Marsh, N. Perrimon, C. A. Poodry, T. M. Rizki, M. J. Simmons and D. F. Woods. P strain stocks were constructed by repeatedly backcrossing flies to a strong P strain (π^2).

Mutagenesis Scheme: X-chromosome lethal mutations were induced using P element mobilization under dysgenic conditions as outlined in Figure 1. The isogenized X-chromosome stock used in the mutagenesis was marked with yellow[1], white[1] and a cryptic weak _bobbed_ allele. The dysgenic F1 males were crossed to P-strain females bearing a balanced X-chromosome lethal mutation. Newly-induced recessive lethal mutations were identified by the absence of male progeny bearing the mutagenized chromosome. These lethal bearing chromosomes were recovered by crossing the

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TABLE 3
X-Chromosome Duplications / Deficiencies.

Duplication Name	X-Chromosome Breakpoints	Ref.	Deficiency Name	X-Chromosome Breakpoints	Ref.
v^+Yy^+	1A1;1B2;Y	1	Df(1)svr	1A1;1B10-13	2
Dp(1;2)Bld	1A1;1C3-4	2	Df(1)A94	1E3;2B15	1
			Df(1)S39	1E4;2B11-12	1
$y^2Y67g19.1$	1A1;2B17-18	1			
Dp(1;3)sta	1D3-E1;2A1	2	Df(1)Pgd^{35}	2D3;2E2-F5	1
			Df(1)64c18	2E1-2;3C2	1
Dp(1;3)w^{vco}	2B17-C1;3C6	2	Df(1)TEM304	2E2-F1;3A4-6	2
			Df(1)JC19	2F3;3C5	1
$B^Sw^+y^+.Y$	2D1;3C7	3			
			Df(1)w$^{258-42}$	3A4-6;3C5-6	2
Dp(1;2)w^{+70h}	3A7-8;3C2-3	2			
			Df(1)w$^{258-48}$	3A9-B1;3C2-3	2
Dp(1;2)w^{+51b7}	3C1-2;3D6-7	1	Df(1)HF366	3E7-8;5A7	2
			Df(1)C149	5A8-9;5C5-6	1
Dp(1;2)w^{+64b13}	3C2;5A1-2	1	Df(1)N73	5C2;5D5-6	1
			Df(1)HA32	6E4-5;7A6	1
Dp(1;2)rb^{+71g}	3F3;5E8	1	Df(1)ct^{78}	6F1-2;7C1-2	2
Dp(1;3)sn^{13a1}	6C11;7C9	1	Df(1)ct^{J4}	7A2;7C1	1
y^+ct^+Y	6E;7C4-6	3	Df(1)ct^{4b1}	7B2;7C4	2
Dp(1;2)sn^{+72d}	7A8;8A5	1	Df(1)C128	7D1;7D5-6	2
			Df(1)RA2	7D10;8A4-5	1
Dp(1;2)v^{+75d}	9A2;10C2	1	Df(1)KA14	7F1-2;8C6	2
Dp(1;2)v^{+63i}	9E1;10A11	1	Df(1)v^{L15}	9B1;10A1	1
			Df(1)HC133	9B9-10;9E-F	2
v^+Yy^+	9F3;10C1-2	1	Df(1)v^{L3}	9F6-9;10A6-7	1
v^+Yy^+3	9F3;10E3-4	1	Df(1)v^{L2}	9F13;10A1	2
Dp(1;2)v^{65b}	10A1;11A7	1	Df(1)RA37	10A6-7;10B15-17	2
			Df(1)KA7	10A9;10F10	1
			Df(1)N71	10B5;10D4	1
Dp(1;4)r$^+f^+$	13F10;16A1-2	2	Df(1)DA622	10B6-8;10D2	2
			Df(1)M13	10B10;11A3-7	5
Dp(1;2)r^{+75c}	14B13;15A9	1	Df(1)N105	10F7;11D1	2
			Df(1)N12	11D1-2;11F1-2	2
Dp(1;3)f^{+71b}	15A4;16C2-3	1	Df(1)sd^{72b26}	13F1;14B1	2
			Df(1)D15	14D1;15C5	4
			Df(1)D17	14F6;15A6	2

1. CRAYMER and ROY (1980).   4. T. K. JOHNSON (PERS. COMM.).
2. LINDSLEY and ZIMM (1987). 5. D. F. WOODS (PERS. COMM.).
3. JOHNSON and JUDD (1979).

$\dfrac{y^1 \; w^1}{y^1 \; w^1}$ _____

(M strain)

X

(P strain)

$y^1 \; w^1$ _____

F1:   (Dysgenic males)

X

FM7 _____

lethal
(P strain)

$y^1 \; w^1$ (1)   **

FM7
F2:   (P cytotype)

X

FM7 _____

(P strain)

$y^1 \; w^1$ (1)   **

F3:   Newly-induced
      recessive lethal,
      males not recovered.

$y^1 \; w^1$ (1)   **

FM7

F3:   Heterozygous females
      bearing newly-induced
      recessive lethal.

X

FM7 _____

(P strain)

STOCK
(P cytotype)
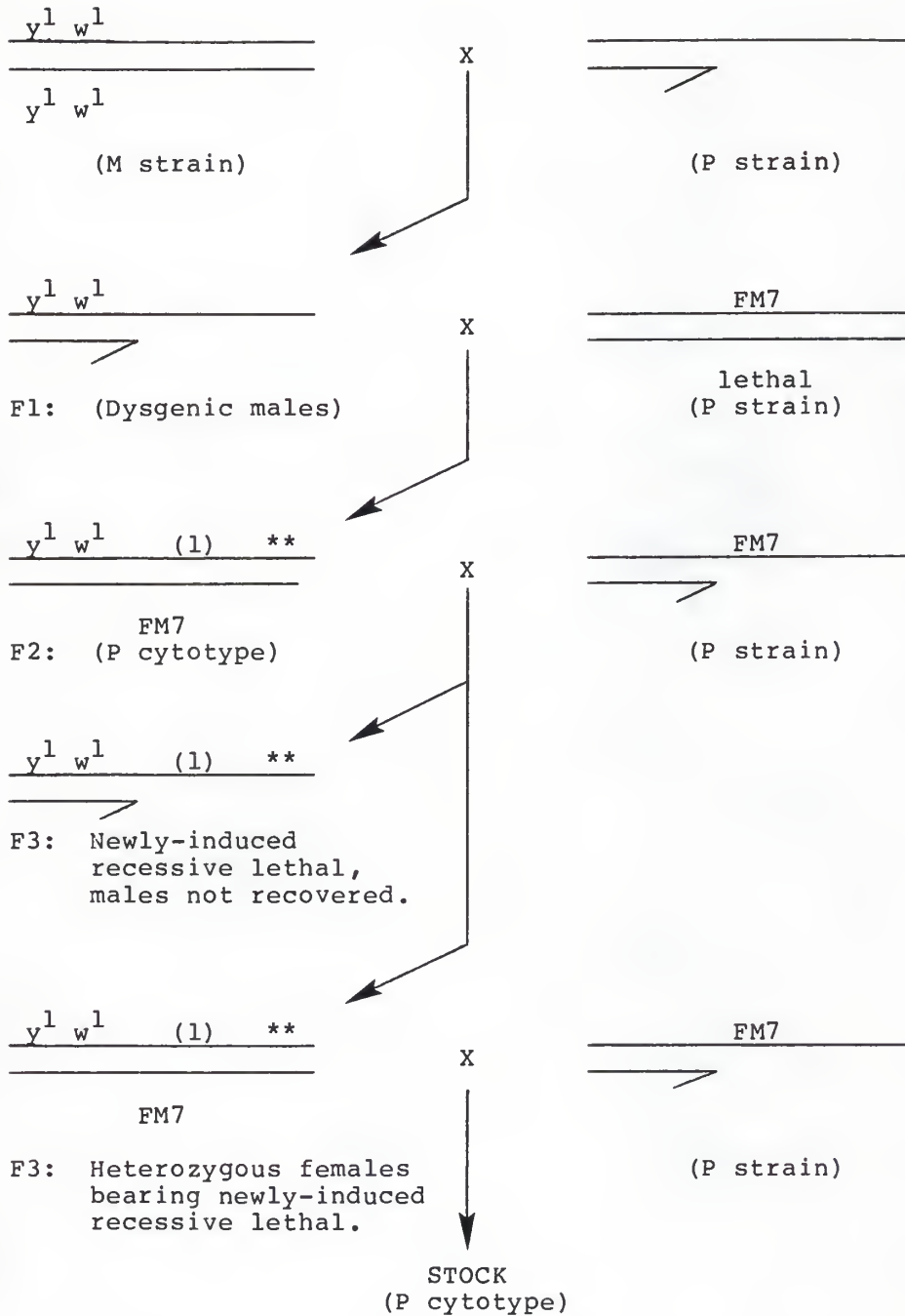
Figure 1.-Mutagenesis Scheme. ** is the mutagenized chromosome.

lethal/balancer heterozygous sibs to P strain males.

Assessment of the Melanotic Tumor Phenotype: A group of
newlyinduced, sex-linked mutations with a terminal lethal
phase during the late third instar or early pupal stage
were assessed to determine whether they caused heritable
"melanotic pseudotumors" (referred to as melanotic tumors
in this article). The hemizygous, mutant larvae from each
lethal stock could be identified due to the existence of a
y^1 marker on the mutagenized chromosome. These mutant
males were examined for melanotic or brown/black masses in
the haemocoele of late third instar larvae. The mutations
described in this study which give rise to melanotic tumors
as a result of a genetic lesion have been designated
aberrant immune response mutations or air. Other lethal
point mutant and deficiency stocks also were examined for
the presence of melanotic tumors during the late third
instar period of development.

Genetic Mapping Protocols: Approximate map positions for
the air mutations were determined initially by
recombination analyses using the following multiply marked
X-chromosomes: sc ec cv ct v g f, y pn cv m f bb^{2r13} and
sc ec cv ct m v f (see Appendices 1, 2, 3 and 4). The
duplications and deficiencies listed in Table 3 were
employed where applicable, to cytologically localize the
air mutations along the X-chromosome. Mutant air males
that had been rescued from lethality were obtained by

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crossing y^1 w^1 air/balancer heterozygous females with males

bearing an appropriate X-chromosome duplication (see

Appendix 5). These duplication-covered male air offspring

were identified by the presence of the y^1 w^1 markers in

adult males. The air mutations were determined to be

within the confines of a deficiency when air/deficiency

heterozygotes were not recovered in the progeny of a cross.

Lethal Complementation Analyses: In some cases, the air

lethals were tested for their ability to complement

functionally, other mutations which had been localized to a

common chromosome subregion. Duplication-covered air males

were crossed to females heterozygous for other air

mutations or lethal point mutations previously isolated by

others, and the progeny were scored for the presence of

lethal/air or air/air females in the absence of the

duplication (see Appendix 6). The inability to recover the

lethal/air or air/air combination in females lacking the

duplication was the criterion used to assign allelism.

Analysis of Lethal Phase: Hemizygous air larvae were

followed from hatching to determine the stage at which

development was arrested. Heterozygous y^1 w^1 air/Binsn (P

cytotype) females from each air stock were allowed to mate

with Binsn (P cytotype) males for 4 days at 25°C, after

which serial egg collections were made using replaceable

petri plates filled with standard Drosophila medium. The

total number of eggs on each plate was determined and the

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percent of larvae hatching was calculated using the formula: (total number of larvae/total number of eggs X 100). Male air larvae had light brown mouthparts due to the y^1 marker on the air chromosome and were easily distinguished from larvae of other genotypes, which had dark brown or black mouthparts (see Appendix 7). The air male larvae were sorted from the other larvae and placed in separate vials to follow their development. Larvae were scored for the most terminal stage of development reached; for simplicity these stages were divided as follows: Early Larvae (EL) = 1st and 2nd instar larvae, Late Larvae (LL) = 3rd instar larvae, Pseudopupae (Psp) = larvae with some pupal characteristics, Early Pupae (EP) and Adults (A) = revertants. Non-mutant larvae also were transferred to vials and followed until eclosion to determine whether the experimental manipulation had affected their survival rate. Mortality in these controls was minimal and it was assumed that the larvae were not adversely affected by the treatment. Lethal periods during the embryonic period of development were tentatively assigned when there were departures from the expected 25% genotypic ratio of air males in the progeny larvae.

Developmental Characteristics of air Larvae: Male larvae from each air stock were subjected to a preliminary characterization of their internal morphology. Larvae were dissected in Ringer's solution and examined with the aid of

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a dissecting microscope. This analysis included a crude morphological examination of the anterior midgut, brain hemispheres, fat body, gastric caeca, imaginal discs, lymph glands, Malpighian tubules, proventriculus, ring gland, salivary gland and ventral ganglion. Since the hemizygous male larvae from all _air_ stocks develop more slowly than do their sibs or wild type counterparts, the stage of development that preceded their most terminal stage was chosen for dissection. Larval characteristics were reported only when all morphological features could be scored on one individual.

Reversion Mutagenesis: Dysgenic hybrids were generated for a few selected _air_ stocks to analyze the frequency of reversion of the lethal phenotype when placed under dysgenic conditions. This provided an indirect assay that P element inserts were responsible for the _air_ mutant phenotype in these stocks. Duplication-covered _air_ males (P strain) were crossed to attached-X females (M strain) bearing the same duplication. Individual F1 dysgenic males then were crossed to attached-X females that lacked the duplication. The total number of F2, attached-X females and any $y^1 \, w^1$ revertant adult males were scored. The reversion frequency was calculated using the formula: (revertant males + attached-X females X 100).

In this study, 20 recessive P element-induced lethal mutations giving rise to melanotic tumor phenotypes were identified and some of their properties were studied. This lethal, melanotic tumor phenotype was observed in approximately 1.2% of all the X-linked lethal mutations generated in our mutagenesis experiment. The characteristic air mutant larval phenotype is shown in Figure 2.

Genetic Mapping: Initially, it was important to determine the number of distinct X-chromosome loci that were represented by the air mutants. Each air mutation was mapped by recombination to provide an approximate map position and also to generate males that were recombinant for the chromosome interval surrounding the air location. Lethality was the phenotypic marker used in all of the recombination mapping experiments, for the melanotic tumor phenotype in every air mutant was characterized by incomplete penetrance and variable expressivity. The lethal and melanotic tumor aspects of each air mutant phenotype were tested to determine whether they were pleiotropic effects of a single gene mutation or two linked mutations. All males recombinant for the marker interval containing the lethal locus were crossed to attached-X females and the stocks were maintained for many

FIGURE 2.- Melanotic tumor phenotype of selected air mutants. A, air1. B, air6. C, D, air8. E, air10. F, air13.
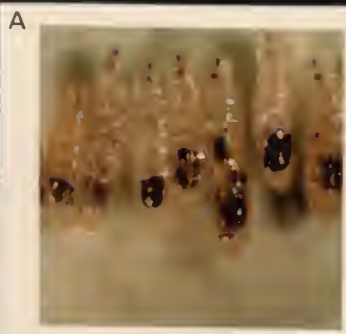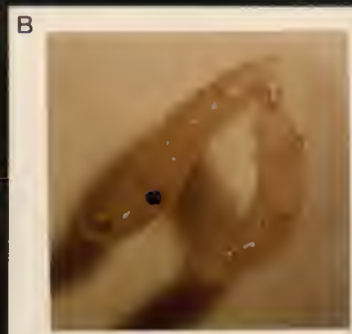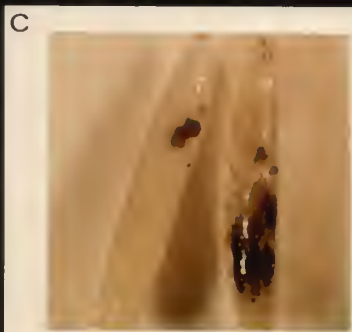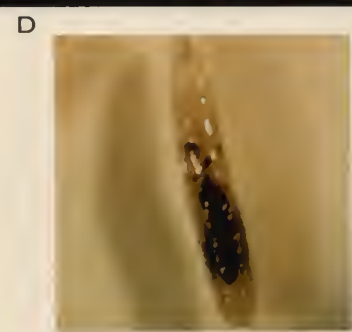
A air1
B air6
C air8
D air8
E air10
F air13

generations. The male offspring from these crosses were examined during late larval stages for any evidence of melanotic tumors, which would indicate that a putative mutation causing this phenotype had been separated from the lethal. The frequency of melanotic tumors observed for any of the recombinant males generated from the air mutant recombination crosses was never greater than that observed in the original M strain (y^1 w^1 stock) used in the mutagenesis (data not shown). Light brown pigmentation was observed at the anterior portion of the fore-/hind-gut junction in the original y^1 w^1 stock and in most of the recombinant male stocks. It should be noted that this pigmentation was distinctly different in appearance from the pigmentation associated with the air melanotic tumor phenotype.

A more precise map position for each air mutation was obtained through the use of numerous X-chromosome duplications and deficiencies (Table 3). A cytogenetic map showing the distribution of duplications, deficiencies and air mutant positions in four subsections of the X chromosome is presented in Figure 3. The cytological locations of air11, air13, air14 and air15 are not displayed in Figure 3, because these mutations could not be rescued from lethality using any of the duplications listed in Table 3. The mutation labelled air16^d was recovered rarely in males bearing the duplication segregant of

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FIGURE 3.- Cytogenetic map of the polytene X-chromosome.
The thin solid bars show the cytological extent of
various duplications and the dashed bars represent the
limits of X-chromosome deficiencies. The genetically-
determined map positions of the air mutants based on
coverage and allelism tests are indicated by thick solid
bars. A, distal region of the X-chromosome including
bands 1-4. B, polytene band interval 5-7. C, polytene
band interval 9-11. D, polytene band interval 13-17.

A

C

$T(1;3)f^{+71b}$, but all of these males were infertile. The

recombination map positions of the mutants: airll, airl3,

airl4, airl5 and airl6[d] are listed in Table 4, along with

their tentative cytological locations derived collectively

from recombination, duplication and deficiency mapping (see

Appendix 9). The cytological location of the other air

mutants also are given in Table 4.

Lethal Complementation Results: Inter se crosses were

performed among those air mutants that had been localized

to coincident chromosome regions and where duplication-

covered, fertile males were available. All heterozygous

combinations of the four independently isolated airl6

mutations failed to complement each other and were assigned

to a single complementation group. No other cases of

allelism between air mutations could be detected. Some air

mutants were tested for their ability to functionally

complement point mutants previously localized to similar

chromosome regions. A compilation of complementation

results is given in Table 5. The mutant air7 was

determined to be allelic to the point mutants: $\underline{l(1)7Ac}^2$,

$\underline{l(1)7Ac}^3$, $\underline{l(1)7Ac}^5$ and $\underline{l(1)7Ac}^6$ previously known as

l(1)HF302, l(1)JA59, l(1)RC57 and l(1)RC61 respectively

(LINDSLEY and ZIMM, 1986). In repeated tests, $\underline{l(1)7Ac}^7$

(l(1)EF465) always complemented air7 and the other members

of the complementation group namely, $\underline{l(1)7Ac}^2$, $\underline{l(1)7Ac}^3$,

$\underline{l(1)7Ac}^5$ and $\underline{l(1)7Ac}^6$ (LINDSLEY and ZIMM, 1986). Thus, the

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TABLE 4

Summary of Map Positions--<u>air</u> Lethals

| Mutant | Map Positions | |
	Recombination	Cytological
<u>air1</u>		1C3-4; 1D3-E1 2B15; 2B17-18
<u>air2</u>		1E3; 1E4
<u>air3</u>		2D1; 2D3
<u>air4</u>		5A1-2; 5E8
<u>air5</u>		5A1-2; 5E8
<u>air6</u>		5A1-2; 5E8
<u>air7</u>		7A6; 7A8
<u>air8</u>		7C4; 7C9
<u>air9</u>		7C9; 7D1 7D5; 7D10
<u>air10</u>		7C9; 7D1 7D5; 8A5
<u>air11</u>	29.0	(8A5; 9A2) (11A7; 13F10)
<u>air12</u>		9E1; 9F3
<u>air13</u>	35.4	(8A5; 9A2) (11A7; 13F10)
<u>hop</u>[air]		10B6; 10B8
<u>air14</u>	44.9	(11A7; 13F10)
<u>air15</u>	55.6	(11A7; 13F10)
<u>air16</u>[a]		16A1-2; 16C2-3
<u>air16</u>[b]		16A1-2; 16C2-3
<u>air16</u>[c]		16A1-2; 16C2-3
<u>air16</u>[d]	56.7	(16A1-2; 16C2-3)

( ) = Approximate cytological locations based on
recombination, duplication and deficiency
mapping. See Appendix 9.

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TABLE 5A

Lethal Complementation Analyses.
(Polytene Band Interval: 1-3).

Mutant (Map Position)	1	2	3	4	5
1. $\underline{dor}^1$ ($\overline{1F1}$;2A2)		-	-		
2. $\underline{air1}$ ($\overline{1C3}$-4;1E3 2B15;2B17-18)	-	-			-
3. $\underline{air2}$ ($\overline{1E3}$;1E4)	-				-
4. $\underline{air3}$ ($\overline{2D1}$;2D3)					-
5. $\underline{1(1)m1}$ ($\overline{3A3}$)		-	-	-	

+ = fails to complement.
- = complements.

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TABLE 5B

Lethal Complementation Analyses.

(Polytene Band Interval: 7).

The map positions listed for the mutants $\underline{l(1)X-6}$ and

$\underline{l(1)26}$ were based on their inclusion within the limits of

the duplication $\underline{y^+ct^+Y}$ (6E;7C4-6) and their exclusion

from $\underline{Df(1)ct}^{4b1}$ (7B2;7C4) (T. K. JOHNSON, PERS. COMM.).

The mutant $\underline{air8}$ was not rescued by the duplication $\underline{y^+ct^+Y}$

nor was it allelic to $\underline{Df(1)ct}^{4b1}$. (+ = fails to

complement, - = complements).

Mutant (Map Position)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. air7 ($\overline{7A6}$;7A8)		−	+	+	+	+	−	−	−	−	−	−	−	−	−	−
2. l(1)25 ($\overline{7A6;7A8}$)	−															
3. l(1)7Ac^2 ($\overline{7A6;7A8}$)	+					+	−									
4. l(1)7Ac^3 ($\overline{7A6;7A8}$)	+															
5. l(1)7Ac^5 ($\overline{7A6;7A8}$)	+						−									
6. l(1)7Ac^6 ($\overline{7A6;7A8}$)	+		+				−									
7. l(1)7Ac^7 ($\overline{7A6;7A8}$)	−		−		−	−										
8. air8 ($\overline{7C4}$-6;7C9)	−								−	−	−	−	−	−		−
9. l(1)X-6 ($\overline{7C4}$;7C6)	−								−			−	−	−		−
10. l(1)26 ($\overline{7C4}$;7C6)	−							−								
11. l(1)7Cc^2 ($\overline{7C6;7D1}$)	−							−	−							
12. l(1)7Cc^4 ($\overline{7C6;7D1}$)	−							−	−							
13. l(1)14 ($\overline{7C6;7D1}$)	−							−	−							
14. l(1)7Ce^1 ($\overline{7C6;7D1}$)	−							−	−							
15. l(1)7Cg^1 ($\overline{7C6;7D1}$)	−							−	−							
16. l(1)7Ch^1 ($\overline{7C6;7D1}$)	−							−	−							

TABLE 5C

Lethal Complementation Analyses.

(Polytene Band Interval 9-10).

a = HANRATTY and RYERSE (1981). The following alleles were also tested: b = $l(1)10Ba^2$; c = $l(1)10Bb^{12}$; d = dsh^{M20}, dsh^{VA153}; e = hop^{VE666}, hop^{HC257}, hop^{L4}, hop^{VA85}, hop^{GA32}, hop^{C111}. The map positions for d and e were obtained from PERRIMON and MAHOWALD (1986, 1987). The map position for f was from D. F. WOODS (PERS. COMM.).

(+ = fails to complement, - = complements).

Mutant (Map Position)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. air12 (9E1;9F3)			−					−					−	−			
2. $l(1)9Fe^1$ (9F9;9F12)								−					−	−			
3. $l(1)9Fh^4$ (9F4;9F8)			−					−					−				
4. $l(1)9Fi^2$ (9F13;10A1)			−					−									
5. $l(1)10Ae^{14}$ (10A6;10A7)			−					−									
6. $l(1)10Ag^8$ (10A9)								−					−				
7. $l(1)10Ah^3$ (10A8;10A11)								−					−				
8. Tum^1 $(1-34.5)^a$			−	−	−	−	−	−		−	−	−	−		−	−	−
9. $l(1)10Ba^1$ $(10B4;10B9)^b$								−					−				
10. $l(1)10Bb^9$ $(10B4;10B9)^c$								−									
11. $l(1)10Bc^1$ (10B4;10B9)								−					−				
12. dsh^1 $(10B5;10B7)^d$								−					−				
13. hop^{air} (10B6;10B8)	−	−	−			−	−	−		−	−	−		+	−	−	−
14. hop^{v48} $(10B6;10B8)^e$	−	−						−					+				
15. dlg^1 $(10B8;10B9)^f$								−					−				
16. $l(1)10Bi^1$ (10B17;10C2)								−					−				
17. $l(1)10Bj^1$ (10B17;10C2)								−					−				

presumed $\underline{1(1)7Ac}^7$ allele did not behave as previously
described (Table 5B). The mutant designated $\underline{hop}^{air}$ was
shown to be an allele of the $\underline{hopscotch}$ locus by its failure
to complement $\underline{hop}^{v48}$, $\underline{hop}^{VE666}$, $\underline{hop}^{HC257}$, $\underline{hop}^{L4}$, $\underline{hop}^{VA85}$,
$\underline{hop}^{GA32}$ and $\underline{hop}^{Clll}$ (Table 5C).

Lethal Phase Analyses: The lethal phase for the $\underline{air}$
mutants was determined by mating $\underline{Binsn}$ males (P cytotype)
to $\underline{y}^1$ $\underline{w}^1$ $\underline{air}/\underline{Binsn}$ females (P cytotype) and following the
development of the hemizygous $\underline{y}^1$ $\underline{w}^1$ males. The first
column in Table 6A gives the percentage of $\underline{air}$ males that
were represented in the total pool of hatched larvae. The
degree of embryonic lethality (Emb.) exhibited by the $\underline{air}$
mutants was calculated by subtracting the percent of $\underline{air}$
male larvae (derived from heterozygous mothers) from the
25% expected for this class, with the difference
representing lethality during the embryonic stage of
development. The larval lethal phases were determined by
scoring the developmental fate of all $\underline{Fl}$, $\underline{air}$ males that
hatched into larvae. The proportion of hatched larvae that
died at Early Larval (1st and 2nd instar), Late Larval (3rd
instar), Pseudopupal (larval and pupal characteristics),
Early Pupal or Adult stages was determined for each $\underline{air}$
mutant. The Pseudopupal (Psp) classification usually was
applied to individuals with shortened larval cuticles, some
cuticular hardening and early signs of pigmentation. The
early pupal (EP) label was applied equally to individuals

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TABLE 6A

Lethal Phases of air Mutations.

Binsn males were crossed to y^1 w^1 air/Binsn females and
their F1's were scored. Embryonic lethality (Emb.) was
calculated by subtracting the percent of hemizygous (y^1
w^1 air) F1 males hatching from the 25% expected for that
progeny class. The larval/adult lethal periods were de-
termined by scoring the stage at which the F1 y^1 w^1 air
males arrested development using the following stages:
A =adults (revertants); EL = 1st & 2nd instar larvae; EP
= early pupae; LL = 3rd instar larvae; Psp = pseudopupae
(larvae with some pupal features).

Mutant	F1: air male larvae (%) Hatched	Emb.	Lethal Phase--air male larvae(%) EL	LL	Psp	EP	A	# Eggs Scored
air1	25.1	---	42.3	55.9	1.8			512
air2	26.5	---	6.7	3.6	-------89.1		0.6	740
air3	19.5	5.5	10.3	17.2	36.8	35.6		568
air4	24.6	0.4	38.8	61.2				445
air5	22.4	2.6	18.4	6.6	55.9	19.1		670
air6	25.5	---	33.6	21.7	44.8			1213
air7	24.2	0.8	51.9	17.6	29.6	0.9		594
air8	21.4	3.6	43.6	13.7	38.5	2.6	1.7	660
air9	22.4	2.6	30.8	20.5	48.7			588
air10	24.6	0.4	50.6	35.4	7.6	6.5		1183
air11	21.6	3.4	67.0	14.0	12.0	5.0	2.0	594
air12	25.2	---	5.2		0.9	93.9		949
air13	23.6	1.4	9.7	1.4	89.0			712
hop[air]	25.0	---	74.9	2.1	2.6	20.0	0.5	928
air14	27.1	---	39.0	48.0	12.4	0.6		707
air15	24.9	0.1	30.7	7.9	0.5	60.8		828
air16[a]	24.1	0.9	59.5	36.7	2.7	1.1		1250
air16[b]	26.2	---	55.7	19.2	11.4	13.8		768
air16[c]	22.4	2.6	37.0	33.3	13.0	16.7		501
air16[d]	22.4	2.6	78.2	14.9	6.9			519

that formed "normal" puparia and then ceased development and to those individuals that actually pupated. Most of the air mutants were characterized by multiphasic lethality (Table 6A). The percent of larval lethality at different stages was based on the ratio: number of air males that reached each stage/total air male larvae that hatched. The predominant lethal phase for each air mutant is listed in Table 6B.

The developmental progression of each air mutant was examined with respect to two parameters. These parameters were: 1) delayed developmental rates and 2) the inability to undergo metamorphosis as evidenced by an extended period of time at the third instar stage. When the air mutants were examined for evidence of extended late larval periods distinct from their delayed developmental rate, there was no indication of heterogeneity in developmental rate for the mutants air4, air5, air12, air16^a and air16^d. A subset of hemizygous air1, air2, air3, air7, air9, air11 and air16^b males were found as active third instar larvae, 7 days after the eclosion date of their non-mutant sibs. Some third instar male larvae that were genotypically air6, air8, air10, air13 or air15 survived up to 14 days after some mutant sibs had ceased development and their non-mutant sibs had eclosed.

Developmental Characteristics of air Mutants: Preliminary characterization was made of the larval anatomy

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TABLE 6B

Predominant Lethal Period--<u>air</u> Mutants.

Early Larvae (EL)	Late Larvae (LL)	Pseudopupae (Psp)	Early Pupae (EP)
<u>air7</u>	<u>air1</u>	<u>air2</u>	<u>air2</u>
<u>air8</u>	<u>air4</u>	<u>air3</u>	<u>air3</u>
<u>air10</u>	<u>air14</u>	<u>air5</u>	<u>air12</u>
<u>air11</u>		<u>air6</u>	<u>air15</u>
<u>hop</u>^{air}		<u>air9</u>	
<u>air16</u>^{a}		<u>air13</u>	
<u>air16</u>^{b}			
<u>air16</u>^{c}			
<u>air16</u>^{d}			

of each air mutant. The ring gland, lymph glands, salivary glands, brain, gut, and imaginal discs were examined for any morphological defects or anomalies. The primary aim of this analysis was to correlate specific defects or tissue involvement with the melanotic tumor phenotype. This was accomplished by recording all melanized structures and melanotic tumors that were observed during the dissections of the mutant larvae. One defect that was common to all air mutants was a small and/or abnormal brain (Table 7). Hemizygous air2, air6, air7, air8, air9, air11, air13 and air15 males were shown to have enlarged lymph gland lobes when compared to the y^1 w^1 (M strain) "wild type" stock. Montages displaying the morphology of the brain complex, ring gland, dorsal vessel and lymph glands of air13 (P strain) and y^1 w^1 (M strain) male larvae are shown in Figure 4. The hypertrophied lymph glands of air6 and air11 males are depicted in Figure 5. Melanotic nodules located in the most anterior pair of the lymph gland lobes can be seen in Figure 5A and C. Melanotic nodules also were observed in the hypertrophied lymph glands of the following mutants: air2, air7, air8, air9, air11 and air13 (Table 7). In addition, the lymph glands (not hypertrophied) of air4 and air12 mutant males also contained melanized nodules. An increase in the total number of lymph gland lobes was observed in air13 mutant larvae (Figure 6). Many air13 individuals contained greater than ten

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TABLE 7

Developmental Characteristics of the air Mutants

Mutant	Ring Gland	Lymph Glands	Salivary Glands	Brain	Gut	Imaginal Discs	Melanized Structures
air1*	sm	norm-lg	sm	Bh-sm Vg-sm	In-mel	Eye/an-m Wing-v	hind-In
air2	sm-norm ab	lg/mel	norm	Bh-ab Vg-sm	AM -v P -lg	Eye/an-ab Wing-v	Lymph gland
air3	norm/ab	norm	sm-norm	Bh-ab Vg-ab	In -mel	Eye/an-ab	ff hind-In Trachea
air4*	sm	sm-norm mel	sm-norm	Bh-sm Vg-sm	AM -lg Gc -m/sm In -mel P -lg/mel	m/ab	hind-In Lymph gland
air5	sm	norm	sm	Bh-ab/sm Vg-sm	In -mel P -ab	v	ff hind-In
air6	sm-norm	lg	sm-norm	Bh-ab Vg-sm	Gar-ab Gc -ab P -lg/mel	Eye/an-ab sm	Proventriculus
air7	sm	lg/mel	sm	Bh-sm Vg-sm	In -mel/ab P -sm	m-sm ab	hind-In Lymph gland Trachea
air8	sm/ab	lg/mel	sm	Bh-ab Vg-ab	AM -lg Gar-ab	ab Wing-v	ff Garland cells

TABLE 7
(continued)

							Gonads / Lymph gland
air9*	sm	v/mel	sm-norm	Bh-ab / Vg-sm	Gc -mel / -sm / P -ab	sm / Wing-v	ff / AM/hind-In / Lymph gland
air10*	sm	norm-lg	norm	Bh-sm / Vg-sm	Gc -mel / P -ab	Wing-lg	hind-In
air11	sm	lg/mel	sm	Bh-ab / Vg-sm	In-mel	Eye/an-ab / m-sm	hind-In / Lymph gland
air12	sm-norm	norm/mel	norm	Bh-ab / Vg-sm	Gar-mel / Gc -v / P -ab	Eye/an-ab / Wing-ab	hind-In / Lymph gland / mid-In
air13	sm	v/mel	sm	Bh-ab / Vg-sm	Gc -v	ab / Wing-ab / -m	ff / Lymph gland
hop air*	sm	norm	norm	sm	Gc -lg / P -ab	sm / Wing-ab	
air14*	sm/ab	norm	sm-norm	Bh-sm / Vg-sm	norm	sm / Wing-lg	Trachea
air15	sm	lg	sm-norm	Bh-sm / Vg-sm	Gc -ab / P -sm	sm/ab	
air16[a]*							

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TABLE 7
(continued)

airl6[b]*	sm	sm-norm	v	Bh-sm Vg-sm	Gar-mel In -mel	sm-m	ff Garland cells hind-In Trachea
airl6[c]*	sm-norm	norm-lg	norm	Bh-sm	In -mel	m	ff hind-In
airl6[d]*	sm	m	sm	Bh-sm	norm	sm-m	

A minimum of 10 (except *) air male larvae were dissected at late third instar or the most terminal developmental stage reached. Abbreviations are: AM = Anterior midgut, Eye/an = Eye-antennal discs, Gar = Garland cells, Gc = Gastric caeca, Bh = brain hemispheres, In = intestine, P = proventriculus, Vg = Ventral ganglion, ab = abnormal, ff = free-floating in haemocoele, lg = large, m = missing, mel = melanized, norm = normal, sm = small, v = variable.

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FIGURE 4.- Montages depicting the brain complex, ring gland, dorsal vessel and lymph gland anatomy of late third instar larvae. A, y^1 w^1 air13 (P strain) mutant exhibiting hypertrophy in the lymph glands and B, y^1 w^1 (M strain) larva with wild type lymph gland morphology. Abbreviations are: BH, brain hemispheres; DV, dorsal vessel; ID, imaginal discs, LG, lymph glands; PC, pericardial cells; RG, ring gland; VG, ventral ganglion. Magnification A, B 15X.

A

LG

LG

RG

DV

BH

VG

LG

DV

LG

LG

y1 w1 air13

B

PC

LG

LG

DV

RG

BH

VG

y1 w1
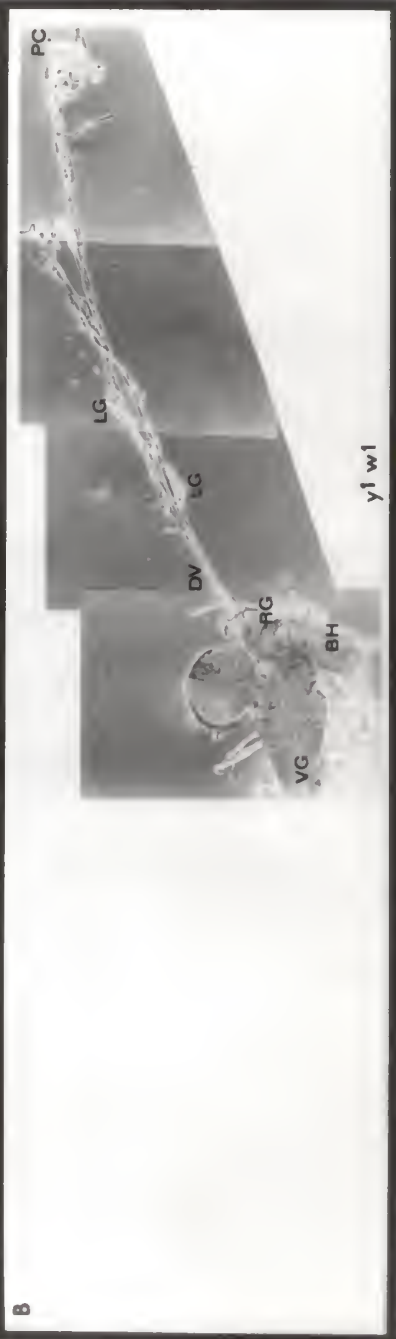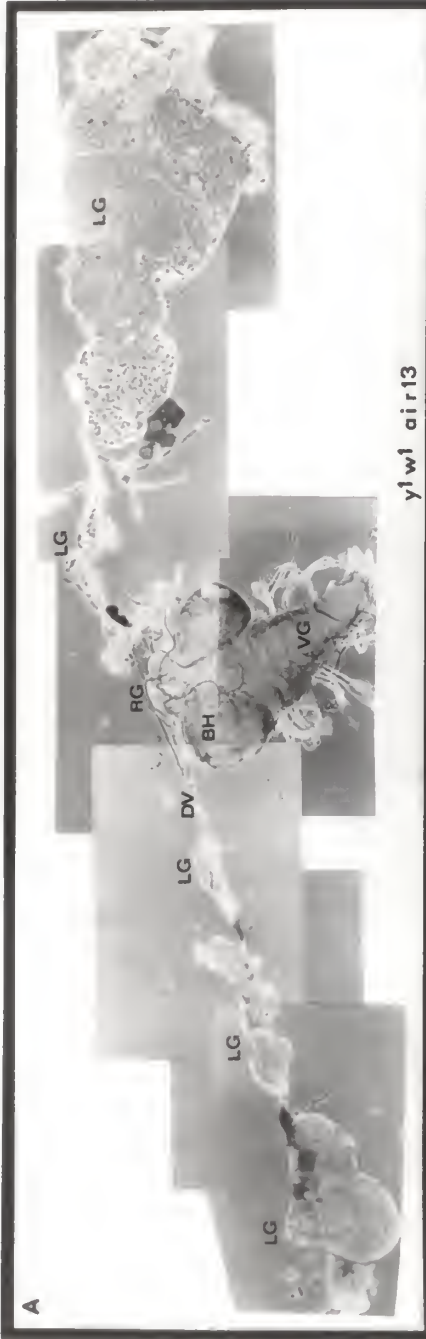
FIGURE 5.- Hypertrophy in the lymph gland lobes of air11
and air16 third instar male larvae. A, Lymph gland
lobes of an air16 larva. Melanotic nodules are located
in the anterior lymph gland lobes. B air16 lymph gland
lobes. The insert depicts the brain complex and lymph
glands of the same individual. C, Lymph gland lobes and
brain-ring gland complex (insert) of an air11 larva.
The anterior pair of lobes contain melanotic nodules. D,
Another air11 mutant larva with hypertrophied lymph
glands. Abbreviations are: BH, brain hemispheres; DV,
dorsal vessel; ID, imaginal discs, LG, lymph glands; RG,
ring gland; VG, ventral ganglion. Magnification A-D
27X, inserts in B and C 7X.
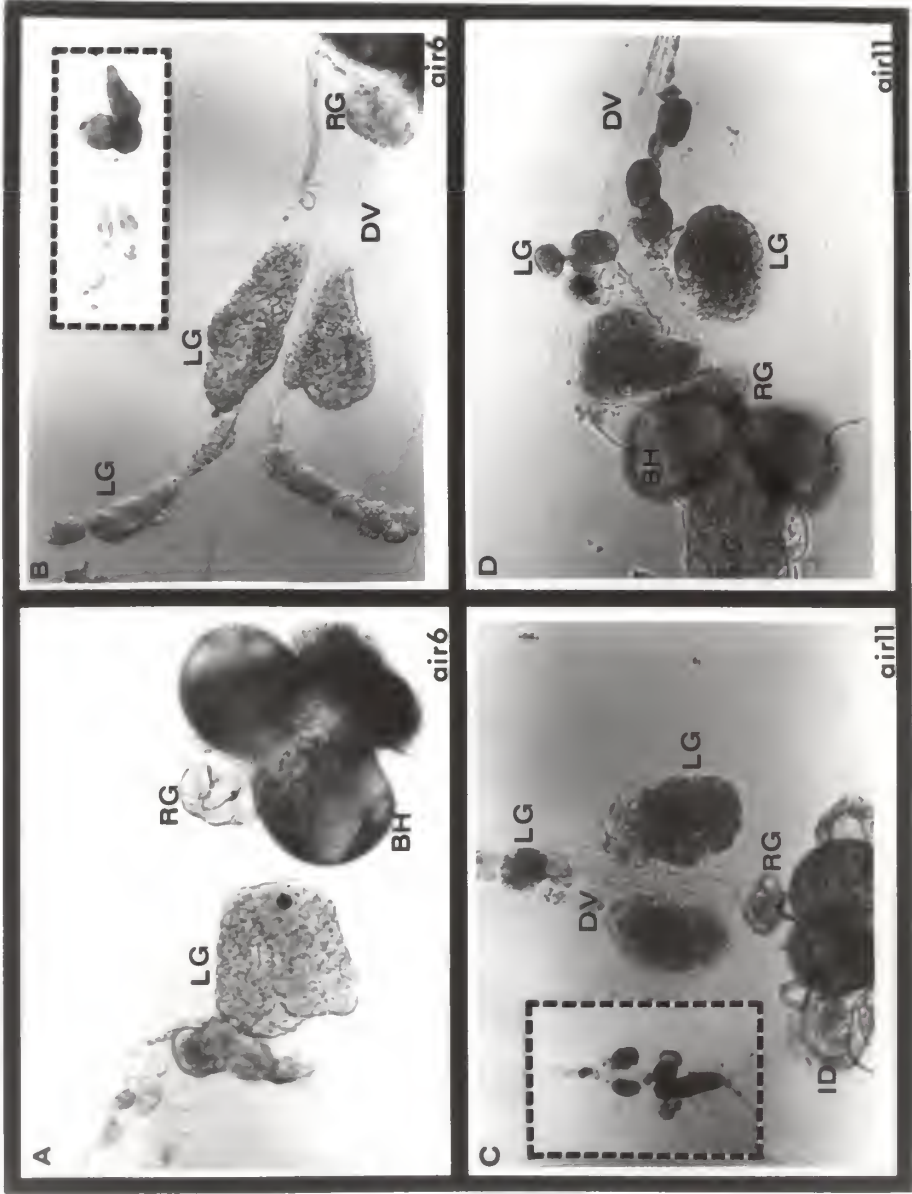
FIGURE 6.- Amplification of lymph gland lobe number in
mutant versus wild type larvae. A, A montage of the
brain and lymph glands in a $\underline{y}^1$ $\underline{w}^1$ (M strain) larva with
"normal" lymph gland morphology. B, An air13 mutant
displaying an increase in the number of lymph gland
lobes. Abbreviations are: BH, brain hemispheres; DV,
dorsal vessel; ID, imaginal discs, LG, lymph glands; RG,
ring gland; VG, ventral ganglion. Magnification A 26X, B
8X.

A

y¹ w¹

LG
AG
VG
BH
ID

B

air13

LG
LG
VG
BH
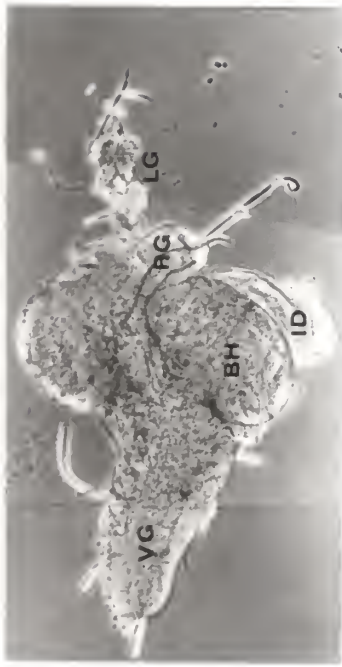
distinguishable lymph gland lobes that were enlarged slightly. It was uncertain in these mutant individuals whether the hypertrophied lobes had fragmented and adhered to the dorsal vessel or whether the lymph glands were associated with hyperplastic growth.

The portion of the alimentary canal that marks the junction between the mid- and hind-gut was melanized in the mutants: air1, air3, air4, air5, air7, air9, air10, air11, air12, air16[b] and air16[c]. Many imaginal disc abnormalities were observed that were subject to considerable variation between individuals bearing the same mutation. Those defects found in the majority of dissected animals were reported as characteristic of that mutant phenotype, otherwise the label "abnormal" was applied.

Observations of Melanotic Phenotypes in Non-air Stocks: To demonstrate that the melanotic phenotype was not merely cytotype-specific or due to an endogenous mutation present in the original mutagenesis stock, an experiment was designed to examine both point lethals and deficiency stocks for the presence of melanotic larvae. The stock vials scored for the presence of tumorous larvae had been aged for a sufficient time to allow representation by all progeny classes including those classes with retarded developmental rates. Positive reports of melanotic larvae were made only when repeated examinations of these stocks revealed a significant number of larvae with melanotic

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tumors or other forms of melanosis. Larvae displaying melanotic tumors were divided into two categories based on the appearance of the tumor phenotype. Category A was characterized by large, melanized tumors or strong tumor expression (Table 8A, B). Those stocks that contained third instar larvae with small melanotic tumors or some form of melanosis were assigned to Category B (Table 8A, B). These phenotypic categories were arbitrary divisions based on the severity of the tumor phenotype and do not necessarily reflect differences in immune responsiveness. The melanotic larvae observed in deficiency stocks which lacked duplications were presumed to represent the hemizygous/deficiency class of male larvae. Deficiency males maintained with Y-borne translocations must have genes/products acting in a dominant manner to give rise to melanotic tumors in these larvae. Those deficiency stocks shown to have third instar larvae with melanotic tumors at a rate higher than would be expected by "spontaneous" tumor events are listed in Table 8A. X-chromosome lethal stocks that were not induced using hybrid dysgenesis were also scored for tumorous larvae. The lethal stocks bearing tumorous larvae are listed in Table 6B. These results must be interpreted with the realization that this phenotype can exist as a normal defense response against wounding or infection and is also consistent with the presence of an unlinked mutation in the background genome.

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TABLE 8A

Deficiency Stocks Containing Melanotic Larvae.

Stock Description   Deleted Interval

CATEGORY A: Large, melanotic tumors.	
Df(1)A94 / FM6	1E3;2B15
Df(1)TEM304, y^2 w^i ct^6 f/ B^S w^+ y^+.Y/ FM6,l	2E2-F1;3A4-6
Df(1)C149 / FM6	5A8-9;5C5-6
Df(1)ct^{J6}, f/ C(1)DX, y w f; Dp(1;3)sn^{13a}	6E1;7C1
Df(1)ct^{J4}, f/ ct^+ Y / FM6, lethal	7A2;7C1
Df(1)v^{L15}, y/ C(1)DX, y w f; Dp(1;2)v^{+75d}	9B1;10A1
Df(1)DA622 / FM7	10B8;10D2
Df(1)M13 / FM7	10B10;11A3-7

CATEGORY B: Small tumors and/or Melanosis.	
Df(1)svr, spl ras^2 fw/ y^2 Y67g19.1/ C(1)DX, y f	1A1;1B10
Df(1)S39 / FM6	1E4;2B11-12
Df(1)w^{258-42} / FM7	3A4-6;3C5-6
Df(1)w^{m4L} rst^{3R}, w^- car/ C(1)DX, y w f; Dp(1;2)w^{+51b7}	3C1-2;3C3-4
Df(1)HA32 / ct^+ Y / FM6, lethal	6E4-5;7A6
Df(1)ct^{78}, y w f/ y^+ ct^+ Y/ C(1)DX, y f	6F1-2;7C1-2
Df(1)ct^{4b1}, t^2 v; Dp(1;3)sn^{13a} (ct^i)/ TM1, Me ri sbd^i	7B2;7C4
Df(1)RA2/ FM7	7D10;8A4-5
Df(1)KA14 / FM7	7F1-2;8C6
Df(1)C52 / FM6	8E4;9A2
Df(1)v^{L3}/ C(1)DX, y w f; Dp(1;2)v^{+63i}/ Cy	9F6-9;10A6-7
Df(1)RA37 / FM7c	10A6-8;10B15-17

TABLE 8B

X-Chromosome Lethal Stocks Containing Melanotic Larvae.

Stock Description   Deleted Interval

CATEGORY A: Large, melanotic tumors.

---

dor^1 / FM6   1F1;2A2

l(1)RC61 / FM7   7A6;7A8
  syn: $l(1)7Ac^6$

$l(1)dsh^{VA153}$ / FM7   10B6;10B7
  syn: l(1)10Bd or dsh

$l(1)hop^{L4}$ / FM7   10B6;10B8
  syn: l(1)10Be or hop

v l(1)v127 fsa f_1/ v^+ B^+ Y y^+ 10B17;10C2
  syn: $l(1)10Bj^1$

CATEGORY B: Small tumors and/or melanosis.

---

l(1)HF302 / FM7   7A6;7A8
  syn: $l(1)7Ac^2$

l(1)GA41 / FM7   7C6;7D1
  syn: $l(1)7Cg^1$

l(1)DF984 / FM7   7C6;7D1
  syn: $l(1)7Ch^1$

l(1)v107 dy / v^+_4 B^+ Y y^+ / FM6   9F4;9F8
  syn: $l(1)9Fh^4$

C(1)DX, y f / y lz g l(1)v24 / v^+ B^+ Y y^+ 10A9

ras v l(1)dsh f / FM7   10B4;10B9
  syn: l(1)10Bd or dsh

$l(1)dsh^{v26}$ / v^+ Y y^+ / FM7   10B6;10B7

$l(1)dsh^{M20}$ / FM7   10B6;10B7

ras $l(1)hop^{v48}$ dy / v^+ B^+ Y y^+ / FM3   10B4;10B9
  syn: l(1)10Be or hop

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hop^{msvl} / FM7	10B6;10B8
l(1)hop^{HC257} / v$^+$ Y y$^+$ / FM7	10B6;10B8
l(1)hop^{VA85} / v$^+$ Y y$^+$ / FM7	10B6;10B8
l(1)hop^{GA32} / FM7	10B6;10B8
l(1)hop^{Clll} / FM7	10B6;10B8
l(1)d.lg-1, y w sn / Binsn syn: l(1)10Bf or <u>dlg</u>	10B8;10B9
l(1)v28 dy / v$^+$ Y y$^+$ / FM6 syn: l(1)10Bi^1	10B17;10C2

Reversion of the air Mutant Lethal Phenotype: Reversion
of air lethality at a rate higher than spontaneous
frequencies would strongly implicate P element inserts as
being responsible for the air mutant phenotype. Five air
mutants that were covered with Y-borne translocations were
tested for their ability to revert under dysgenic
conditions (Table 9). The mutants air2, air3 and hop^{air}
reverted at frequencies that were comparable or higher than
2.6-6.5% that was reported for reversion to non-lethality
of P element-induced mutations in the dysgenic state
(VOELKER et al. 1984). Since some P element transpositions
are known to occur premeiotically (ENGELS, 1983), the high
reversion frequencies observed for air2, air3 and hop^{air}
stocks may be due to clusters generated by a single
premeiotic event. No dysgenic revertants were obtained for
air1 and air7, which might be the result of a small sample
size. Since the tested mutants did not represent selected
variants within the air collection, it can be assumed that
the majority of air mutants contain P elements capable of
transposing under dysgenic conditions.

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TABLE 9

Dysgenesis-Induced Reversion of <u>air</u> Lethality.

<u>Scheme:</u>

Score progeny
($\underline{y}^1$ $\underline{w}^1$ males = revertants)
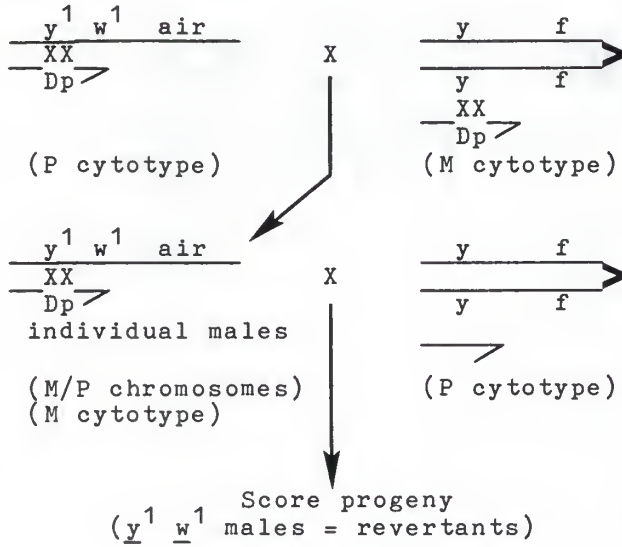
Results:

Mutant	Number of Revertant males	Total Offspring	Reversion Frequency (%)
<u>air1</u>	0	8	0
	0	8	0
<u>air2</u>	19	38	50.0
	21	35	60.0
<u>air3</u>	3	26	11.5
	1	10	10.0
<u>air7</u>	0	7	0
	0	4	0
	0	11	0
	0	6	0
<u>hop</u>^{air}	1	19	5.3

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DISCUSSION

Cellular immunity in _Drosophila_ involves a series of
integrated reactions leading to the encapsulation of
foreign objects that are too large to be phagocytosed. The
reactions include: recognition of "non-self",
developmentally-regulated transformation of plasmatocytes
to lamellocytes, aggregation and layering of haemocytes to
form a cellular capsule, and the deposition of melanin to
render the immune capsule and its contents physiologically
inert (CHADWICK, 1975; GATEFF, 1984; GOTZ, 1986; NAPPI,
1975; RIZKI, 1978). It was suggested that tumorigenesis
(haemocytic encapsulation) in _Drosophila_ was under the
control of two polygenic systems (CORWIN and HANRATTY,
1976). The two systems were involved in regulation of
haemocyte differentiation and transformation and in
rections leading to the initiation of melanization. Since
genetic control of the cellular defense reactions could
operate at many steps in this process, it is not surprising
that great variability in the expression and penetrance of
the tumorous phenotype exists in the known melanotic tumor
mutants (SPARROW, 1978). The assessment of variable
expression and penetrance of the tumor phenotype usually is
based only on the appearance of melanotic tumors and not on
the presence or absence of the haemocytic capsule. RIZKI
and RIZKI (1986) have demonstrated that amelanotic capsules

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are formed in some tumor mutants supporting the hypothesis that the deposition of melanin is under different regulatory control than the encapsulation reactions. In addition, melanin formation is closely associated with a variety of physiological reactions involving phenoloxidase enzymes that require careful regulation to avoid exposing the larvae to the toxic intermediates generated in these reactions (SODERHALL and SMITH). It may well be that fluctuations in the deposition of melanin are responsible for most of the variability in expression and penetrance that is associated with the melanotic tumor phenotype.

The air mutations described in these studies have genetic lesions which trigger some form of autoimmune response leading ultimately to the development of melanotic tumors. It was important to determine whether the lethal and melanotic tumor phenotypes expressed by the air mutants were the consequence of a single mutational event or due to multiple mutational events. We attempted to separate tumor formation from the lethal phenotype by examining third instar male larvae bearing: 1) non-lethal recombinant chromosomes containing material which flanked but did not include the lethal air locus 2) dysgenically-induced, non-lethal revertant air chromosomes and 3) duplication-covered air chromosomes. All strategies failed to separate the lethal and tumorous aspects of the air mutant phenotype suggesting that both traits were caused by a single genetic

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lesion. The phenotype of the X-linked, neoplastic mutant tumorous-lethal (Tum^1) consists of many distinct pleiotropic effects including lethality, lymph gland neoplasia and a melanotic tumor phenotype (HANRATTY and RYERSE, 1981). Experiments that were designed to separate the lethal and tumor phenotypes in the Tum^1 mutant failed to reveal any evidence that the two characteristics assorted or segregated independently. Both Tum^1 and another well-characterized, X-linked tumor mutation $tu(1)Sz^{ts}$ exhibit a temperature-sensitive, tumor phenotype. Unlike these mutations, the air mutants did not show a temperature-sensitive adult tumor phenotype (data, not shown), and any temperature-influenced effects on larval pathology were not investigated. Heterozygous Tum^1 females at the restrictive temperature express a dominant melanotic tumor phenotype in both larvae and adults. We have noticed dominant melanotic tumor expression in heterozygous larvae from many of the air mutant stocks.

The 20, P element-induced air mutations resolved into a minimum of 14 separate complementation groups that were distributed along the length of the X-chromosome. Confirmation of additional allelic relationships between air mutants covered by a common duplication, was not possible due to the infertility of some duplication-covered air males. Three of the air loci had additional allelic variants associated with them, namely: the complementation

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group at l(1)7Ac (LINDSLEY and ZIMM, 1987), hopscotch (hop, PERRIMON and MAHOWALD, 1986) and air16 (this study). The lethal period associated with each air mutant was multi-phasic suggesting that the wild type gene products of the air loci may be required at many distinct developmental steps or continuously throughout larval development. The multi-phasic lethality might indicate that air products are required early in development, and mutant products will induce melanotic tumor formation if the individual survives to later stages of development.

It was interesting to note that stocks bearing X-chromosome deficiencies, whose cytological limits included air loci, frequently contained tumorous larvae. Larvae from the deficency stocks Df(1)TEM304, Df(1)DA622 and Df(1)M13 exhibited melanotic tumors characteristic of Category A (Table 8A, B) or a strong melanotic tumor phenotype, but these deficiencies did not include any of the air mutant loci. However, the melanotic tumor mutant, l(1)ml was previously mapped to region that falls within the limits defined by Df(1)TEM304 (LINDSLEY and GRELL, 1968). The other two deficiency stocks may uncover additional loci in which a loss-of-function leads to melanotic tumor formation. Another dysgenesis-induced lethal with an air mutant phenotype was mapped within the boundaries of Df(1)A94 but was not allelic to air2 suggesting the existence of additional tumorous loci (A. L.

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Johnson, unpublished results). This explanation is supported by the high correlation that existed between tumorous larvae observed in lethal point mutant stocks (not previously associated with a melanotic phenotype) and those observed in deficiency stocks that included the point mutant loci within their cytological boundaries. For example, the deficiency Df(1)DA622 includes l(1)disc-large (l(1)dlg) (PERRIMON and MAHOWALD, 1986) and the deficiency Df(1)M13 includes both $l(1)10Bi^1$ and $l(1)10Bj^1$ (LINDSLEY AND ZIMM, 1987). All of these deficiency and lethal point mutant stocks contained tumorous larvae. The presence of larvae with a strong melanotic tumor phenotype was noted in the $Df(1)v^{L15}$ stock and this deficiency fails to complement the melanotic tumor mutant airl2. All of the deficiency stocks that exhibited small tumors or some other form of melanosis (Category B, Table 8) also contained melanotic tumor loci within their deficiency interval except the following: Df(1)S39, $Df(1)w^{258-42}$, $Df(1)w^{m4L}$ and Df(1)HA32. We expect that these deficiency stocks include unidentified tumor genes or genes which are capable of influencing tumor formation at other loci. Although the melanotic tumor phenotype could have been caused by mutations at different sites in the genome it seems unlikely that there would be such a high correlation between lethal point mutant stocks bearing tumorous larvae, the air mutant loci and allelic deficiency stocks that also

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give tumorous larvae.

Various lethal point mutant stocks bore tumorous larvae (Table 8B), including two alleles of air7, seven variants of the hopscotch locus, and four dishevelled (dsh) alleles. Developmental and genetic analyses of the hop locus have indicated that products of this locus are required both maternally for proper embryonic development and zygotically for the proper regulation of cell division in late larval stages (PERRIMON and MAHOWALD, 1986). Mutations of the dsh locus are associated with extreme segment polarity defects in developing embryos, presumably caused by the inability of the mutant embryos to maintain or express a determined state (PERRIMON and MAHOWALD, 1987). The dsh gene product was also required late in larval development for the proper differentiation of imaginal discs (PERRIMON and MAHOWALD, 1987). It is interesting that mutations at these two loci were associated with tumor expression during the late larval stage, which coincided with the time that the wild type products of these loci are required to complete proper development.

We have cytologically mapped the melanotic tumor mutation Tum^1 to 10A1;10A6-7 which is a region reported to contain approximately four distinct complementation groups (GEER, LISCHWE and MURPHY, 1983; ZHIMULEV et al. 1981). This map position was derived from coverage by the duplications v^+y y^+3 (9F3;10E3-4) and $v\ y\ y^+$ (9F3;10C1-2) and exclusion from

the deficiencies $Df(1)v^{L15}$, $Df(1)RA37$, $Df(1)KA7$, $Df(1)N71$,
and $Df(1)M13$. It was noted that Tum^1 females in
heterozygous combination with chromosomes containing the
genetic variants: hop^{air}, hop^{v48}, dlg, $l(1)v28$
($l(1)10Bi^1$), $l(1)V178$ ($l(1)10Bb^{12}$, LINDSLEY and ZIMM,
1987), $Df(1)v^{L15}$, $Df(1)v^{L2}$, $Df(1)RA37$, $Df(1)KA7$, $Df(1)N71$,
$Df(1)DA622$ and $Df(1)N12$ showed enhanced melanotic tumor
expression at the restrictive temperature. Since the Tum^1
mutation is associated with a dominant tumor phenotype in
females at 29^oC (NAPPI and CARTON, 1986), the enhanced
tumor expression was assessed by comparing Tum^1/deficiency
(or Tum^1/lethal) females to Tum^1/balancer sibling females
generated from the same cross. The non-lethal melanotic
tumor mutant $tu(1)Sz^{ts}$ (RIZKI and RIZKI, 1980b) when reared
at the restrictive temperature exhibited enhanced tumor
expression in heterozygous combination with $Df(1)v^{L15}$,
$Df(1)v^{L3}$, $Df(1)v^{L2}$, the deficiency segregant of $T(1;2)v^{65b}$,
$Df(1)DA622$, $Df(1)KA7$, $l(1)Q54$ ($l(1)9Fe^1$, LINDSLEY and ZIMM,
1987) and hop^{v48}. The $tu-Sz^{ts}$ mutation was previously
localized to 10A10-10A11 (RIZKI and RIZKI, 1980b) and is
not allelic Tum^1 based on our cytological map position
(10A1; 10A6-7). These results strongly suggest that some
melanotic tumor loci can interact synergistically or
quantitatively with other genes to produce a tumor
phenotype.

We propose that melanotic tumor mutations can be

classified into two general groups based on how the immune
responses are triggered. The first group includes those
genetic lesions which cause abnormal cell surface
components to be expressed or presented to the immune
surveillance system. The second group is represented by
mutaions which non-specifically induce defense responses
because of the abnormal temporal or spatial distribution of
haemocytes (ie. lymph gland neoplasia; HANRATTY and RYERSE,
1981). RIZKI and co-workers (RIZKI and RIZKI, 1974a, b;
RIZKI and RIZKI, 1980a, b) have demonstrated that the
specific encapsulation of caudal adipose cells in the tu-W
and tu-Sz^{ts} mutants that coincides with basement membrane
degeneration in the fat body tissue. This mutation is
representative of the first group of melanotic tumor
mutant, in which a specific tissue defect induces the
defense response. Recent studies by KNIEBIEHLER et al.
(1987) have ascribed a role for haemocytes in basement
membrane deposition based on the production and
accumulation of type lV collagen gene transcripts by the
haemocytes. Prior to these studies EL SHATOURY (1957a,b,c)
had reported that a relationship existed between the cycles
of hypertrophy and regeneration occurring in the lymph
glands at each larval molt, and the proper differentiation
of the testes, hind-gut primordia, proventriculus and the
salivary gland primordia. Migratory cells released from
the lymph glands (presumably the haemocytes) were the

mediators in the differentiation or morphogenesis of these developing tissues. It is still unclear what role the haemocytes may play in various morphogenetic or differentiation processes.

We tried to determine whether specific developmental abnormalities might have triggered the immune response system in the air mutants. The rationale for this study was to identify specific morphological defects observed in mutant larvae and correlate these with the sites of melanotic encapsulation. We used two approaches 1) larval anatomy was scored for the presence of developmental defects or melanization and tumor formationa and 2) the lymph glands were examined specifically for any evidence of hypertrophy that might suggest neoplastic growth. The first approach allowed us to identify some consistent anatomical anomalies that were sometimes associated with melanotic tumor formation and/or melanosis. Melanotic tumors or melanosis were observed only infrequently in some air mutants due to the variability that existed in expression and penetrance of the melanotic tumor phenotype. One feature common to all of the air mutants was a small brain phenotype. Although the morphology of the brain was often abnormal the small size applied to both the formation centers of the optic lobes and the ventral ganglion. The ring gland in most of the air mutant larvae examined was proportional to the size of the brain. In mutants

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containing ring glands that approached the size proportions expected for wild type larvae, the ring glands were abnormally thin and diffuse in appearance. Defects in the ring gland may have been responsible for the extended larval life observed in some of the air larvae. A portion of the intestine which corresponded to the hind-gut imaginal ring (BRYANT and LEVINSON, 1985), was frequently melanized in the mutants: air1, air3, air4, air5, air7, air9, air10, air11, air12, air16[b] and air16[c]. Melanosis in a region of the proventriculus that may represent the fore-gut imaginal ring was observed in air6 mutant larvae. The imaginal primordia of the gut (imaginal rings), are diploid cells found in larvae that grow by cell proliferation until metamorphosis, at which time point they give rise to portions of the adult intestine (BRYANT and LEVINSON, 1985). Although abnormal proliferation in the imaginal ring cells had been reported for some mutants with extended larval periods (BRYANT and LEVINSON, 1985), we found no evidence that air mutants with prolonged larval periods were more likely to exhibit melanized imaginal rings. It may be significant that the observed gut melanizations were confined to the imaginal ring centers, which are one of the few cell populations in a larva that retains the ability to divide.

Another interesting characteristic associated with the mutants air8 and air16[b] was the presence of melanotic

garland cells (wreath cells). The garland cells are found in a clumped mass at the junction between the oesophagus and the proventriculus and they function to remove toxic wastes from the haemolymph (RIZKI, 1978). The observed melanization in the garland cells may have been caused by the absorption and/or accumulation of melanin obtained from the haemolymph. This may imply that abnormal humoral reactions created these melanotic products, perhaps in response to an inappropriate signal caused by the air mutations.

The second approach that was used to identify specific developmental defects associated with the air mutant phenotype was the examination of mutant male lymph glands. This analysis revealed two types of lymph gland defects. One defect was the extreme hypertrophy of lymph gland lobes that often resulted in rupture and dispersal of the lymph gland fragments throughout the haemocoele (not an artefact of dissection). The mutants: air2, air6, air7, air8, air9, air11, air13 and air15 showed varying degrees of such lymph gland hypertrophy, with small melanotic nodules found in the majority of hypertrophied lymph gland lobes. Total melanotic encapsulation of some lymph gland lobes was observed in many of these mutants. Confirmation that the free-floating masses found in the haemocoele were primarily of lymph gland origin came from observations of incompletely melanized capsules. This was an extremely

interesting observation since lymph gland hypertrophy in other mutants has been associated with a transplantable neoplasm of this gland (ie. Tum[1], HANRATTY and RYERSE, 1981; NAPPI and CARTON, 1986). Some air mutant individuals also displayed variability in the size of the imaginal discs, ring gland and other structures suggesting that there were more global defects in growth regulation.

The other lymph gland defect that was observed in airl3 was an amplification in the total number of lymph gland lobes. This phenotype also was found in conjunction with lymph gland hypertrophy. Lymph glands normally consist of 4-6 pairs of lobes with a predictable morphology that is dependent on the position that the lobes occupy along the length of the dorsal vessel (SHRESTHA and GATEFF, 1982). The airl3 mutant lymph glands were observed with up to 16 separate lobes of variable sizes that were independent of their position along the dorsal vessel. These lobes also were larger than wild type lymph gland lobes.

The present study has provided evidence that at least 14, distinct X-chromosome loci can be mutated to give a lethal melanotic tumor phenotype. We have made a preliminary characterization of the mutant syndrome associated with each aberrant immune response (air) mutation and identified a number of developmental defects that may contribute to the melanotic tumor phenotype. Based on these preliminary observations, the air mutations appear to be equally

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distributed between the proposed classes of melanotic tumor mutants. The consistent melanization in cells or tissues caused by some <u>air</u> mutations, suggests that a specific defect in these cells/tissues triggers melanotic tumor formation (Class 1). Eight <u>air</u> loci were associated with hypertrophy of the lymph gland lobes in mutant male larvae. These eight <u>air</u> mutations may contain neoplastic or abnormal functioning lymph glands that initiate melanotic tumor formation (Class 2).

LITERATURE CITED

BRYANT, P. J. and P. LEVINSON, 1985 Intrinsic Growth
    Control in the Imaginal Primordia of Drosophila, and the
    Autonomous Action of a Lethal Mutation Causing
    Overgrowth. Dev. Biol. 107: 355-363.

CHADWICK, J. S., 1975 Hemolymph Changes with Infection or
    Induced Immunity in Insects and Ticks, pp 241-271. In:
    Invertebrate Immunity, Mechanisms of Invertebrate
    Vector-Parasite Relations. Edited by K. Maramorosch & R.
    E. Shoppe. Academic Press, New York.

CORWIN, H. O. and W. P. HANRATTY, 1976 Characterization of
    a Unique Lethal Tumorous Mutation in Drosophila. Mol.
    Gen. Genet. 144: 345-347.

CRAYMER, L. and E. ROY, 1980 New Mutants-Drosophila
    melanogaster. Drosophila Inform. Serv. 55: 200-203.

EL SHATOURY, H. H. and C. H. WADDINGTON, 1957a Functions
    of the Lymph Gland Cells during the Larval Period in
    Drosophila. J. Embryol. exp. Morph. 5: 122-133.

EL SHATOURY, H. H. and C. H. WADDINGTON, 1957b Development
    of the Intestinal Tract during the Larval Period of
    Drosophila. J. Embryol. exp. Morph. 5: 134-12.

EL SHATOURY, H. H. and C. H. WADDINGTON, 1957c The
    Development of Gastric Tumors in Drosophila Larvae. J.
    Embryol. exp. Morph. 5: 143-152.

ENGELS, W. R., 1983 The P family of transposable elements

in Drosophila. Annu. Rev. Genet. 17: 315-344.

GATEFF, E., 1984 Comparative Ultrastructure of Wild-Type and Tumorous Cells of Drosophila, pp 559-578. In: Insect Ultrastructure, vol. 2. Edited by R. C. King & H. Akai. Plenum Publishers.

GEER, B. W., T. D. LISCHWE and K. G. MURPHY, 1983 Male Fertility in Drosophila melanogaster: Genetics of the vermilion Region. J. Exp. Zool. 225: 107-118.

GOTZ, P., 1986 Encapsulation in Arthropods, pp 153-170. In: Immunity in Invertebrates. Edited by M. Brehelin. Springer-Verlag, Berlin.

HANRATTY, W. P. and J. S. RYERSE, 1981 A Genetic Melanotic Neoplasm of Drosophila melanogaster. Dev. Biol. 83: 238-249.

JOHNSON, T. K. and B. H. JUDD, 1979 Analysis of the cut locus of Drosophila melanogaster. Genetics 92: 485-502.

KNIBIEHLER, B., C. MIRRE, J-P. CECCHINI and Y. LE PARCO, 1987 Haemocytes accumulate collagen transcripts during Drosophila melanogaster metamorphosis. Roux's Arch. Dev. Biol. 196: 243-247.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic Variations of Drosophila melanogaster. Carnegie Inst. Washington Publ. 627.

LINDSLEY, D. L. and G. ZIMM, 1986 The Genome of Drosophila melanogaster. Part 2: lethals; maps. Drosophila Inform. Serv. 64: 1-158.

LINDSLEY, D. L. and G. ZIMM, 1987 The Genome of <u>Drosophila</u>
    <u>melanogaster</u>. Part 3: rearrangements. Drosophila
    Inform. Serv. 65: 1-224.

NAPPI, A. J., 1975 Parasite Encapsulation in Insects, pp
    293-326. In: <u>Invertebrate Immunity, Mechanisms of</u>
    <u>Invertebrate Vector-Parasite Relations</u>. Edited by K.
    Maramorosch & R. E. Shoppe. Academic Press, New York.

NAPPI, A. J., 1977 Comparative Ultrastructural Studies of
    Cellular Immune Reactions and Tumorigenesis in
    <u>Drosophila</u>, pp 155-188. In: <u>Comparative Pathobiology,</u>
    <u>Vol. 3: Invertebrate Immune Responses</u>. Edited by L. A.
    Bulla Jr. & T. C. Cheng. Plenum Press, New York.

NAPPI, A. J. and Y. CARTON, 1986 Cellular Immune Responses
    and Their Genetic Aspects in Drosophila, pp 171-187. In:
    <u>Immunity in Invertebrates</u>. Edited by M. Brehelin.
    Springer-Verlag, Berlin.

PERRIMON, N. and A. P. MAHOWALD, 1986 <u>l(1)hopscotch</u>, a
    Larval-Pupal Zygotic Lethal with a Specific Maternal
    Effect on Segmentation in <u>Drosophila</u>. Dev. Biol. 118:
    28-41.

PERRIMON, N. and A. P. MAHOWALD, 1987 Multiple Functions
    of Segment Polarity Genes in <u>Drosophila</u>. Dev. Biol. 119:
    587-600.

RIZKI, T. M., 1978 The Circulatory System and Associated
    Cells and Tissues, pp 398-452. In: <u>The Genetics and</u>
    <u>Biology of Drosophila</u>, vol. 2B. Edited by M. Ashburner

& T. R. F. Wright. Academic Press, New York.

RIZKI, R. M. and T. M. RIZKI, 1974a Basement membrane abnormalities in melanotic tumor formation. Experientia 30: 543-546.

RIZKI, T. M. and R. M. RIZKI, 1974b Topology of the Caudal Fat Body of the Tumor^W Mutation of Drosophila melanogaster. J. Invert. Pathol. 24: 37-40.

RIZKI, R. M. and T. M. RIZKI, 1980a Hemocyte responses to implanted tissues in Drosophila melanogaster larvae. Wilhelm Roux's Arch. Dev. Biol. 189: 207-213.

RIZKI, T. M. and R. M. RIZKI, 1980b Developmental Analysis of a Temperature-Sensitive Melanotic Tumor Mutant in Drosophila melanogaster. Wilhelm Roux's Arch. Dev. Biol. 189: 197-206.

RIZKI, T. M. and R. M. RIZKI, 1986 Surface Changes on Hemocytes during Encapsulation in Drosophila melanogaster Meigen, pp 157-190. In: Hemocytic and Humoral Immunity in Arthropods. Edited by A. P. Gupta. John Wiley & Sons, New York.

SHRESTHA, R. and E. GATEFF, 1982 Ultrastructure and Cytochemistry of the Cell Types in the Larval Hematopoietic Organs and Hemolymph of Drosophila melanogaster. Develop. Growth and Differ. 24: 65-82.

SODERHALL, K. and V. J. SMITH, 1986 The Prophenoloxidase System: The Biochemistry of Its Activation and Role in Arthropod Cellular Immunity with Special Reference to

Crustaceans, pp 208-223. In: Immunity in Invertebrates.

Edited by M. Brehelin. Springer-Verlag, Berlin.

SPARROW, J. C., 1978 Melanotic Tumours, pp 277-313. In:

The Genetics and Biology of Drosophila, vol. 2B. Edited

by M. Ashburner & T. R. F. Wright. Academic Press, New

York.

VOELKER, R. A., A. L. GREENLEAF, H. GYURKOVICS, B. B.

WISELY, S. HUANG and L. L. SEARLES, 1984 Frequent

imprecise excision among reversions of a P-element-

caused lethal mutation in Drosophila. Genetics 107:

279-294.

ZHIMULEV, I. F., G. V. POKHOLKOVA, A. V. BGATOV, V. F.

SEMESHIN and E. S. BELYAEVA, 1981 Fine Cytogenetical

Analysis of the Band 10A1-2 and the Adjoining Regions in

the Drosophila melanogaster X Chromosome II. Genetical

Analysis. Chromosoma 82: 25-40.

Appendix 1:   Recombination Mapping--air Lethals.
             (sc ec cv ct v g f).

Mapping Stock: sc ec cv ct v g f
                    _____
                    _____

                    FM7 (w^a v^{Of} g B), l
                    (Heterozygous females have orange eyes)

air Lethals: y w   air bb^w
                    _____
                    _____

                    FM7 (w^+ B)

Cross 1:

   y w   air bb^w X   sc ec cv ct v g f
   _____   _____
   _____   _____ /

       FM7 (w^+ B)   Y   /

   y w   air bb^w X   sc ec cv ct v g f
   _____   _____
   _____   _____ /

   sc ec cv ct v g f   Y   /

                          Score males
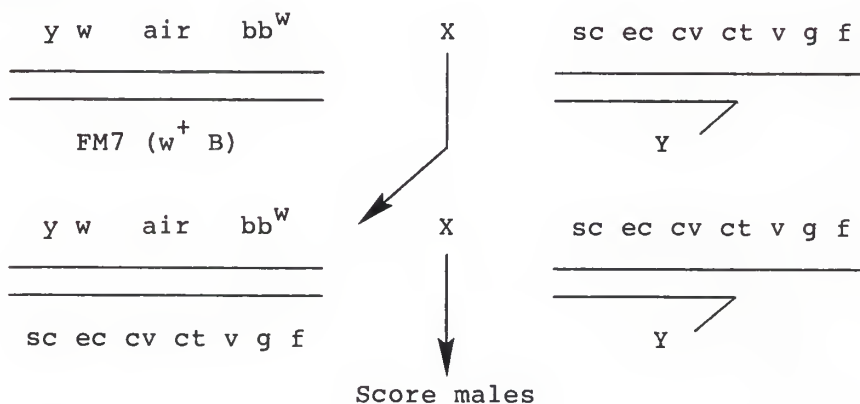
Marker Mutations:

air	=	X	lethal, melanotic tumor mutation
y	=	0.0	yellow
sc	=	0.0	scute
w	=	1.5	white
w^a	=	1.5	white-apricot
ec	=	5.5	echinus
cv	=	13.7	crossveinless
v	=	33.0	vermilion
v^{Of}	=	33.0	vermilion of Offermann
g	=	44.4	garnet
f	=	56.7	forked
B	=	57.0	Bar
bb^w	=	66.0	bobbed (weak)
FM7	=	X	Balancer Chromosome

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Appendix 2: Recombination Mapping--<u>air</u> Lethals
            (<u>y</u> <u>pn</u> <u>cv</u> <u>m</u> <u>f</u> <u>bb</u>^{2rl3}).

<u>Mapping Stock:</u>   y pn cv m f bb^{2rl3}

P: y w   air   bb^w X   y pn cv m f bb^{2rl3}
   ─────────────────────────   ─────────────────────────
      FM7, B y^{31d} sn^{X2} B^S ⁄
      (P females)   (M males)

F1:   y w   air   bb^w X   y pn cv m f bb^{2rl3}
   ─────────────────────────   ─────────────────────────
      y pn cv m f bb^{2rl3} B^S ⁄
      (P females)   (M males)

F2:   Score males
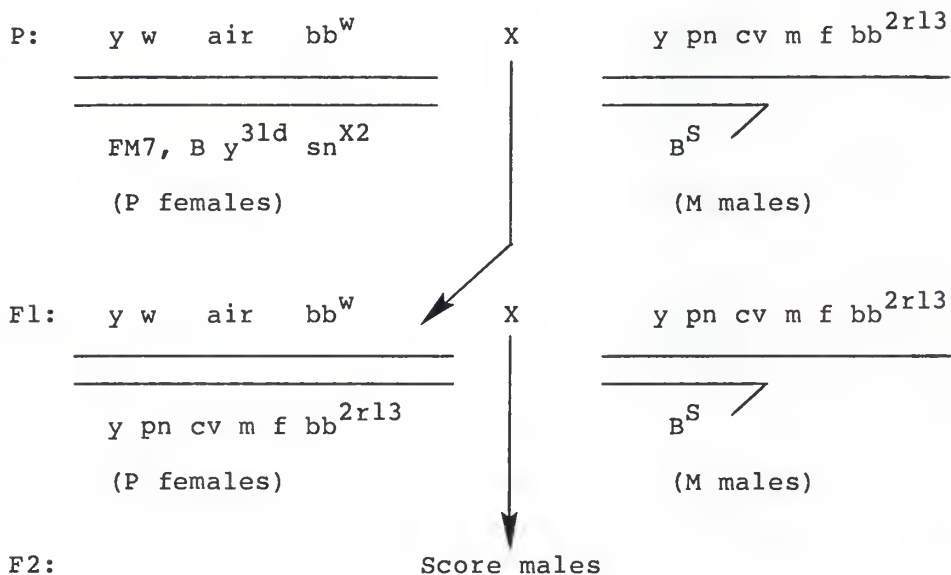
       Marker Mutations:
   ┌───┐
   │   air   =   X   lethal, melanotic tumor mutation   │
   │   y   = 0.0   yellow   │
   │   pn   = 0.8   prune   │
   │   w   = 1.5   white   │
   │   cv   = 13.7   crossveinless   │
   │   sn   = 21.0   singed   │
   │   m   = 36.1   miniature   │
   │   f   = 56.7   forked   │
   │   B$_S$ = 57.0   Bar   │
   │   B   = 57.0   Bar of Stone   │
   │   bb^w = 66.0   bobbed (weak)   │
   │   bb^{2rl3} = 66.0   bobbed (lethal allele)   │
   │   FM7   =   X   Balancer Chromosome   │
   │   P   = P cytotype   │
   │   M   = M cytotype   │
   └───┘

Appendix 3: Construction of an Additional Mapping Stock:
$\underline{sc}$ $\underline{ec}$ $\underline{cv}$ $\underline{ct}$ $\underline{v}$ $\underline{m}$ $\underline{f}$.

<u>Mapping Stock a</u>   <u>Mapping Stock **b**</u>

$$\frac{sc\ ec\ cv\ ct\ v\ g\ f}{1,\ FM7(w^a\ B\ sn^{X2}\ y\ v\ g)}\qquad\frac{C(1)DX\ /\ B^S\ Y}{y\ pn\ cv\ m\ f\ bb^{2r13}}$$

<u>1st Cross:</u>
P1:

$$\frac{sc\ ec\ cv\ ct\ v\ g\ f}{1,\ FM7}\qquad X\qquad \frac{y\ pn\ cv\ m\ f\ bb^{2r13}}{B^S}\diagup$$

(B, g, females)   (males)

<u>2nd Cross:</u>

$$\frac{sc\ ec\ cv\ ct\ v\ g\ f}{y_+pn\ cv\ m\ bb^{2r13}}\qquad X\qquad \frac{sc\ ec\ cv\ ct\ v\ g\ f}{Y}\diagup$$

$(y^+$ cv f females)   (males)

Collect non-bobbed males: $\dfrac{sc\ ec\ cv\ ct\ v\ m\ f}{Y}\diagup$
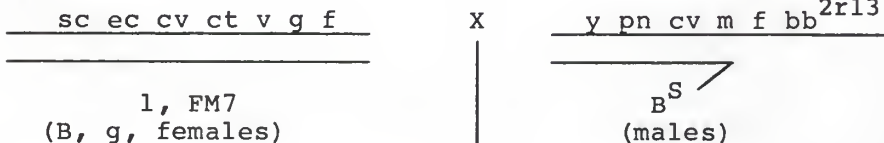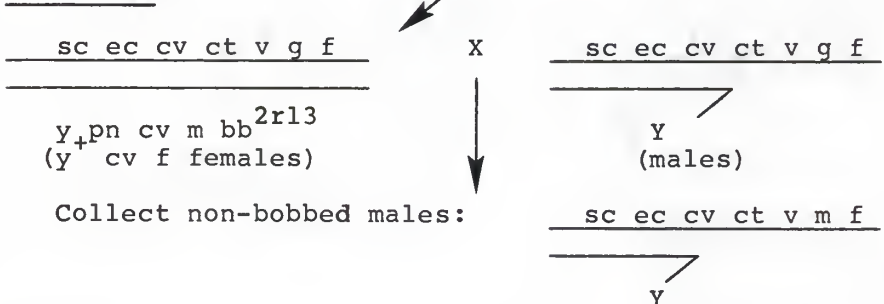
<u>3rd Cross:</u> Cross males to attached-X females to maintain the X-chromosome in males.

<u>4th Cross:</u> Recombination Mapping Crosses.

$$\frac{sc\ ec\ cv\ ct\ v\ m\ f}{Y}\diagup\qquad X\qquad \frac{air}{FM7\ (B)}$$

$$\frac{sc\ ec\ cv\ ct\ v\ m\ f}{air}\qquad X\qquad \frac{sc\ ec\ cv\ ct\ v\ m\ f}{Y}\diagup$$
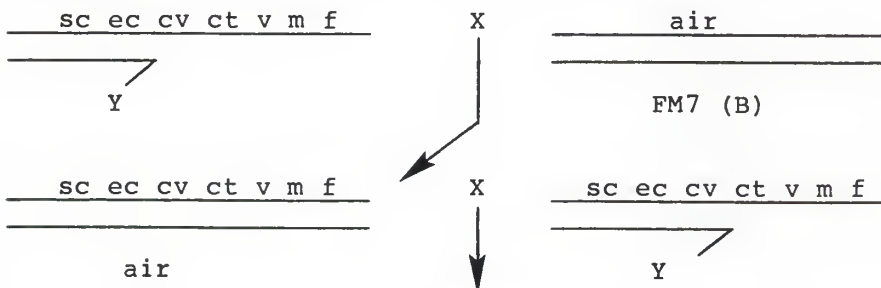
<u>SCORE MALES</u>
<u>Selected Mapping Stock Generated Using this Protocol:</u>

sc ec cv ct v m f / C(1)DX, y w f

Appendix 4:   Recombination Mapping Results--air Lethals

Mutant	Mapping Stock	Marker Interval	Total males Scored	Map Position
air1	1 2	sc-w pn-cv	491 376	0.5 2.1
air2	1 2	w -ec pn-cv	72 276	2.8 4.3
air3	1 2	sc-w w -cv	213 121	1.0 1.7
air4	2	cv-m	529	13.9
air5	2	cv-m	277	15.6
air6	3	cv-ct	104	15.0
air7	2	cv-m	308	15.9
air8	2	cv-m	426	22.3
air9	2	cv-m	125	20.0
air10	2	cv-m	110	21.9
air11	2	cv-m	153	29.0
air12	2	cv-m	246	33.4
air13	2	cv-m	259	35.4
hop[air]	2	cv-m	227	35.3
air14	3	m -f	107	44.9
air15	2	m -f	110	55.6
air16[a]	2	m -f -+	296	56.2
air16[b]	3	m -f -+	219	56.7
air16[c]	3	m -f -+	105	56.7
air16[d]	2	m -f -+	288	56.7

1 = sc ec cv ct v g f   2 = y pn cv m f bb[2rl3]

3 = sc ec cv ct v m f

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Appendix 5: Duplication Mapping the <u>air</u> Lethals

(1) <u>Autosomal-Borne Duplications</u>:

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{FM7,\ B\ y^{31d}\ sn^{X2}}\ \ \frac{+}{+}\quad X\quad \frac{\ \ \ Df}{\diagup}\ \frac{Dp}{+}$$

Lethal chromosome-bearing females:

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{\underset{Df}{(\ \ )}}\ \ \frac{+}{+}\quad -Dp = \text{viable(?) females}$$

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{\underset{Df}{(\ \ )}}\ \ \frac{+}{\underset{Dp}{xx}}\quad +Dp = \text{viable females}$$

Lethal chromosome-bearing males:

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{Y\diagup}\ \ \frac{+}{+}\quad -Dp = \text{lethal males}$$

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{Y\diagup}\ \ \frac{+}{\underset{Dp}{xx}}\quad +Dp = y^1\ w^1\ \text{males}$$
$$\qquad\qquad\qquad\qquad\qquad\text{lethal covered}$$

(2) <u>Y-Borne Duplications</u>:

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{FM7,\ B\ y^{31d}\ sn^{X2}}\quad X\quad \frac{+}{\underset{Dp}{xxx}\diagup}$$

Lethal chromosome-bearing females:

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{+}\quad -Dp = \text{viable females}$$

Lethal chromosome-bearing males:

$$\frac{y^1\ w^1\ \ air\ \ bb^w}{\underset{Dp}{xxx}\diagup}\qquad$$

+Dp = viable $y^1\ w^1$ males,
if the Dp covers the
lethal locus
= lethal, if Dp does not
cover the lethal locus

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Appendix 6: air Lethals--Allelism Tests

(1) Covered Males with Autosomal-Borne Duplications:

$$\frac{y^1\ w^1\quad airA}{FM7,\ y^{31d}\ sn^{X2}\ B} \quad \frac{+}{+} \quad X \quad \frac{y^1\ w^1\quad airB}{Covered\ Males}\diagup \quad \frac{Dp}{+}^{xx}$$

Fl females bearing lethal chromosomes:

$$\frac{y^1\ w^1\quad airA}{y^1\ w^1\quad airB} \quad \frac{+}{+}$$

-Dp & viable = mutations,
                not allelic.
-Dp & lethal = mutations,
                allelic
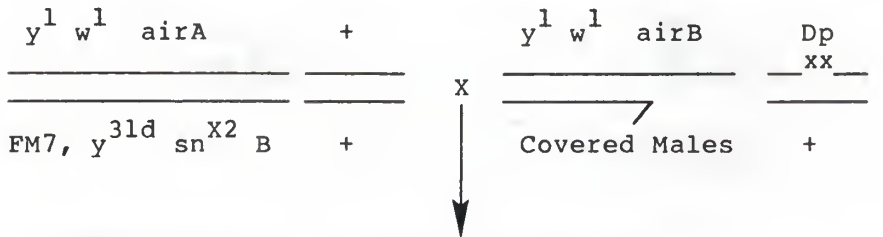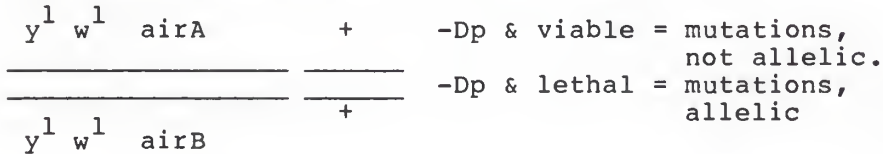
NB: The homologous autosome is usually marked.

(2) Covered Males with Y-Borne Duplications:

$$\frac{y^1\ w^1\quad airA}{FM7,\ y^{31d}\ sn^{X2}\ B} \quad X \quad \frac{y^1\ w^1\quad airB}{\underset{Dp}{xxx}\diagup\ Covered\ Males}$$

Fl females bearing lethal chromosomes:

$$\frac{y^1\ w^1\quad airA}{y^1\ w^1\quad airB}$$

-Dp & viable = mutations,
                not allelic
-Dp & lethal = mutations,
                allelic
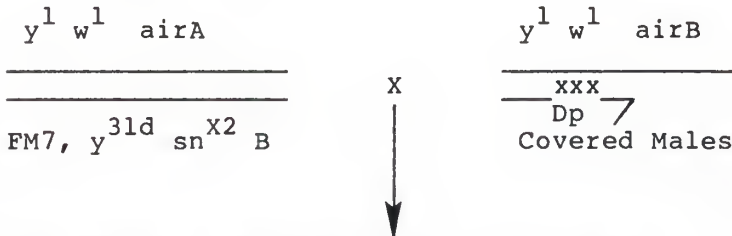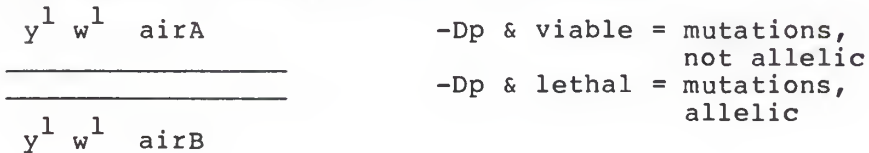
A, B = two different air mutants.

Appendix 7: <u>air</u> Lethals Placed Over a Binsn Balancer

<u>Cross 1:</u>

$$\frac{y^1 \ w^1 \quad air \ bb^W}{FM7, \ y^{31d} \ w^1 \ B}$$
(P cytotype)

X

$$\frac{y^+ \ Binsn}{Y\diagup}$$
(P cytotype)
Derived from repeated
backcrosses to a π^2 strain.

<u>Cross 2:</u>

$$\frac{y^1 \ w^1 \quad air \ bb^W}{y^+ \quad Binsn}$$
(P cytotype)

X
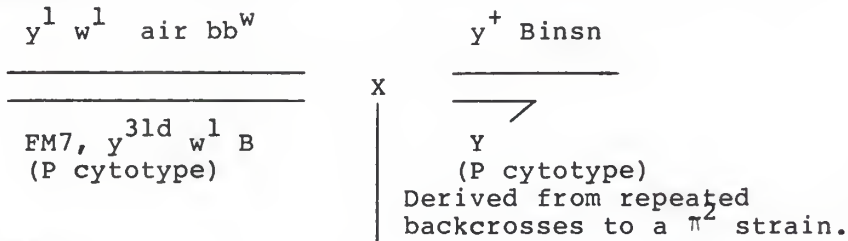
$$\frac{y^+ \ Binsn}{Y\diagup}$$
(P cytotype)
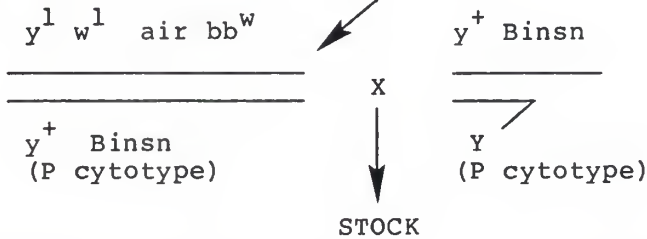
STOCK

Larval Mouthparts in Stock (P cytotype):

$$\frac{y^+ \ Binsn}{y^+ \ Binsn}$$
larvae = black mouthparts ($y^+/ \ y^+$)

$$\frac{y^+ \ Binsn}{Y\diagup}$$
larvae = black mouthparts (y^+)

$$\frac{y^1 \ w^1 \quad air \ bb^W}{y^+ \quad Binsn}$$
larvae = black mouthparts ($y^+/ \ y^1$)

$$\frac{y^1 \ w^1 \quad air \ bb^W}{Y\diagup}$$
larvae = brown mouthparts (y^1)

Appendix 8: Analysis of Recombinant Males that Contain
             Flanking X-Chromosome Material.

$$\frac{y\ w\ \ air\ \ \ bb^W}{y\ pn\ cv\ m\ f\ bb^{2rl3}}\quad X\quad \frac{y\ pn\ cv\ m\ f\ bb^{2rl3}}{B^S}$$

   (M/P chromosomes)   (M chromosomes)
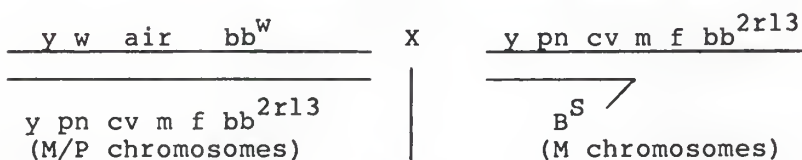
Recombinant males containing markers which flank the
presumed lethal locus were collected and mated to
attached-X females (M cytotype).

Vials of third instar larvae were scored for the presence
or absence of melanotic tumors.

Results

Mutant	Recombinant air Chromosome Constitution	# Vials Examined	# Vials with Tumors *
<u>air1</u>	y pn $cv_+\ m_+$	8	0
	y w cv m_+	6	2
<u>air2</u>	y w cv_+ m f	8	0
	y w cv_+	6	6
<u>air4</u>	y pn cv m^+	8	4
<u>air7</u>	cv m^+	8	4
<u>air8</u>	y pn $cv_+\ m^+$	4	4
	y w cv_+ m	8	0
<u>air9</u>	y pn $cv_+\ m^+\ f^+$	8	0
	y w cv^+ m f	8	0
	y w cv m f	8	0
<u>air10</u>	y w $cv^+\ m_+$	8	0
	cv m^+	4	2
<u>air11</u>	y pn $cv_+\ m^+$	8	0
	y w cv_+ m	4	2
<u>air13</u>	$cv_+\ m_+$	8	0
	cv^+ m	8	0
<u>hop^{air}</u>	$cv_+\ m^+$	8	0
	cv^+ m	8	0

Appendix 8:   (continued)

$\underline{air15}$	y	w cv$^+$ m$^+$ f		8	0
	y pn	cv$^+$ m$^+$ f		8	0
$\underline{air16}^d$		m$^+$ f		6	2
Mapping Stock (M cytotype):					
	y pn	cv m f bb^{2}rl3		8	0
$\underline{y}^1\ \underline{w}^1$ Stock (M cytotype):					
	y^1 w^1	bb^w		6	4

* = The tumors observed in these experiments consisted of
small-medium sized, light brown pigmented material located
at the anterior junction of the mid- and hind-intestine.
In addition, small light brown pigmented nodules were
found lining the dorsal vessel. The melanosis observed in
these stocks was distinctly different from the tumor
phenotype associated with the air mutations.

Appendix 9:   Duplication/Deficiency Results--<u>air</u> Lethals.

Duplication Name (Breakpoints)		air11	air13	air14	air15	air16^d
sn^{+72d}	(7A8;8A5)	-	-			
v^{+75d}	(9A2;10C2)	-	-		-	
v^{+63i}	(9E11;10A11)	-	-		-	-
v$^+$Yy$^+$	(9F3;10C1-2)	-	-	-	-	
v$^+$Yy$^+$3	(9F3;10E3-4)	-	-	-	-	
v^{65b}	(10A1;11A7)	-	-	-	-	
r$^+$f$^+$	(13F10;16A1-2)	-	-	-	-	-
r^{+75c}	(14B13;15A9)	-	-	-	-	-
f^{+71b}	(15A4;16C2-3)	-	-	-	-	+/-

Deficiency Name (Breakpoints)		air11	air13	air14	air15	air16^d
v^{L15}	(9B1;10A1)	-	-		-	
v^{L3}	(9F6-9;10A6-7)	-			-	-
v^{L2}	(9F13;10A1)	-	-			
KA7	(10A9;10F10)	-	-	-	-	
N71	(10B5;10D4)	-	-		-	
DA622	(10B8;10D2)			-	-	
sd^{72b26}	(13F1;14B1)				-	-
D15	(14D1;15C5)	-	-	-	-	-
D17	(14F6;15A6)				-	-

- = complements ie. not allelic.
+ = fails to complement ie. allelic.

P Element-Induced, X-Linked Lethal Mutations Causing

Melanotic Tumors in <u>Drosophila</u> <u>melanogaster</u>

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

Twenty, X-linked lethal mutations exhibiting melanotic
tumor phenotypes were isolated in a P element mutagenesis
experiment. These aberrant immune response (air) mutations
resolved into 14 separate complementation groups that were
distributed along the X-chromosome. Alleles of the
hopscotch locus and the l(1)7Ac complementation group were
represented in the air mutant collection. The lethal period
associated with each air mutation was multi-phasic with
lethality occurring predominantly at the late larval-early
pupal boundary. The air mutant, lethal syndrome included a
small brain phenotype in addition to other morphological
defects which may have contributed to the observed melanotic
tumor phenotype. Seven air mutations gave rise to
hypertrophied lymph glands (hematopoietic organs) that are
characteristic of mutants with invasive lymph gland
neoplasms. The phenotype expressed by the air mutants is
consistent with the suggestion that abnormal cellular immune
responses are implicated in melanotic tumor formation