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# The genetics of some second chromosome melanotic tumour mutants of *Drosophila melanogaster*

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SUMMARY

The genetic relationships of the available second chromosome melanotic tumour mutants in *Drosophila melanogaster* have been investigated. Complementation tests demonstrate the existence of new alleles of the *tu bw* locus and show that *tu-W* and *tu-g* are alleles. The data suggest that there is a minimum of three major gene loci on the second chromosome involved in tumorigenesis. A number of modifier genes were found which affect the penetrance of the major tumour genes analysed. These and the problems they cause in mapping the low penetrant tumour genes are discussed. It has not been possible to map *tu-48a*, *tu-W* and *tu-g* accurately, due largely to the presence of modifier genes. It appears that the genetic basis for melanotic tumour formation is complex.

## 1. INTRODUCTION

About 40 melanotic tumour mutants have been described in *Drosophila melanogaster*, and many normal strains carry subthreshold mutations which can be exposed by culturing larvae in appropriate environments (see Harshbarger & Taylor, 1968, and Sang, 1968, for details). Despite this genetic diversity, the phenotype is strikingly similar for all mutants, and has been well characterized. The tumours are caused by a precocious transformation of haemocytes, from the plasmatocyte to the lamellocyte form. The aggregates of lamellocytes either associate with the fat-body, or form free-floating masses in the haemocoel and melanize just prior to pupation to give the so-called melanoma (Rizki, 1960; Sang & Burnet, 1963). The primary change is thus a simple one, and it is surprising that so many mutations produce the same effect. This raises the problem of how many genes are involved, which is one of the concerns of this paper. Unfortunately over the years many tumorous strains have been lost and the present study involves only those second-chromosome mutants currently available.

Melanotic tumour genes show incomplete penetrance and variable expressivity, and many strains also carry enhancing or suppressing modifier genes (Herskowitz & Burdette, 1951; Wilson, King & Lowry, 1955; Glass, 1957; Ghelelovitch, 1958; Burdette, 1959; Burnet & Sang, 1964; Barigozzi, 1968). Consequently, conventional techniques of localization and analysis have to be modified. None the less, the pattern of inheritance is easily followed if susceptibility to tumorigenesis is

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scored rather than the actual incidence of tumours. In those instances which have been studied the phenotype is usually found to be due to a major recessive gene behaving in a mendelian fashion (Lindsley & Grell, 1968). Surprisingly, most are second chromosome genes, with the exceptions of *tu-53* and *tu-R* which are sex-linked, and *tu-lb* which is on the third chromosome.

This paper reports the results of a series of experiments performed to examine the genetic relationships of second-chromosome melanotic tumour mutants.

## 2. MATERIALS AND METHODS

All flies were grown on yeasted Lewis medium (Lewis, 1960). After eclosion adults were etherized and cleared in 150% (w/v) fructose solution containing Tween 80 as a wetting agent and thymol as an antiseptic (Sang, 1966). After clearing for 24 h melanotic tumours are easily scored without dissection using $\times 25$ magnification.

Genetic details of the strains used are given in the text. The balancer chromosomes *SM5* and *TM3* were used in some of the experiments because they effectively suppress crossing over in the second and third chromosomes. All gene symbols and locations used are as given in Lindsley & Grell (1968). The *all* chromosome, *al dp b pr c px sp*, was checked for gene order before use.

## 3. RESULTS

### (i) *Allelism of melanotic tumour genes*

Complementation tests were performed with the different melanotic tumour strains available and their $F_1$ progeny were classified by the criteria of Erk & Sang (1966). The occurrence of less than 10% tumorous individuals was taken to imply that the major tumour genes of the parental strains were not allelic (Table 1). The results confirm those of Wilson *et al.* (1955), Erk & Sang (1966) and Burnet (1966). The *tu bw*, *tu-B3* and *tu-50d* strains carry allelic tumour mutations which are not allelic to *tu-W* or *tu-48a*, which are not themselves alleles. These five strains therefore represent at least three major tumour loci. The lack of tumorous $F_1$ progeny from crosses between *Samarkand*, a wild-type strain, and the tumour stocks confirms that these tumour genes are recessive, and are not sex-linked.

The three non-allelic strains, *tu bw*, *tu-W* and *tu-48a* were used to test other available tumour mutants. Table 2 shows that *tu-g* is an allele of *tu-W* and that *tu-36a* is an allele of *tu bw*. The other tumour strains, *tu-36e* and *tu-47*, had very low tumour penetrance, which was not significantly increased by crossing to any of the other strains.

Two further features emerge from examination of Tables 1 and 2. First, reciprocal crosses between *tu-B3* and *tu-50d* give significantly different levels of tumour penetrance. Secondly, crosses between *tu bw* and *tu-36a* give $F_1$ progeny with higher tumour levels than the *tu-36a* parental stock. Penetrance data for the crosses between *tu bw*, *tu-B3* and *tu-50d* are presented in Table 3. The progeny from matings between *tu bw* and *tu-B3* or *tu-50d* show consistently high tumour

Table 1. *Complementation tests between various melanotic tumour mutants and a non-tumorous wild type*

| ♂ \ ♀ | *tu bw* | *tu-B3* | *tu-50d* | *tu-W* | *tu-48a* | Samarkand |
|---|---|---|---|---|---|---|
| *tu bw* | + + | + + | + + | − | − | − |
| *tu-B3* | + + | + + | + + | − | − | − |
| *tu-50d* | + + | + | + + | − | − | − |
| *tu-W* | − | − | − | + + | − | − |
| *tu-48a* | − | − | − | − | + + | − |
| Samarkand | − | − | − | − | − | − |

+ +, Tumour penetrance greater than 41%.
+, Tumour penetrance between 11% and 40%.
−, Penetrance less than 10%.

Table 2. *Complementation tests between various melanotic tumour mutants*

| | *tu bw* | *tu-W* | *tu-48a* | *tu-36a* | *tu-g* | *tu-47* | *tu-36e* | Samarkand |
|---|---|---|---|---|---|---|---|---|
| *tu bw* | + + | − | − | + + | − | − | − | − |
| *tu-W* | − | + + | − | − | + + | − | − | − |
| *tu-48a* | − | − | + + | − | − | − | − | − |
| *tu-36a* | + + | − | − | + | − | − | − | − |
| *tu-g* | − | + + | − | − | + + | − | − | − |
| *tu-47* | − | − | − | − | − | − | − | − |
| *tu-36e* | − | − | − | − | − | − | − | − |
| Samarkand | − | − | − | − | − | − | − | − |

Symbols as in Table 1.

Table 3. *Tumour penetrance of the* $F_1$ *progeny from the reciprocal crosses between* tu bw, tu-B3 *and* tu-50d

| ♂ \ ♀ | *tu-B3* | *tu-50d* | *tu bw* |
|---|---|---|---|
| *tu-B3* | 49·7 (117) | 66·2 (148) | 79·5 (88) |
| *tu-50d* | 17·2 (395) | 81·9 (243) | 90·7 (356) |
| *tu bw* | 84·3 (430) | 95·8 (243) | 96·8 (573) |

Figures in parenthesis denote the number of each progeny type scored.

levels, but those between *tu-B3* and *tu-50d* show non-reciprocity. These two strains have significantly different levels of tumour penetrance, and the cross

♀*tu-B3* × ♂*tu-50d*

has a tumour penetrance very much lower than either parental strain or the reciprocal cross. When the penetrance data from a replicate experiment are classified by sexes (Table 4) there are no differences in tumour penetrance between the sexes of a given progeny class, except in the case of the cross

♀*tu-50d* × ♂*tu-B3*.



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Table 4. *The tumour penetrance of* $F_1$ *males and females from reciprocal crosses between* tu-B3 *and* tu-50d

| ♀ / ♂ | *tu-B3* ♀ | *tu-B3* ♂ | *tu-50d* ♀ | *tu-50d* ♂ |
|---|---|---|---|---|
| *tu-B3* | 45·8 (59) | 53·5 (58) | 30·7 (62) | 91·9 (86) |
| *tu-50d* | 18·9 (185) | 16·2 (210) | 81·9 (132) | 82·0 (111) |

In this case the difference between the sexes is large. The female progeny of this cross show a tumour penetrance which is just significantly different from the females of the reciprocal cross (chi-square = 3·86, D.F. = 1). Since the females are genetically identical, this difference may be due to maternal effects. Comparison of the male progeny of the two reciprocal crosses shows that the males from the cross ♀*tu-50d* × ♂*tu-B3* have a very high tumour penetrance. The fact that males hemizygous for the *X*-chromosome from the *tu-50d* female parent have an abnormally high tumour penetrance compared to their sibling females, suggests that this *X*-chromosome carries either a major melanotic tumour mutation or an enhancer mutation affecting the expression of the *tu bw* alleles (*tu-50d/tu-B3*). The former possibility can probably be discounted since crosses between *tu-50d* and a non-tumour strain, *Samarkand*, do not give rise to tumourous male progeny. The presence of a positive modifier on the *X*-chromosome of the *tu-50d* strain might account for this strain having a higher tumour penetrance than *tu-B3*. There is no significant interaction between this modifier and *tu bw*. However, the penetrance scale is inaccurate near its upper and lower limits (Sang, 1963), and it is therefore not possible to decide if the *tu-50d X*-chromosome modifier affects the expression of all the *tu bw* alleles.

(ii) *Chromosomal location of melanotic tumour mutants*

Although it is known that the major genes of the *tu bw*, *tu-48a* and *tu-W* strains are located on the second chromosome (Lindsley & Grell, 1968), an experiment was performed to confirm these findings, and to investigate the possible presence of modifiers in these strains. Each of the tumour strains was crossed to a strain carrying the second chromosome balancer *SM5* and the third chromosome *TM3* (Lindsley & Grell, 1968). From the progeny, the cross

$$SM5/tu;TM3/+ ♀ \times SM5/tu;Sb/+ ♂$$

was made which gave the $F_2$ progeny and results shown in Table 5. None of the $F_1$ progeny showed the tumour phenotype, again excluding the possibility of *X*-linked tumour genes. The results with the *tu bw*, *tu-W*, *tu*-48*a* and *tu-g* strains show that for the tumour phenotype to occur the presence of the original second chromosome (signified by the symbol *tu* in Table 5) is required, the genotype *tu-48a/tu48a*;+/+ showed a high level of tumour penetrance, yet in these crosses not all the *tu-48a/tu-48a* genotypes had this high penetrance. The presence of the

*TM3* chromosome apparently lowers the penetrance of *tu-48a*. Since this effect is not changed by the presence of the original *tu-48a* strain third chromosome, or the *Sb* chromosome, in the heterozygous combinations with *TM3* it seems likely that the *TM3* chromosome carries a dominant suppressor of the *tu-48a* gene. This is apparently a different suppressor from the recessive suppressor described by Sang & Burnet (1967), which suppressed the action of the *tu bw* gene in the genotype *tu bw/tu bw; TM3/su-tu*. The *su-tu* gene is a specific recessive suppressor of *tu bw* (Glass, 1957), and does not suppress the action of *tu-48a* (Burnet, 1966).

Table 5. *Chromosomal location of tumour mutants*

| | Original strain | | | |
|---|---|---|---|---|
| Genotype | *tu bw* | *tu-g* | *tu-W* | *tu-48a* |
| *tu/tu*; +/+ | + + | + + | + + | + + |
| *tu/tu*; *TM3*/+ | + + | + | + + | − |
| *tu/tu*; *Sb*/+ | + + | + + | + + | + + |
| *tu/tu*; *TM3/Sb* | + + | + + | + + | − |

All heterozygotes with SM5 were free of tumours.
Symbols as in Table 1.

### (iii) *Mapping of melanotic tumour mutants*

Only two of the melanotic tumour mutants used in this study have been previously mapped, *tu bw* and *tu-48a*. Recently Grell used three marker genes and mapped *tu bw* at 80·5 (Lindsley & Grell, 1968). The *tu-48a* gene has been mapped at 29·5 on the second chromosome (Ghelelovitch, 1958). The *tu-W* and *tu-g* genes have not been mapped, though the evidence (Wilson, *et al.* 1955) suggests that tumorigenesis in the *tu-W* strain may be due to the presence of two second-chromosome tumour mutants.

Attempts to map *tu-W*, *tu-48a* and *tu-50d* by the usual method of recombination frequency between the tumour genes and mutants in the *al dp b pr c px sp* chromosome gave ambiguous results. This was apparently due to the low penetrance of the tumour genes after outcrossing to the marker stocks, which may demonstrate the presence of tumour modifier genes in the various stocks, *tu bw*, *tu-48a*, *tu-g* and *tu-W*. To circumvent this difficulty, the mating scheme in Fig. 1 was used. Single recombinants carrying successively larger fragments of the marked chromosomes were recovered and backcrossed to the original tumour strain. The tumour penetrance of the resulting progeny were scored (Table 6). The significance of the change in tumour incidence between recombinant classes was tested by a chi-square test, and only significant changes are considered below.

The tests on the *tu bw* strain show that the major tumour gene is located between *c* (75·5) and *px* (100·5). Since it is already known that *tu bw* maps at 80·5 (Lindsley & Grell, 1968), this experiment serves as a control and demonstrates the absence of any second chromosome tumour modifier genes in the strain.

The results obtained with *tu-48a* show that after the addition of *dp* to the left

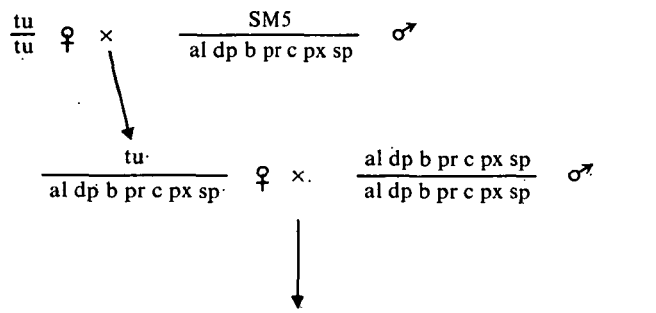


Fig. 1. Matings made to map the tumour genes *tu bw*, *tu-48a*, *tu-g* and *tu-W*.

Table 6. *Results of mapping the tumour genes* tu bw, tu-48a, tu-g *and* tu-W

| Recombinant | | | | | | | *tu bw* | | *tu-48a* | | *tu-g* | | *tu-W* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | tu (%) | *N* | tu (%) | *N* | tu (%) | *N* | tu (%) | *N* |
| *al* | + | + | + | + | + | + | 47·5 | 40 | 32·1 | 250 | 9·6 | 167 | 12·4 | 161 |
| *al* | *dp* | + | + | + | + | + | 45·4 | 33 | 13·4 | 252 | 13·9 | 144 | 2·0 | 247 |
| *al* | *dp* | *b* | + | + | + | + | 44·9 | 40 | 20·4 | 230 | 7·9 | 164 | 0·0 | 144 |
| *al* | *dp* | *b* | *pr* | + | + | + | 39·0 | 40 | 7·5 | 358 | 0·0 | 129 | 1·1 | 94 |
| *al* | *dp* | *b* | *pr* | *c* | + | + | 40·5 | 37 | 0·7 | 144 | 0·0 | 121 | 0·0 | 83 |
| *al* | *dp* | *b* | *pr* | *c* | *px* | + | 0·0 | 40 | 0·5 | 123 | 0·0 | 84 | 0·0 | 123 |
| *al* | *dp* | *b* | *pr* | *c* | *px* | *sp* | 0·0 | 40 | 0·0 | 234 | 0·0 | 106 | 0·0 | 121 |
| + | *dp* | *b* | *pr* | *c* | *px* | *sp* | 0·0 | 41 | 4·1 | 72 | 0·0 | 68 | 0·0 | 41 |
| + | + | *b* | *pr* | *c* | *px* | *sp* | 0·0 | 45 | 3·0 | 135 | 0·0 | 83 | 0·9 | 110 |
| + | + | + | *pr* | *c* | *px* | *sp* | 0·0 | 40 | 0·0 | 56 | 1·5 | 69 | 0·0 | 56 |
| + | + | + | + | *c* | *px* | *sp* | 0·0 | 45 | 3·7 | 243 | 0·0 | 92 | 1·6 | 63 |
| + | + | + | + | + | *px* | *sp* | 27·5 | 40 | 27·4 | 131 | 1·3 | 153 | 18·4 | 71 |
| + | + | + | + | + | + | *sp* | 42·5 | 40 | 35·0 | 140 | 14·3 | 42 | 30·0 | 213 |
| + | + | + | + | + | + | + | 38·7 | 62 | 32·4 | 154 | 13·6 | 96 | 38·5 | 26 |

end of the chromosome there is a significant decrease in tumour penetrance which does not drop to zero until the markers *pr* or *c* are added. This suggests that to the left of *dp* there is a tumour enhancer of *tu-48a* expression in the original tumour stock, or a tumour suppressor in the *all* chromosome. The data imply that *tu-48a* is located between *pr* (54·4) and *px* (100·5), which is not consistent with the earlier mapping of this mutant (Ghelelovitch, 1958). The reason for this disparity is not apparent. A more precise location of *tu-48a* is not possible from this experiment.

The results obtained with the *tu-W* strain are not consistent with the hypothesis that tumorigenesis is due to a single gene. There must be a minimum of two tumour genes present, one at either end of the chromosome. Both genes are apparently

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required for significant levels of tumours, such that neither alone can be called the major tumour gene.

The *tu-g* strain gave very similar results to the *tu-W* strain, which it does not complement. Again there appears to be a minimum of two genes required for tumorigenesis but they are confined to a smaller segment of the chromosome, between *b* (48·5) and *px* (100·5). This implies that the locus or loci at which *tu-W* and *tu-g* are apparently allelic is confined to the chromosome segment between *b* and *px*.

## 4. DISCUSSION

Complementation tests performed on the presently available second chromosome melanotic tumour mutants indicate that they can be assigned to a minimum of three allelic groups. This suggests that there may not be many genes involved in tumorigenesis, especially since two strains previously untested, *tu-W* and *tu-g*, were found to be allelic. Two new alleles of the *tu bw* locus were also discovered, bringing the known number of alleles at this locus to over twelve, a large proportion of the 50–60 tumour mutants ever described (Harshbarger & Taylor, 1968). In this study complementation tests were assessed on the basis of a tumour penetrance of over 10% in the $F_1$ progeny. However, it is well known that a number of isogenic tumour strains show tumour penetrance lower than this (Burdette, 1959). A limited survey of putative wild-type strains (Erk & Sang, unpublished data – see Sang, 1968) revealed that many of them carried subthreshold tumour genes which could be exposed by some of the dietary treatments known to affect tumorigenesis in the *tu bw* strain. Sang (1968) concluded that the tumour genes so far studied represent only part of the tumour gene population carried by *Drosophila*; the number of tumour genes must be large and many are unlikely to be readily revealed even by exhaustive use of the axenic culture technique.

The low and variable penetrance of many tumour genes in different genetic backgrounds presents many difficulties in the design of experiments. The low penetrance of most tumour strains, when outcrossed and backcrossed in the standard manner for mapping genes by recombination frequency, precludes the use of such an experimental design. This is probably due to the interactions of subthreshold genes in some backgrounds and the presence of modifier systems, which are clearly illustrated in this study. Algebraic functions have been derived for analysis of three-point linkage tests involving a gene of low penetrance (Kojima & Dalebroux, 1965), but these assume an absence of linked modifiers; an expectation that is rarely justified. The technique of mapping the major gene using recombinant chromosomes formed as a result of single crossover events between successive markers of a multiply-marked strain and the tumour chromosome is useful for assigning the major loci to particular chromosome segments and for detecting the presence of modifier genes. It suffers from the disadvantage that precise location is not possible.

The results of these experiments show that the genetic basis of tumour formation in the *tu bw* strain is a single major gene and assign the *tu bw* gene to the

chromosome segment 75·5–100·5. Since *tu bw*, which has a high tumour penetrance and can be mapped by standard mapping procedures, has been mapped accurately to 80·5 on the second chromosome (Linsley & Grell, 1968) this justifies the use of this mapping technique. Tumorigenesis in the *tu-48a* strain is apparently also due to a single major gene, although some evidence was found for a tumour enhancer gene on the left chromosome arm. *tu-W* and *tu-g* are not single major genes but depend on two genes for their full expression.

Although the present study has shown that some *tu* genes are alleles, the genetic basis of tumour formation is still demonstrably complex and readily modifiable by mutations at loci other than those of the major genes. Perhaps this second consequence might be expected from the susceptibility of some mutations to penetrance variations caused by metabolic stresses (Sang, 1968). Clearly, this combined genetic and environmental instability of the phenotype makes it difficult to carry out the usual genetic analyses, and the few strains which show very low tumour penetrance (e.g. Table 2) cannot be studied in the conventional way. It follows that the conclusions based only on genes of high penetrance may not apply in these instances.

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