MELANOTIC TUMOURS OF DROSOPHILA: A PARTIALLY-MENDELIAN CHARACTER

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1. INTRODUCTION

MELANOTIC tumours (*i.e.* presence of masses of melanin) have been described in many insects and especially in *Drosophila* as a heritable trait. Investigations by several authors (see Barigozzi, 1962) and especially by Barigozzi, Castiglioni and Di Pasquale (1960) have shown that the production of melanin is controlled by several factors, which in many stocks are located in the 2nd chromosome (*tu* factors, mostly recessive). These factors, however, act only when two conditions are present, which are also controlled by several genes located on different chromosomes: a sufficient amount of a particular type of hæmolymph cells in the lymph gland, and the disintegration of the gland during the larval stage. The behaviour of all steps (*i.e.* the control of the amount of large cells, of the lymph gland and of the melanin production) appears to be Mendelian in some experiments, and non-Mendelian (cytoplasmic) in some others. For this reason "melanotic tumours" will be referred to as being a partially-Mendelian character.

The first evidence that—in some individuals—the character is controlled by the chromosomes and by the cytoplasm at the same time was given by Di Pasquale (1956). This author replaced the chromosomes of 4 tumorous stocks with those of a tumourless one while the cytoplasm came from the tumorous stock: in spite of the replacement of the chromosomes a proportion of flies still showed a percentage of tumours (from 2·2 per cent. up to 9·7 per cent.). Although the technique available for replacing chromosomes (based on the use of a balanced lethal stock) is not completely satisfactory the results seem to be in favour of the existence of a dual mode of transmission. Moreover there is a difference of tumour incidence in reciprocal crosses between different tumorous stocks.

With the progress of the investigations, other facts appeared unexplainable in Mendelian terms, the most relevant of which are summarised here below: (1) Instability of isogenic lines propagated through single pairs. In stock *tu* B_3, out of 23 lines only 8 behaved as stable (the frequency of tumorous individuals oscillated, in subsequent generations, less than 10 per cent.), while 3 showed irregular oscillations of more than 10 per cent., 2 increased gradually up to a high percentage and 10 decreased gradually down to nearly 0 (Barigozzi,

1962). (2) In two stocks having the combination *tumorous cytoplasm* + *tumourless chromosomes* no tumours appeared for years. Later on, the stocks became tumorous. The anatomical location of the tumours was thoracic in the combination having the cytoplasm from stock *tu* A_2, where tumours are frequently thoracic. In the combination with cytoplasm from *tu* B_3 (where tumours are nearly always abdominal) only abdominal tumours appeared (Di Pasquale, 1959). These observations led us to the working hypothesis that the cytoplasm might exert a transmissible influence on the chromosomes.

In order to test this hypothesis a first experiment was devised, consisting in combining the cytoplasm from *tu* B_3 (100 per cent. of tumours generally located in the abdomen) with the chromosomes from the almost tumourless stock Chieti-vermilion.

According to the hypothesis, if the chromosomes of the combination fail to yield tumours, the experiment could not prove any influence exerted by the cytoplasm on the chromosomes; if the combination yielded significant frequency of tumours chromosomally controlled then the influence could be considered as experimentally proved.

The technique used for producing the combination (cyt. *tu* B_3 + chrom. Chieti-vermilion) comprised the following procedures: (1) Preliminary analysis of the genotype of Chieti-vermilion, by means of tumour countings in lines isogenic for the 2nd chromosome. All lines gave a very low tumour incidence; and out of 36 lines, 15 proved to be tumourless, while the highest frequency found was 7·2 per cent. (2) Replacement of chromosomes in the stock *tu* B_3 by means of repeated backcrosses, according to the following scheme:

$$tu\ B_3 \times \text{Chieti-}v$$

$$\frac{tu\ B_3}{\text{Chieti}} \times \text{Chieti-}v \text{ etc. for 7 or 9 times.}$$

Out of 72 sublines, many have been tested for *tu* genes, using appropriated crosses with *tu* B_3 where *tu* genes are located near the right end of the chromosome and are recessive. The majority proved to carry no *tu* factors or very weak ones, as in the Chieti-*v* stock. Four exceptions, on the other hand, have been found, namely sublines 6*f*, 3*c*, 8*c* and 11*e*. Subline 6*f*, showing a low tumour frequency (around 5 per cent.), when crossed to *tu* B_3 gave a very high percentage of tumours: several isogenic lines derived from 6*f* yielded nearly 100 per cent. of very large abdominal tumours. Subline 3*c* gave rise to one isogenic genotype, with as many as 43·3 per cent. of tumours. Crossed to *tu* B_3, a nearly tumourless offspring resulted. Hence, we conclude that the *tu* factors of 3*c* are different from those of *tu* B_3. Similar findings resulted from sublines 8*c* and 11*e*.

One may conclude that it is highly improbable that the occurrence of so many tumorous sublines is due to chance, after so many backcrosses (the probability should be of the order of magnitude of $(\frac{1}{2})^9 = \frac{1}{512}$

after 9 back-crosses, while tumours appeared in 4 lines out of 72). The difference between the *tu* factors of 3*c* and those of *tu* B_3 might be explicable only as an effect of some chromosome rearrangements, which, in fact, have never been detected cytologically (Barigozzi, 1962). All these facts lead to the conclusion that some extra-Mendelian mechanism is at work.

TABLE 1

Frequency of tumours in lines isogenic for the 2nd chromosome of Chieti-v. All tumours abdominal (Winter, 1960)

Lines frequency	Per cent. tumours
1	0-2·0
5	2·0-4·0
2	4·0-6·0
2	6·0-8·0
10	
Maximal frequency : 7·2	Mean : 4·0±0·25
Counted flies No. 5738 (in five subsequent generations)	

Frequency of tumours in lines isogenic for the 2nd chromosome of vermilion. All tumours abdominal (Spring, 1961)

Lines frequency	Per cent. tumours
15	0-2·0
22	2·0-4·0
3	4·0-6·0
—	6·0-8·0
—	6·0-8·0
40	
Maximal frequency : 6·0	Mean : 2·6±0·17
Counted flies No. 8394 (in three subsequent generations)	

Unfortunately, the technique of replacing chromosomes and of producing the desired combination of given chromosomes with a given cytoplasm is not completely satisfactory, because the suppression of recombination is never absolute. To overcome this drawback, a completely different technique has been adopted, *i.e.* that of grafting ovaries from an almost tumourless stock into genotypically tumorous larvæ. The principles on which the experiment is based are that, using a suitable marker, it is possible to recognise individually each egg produced from the implanted ovary (it must be borne in mind that the ovaries of the recipient larva are also able to deliver eggs,

so that a correct classification of the eggs in relation to the ovary which has produced them is imperative. The developing oocytes and eggs within the grafted ovary are in contact with the internal environment, keeping their nuclear and cytoplasmic integrity. If in the recipient individual there is a factor capable of influencing in a stable or persistent manner the genotype of the graft, it is conceivable that the grafted oocytes of a tumourless genotype (duly marked), developing in the internal environment of a tumorous genotype, might have some chromosomes influenced, *i.e.* changed in a way which could be

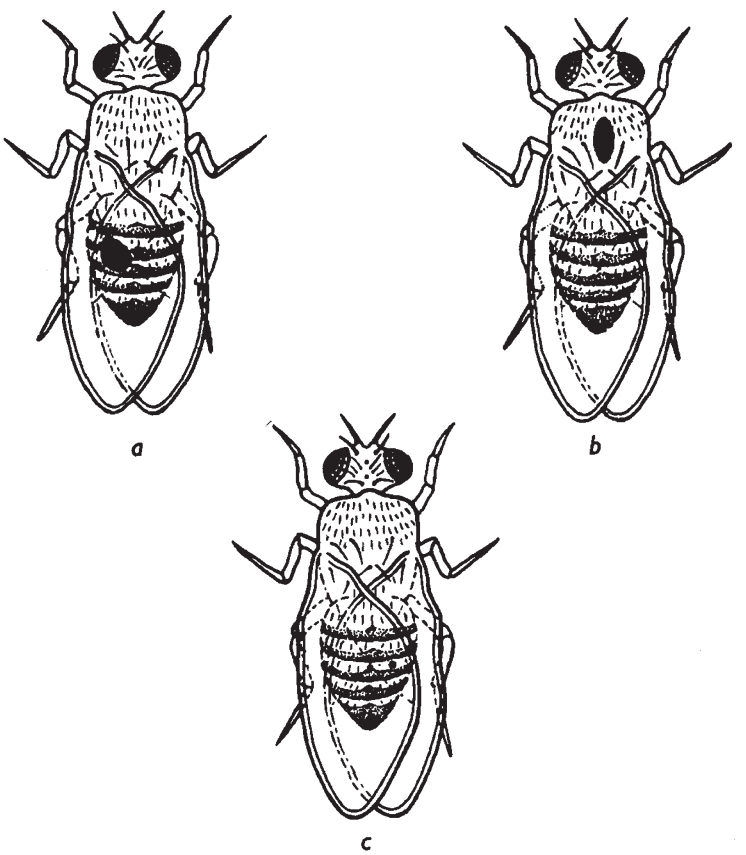

FIG. 1—Phenotypes of the stocks used :
1*a*—Large abdominal tumour, typical of *tu* B_3
1*b*—Large thoracic tumour, typical of *tu* A_2
1*c*—Small abdominal tumour, typical of Chieti-*v* and *v*.

genetically analysed. In an experiment of this type, a negative answer has no significance for acceptance or rejection of the working hypothesis, but a positive answer is critical.

2. MATERIAL AND METHODS

As donors of ovaries, two stocks have been selected, both marked with vermilion and both showing a low frequency of tumours. The first was Chieti-vermilion (tumour

incidence : 4·±0·25 per cent. calculated on isogenic chromosomes ; tumours very small, all located in the *abdomen*). The second was vermilion (the same mutant, on a different genetic background) having a tumour incidence of 2·64±0·17 per cent. (see table 1) (calculated on 40 isogenic chromosomes), showing also small and *only abdominal* melanotic masses(*) (see fig. 1*c*). Two stocks have been used as recipients: *tu* A_2, showing 30-40 per cent. of big tumours, located in the abdomen *and in the thorax* (16-17 per cent. of the total tumour percentage are thoracic) (see fig. 1*b*), and *tu* B_1 (100 per cent. of the individuals) where tumours are nearly always localised in the abdomen, and only exceptionally in the thorax (see fig. 1*a*). For implanting ovaries the usual technique originally found by Hadorn and Niggli (1946) has been

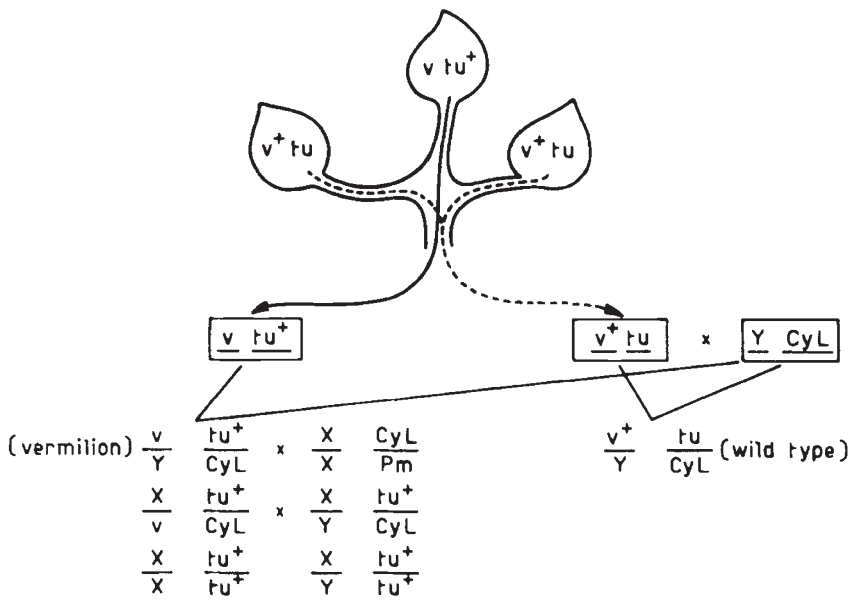

FIG. 2.—Scheme of the experiments based on ovary implantations. In the fly where the implanted ovary functions, three ovaries are present, all producing eggs.

used. Implants were made in third instar larvæ. Obviously, an accurate control of the manifestation can be made only when lines are produced isogenic for the 2nd chromosome, where the *tu* genes are located. According to the working hypothesis, in the genotypes derived from oocytes developed in stock *tu* A_2 some thoracic localisation should result, but only abdominal tumours from oocytes implanted into *tu* B_2. In the absence of a cytoplasmic effect, the manifestation of the implanted genotype should not be different from that of the stock from which the ovary derived. The results must thus take the form of a statistical comparison between the frequency distributions of tumours in a number of genotypes, isogenic for the 2nd chromosome, derived from the donor stock and from ovaries of the same origin, implanted in *tu* A_2 and in *tu* B_2 (see fig. 2). Since the highest tumour incidence obtained in isogenic lines of Chieti-*v* has been 7 per cent., and 6 per cent. in those of *v*, so 14 per cent. and 12 per cent. respectively have been taken as the lowest limit of significance.

3. RESULTS

(i) *First series of implantations* (ovaries implanted from Chieti-*v*). The results can be summarised as follows (see tables 2 and 3).

* Larvæ from mass cultures have been preferred to those from isogenic lines.

(*a*) Thoracic tumours appeared in the progeny derived from 3 ovaries implanted in *tu* A_2, while they never appeared in the progeny from implantation in *tu* B_3. (*b*) Two out of 26 chromosomes of implants in *tu* A_2 and 6 out of 26 chromosomes of implants in *tu* B_3 manifested persistently for several generations more tumours than Chieti-*v*. (*c*) The overall tumour percentage is higher in the descendants from implanted oocytes than in the control (table 4).

TABLE 2

Total frequency of tumours in the first 5 generations of isogenic lines derived from Chieti-v ovaries implanted in tu A_2. *The frequencies italicised are considered to be significantly higher than the control.*

Ovary	Lines	Frequency	Thoracic *tu*	No. individuals
1	1	9·1	—	276
	2	4·6	—	263
	3	8·7	—	252
	4	8·9	—	292
	5	2·2	—	185
	6	6·9	—	159
	7	10·8	—	223
2	1	9·8	—	245
	2	6·3	—	208
3	1	*16·0*	6/58	363
	2	13·8	5/45	325
	3	12·3	—	235
	4	2·1	—	232
	5	10·0	—	170
	6	9·8	—	264
	7	*18·4*	4/49	266
4	1	4·6	—	262
	2	7·5	—	308
	3	6·8	—	264
	4	4·7	—	148
5	1	4·4	—	159
6	1	5·0	—	258
	2	6·0	—	301
	3	7·7	—	207
	4	6·8	—	192
	5	7·0	—	272

These results seem to indicate that an intercellular "influence" has been exerted on the 2nd chromosome, and transmitted through several generations. Some objections can be raised, such as the small number of chromosomes analysed.

For these reasons a second series of implantations has been carried out.

(ii) *Second series of implantations* (ovaries from implanted *v*). The technique used was exactly the same as in the first series. The new donor stock behaved completely normally, never showing thoracic tumours (for tumour frequency, see table 1). The abdominal masses were always extremely small. The counts from *three* subsequent generations are pooled in the data. The data of tables 5, 6 and 7 show a

TABLE 3

Total frequency of tumours in the first 5 generations of isogenic lines derived from Chieti-v ovaries implanted in tu B_1. *The frequency of line 16, italicised, is significantly higher than Chieti-v.*

Ovary	Line	Frequency	No. individuals
1	1	8·2	306
	2	5·6	252
	3	5·2	386
	4	11·6	251
	5	6·6	287
	6	1·5	329
	7	11·1	298
	8	7·8	269
	9	4·0	298
	10	11·6	267
	11	2·6	234
	12	3·8	312
	13	3·1	228
	14	6·8	278
	15	9·0	343
	16	*20·6*	233
	17	5·3	244
	18	5·4	276
	19	7·9	305
	20	4·8	247
	21	3·7	189
	22	2·9	171
	23	1·5	200
	24	2·2	135
	25	6·6	256
	26	8·9	214

TABLE 4

Summarised data and significance tests of tables 1, 2 and 3.

	With tumours	Total individuals	Per cent. tumour
Chieti-*v*→*tu* A_2	536	6329	8·46±0·349
Chieti-*v*→*tu* B_3	450	6808	6·60±0·300
Chieti-*v*	229	5738	4·00±0·252

$\chi^2_{(1)}$(Chieti-*v*→*tu* A_2 ; Chieti-*v*) = 101·20, P<0·01
$\chi^2_{(1)}$(Chieti-*v*→*tu* B_3 ; Chieti-*v*) = 41·30, P<0·01
$\chi^2_{(1)}$(Chieti-*v*→*tu* A_2 ; Chieti-*v*→*tu* B_3) = 16·062, P<0·01

great resemblance to those of the first series. First of all, thoracic tumours appeared again from implanted ovaries in *tu* A_2 (5 lines out of 28): secondly, in both combinations an increase in tumour incidence occurred, which seems to be very significant in some lines.

The final conclusion is that in both experiments a stable influence has been exerted by the genotype of the host upon the genotype of the implanted ovaries, and that the results from the two series are practically the same: the only difference which we can observe here refers to the lesser activity of *tu* A_2 in the second series. The character has been transmitted to the progeny (the observations lasted for several

TABLE 5

Total frequency of tumours in the first 3 generations of isogenic lines derived from vermilion ovaries implanted in tu A_2. *The frequency considered as significantly higher than in the control is italicised*

Ovary	Line	Frequency	Thoracic *tu*	No. individuals
1	1	4·0	—	176
	2	5·0	—	100
2	1	*20·2*	2/17	84
	2	1·8	1/6	324
3	1	3·4	—	203
	2	4·8	—	125
	3	5·0	—	220
	4	6·3	—	127
	5	3·8	—	186
	6	1·9	—	154
	7	8·7	1/21	241
	8	4·0	—	201
	9	4·8	—	230
	10	2·1	—	235
	11	2·4	—	123
4	1	2·1	—	183
5	1	2.1	—	183
	2	3·9	—	203
	3	2·7	—	224
	4	1·6	—	189
	5	1·5	—	478
	6	2·8	—	178
	7	6·1	1/6	103
6	1	8·3	1/10	121
	2	2·1	—	187
	3	4·2	—	165
	4	2·4	—	247
	5	3·7	—	81

generations, propagated through single pairs of brother and sister), so that the question arises of the genetical relationship between the *tu*-loci of the implanted oocytes and the loci of the oocytes of *tu* A_2 and of *tu* B_3.

This analysis has been based on the following scheme. Heterozygotes of the type $\frac{v \rightarrow tu\ A_2 \text{ (or } tu\ B_3)^*}{tu\ A_2 \text{ (or } tu\ B_3)}$ can show either a proportion of tumours, or no tumours. If tumours appear it is assumed (since the tumour genes are always recessive) that the factors located in the chromosomes of the implanted oocytes are the same as in *tu* A_2 or in

* $v\rightarrow$ means : genotype v implanted in . . .

TABLE 6

Total frequency of tumours in the first 3 generations of isogenic lines derived from vermilion ovaries implanted in tu B_3. *The frequencies considered as significantly higher than in the control are italicised*

Ovary	Line	Frequency	No. individuals	Ovary	Line	Frequency	No. individuals
1	1	9·2	262	7	1	5·2	77
	2	5·6	196		2	9·9	243
	3	9·7	82		3	5·4	111
	4	6·8	322		4	3·8	52
	5	4·8	229		5	8·2	220
	6	9·5	253		6	0·0	46
	7	6·1	165		7	4·3	162
	8	9·3	97	8	1	6·1	65
	9	3·2	124		2	13·8	195
	10	1·9	212	9	1	2·0	203
	11	6·7	149		2	2·9	173
	12	3·7	189		3	6·2	80
	13	*20·8*	154		4	0·0	56
	14	*21·5*	181		5	0·0	74
	15	6·8	132		6	4·9	184
	16	2·0	50		7	8·7	127
	17	10·7	224		8	5·0	219
	18	3·9	51		9	5·2	271
	19	11·0	237		10	13·1	160
2	1	4·3	140		11	4·2	144
3	1	5·3	38		12	3·0	101
	2	*12·5*	32		13	16·5	164
4	1	1·5	129		14	2·4	295
	2	11·7	188		15	2·4	166
	3	2·8	105		16	6·3	142
	4	2·5	162		17	3·5	259
	5	5·8	242		18	5·2	213
	6	4·4	248	10	1	1·8	167
	7	19·5	220		2	6·3	142
5	1	4·2	143		3	3·1	129
	2	10·5	181		4	7·4	161
6	1	4·9	81		5	7·4	54
	2	2·3	130	11	1	3·3	182
	3	8·5	211		2	3·3	181
	4	1·3	74				

TABLE 7

Summarised data and significance tests of tables 5 and 6

	With tumour	Total individuals	Per cent. tumour
v→*tu* A_2 . .	202	5317	3·8±0·261
v→*tu* B_3 . .	730	10851	6·7±0·240
v . . .	222	8324	2·6±0·174

$\chi^2_{(1)}$ (*v*→*tu* A_2 ; *v*) = 1·409, 0·3<P<0·2

$\chi^2_{(1)}$ (*v*→*tu* B_3 ; *v*) = 166·912, P<0·01

$\chi^2_{(1)}$ (*v*→*tu* A_2 ; *v tu* B_3) = 55·794, P<0·01

tu B_3 respectively, *i.e.* located in almost the same chromosome section, perhaps in the same cistron. If no tumours appear the "new" factors (or the "new" loci) could be also located on a different chromosome, but the method does not allow them to be localised.

Only 2nd chromosomes have been considered; thus the genotype $\frac{+ \; tu\ A_2 \text{ (or } tu\ B_3)}{+ \; tu\ A_2 \text{ (or } tu\ B_3)} \frac{H}{Sb\ Me}$ has been used as tester stock.

As regards the implanted oocytes of the first series, the conclusion was drawn that the thoracic manifestation of Chieti-*v* implanted in *tu* A_2 is identical to that of *tu* A_2, because 2nd chromosome from implanted oocytes × 2nd chromosomes from *tu* A_2 gave some thoracic tumours.

TABLE 8

Comparison between percentages of tumours in 10 lines vermilion and in heterozygotes $\frac{v}{tu\ B_3}$ *and* $\frac{v}{tu\ A_2}$

Chrom. *v*/*v*	Per cent. *tu* $F_1+F_2+F_3$	Per cent. *tu v*/*tu* B_3	Per cent. *tu v*/*tu* A_2
2*d* 1 . .	6·4±2·058	0·9±0·896	0·0
3*c* 1 . .	15·3±3·903	0·0	6·7±2·151
3*e* 3 . .	0·0	6·9±3·325	0·0
4*a* 3 . .	9·0±3·235	1·6±1·440	3·1±1·774
6*b* 2 . .	0·0	0·0	2·9±1·175
7*d* 1 . .	0·0	0·8±0·800	1·4±0·300
8*a* 1 . .	0·0	3·8±1·865	0·9±0·657
8*d* 1 . .	5·0±1·841	3·4±2·393	3·3±1·891
9*c* 1 . .	4·4±1·526	2·0±1·386	10·9±2·584
9*d* 3 . .	0·0	0·0	0·0

Means 1·9±0.473 3·5±0·534

$\chi^2_{(1)} = 5{\cdot}01$; P = 0·05-0·02

A more precise investigation has been carried out concerning the second experiment. One isogenic line has been secured from both *tu* A_2 and from *tu* B_3 (tumour percentage: 17·8±3·15 with 19·2 of thoracic tumours for *tu* A_2 and 36·8±5·53 and no thoracic tumours for *tu* B_3) and used as testers. As a control, 10 isogenic lines from *v* have been produced, and crossed each to the isogenic *tu* A_2 and *tu* B_3 (table 8). From these crosses it turns out that such ability as *v* possesses of producing tumours is genetically more similar to that of *tu* A_2 than to that of *tu* B_3, since the tumour incidence is higher in crosses with *tu* A_2 than in those with *tu* B_3. The results from the heterozygotes between testers and chromosomes to be tested $\left(\frac{v \rightarrow tu\ B_3 \text{ (or } tu\ A_2)}{tu\ B_3 \quad \text{(or } tu\ A_2)}\right)$ for 10 different isogenic lines (= 10 chromosomes, 5 with low and 5 with high manifestation) are recorded on tables 9 and 10. Table 9 shows that, in 10 isogenic lines of *v*→*tu* B_3, 5 manifesting a high and 5 a low

percentage of tumours, there is a general tendency to produce less tumours in the heterozygotes with *tu* B_3 than in homozygous condition or to show the same rate. The only possible exception is line 5*c* 1 where there is an increase.

TABLE 9

Comparison between tumour percentages in isogenic lines from v→tu B_3 *and in heterozygotes* $\frac{\text{v→tu } B_3 \text{ isog.}}{\text{tu } B_3(A_2)}$

Chrom. *v→tu* B_3	Per cent. $F_1+F_2+F_3$	Per cent. *tu v→tu* B_3/*tu* B_3	Per cent. *tu v→tu* B_3/*tu* A_2
2*a* 10 . .	1·9±0·932	4·9±2·376	4·3±2·323
2*a* 13 . .	20·8±3·268	9·7±1·683	3·2±1·113
2*a* 14 . .	21·5±3·055	1·1±1·126	0·9±0·606
2*a* 16 . .	2·0±1·977	0·0	5·4±2·145
3*f* 8 . .	19·5±2·673	4·3±1·605	8·3±1·617
5*c* 1 . .	2·0±0·982	6·7±2·035	1·5±1·059
5*c* 6 . .	0·0	0·7±0·670	4·1±1·523
5*c* 14 . .	13·1±2·662	18·7±3·448	1·9±1·319
5*c* 17 . .	16·5±2·890	2·5±1·245	2·6±1·788
6*a* 4 . .	1·8±1·017	0·0	0·0

Means 5·6±0·603 3·5±0·466

$\chi^2_{[1]} = 7{\cdot}7$; P<1 per cent.

TABLE 10

Comparison between tumour percentages in isogenic lines from v→tu A_2 *and in heterozygotes* $\frac{\text{v→tu } A_2 \text{ isog.}}{\text{tu } A_2(B_3)}$

Chrom. *v→tu* A_2	Per cent. *tu* $F_1+F_2+F_3$	Per cent. *tu v→tu* A_2/*tu* A_2	Per cent. *tu v→tu* A_2/*tu* B_3
3*c* 1 . .	20·2±4·383	7·8±1·995	0·0
3*c* 2 . .	1·8±0·748	4·2±1·664	2·5±2·468
6*d* 4 . .	5·0±1·463	8·4±2·137	0·0
6*d* 7 . .	1·9±1·103	3·9±1·724	0·0
6*d* 8 . .	8·7±1·813	13·8±3·186	0·0
6*d* 14 . .	2·1±0·931	2·7±1·884	0·0
11*a* 6 . .	1·6±0·899	7·7±3·675	1·4±0·975
11*a* 7 . .	1·5±0·543	4·3±1·216	0·0
11*a* 9 . .	5·8±2·330	3·3±1·322	0·0
11*b* 1 . .	8·3±2·487	7·8±2·621	3·5±2·434

Means 6·2±0·625 0·6±0·256

$\chi^2_{(1)} = 44{\cdot}9$; P very small

The lower incidence of tumours in the heterozygous flies can be interpreted as an indication of the fact that some *v→tu* B_3 2nd chromosomes are not identical with those of the tester stock *tu* B_3. The cases where there is almost the same frequency are interpretable as springing from a closer similarity between the two kinds of 2nd chromosomes.

The case of line 5*c* 1 is inexplicable. In the heterozygotes $\frac{v \rightarrow tu\ B_3}{tu\ A_2}$, we find significantly fewer tumours than in the heterozygotes $\frac{v \rightarrow tu\ B_3}{tu\ B_3}$, proving that the original similarity between *v* and *tu* A_2 (see table 8) now fails to appear.

Table 10 gives us the corresponding picture of what happens when $v \rightarrow tu\ A_2$ chromosomes are considered, again in isogenic and in heterozygous condition, *viz.* $\frac{v \rightarrow tu\ A_2}{tu\ A_2}$, and $\frac{v \rightarrow tu\ A_2}{tu\ B_3}$. The results confirm those of table 9. Here the difference between the two heterozygous conditions is in favour of $\frac{v \rightarrow tu\ A_2}{tu\ A_2}$ and is very marked, the tumour frequency in the heterozygotes with *tu* B_3 being nearly zero. In conclusion the 2nd chromosomes of *v* show a reverse condition in heterozygosity with *tu* A_2 and *tu* B_3 according to grafting in *tu* A_2 and in *tu* B_3.

4. DISCUSSION

The data presented here show that, besides several phenomena of difficult interpretation which lead us to consider the possibility that genic and cytoplasmic factors are operating in the same cell and controlling the production of melanotic masses in *Drosophila*, a persistent influence on given sections of the 2nd chromosome can be exerted by internal environment. Although the maturation of oocytes from low tumorous stocks in a tumorous environment does not correspond to the combination: *tumourless chromosomes + tumorous cytoplasm* as investigated in previous work mentioned above, the positive results obtained by transplantation make more likely the interpretation that a persistent influence can be exerted by the cytoplasm upon the chromosomes of the same cell.

It is now desirable to see more precisely what kind of change can occur in the 2nd chromosome, taking tables 9 and 10 especially into account. The recessiveness of the genes controlling melanotic tumours makes the character appear in a cross only when at least one factor or allele is present in both homologous chromosomes. Whenever a cross yields fewer tumours than the parent genotypes, a lack of genotypic identity is revealed. The following argument is based on this assumption.

Now if we compare the same chromosome from grafted oocytes in heterozygous condition with *tu* B_3 and, in turn with *tu* A_2, we find a closer similarity to the chromosome of the host than to that of the donor, bringing about a reversal of incidence of tumours with the change of host (compare table 9 with table 10). It is also interesting to notice that the difference between the two heterozygous conditions is greater when *v* oocytes have been implanted into *tu* A_2. Hence, we can conclude that the persistent influence might be visualised as an increase in identical

loci in the 2nd chromosomes of the host and of the donor. Almost complete non-identity between the loci influencing the tumours in *v→tu* A_2 and *tu* B_3 might even mean a loss of factors identical with those of *tu* B_3 in *v→tu* A_2 which were present in the original *v* (see table 8). Finally the factor responsible for thoracic location seems to be different in *tu* A_2 and in *v→tu* A_2, since heterozygotes between them fail to show thoracic tumours.

We may conclude that after grafting a change occurs in definite genetic factors, which might be interpreted as of a mutational type. If we accept this view, the unavoidable consequences are: (i) the mutations are caused by a mutator which can move from tissue to tissue and not only from cell to cell or act from a distance; (ii) the mutations are directed, because they copy the phenotype of the host, from which the mutator derives; (iii) the mutations occur at a very high rate.

These consequences give a peculiar aspect to the phenomenon. An alternative explanation is supplied by the findings in bacteria, besides some analogies found in maize and other organisms (Jacob, Schaeffer and Wollman, 1960) which lead to the postulation of the existence of particles (episomes) which can be added to and removed from distinct and different chromosomal loci, changing site between the gene string and the cytoplasm.

On this basis the changes from one allelic stage to the other (for example from *tu*$^+$ to *tu*) might be visualised hypothetically as an integration of a particle which, in the present case, should be capable of migrating not only from the cytoplasm to the nucleus and of being released from the chromosomes to the cytoplasma, but also of moving from cell to cell.

If we assume that the non-Mendelian side of the genetic alphenomena shown by the transmission of the melanotic tumours is of episome-like type, several facts immediately become understandable: the existence of so many different sets of *tu*-factors in different stocks (episomes have no obligatory fixed location); the reappearing of tumours in chromosomally tumourless genotypes where the cytoplasm is tumorous (Di Pasquale, 1959); and the genic instability invoked by Di Pasquale and Koref Santibañez (1961) to explain the frequent occurrence of tumours in wild populations which can now be understood in terms of displacement of episomes. The episome-like explanation is put forward as the most useful one for visualising the process at the present stage of our knowledge. Nonetheless it leaves unsolved for investigation in the future many problems such as the frequency of the cytoplasmic influence (which seems to be very high), the type of insertion of the episome in the gene string, and the degree of persistency.

On the other hand our observations may perhaps be interpreted by a more orthodox mechanism. It might be possible that a tendency to higher production of tumours may be functionally linked with higher probability for the oocyte to be fertilised; thus selection might explain the higher tumour frequency found in the offspring from grafted

oocytes. The appearance of thoracic tumours in *v*→*tu* A_2, could not, however, be explained in this way when *v* completely lacks this pattern. Thus intra-oocytes selection does not seem to be a good alternative to the episomic explanation. A possible similarity with the nuclear-cytoplasmic control of mating-types in *Paramecium* (Nanney, 1957), for which the existence of episomes is not invoked, may represent an alternative interpretation. Whatever may be the explanation of the phenomena we have described, an important point is that we now have available for further studies a case of a character endowed with dual genetical control. This may help our understanding of the formal relationships between cytoplasm and genes in its bearing on evolution.

5. SUMMARY

1. Since previous investigations had shown that the transmission of melanotic tumours of Drosophila is not fully understandable in mendelian terms, both cytoplasm and chromosomes being involved, an experiment has been devised to test a possible influence of the cytoplasm on the genes. Ovaries of nearly tumourless individuals were grafted into tumorous females. As donors two stocks have been selected, each marked with vermilion and characterised by a low incidence of very small abdominal tumours. As recipient, two stocks were taken, one of them (*tu* A_2) showing a proportion of large thoracic tumours, and the other large tumours, generally abdominal and only exceptionally thoracic.

2. If that is a persistent influence of the cytoplasm on the chromosomes, the expectation is the appearance of thoracic tumours in the progeny of donor ovaries grafted into *tu* A_2, and of abdominal tumours (exceptionally thoracic) in that of donor ovaries in *tu* B_3.

The results agreed with this expectation. Thoracic tumours appeared, and have been transmitted, in the progeny (isogenic for the 2nd chromosome) from grafts in *tu* A_2, while from grafts in *tu* B_3 only abdominal tumours appeared, with a thoracic one in a single fly. Beside this, an overall tendency to produce more tumours has been observed in both types of progeny. These progenies have been tested by means of crosses with 2nd chromosomes of the donor stock vermilion, as well as with 2nd chromosomes of *tu* A_2 and *tu* B_3. It was found that while the original stock *v* is genetically more similar (as far as the 2nd chromosome is concerned) to *tu* A_2 than to *tu* B_3, the 2nd chromosomes of the progeny from grafted ovaries proved more similar to *tu* B_3, when the graft had been made in this particular stock.

3. It thus seems justified to speak of an influence exerted by the internal environment on the chromosomes and lasting several generations. This may also explain the observations made in previous works. This influence may be visualised hypothetically as caused by transmissible particles characterised by a dual phase, the one cytoplasmic and the other chromosomal, although other possibilities cannot be

excluded—a picture which is similar to that of an episome, so far described only in bacteria.

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