

Experimental modification of the dominance relations of a melanotic tumour gene in *Drosophila melanogaster*

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SUMMARY

The melanotic tumour gene *tu-C4* in *Drosophila melanogaster* shows incomplete dominance, together with variable penetrance and expressivity. It is tentatively located in the region of locus 52–53 on the third chromosome. Tumour formation in mutant homozygotes involves a precocious haemocyte transformation leading to the appearance of lamellocytes at the beginning of the third larval instar. These aggregate to form tumour-like masses which subsequently melanize. The process of tumour formation is in broad outline similar to that found in other tumour strains. Melanotic tumour formation is treated as a dichotomous threshold character, assuming an underlying normal distribution of liability relative to a fixed threshold. The expression of the tumour gene can be influenced by the levels of protein, phospholipid, nucleic acid and carbohydrate in the larval food medium, and changes in dominance and penetrance induced by sub-optimal environments deficient in these nutrients are positively correlated. Reinforcement by selection of the dominance relations of *tu-C4* was accompanied by correlated changes in penetrance. Conversely, selection for increased penetrance was accompanied by correlated changes in dominance. Dominance and penetrance, it is concluded, are fundamentally related aspects of tumour gene expression. Recruitment of dominance modifiers linked to the tumour gene was excluded by the mating scheme employed, and the observed changes in dominance relations in response to selection were due largely to modifiers located on the second chromosome. Changes in dominance relations produced by selection could be significantly reinforced, or reversed, by environmental factors and consequently show a substantial genotype – environment interaction effect. These facts are relevant to current theories of dominance evolution.

INTRODUCTION

Tumours of *Drosophila* can be grouped into three categories. The neuroblastoma of the *lethal giant larva* mutant described by Gateff & Schneiderman (1969) closely resembles the invasive neoplasia of vertebrates. This tumour which originates in the larval brain shows uncontrolled cell division and the tumour cells invade other tissues. Typically ovarian tumours described by King (1970) occur in certain *female-sterile* mutants. They are confined to the ovarian cysts of female flies. In *female-sterile* mutants there is proliferation of cystocyte cell divisions and failure of oogonial differentiation but no invasive growth. Melanotic ‘tumours’ form the

third category. They are formed from larval blood cells and differ from the other tumours in showing no convincing evidence of uncontrolled cell division. Tumour formation in this case appears to be a consequence of the mistiming of a normal process of cell differentiation (Rizki, 1957*b*), and consists of the aggregation of larval haemocytes into tumour-like nodules which are subsequently melanized. The melanized tumours persist through pupation into the adult stage and usually have no obvious effect on the viability of affected individuals.

Many different mutant genes are known to be capable of initiating melanotic tumour formation in *Drosophila* (Harshbarger & Taylor, 1968). Most of these mutant genes are recessive and located on the second chromosome. They have in common the property that the incidence of tumorous individuals in inbred mutant strains is strikingly sensitive to influence by different nutritional and other factors (Burnet & Sang, 1968; Sang & Burnet, 1967) and is consequently the result of a complex genotype-environment interaction. The melanotic tumour gene *tu-C4* described here gives rise to a mutant phenotype which is indistinguishable from that produced by the recessive second chromosome mutants. It shows variable penetrance and expressivity, but is unusual in that it also shows partial dominance. The mutant consequently provides a suitable system for investigating the extent to which genetic and environmental factors are capable of modifying the dominance relations of a major gene mutation.

2. MATERIALS AND METHODS

Strains. The *tu-C4* strain was kindly supplied by Professor C. Barigozzi from the stock collection of the Institute of Genetics, University of Milan. A full description of mutants and balancer chromosomes is given in Lindsley & Grell (1967).

Culture media. The flies were cultured on a standard agar-oatmeal-molasses medium liberally seeded with live bakers yeast. Selection for dominance and penetrance was carried out using this medium. The effect of nutritionally sub-optimal environments was examined using a modification of Sang's medium C described by Burnet & Sang (1968). Eggs were collected over a 4 hr period and sterilized using the procedure described by Sang (1956). Germ-free first-instar larvae hatching 20–24 h later were inoculated into 6 × 1 in. boiling tubes each containing 5 ml of defined medium. Fifty larvae were placed in each culture, using 8–10 cultures for each nutritional treatment. Infected cultures were discarded. All experiments were carried out at 25° C.

Tumours. Tumour incidence was scored as the percentage of hatched adults and unhatched pupae with tumours, and differences in incidence compared using the usual 2 × 2 χ^2 test.

3. RESULTS

(i) Genetic basis of tumour formation in the *tu-C4* strain

In standard live yeast bottle cultures the *tu-C4* strain shows a tumour incidence which is variable but close to 100%. *Prima facie* evidence for dominance of the relevant tumour gene is given by the presence of tumorous individuals in the F_1

Table 1. Tumour incidence (percentage) in the F_1 offspring of crosses between *tu-C4* and different massbred wild stocks

| Strain | Tumour incidence | |
|-----------|-------------------------|-------------------------|
| | ♀ <i>tu-C4</i> × ♂ wild | ♀ wild × ♂ <i>tu-C4</i> |
| Florida | 9.8 | 1.7* |
| Edinburgh | 15.2 | 14.4 |
| Oregon | 53.4 | 30.7* |
| Pacific | 58.5 | 62.9 |

(* Denotes differences between reciprocal crosses which are significant at the 1% level of probability.)

Table 2. Tumour incidence (percentage) in five strains homozygous for different combinations of chromosomes from the *tu-C4* and Edinburgh wild-type strains

| Strain | Chromosome | | | Tumour incidence (%) |
|--------|------------|-----------|-----------|----------------------|
| | 1 | 2 | 3 | |
| (i) | <i>tu</i> | <i>tu</i> | <i>tu</i> | 98.3 |
| (ii) | + | <i>tu</i> | + | 0.7 |
| (iii) | <i>tu</i> | <i>tu</i> | + | 0.3 |
| (iv) | + | + | <i>tu</i> | 85.5 |
| (v) | + | + | + | 0.2 |

offspring of crosses between *tu-C4* and a series of mass-bred wild-type strains (Table 1). Although there are no significant differences between sexes *within* reciprocal crosses – and hence no evidence of sex-linked inheritance, in two cases there is a maternal effect indicated by a significant difference *between* reciprocals. In both instances tumour incidence is higher in the offspring of *tu-C4* females than in the offspring of wild-type females. Results for F_2 and backcrosses of the F_1 offspring to the respective parental strains were in each case consistent with the hypothesis that tumour formation in the *tu-C4* strain is due to the presence of a single partially dominant mutant gene, and that the level of dominance, as suggested by the results summarized in Table 1, is markedly influenced by the genetic background.

The chromosomal location of the *tu-C4* gene was determined by synthesis of a series of five strains homozygous for different combinations of chromosomes derived from the *tu-C4* and Edinburgh wild strains, using a *B*; SM5; TM3 strain with three inversion-containing balancer chromosomes carrying dominant marker genes. No account was taken of the fourth chromosomes. Tumour incidence in each of these strains is shown in Table 2. Strain (iii), which has chromosomes 1 and 2 derived from the *tu-C4* strain, together with the Edinburgh wild third chromosome, shows a very low tumour incidence – not significantly higher than in strain v which has all three Edinburgh chromosomes. Strain (iv), homozygous for the *tu-C4* third chromosome in combination with chromosomes 1 and 2 from the Edinburgh strain, shows a high tumour incidence which approaches that in strain (i) where all

Table 3. *Maximum likelihood estimates of recombination between tu-C4, scarlet and stripe in the backcross*

| | <i>st-tu-C4</i> | <i>tu-C4-sr</i> |
|--|-------------------|-------------------|
| Recombination fraction (y) | 0.085 ± 0.025 | 0.099 ± 0.025 |
| Proportion misclassified (λ) | 0.752 ± 0.020 | 0.744 ± 0.020 |

(Both values of y differ significantly from 0.50, and both values of λ differ significantly from zero, respectively, at the 1% level of probability.)

three major chromosomes are derived from the tumour strain. Tumour formation in the *tu-C4* strain is evidently due to the presence of one or more semi-dominant mutant genes on the third chromosome. Consequently the report of Barigozzi & Di Pasquale (1956) that there is a multichromosomal basis for tumour formation in the *tu-C4* stock, with mutant genes on the first and second chromosomes, finds no support in our data.

The location of the *tu-C4* gene to a precise position on chromosome three is complicated by the incomplete penetrance and semi-dominance of the mutant. The *tu-C4* strain was crossed to a multiple marked strain *ruhthstcusre^sca*. The heterozygous F_1 females were backcrossed to males homozygous for the eight recessive third-chromosome markers. Analysis of the progeny of this backcross shows that the *tu-C4* third chromosome carries a single semi-dominant tumour gene located between *st* and *sr*. Computation of the map position was made using the maximum likelihood method described by Bailey (1961), taking into account the proportion (λ) of tumorous individuals misclassified as wild-types because of incomplete manifestation of the tumour gene. As shown in Table 3, *tu-C4* showed 8.5% recombination with *st* and 9.9% with *sr*, placing the tumour gene provisionally in the region of locus 52–53 on the standard map.

Of the other melanotic tumour genes known to be located on the third chromosome the only one which has been mapped to a precise locus is *benign* (*be-3*) at 25.0 (Stark & Bridges, 1926). This mutant is completely recessive. As far as we are aware, no dominant melanotic tumour gene other than *tu-C4* has previously been recorded in *D. melanogaster*.

(ii) *Melanotic tumour formation in tu-C4 mutants*

Melanotic tumours of *Drosophila* consist of aggregations of larval blood cells or haemocytes which are produced by the lymph glands, or pericardial bodies. The haemocyte population of wild-type larvae consists of spherical plasmatocytes and larger crystal cells. Towards the time of pupation the plasmatocyte undergoes a change in morphology characterized by the appearance of pseudopodia-like projections of the cell surface to give the podocyte variant. In this process the podocyte cells become flattened as extension of the marginal projections continues, to yield a large disk-shaped cell known as the lamellocyte. Detailed description of the haemocyte transformation is given by Rizki (1957a).

Table 4. *The changes in haemocyte morphology at 25 °C in tumorous and wild-type larvae*

| Strain | Larval age (h) | Percentage of cells | | |
|--------------|----------------|---------------------|-----------|--------------|
| | | Plasmatocytes | Podocytes | Lamellocytes |
| Edinburgh | 35 | 92.9 | 7.1 | 0 |
| | 51 | 79.1 | 20.8 | 0 |
| | 64 | 78.2 | 21.6 | 0.4 |
| | 81 | 40.8 | 59.0 | 0.1 |
| <i>tu-C4</i> | 38 | 83.2 | 16.8 | 0 |
| | 50 | 56.3 | 38.9 | 4.7 |
| | 62 | 26.1 | 68.9 | 4.9 |
| | 78 | 6.3 | 91.9 | 1.8 |

(Larval age is expressed in hours after eclosion from the egg and standardized for each strain to a 96 h larval period.)

Changes in haemocyte morphology in the Edinburgh wild and *tu-C4* strains are shown in Table 4. These observations are in general agreement with those previously described by Rizki (1957*b*), and Burnet & Sang (1964), in showing that in tumour strains the haemocyte transformation takes place earlier than in the wild-type. Lamellocytes are present in *tu-C4* larvae at the beginning of the third-larval instar, whereas in Edinburgh wild-type they do not appear at a comparable frequency until after pupation. In contrast with the situation described by Rizki (1957*b*), in neither strain is there an abrupt change from plasmatocyte to podocyte. Rather, there is a gradual change in the proportions of these blood cell types throughout the third-larval stadium.

In sectioned material, amelanotic tumours formed by aggregations of lamellocytes are present towards the posterior end of the abdominal haemocoel by the middle of the third instar. Melanization of the tumour appears to begin simultaneously at many different foci and rapidly spreads so that the entire tumour consists of a dense mass of inert melanin. As shown in Fig. 1 pigmentation of the tumours occurs between 80 and 95 h after eclosion from the egg. The body colour mutant *straw* has no effect on the proportion of individuals with melanized tumours at pupation (Fig. 1). Tumour incidence was reduced to only 64% when the tumour gene was combined in a double homozygote with the body colour mutant *black*. Sectioned material confirms, however, that this is due to lower penetrance of *tu-C4* since there was no evidence for the presence of unpigmented tumours in these flies. These facts confirm the view (Burnet & Sang, 1968) that cuticular and tumour melanin are synthesized by separate biosynthetic pathways.

Fig. 1 shows that in the *stw,tu-C4* and *b,tu-C4* strains the proportion of individuals with melanized tumours at the time of emergence of the adult fly is actually lower than at pupation. This phenomenon is background dependent, occurring consistently in some *tu-C4* strains, but not in others, and appears to be caused by histolysis of small or loosely aggregated tumours during the pupal stage. It may well explain why, in certain instances, experimental modification of the

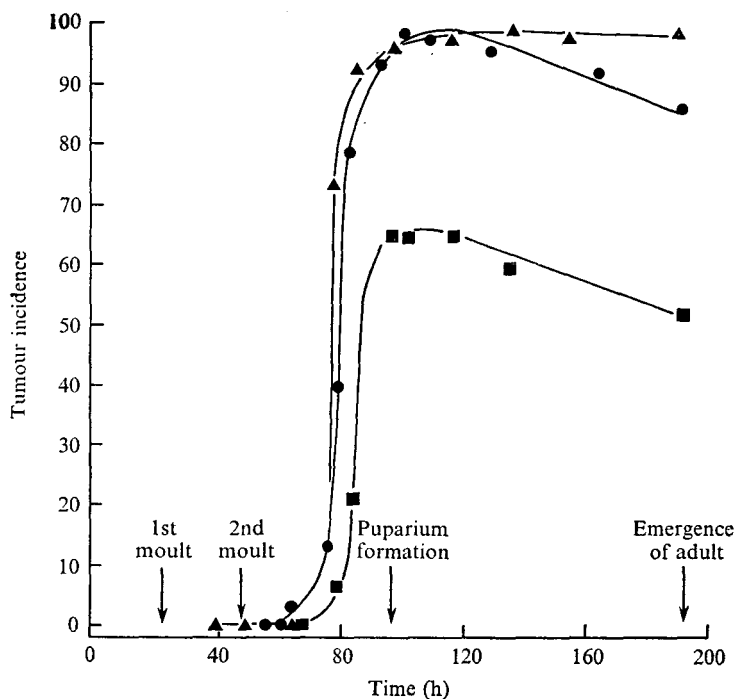


Fig. 1. The frequency of melanized tumours in relation to age (expressed in hours from the time of eclosion from the egg) on a standardized 96 h larval period. The strains are: *tu-C4* (●) *b;tu-C4* (■) and *stw⁵;tu-C4* (▲).

dominance or penetrance of *tu-C4* may apparently fall short of achieving expression of the tumorous phenotype in every individual fly.

(iii) Genotype-environment interactions involving *tu-C4*

Fifteen pairs of flies homozygous for *tu-C4* were introduced into a half-pint bottle culture containing oatmeal medium seeded with a suspension of live yeast. The flies, which mated at random, were allowed to lay eggs for a period of 8 days until the first progeny were about to hatch. The newly emerged flies were then collected at daily intervals for the following 9 days and classified for tumours. The results are shown in Fig. 2. The incidence of individuals with tumours remained constant for the first 3 days of emergence and thereafter declined steadily until, by the sixth day, only 50% of emerging individuals showed the mutant phenotype. Another group of 15 pairs of flies was introduced into a culture bottle made from the same batch of medium. The flies were allowed to lay eggs for 24 h and transferred to a fresh culture bottle on each of 8 consecutive days. As shown in Fig. 2, flies emerging from cultures in which parents were allowed only a 24 h oviposition period show no significant changes in tumour incidence. The penetrance of *tu-C4* in mutant homozygotes, as shown by the emergence effect in mass culture, is susceptible to influence by the environment. Since no maternal age effect can be

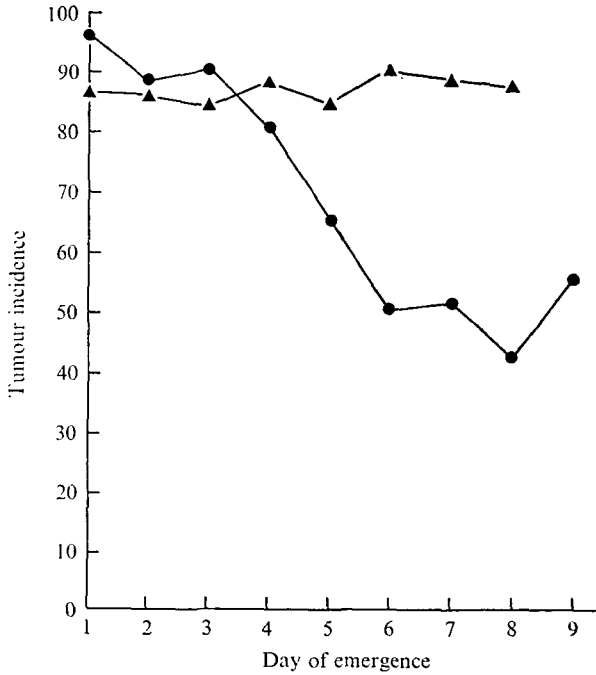


Fig. 2. The relationship between culture age (●) maternal age (▲) and tumour incidence in the *tu-C4* strain.

Table 5. The effect of suboptimal environments on the expression of *tu-C4* in the base population

| | Heterozygotes, <i>tu-C4</i> /TM 3 | Homozygotes, <i>tu-C4</i> / <i>tu-C4</i> |
|------------------------------------|--------------------------------------|---|
| Control tumour incidence (%) | 49.2 | 88.4 |
| Major nutrient deficiencies | | |
| Casein 2.5 % | - 31.1** | - 24.8** |
| Cholesterol 0.0016 % | - 11.1 | - 8.9** |
| Lecithin 0.0125 % | - 25.8** | - 18.1** |
| Ribonucleic acid nil | - 35.5** | - 68.6** |
| Sucrose nil | - 13.4** | - 8.1** |
| Vitamin deficiencies | | |
| Biotin 0.005 µg | - 8.4 | - 5.0 |
| Niacin 5.0 µg | - 7.2 | - 4.2 |
| Pantothenate 12.0 µg | - 0.2 | + 3.8 |
| Pyridoxine 0.8 µg | - 3.7 | - 0.6 |
| Riboflavin 4.0 µg | - 4.9 | - 8.4* |
| Thiamine 0.4 µg | + 37.4** | + 13.1** |

(Tumour incidence on each treatment medium is expressed as the deviation from the intra-experiment control. * and ** denote differences which are significant at the 5% and 1% levels of probability respectively.)

demonstrated, the fall in penetrance must be caused by changes in the culture bottle environment. Larvae from the first eggs to be laid complete the larval period under nearly optimal conditions, whereas younger larvae encounter an increasing intensity of competition for the resources of a limited and progressively deteriorating environment. The situation is similar to that described for *antennal-less* by Gordon & Sang (1941).

A detailed survey of the effect of dietary environmental factors on the expression of *tu-C4* was made using germ-free techniques for culturing larvae on defined culture media. The strain used in this instance was the control base population used for the selection experiments to be described below. This permits a direct comparison of the action of suboptimal environments, represented by individual nutritional deficiencies, on the expression of the tumour gene in homozygous and in heterozygous full-sibs cultured together in the same series of replicate cultures. The results are summarized in Table 5.

With the possible exceptions of cholesterol and riboflavin, the effects of individual nutrient deficiencies on the expression of *tu-C4* are in the same direction in heterozygotes and homozygotes. In other words, environmental components which are capable of influencing the expression of the tumour gene influence dominance and penetrance in the same way in the base population.

(iv) *Changes in dominance and penetrance under selection*

Different wild-type backgrounds are capable of influencing the dominance relations of *tu-C4*. In the presence of variation provided by these backgrounds it should be possible to change the dominance relations of *tu-C4*, relative to its wild-type allelomorph, by selection.

The complex inversion-containing third chromosome TM3 (Lewis, 1960) was used to balance the third chromosome carrying *tu-C4*. TM3 is marked by the

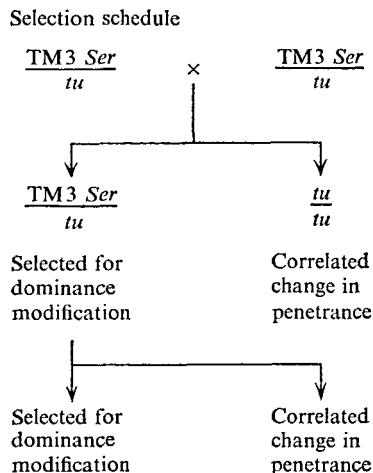


Fig. 3. The system of mating used in the selection experiment.

dominant mutant *Serrate* and is lethal when homozygous. The enclosed series of overlapping inversions prevents recombination throughout the third chromosome in TM3 *Ser/tu-C4* heterozygotes. The base population consisted of TM3 *Ser/tu-C4* heterozygotes with a mixed background of chromosomes 1, 2 and 4 derived from the Edinburgh, Oregon and Pacific wild-type strains. As shown in Fig. 3 the progeny of these heterozygotes consisted of: (i) TM3 *Ser/tu-C4* heterozygotes which were subjected directly to selection for and against dominance; and (ii) homozygous *tu-C4* individuals which provide in each generation a check on the presence of correlated changes in penetrance of the tumour gene.

Selection for dominance was made in late third-instar larvae and white pupae on the basis of presence or absence of a pigmented tumour. Virgin selected individuals were separated into homozygotes and heterozygotes, using the *Serrate* marker, after eclosion from the pupa. In the down selected line adult flies were checked again for absence of tumours in order to avoid misclassification due to late tumour pigmentation.

A proportion of the homozygotes segregating from the base population was used to establish two further lines which were subjected to selection for high or low penetrance of the tumour gene.

The response to selection for dominance, illustrated in Fig. 4, was immediate and rapid. The proportion of heterozygotes with tumours increased from 13.7% in the base population to 35.8% in one generation of selection, and exceeded 90% in the base population to 35.8% in one generation of selection, and exceeded 90%

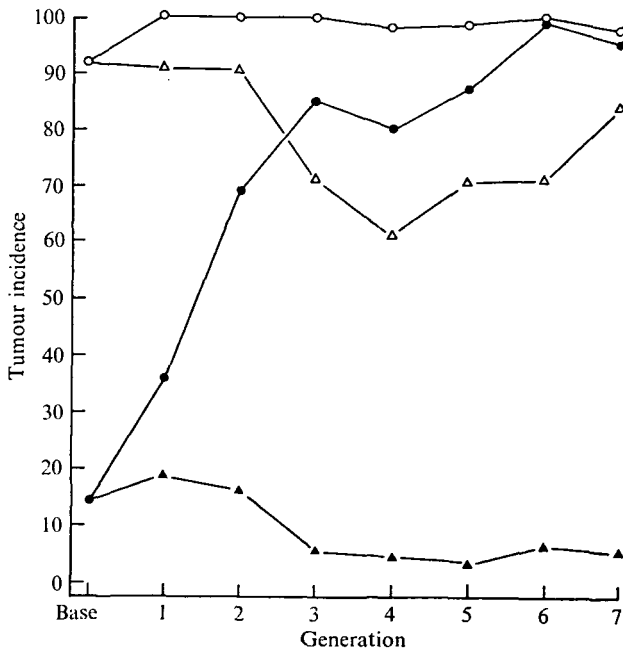


Fig. 4. Response to selection for high (●) and low (▲) dominance and the correlated change in penetrance observed in the high (○) and low (△) selected lines respectively. In both selected lines ten pairs of flies on average were used as parents in each generation. Each plotted point is based on approximately 400 individuals.

by the 6th–7th generation. The rapidity of the response seems to suggest that relatively few genes with major effect are largely responsible for controlling the dominance relations of the tumour gene in the selected population. Melanotic tumours were present in 92% of the homozygotes for *tu-C4* in the base population. This rose to 100% in the first generation of selection for dominance and remained between 97 and 100% in subsequent generations.

Selection for low dominance of *tu-C4* was also effective. The proportion of heterozygotes with tumours fell from 13.7% in the base population to 4.7% after seven generations, but the response was slow and irregular. This may well be due to the very low selection differential which could be applied in the down direction. Since we are dealing with a threshold character, every individual lacking tumours is used as a parent for the next generation. This means that after generation 3 some 95–97% of the flies in the down selection line were used as parents. Correlated changes in penetrance were also observed in the homozygotes segregating in the low dominance selection line. The response was quite rapid up to generation 4, but thereafter drifted upwards again, suggesting that by this time the selection differential was so low that selection intensity must have been greatly attenuated.

The response to selection for high and low penetrance in the homozygous lines derived from the base population is shown in Fig. 5. Penetrance rose from 92% initially to remain consistently around 97%. Selection for low penetrance was rapid though irregular up to the fourth generation, but levelled out at just about 30% subsequently.

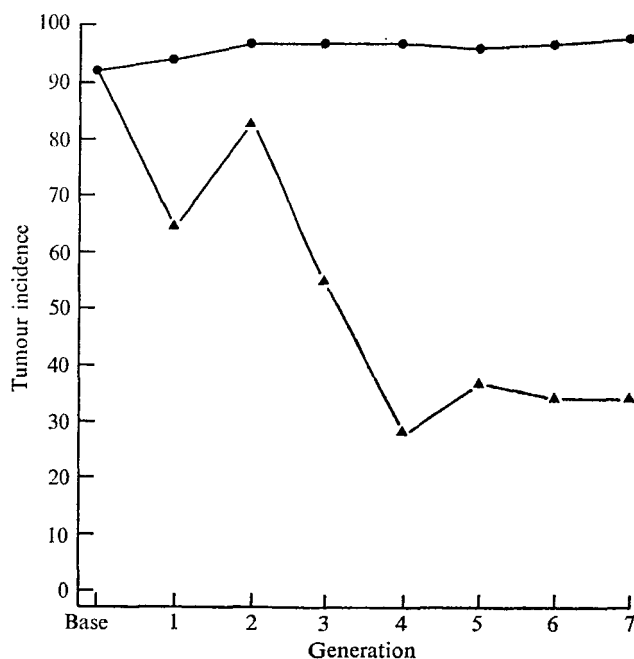


Fig. 5. Response to selection for high (●) and low (▲) penetrance of *tu-C4* in the homozygous population. In both selected lines 15 pairs of flies on average were used as parents in each generation. Each plotted point is based on approximately 600 individuals.

Table 6. Tumour incidence (percentage) in the F_1 offspring of crosses between three inbred 'non-tumour' strains and the lines selected for penetrance and dominance

| Strain | Penetrance | | Dominance | | | |
|---------------|-----------------|-----------------|-----------------|-------|-----------------|-------|
| | Low | High | Low | | High | |
| | <i>tu-C4</i> /+ | <i>tu-C4</i> /+ | <i>tu-C4</i> /+ | TM3/+ | <i>tu-C4</i> /+ | TM3/+ |
| <i>purple</i> | 14.3 | 32.5 | 8.3 | 0.6 | 68.4 | 2.0 |
| <i>sepia</i> | 11.9 | 47.3 | 21.0 | 1.4 | 36.8 | 2.3 |
| Oregon | 3.6 | 10.6 | 3.4 | 0 | 15.1 | 0 |

As we have seen, changes in the dominance relations of the tumour gene in response to selection were accompanied by correlated changes in penetrance in the homozygotes. This naturally poses the question: are gains in selection for penetrance accompanied by changes in dominance relations? To test this point, the lines selected for high and low penetrance were crossed to each of the three inbred 'non-tumour' stocks shown in Table 6. There is a consistent difference in the proportions of tumorous individuals between F_1 offspring of the two selected lines on each of the three backgrounds, indicating that selection for penetrance has been accompanied by changes in dominance relations in these lines.

(v) Chromosome location of dominance and penetrance modifiers

In the dominance selection lines heterozygotes were recognized by the presence of the dominant mutant gene *Serrate* marking the complex inversion-containing balancer chromosome TM3. The presence of this chromosome in TM3 *Ser/tu-C4* individuals excludes the possibility of any contribution to selection progress arising from recombination in the third chromosome. Changes in dominance relations produced by selection must therefore be attributable to variation located elsewhere in the genome. The relative importance of the first and second chromosomes was examined by crossing TM3 *Ser/tu-C4* heterozygotes of the high dominance selected line to a triple balancer strain *B;SM5;TM3*. Heterozygous females were then backcrossed to TM3 *Ser/tu-C4* heterozygotes of the high dominance line. The results are summarized in Table 7. Attempts to analyse the effect of the dot-like fourth chromosome were abandoned because of the difficulty of getting sufficient progeny from flies heterozygous for the marked fourth chromosomes *ci^D* or *ey^D* in combination with the other three dominant-marked balancer chromosomes. The backcross offspring consist of TM3 *Ser/tu-C4* heterozygotes together with homozygotes for the *tu-C4* chromosome, thus allowing us to assess the effects of the two other major chromosomes on dominance and penetrance simultaneously within the same group of full sibs reared together.

The results (Table 7) show that the first and second chromosomes of the selected line both have an effect on dominance and penetrance. In both cases it is the second chromosome which had the greatest effect. The effect on dominance of the first and second chromosomes in combination is approximately equal to the sum of

Table 7. *Effects of the first and second chromosomes of the high dominance selection line on the expression of tu-C4 in heterozygous and homozygous states*

| Heterozygotes <i>tu-C4/TM3</i> | | Homozygotes <i>tu-C4/tu-C4</i> | |
|-----------------------------------|----------------------|-----------------------------------|----------------------|
| Selected chromosome homozygous | Tumour incidence (%) | Selected chromosome homozygous | Tumour incidence (%) |
| 0 | 18.7 | 0 | 58.7 |
| 1 | 28.5 | 1 | 63.8 |
| 2 | 71.2 | 2 | 94.4 |
| 1+2 | 79.6 | 1+2 | 95.7 |

(Details of the relevant backcross are given in the text. Possible effects of chromosome four have been ignored.)

Table 8. *The effect of suboptimal environments on the expression of tu-C4 in the high dominance selected line*

| | Heterozygotes <i>tu-C4/TM3</i> | Homozygotes <i>tu-C4/tu-C4</i> |
|------------------------------|-----------------------------------|-----------------------------------|
| Control tumour incidence (%) | 90.06 | 98.9 |
| Major nutrient deficiencies | | |
| Casein 2.5% | - 15.9** | + 0.3 |
| Cholesterol 0.0016% | + 6.4 | + 0.6 |
| Lecithin 0.0125% | + 3.6 | + 1.3 |
| Ribonucleic acid nil | - 19.8** | - 1.9 |
| Sucrose nil | - 9.7* | + 1.1 |
| Vitamin deficiencies | | |
| Biotin 0.005 µg | + 3.69 | + 0.65 |
| Niacin 5.0 µg | - 10.1 | + 0.9 |
| Pantothenate 12.0 µg | - 4.3 | - 0.9 |
| Pyridoxine 0.8 µg | + 1.0 | - 0.1 |
| Riboflavin 4.0 µg | - 2.2 | 0 |
| Thiamine 0.4 µg | + 9.1** | + 0.9 |

(Tumour incidence on each treatment medium is expressed as the deviation from the intra-experiment control. * and ** denote differences which are significant at the 5% and 1% level of probability respectively.)

their independent contributions. This is less apparent in the case of penetrance due, probably, to the limitations imposed by the percentage scale together with the effect of tumour resorption described earlier.

(vi) *Genotype-environment interaction and the response to selection*

The effects of suboptimal environments on the expression of *tu-C4* in the high dominance selection line are shown in Table 8. The results may be compared with those for the base population shown in Table 5. Phospholipid deficiency no longer has any significant effect on the proportion of TM3 *Ser/tu-C4* heterozygotes with tumours in the high dominance line, and the effects of protein, RNA, sugar, and thiamine deficiency are reduced, as compared with the responses of the base popula-

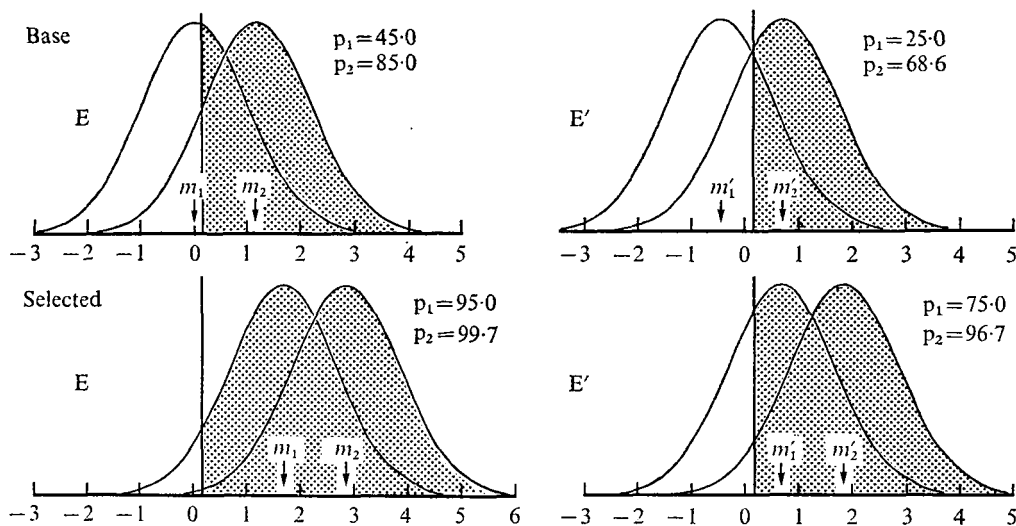


Fig. 6. Tumour incidence in *tu-C4* homozygotes and *+|tu-C4* heterozygotes on the assumption that the distribution of liability is represented by a pair of unit normal curves transected by a fixed threshold. The mean (m_2) for homozygotes is in each case 1.16σ to the right of the mean (m_1) for the heterozygotes. The proportions of individuals with tumours (p_1 and p_2) are related to the shaded areas under the curves lying to the right of the threshold. The effect of selection for increased dominance in heterozygotes (compare base E with selected E) is to move the distributions for both genotypes in tandem to the right relative to the threshold. Due to a scaling effect, equivalent shifts in the position of the means for the two genotypes do not give rise to equivalent changes of percentage tumour incidence. Reduction in tumour incidence of 20% in heterozygotes of the base population in response to environment E' corresponds to a reduction of 16.4% in homozygotes. In the selected population a reduction in tumour incidence in response to E' of 20% in heterozygotes corresponds to a reduction of only 3% in homozygotes.

tion. What is striking, perhaps, is that the segregating *tu-C4* homozygotes of the high dominance line do not respond significantly to any of the nutritional treatments. Penetrance of the tumour gene is almost complete and remains relatively stable over the entire range of nutritionally suboptimal environments.

The differences in gene expression between heterozygotes and homozygotes are made more meaningful if, instead of the proportion of affected individuals we think in terms of an underlying distribution of liability to produce the tumorous phenotype. Liability may be thought of as something which is determined by the concentration of some morphogenetic substance – the *phenodeterminant* – relative to a fixed threshold. The situation is discussed by Falconer (1960). We assume that variation in the amount of phenodeterminant between individuals is genetic and environmental in origin, and that the distribution is normal about the mean value in the population. This is illustrated in Fig. 6 which shows the distributions of liability to melanotic tumour formation in heterozygotes and homozygotes of the base population as two unit normal curves.

If the assumption of normality is well founded, the position of the mean for each genotype relative to the threshold may be expressed in terms of normal

Table 9. *The distance ($m_1 - m_2$) in normal equivalent deviates (\pm standard error) between the means of the underlying distributions of liability to tumour formation for heterozygotes and homozygotes in the base and selected population*

| | Population | |
|-----------------------------|--|-------------------|
| | Base | Selected |
| Control | 1.256 \pm 0.151 | 1.003 \pm 0.140 |
| Major nutrient deficiencies | | |
| Casein | 1.115 | 1.462 |
| Cholesterol | 1.427 | — |
| Lecithin | 1.136 | 0.888 |
| Ribonucleic acid | 0.046** | 1.232 |
| Sucrose | 1.126 | 1.448 |
| Vitamin deficiencies | | |
| Biotin | 1.516 | — |
| Niacin | 1.068 | — |
| Pantothenate | 1.239 | 1.049 |
| Pyridoxine | 1.121 | 0.979 |
| Riboflavin | 0.864 | 1.239 |
| Thiamine | 0.936 | — |
| Average | 1.164 \pm 0.058 <i>1.071 \pm 0.107</i> | 1.163 \pm 0.076 |

(The figures in italics denote the average for the base population if the RNA-deficiency group is included. Missing values occur where homozygotes of the selected population had a tumour incidence of 100% so that the position of m_2 relative to the threshold cannot be calculated. ** Denotes values which differ significantly from the control at the 1% level of probability.)

equivalent deviates, and the difference between the means m_1 and m_2 for heterozygotes and homozygotes, respectively, is readily calculated as shown in Table 9. Since the observations consist only of the proportion of affected individuals in a given sample, the dual assumption of (i) normality and (ii) equivalent variance for the two distributions is difficult to check directly. However, in neither the base nor the selected population is there any indication that the distance between means $m_1 - m_2$ is significantly correlated with the proportion of affected heterozygotes. This could not be the case if either assumption were seriously violated.

The head of Table 9 shows the mean \pm standard error for the distance $m_1 - m_2$ using the series of replicate control groups in the base and selected population. The difference between mean distances (1.256 compared with 1.003) is not significant ($t = 1.18$ with 4 degrees of freedom). This suggests that the difference in mean liability between heterozygotes and homozygotes has remained relatively constant, and that the two distributions have been moved in tandem up the scale in response to selection for increased dominance, as illustrated in Fig. 6.

With the exception of RNA-deficiency, the estimates of $m_1 - m_2$ for the base population in the series of suboptimal environments each fall within two standard deviations ($s = 0.262$) of the mean value for the controls. This suggests that the distance between heterozygous and homozygous genotypes remains relatively

constant with the means m_1 and m_2 moving in tandem along the scale in response to the different environments. The results for the selected population lead to essentially the same conclusion. Where values for $m_1 - m_2$ can be calculated, they fall within two standard deviations ($s = 0.243$) of the mean for the control selected population, the RNA-deficiency group being in line with the others. The average values for $m_1 - m_2$ over all environments, including the control, can be used to provide better estimates for the two respective populations as shown at the foot of Table 9. The difference between the averages for the base and selected population (1.071 versus 1.163) is not significant ($t = 0.68$ with 18 degrees of freedom), and nearly all attributable to the value for RNA-deficiency in the base population. If this is set aside we arrive at an estimate of $m_1 - m_2 = 1.16\sigma$ for the base and selected population. Proceeding from the assumption of unit normal distributions of liability, with $m_1 - m_2 = 1.16\sigma$, the expected proportion of *tu-C4* homozygotes with tumours (p) corresponding to any observed proportion of *+tu-C4* heterozygotes with tumours (p') is readily calculated from the normal equivalent deviates

$$\text{n.e.d. (p)} = \text{n.e.d. (p')} + 1.16\sigma.$$

Consequently, for a given environmentally induced deviation of the heterozygote from its control, the corresponding expected deviation from control of the homozygote can be calculated, as shown in Fig. 7. In the base population, only the deviation from control of the homozygotes in the RNA-deficient environment is significantly greater than the expectation. None of the points for the other suboptimal environments differs significantly from the expected value. Similarly, none of the points for the selected population, over the same array of suboptimal

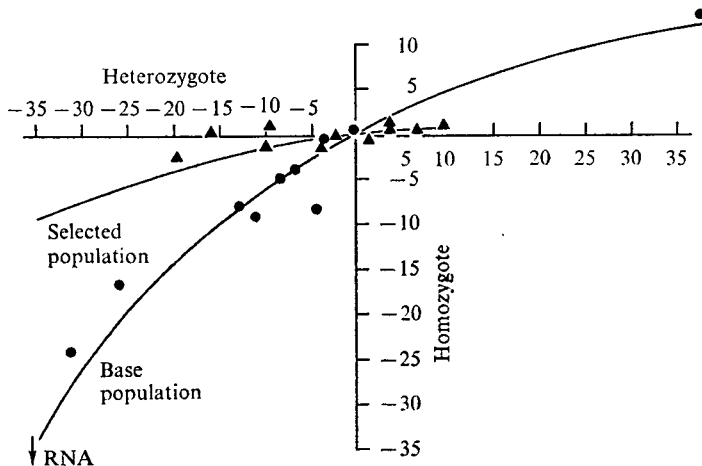


Fig. 7. Plotted lines show the expected deviation from control of the percentage of individuals with melanotic tumours in *tu-C4* homozygotes corresponding to a given deviation from control of *+tu-C4* heterozygotes. The control values for heterozygotes are 49.2 and 90.1% in the base and selected population respectively. Deviations over all the suboptimal environments observed in the base (points) and selected population (triangles) respectively are taken from the data summarized in Tables 5 and 8.

Table 10. *The effect of environment on dominance expressed as the difference (in normal equivalent deviates) between the means for heterozygotes in the control and each of the series of suboptimal environments*

| | Environment | | Selection |
|--------------|-------------|----------|-----------|
| | Base | Selected | |
| Control | — | — | 1.307 |
| Casein | -0.712** | -0.653** | 1.366 |
| Cholesterol | -0.484 | +0.565 | 2.357 |
| Lecithin | -0.548** | +0.212 | 2.067 |
| RNA | -0.867** | -0.769** | 1.406 |
| Sucrose | -0.209** | -0.446* | 1.071 |
| Biotin | -0.416 | +0.285 | 2.003 |
| Niacin | -0.174 | -0.446 | 1.036 |
| Pantothenate | +0.003 | -0.216 | 1.089 |
| Pyridoxine | +0.085 | +0.059 | 1.452 |
| Riboflavin | -0.116 | -0.117 | 1.306 |
| Thiamine | +1.142** | +1.122** | 1.287 |

(The column on the right measures the effect of selection as the difference in *n.e.d.* between the means for heterozygotes in the base and selected population in each environment. * and ** denotes those cases in which tumour incidence differs significantly from the control at the 5% or 1% levels respectively as shown in Tables 5 and 8.)

environments, differs significantly from expectation. The relative constancy over the different environments of the high proportion of homozygous individuals with tumours in the population selected for high dominance is evidently due to a scaling effect.

The relative effect of environment on dominance relations can be assessed from the differences in normal equivalent deviates listed in Table 10. With the exception of the response to lecithin, where there are significant differences in tumour incidence in heterozygotes, the shift in mean is similar in direction and magnitude in the base and selected population. The reduced response of the heterozygotes of the selected line to protein, RNA, sugar, and thiamine deficiency suggested by the deviations from control in percentage affected, shown in Table 8, is therefore attributable to the same scaling phenomenon discussed above. Consequently there is no good evidence that heterozygotes of the selected population are less affected by a given change of environment than unselected heterozygotes, and to this extent dominance does not appear to be a by-product of canalizing selection. The greatest changes in mean are: -0.867σ for RNA deficiency and $+1.142\sigma$ for thiamine deficiency in the base population. This represents movement over a range of 2.009σ in response to changes in environment.

The change attributable to selection shown in the right hand-column of Table 10 is the difference between means for heterozygotes of the base and the selected population in each of the suboptimal environments. The average difference must be treated with caution. Where we are comparing distributions of liability for heterozygous and homozygous genotypes *within* the base or selected strain the

Table 11. *Distribution of tumour number per individual in heterozygotes of the base population and of the line selected for high dominance, shown as the percentage of individuals with one or more tumours*

| Tumours/individual | 1 | 2 | 3 | 4 | 5 | <i>n</i> |
|--------------------|------|------|-----|-----|-----|----------|
| Base | 80.4 | 16.7 | 1.5 | 0.7 | 0.7 | 138 |
| High dominance | 59.7 | 32.3 | 5.8 | 1.3 | 0.9 | 226 |

(*n* = number of individuals classified.)

assumption of unit normality is reasonable. However, there are theoretical grounds for the expectation that selection may change the variance of liability in the high dominance line. In this case the difference between means for heterozygotes of the base and selected populations will have little meaning because the respective means would then be measured in different units of scale.

(vii) *Expression of the tumorous phenotype in heterozygotes of the selected line*

Comparisons of the degree of expression of melanotic tumour genes are complicated by the difficulty of deciding upon a suitable scale of measurement. The question as to whether the number or volume of tumours provides the best measure remains to be settled. Notwithstanding this difficulty, Table 11 shows that reinforcement of the dominance of *tu-C4* in heterozygotes has been accompanied by an increase in the proportion of heterozygous individuals with multiple tumours, that is, individuals with more extreme expression of the mutant phenotype. This is analogous to the correlation between penetrance and expressivity familiar in mutant homozygotes and discussed in detail by Sang (1963).

4. DISCUSSION

Fisher (1928) presented a comprehensive argument to show that the dominance of wild-type over deleterious mutant characters, such as those which are familiar in *Drosophila*, is the product of natural selection. The essence of his theory is that selection acts upon modifier genes with effects restricted to the mutant heterozygotes, in such a manner as to shift the phenotype of the heterozygote towards that of the homozygous wild-type.

The proposition – that the dominance of wild-type characters has evolved – seems to be generally accepted (for an alternative view see Crosby 1963), but Fisher's suggestion as to the mechanism by which dominance has evolved has been criticized by several authors. The point most at issue is the probable effectiveness of the second-order selection necessary to cause any change in the frequency of modifier genes with effects restricted to rare mutant heterozygotes. The details of this controversy, which has continued sporadically to the present time, are well reviewed by Sved & Mayo (1970) and need not concern us here.

Muller (1932) and Plunkett (1933) independently suggested that dominance of wild-type alleles would arise as a by-product of selection for stability of expression of the wild-type phenotype. Muller (1932) postulated that the mutations favouring

dominance – the genes or genetic conditions which tend to make the heterozygote like the homozygote – have been selected and are maintained, not so much for their specific protection against heterozygosis at the locus in question, as to provide a margin of stability and security to insure the organism against weakening or excessive variability of the character by environmental influences and variation in the residual genotype. This idea provides for the build-up by selection of a co-adapted system of modifier genes which buffers the expression of the wild-type allele above a certain threshold necessary for expression of the wild-type phenotype in a fluctuating environment. Since selection would operate on wild-type homozygotes which are the commonest genotype in the population, as well as on the heterozygotes for rare mutant alleles, the main difficulty in Fisher's theory is here avoided.

There is ample evidence for the presence of coadapted combinations of genes modifying the dominance relations of particular characters in natural populations, such as the different colour phases in the moth *Triphaena comes* described by Ford (1955). Gordon & Gordon (1957) give a detailed account of geographical variation in the genetic background modifying the dominance of a potentially injurious macromelanophore pattern gene in the fish *Xiphophorus maculatus*, and locally coadapted systems of modifier genes control the dominance relations of different mimetic forms of *Papilio dardanus* (Clarke & Sheppard, 1960).

Each of these theories of dominance evolution have in common the assumption that dominance relations in heterozygotes are modifiable by selection. This expectation is amply confirmed experimentally by Ford (1940), and by Fisher & Holt (1944). The response to selection in *tu-C4* heterozygotes is in general accord with these findings, and indicates the ready availability of genetic variation capable of influencing the dominance relations of this mutant. The response to selection for high dominance of the mutant was accompanied by a correlated increase in penetrance in the mutant homozygotes as elsewhere reported. The converse expectation which follows from the theory of dominance suggested by Plunkett & Muller was also found: selection progress towards high penetrance in mutant homozygotes was accompanied by an increase in dominance of the tumour gene in heterozygotes. Such correlated changes show that dominance and penetrance are fundamentally related aspects of the expression of this dichotomous threshold character. Consequently, the response to selection for increased dominance of *tu-C4* cannot be ascribed in any large part to selection for modifier genes with effects restricted only to heterozygotes.

Whitten (1968) found that genes modifying the dominance relations of the mutant *witty* in *D. melanogaster* are linked to the *wi* mutant itself, whilst penetrance modifiers are scattered throughout the genome. The experimental design which we used precludes recruitment of genetic variation from within the *tu-C4* containing third chromosome, so that dominance modifiers need not be linked to the major gene whose dominance relations they modify. However, it is doubtful in our view, whether the distinction between modifiers of dominance and penetrance made by Whitten is altogether a real one.

The fact that the *tu-C4* containing third chromosomes of the base population were coisogenic, being derived from a highly inbred strain, also permits us to exclude another possibility: that the change in dominance relations was due to selection of isoalleles of different potency at the major locus itself (Haldane, 1930).

Crosby (1963) has questioned whether selection for phenotypic stability in wild-type homozygotes would lead to dominance as a by-product as Plunkett & Muller suggested. Thus he argues that selection would not produce a safety factor greater than that needed by the homozygous wild-type for the most adverse conditions experienced in the history of the species. There is no reason for supposing that under such conditions the heterozygote would achieve the normal phenotype. Consequently, evolution of dominance in this way would lead to a situation in which, under many abnormal environmental conditions, dominance would be imperfect or lacking, and the phenotypic expression of heterozygotes variable. In Crosby's view, there is no substantial evidence for this.

The question posed here is a general one: to what extent can dominance relations be modified by environmental factors? The evidence, for major gene mutations, from well analysed cases such as the response of the *eyeless* mutant to boron salts described by Sang & McDonald (1954), is that dominance of the wild-type allele is indeed incomplete when mutant heterozygotes are exposed to abnormal environmental conditions. Burnet & Sang (1964) describe another instance involving melanotic tumour genes. The results for the mutant *tu-C4* take the evidence a stage further by showing that changes in gene expression in heterozygotes produced by selection can be significantly reinforced, or reversed, by environmental factors. Dominance relations in this case show substantial genotype-environment interaction, and selection for expression of the tumorous phenotype in different suboptimal environments would be expected to reinforce the dominance relations of the tumour gene.

The Plunkett & Muller theory, in common with other early theories of dominance, is incomplete in that it is essentially a single threshold model. Selection was thought simply to result in movement of the distribution of the phenodeterminant in mutant heterozygotes above some minimum threshold necessary for realisation of the wild-type phenotype. However, as Rendel (1967) has pointed out, there is a fundamental distinction between dominance of the wild-type characterized by its invariance of phenotype, and mutant dominance. The former is a by-product of canalizing selection, and involves the existence of two thresholds, together with a non-linear function relating the mean of the distribution of phenodeterminant (*make* in Rendel's (1967) terminology) to the observed phenotype scale. The wild-type segment of this function subtends a flat portion, within which an increment of change in phenodeterminant corresponds to zero change in phenotype. The *tu-C4* allele, which is either neomorphic or antimorphic on Muller's (1932) classification, shows typical mutant dominance. It shifts the distribution of phenodeterminant in heterozygotes and mutant homozygotes out of the zone of canalization of the wild-type and beyond the threshold of mutant gene expression, where we observe that phenotypic variability which is characteristic of an uncanalized

phenotype. Selection for *constancy of expression* of *tu-C4* in different suboptimal environments would in effect, therefore, be selection for canalization of the melanotic tumour phenotype, and should be associated with a change from mutant type to wild-type dominance.

From these considerations we can see that in a situation involving the occurrence within a population of a new mutant allele favoured by selection, and destined to replace the existing wild-type allele, dominance evolution according to the model proposed by Fisher would not be likely initially, even when the mutant heterozygotes are relatively frequent. This is because, unless the mutant allele in question was an antimorph or a neomorph, it would not in normal circumstances be able to escape from the zone of canalization of the wild-type. Since canalization of the new mutant phenotype involves the building of a coadapted system of modifier genes interacting with the new mutant allele, the process of establishing a new canalization plateau is likely to require a longer time scale than that required for spread of the mutant allele in the population, and seems most likely to commence in mutant homozygotes after the mutant allele has already reached relatively high frequency. Reinforcement of the dominance relations of the mutant allele by selection acting on heterozygotes would be expected to occur subsequently, as the formerly wild-type allele declined to relative rarity in the population. Where the novel allele favoured by selection is an antimorph, or a neomorph, the process is likely to be much more rapid since the partial dominance displayed by this kind of mutant would enable canalizing selection to commence in heterozygotes at an earlier stage in the progress of the mutant allele toward fixation.

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