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Chromosomal and extrachromosomal transmission of cellular melanization in the Freckled phenotype in *Drosophila melanogaster*

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In previous investigations Barigozzi and collaborators (for a summary of conclusions, see Barigozzi, 1963) have demonstrated that in melanotic stocks of *D. melanogaster* (i.e. stocks showing masses of melanine generally referred to as tumours in the abdomen, seldom in the thorax, practically never in the head) the transmission of the character seems to have a dual genetic control: a factor (or some factors) chiefly located in the 2nd chromosome and nearly always recessive (the *tu* genes), and an entity not clearly determined, which seems to be extrachromosomal.

However, previously studied stocks had not the best requisites to provide a definite description of the phenomena, the penetrance of the character generally being less than 100% and often fluctuating; also the localization of the chromosomal factor or factors within the chromosome was not clear-cut.

The present results refer to a new melanotic stock (*Freckled, Frd*) which meets all requirements for a more accurate investigation of the phenomena mentioned above. It will be shown that *Frd*, which clearly differs from the other melanotic stocks previously studied as far as both phenotype and genotype are concerned, shows more clearly all the characteristics of transmission typical of the other stocks.

1. MATERIALS AND METHODS

In the present investigation the following stocks were used:

Freckled (*Frd*), characterized by a number of black masses scattered throughout the whole body of the adult flies, including head, thorax and legs. The abdomen shows, in the freshly emerged fly, a uniform brown colour; older flies frequently show two dark beadlike rows of dots formed by the pericardial cells. According to Halfer, Piccinelli & Torri Tarelli (1965) melanization occurs mostly in the fat cells. In nearly 12% of the flies, melanotic masses are also formed by haemocytes, as in all common tumorous stocks. Melanization of fat cells (which are isolated or in small groups) starts around the nucleus (data confirmed by electron microscopy, Perotti & Bairati, 1967) and spreads throughout the whole cell. The fat cells which form a layer at the lower wall of the abdomen melanize less strongly. Pericardial cells melanize secondarily, as a consequence of storage of small melanotic debris, probably released by the fat cells (Perotti & Bairati, 1967).

Frd is 100% penetrant at 25 °C, if fresh yeast is added to the food. However, at 20 °C and with no fresh yeast both penetrance and expressivity are strongly reduced. All experiments referred to here were based on rearing flies at 25 °C and supplying them with food supplemented with fresh yeast.

It is, on the other hand, worth while to mention an experiment (A. Innocenti, personal communication) made on the manifestation of the *Frd* phenotype after a long-term effect of temperature of 20 °C and food without fresh yeast. These conditions lasted without interruption for ten generations and during this period (comprising nearly 7 months) the phenotype was reduced to a few melanization traces in only 47·5% of the individuals, the remainder being totally normal. Normal flies led to ovoposit on complete food at 25 °C produced an offspring which was 100% *Freckled*. This result indicates that nothing had changed in the genotype.

Frd gene is localized in the 2nd chromosome; it is fully dominant and lethal when homozygous; lethality occurs in the embryo. The salivary chromosomes (B. Nicoletti, personal communication) appear normal.

All stocks listed below have been used, since a careful investigation proved that they are devoid of transmissible melanization of any type.

(A) *CyL/Pm* has been tested for its efficiency in eliminating crossovers (which proved excellent) and for other purposes. During these crosses a total of 28788 individuals were scored for tumours. Not a single tumour either similar to *Frd* or of any other type was found. Thus the stock must be considered as absolutely free of dominant tumour genes.

(B) *yw*. This stock proved very successful for detecting the manifestation of *weak Frd* (see later), therefore it was submitted to careful control for presence of tumours. Two different countings (mass cultures) were made: one, made in 1965, gave one individual with one single small abdominal tumour out of 1128 flies (0·09%); the second one (1966) gave six individuals each showing one small abdominal tumour out of 4926 flies (0·12%). We have not been satisfied with this control, because we wanted to make it clear whether the 2nd and the 3rd chromosome carry some recessive *tu* gene, the manifestation of which could be revealed only in isogenic condition. Thirty-four lines isogenic for both chromosomes (obtained by using *CyL/Pm;Sb Mé/H* for chromosome replacements) gave a total of 1162 individuals (counted in fifty-eight single-pair cultures), among which only two had a small abdominal tumour (0·17%). Thus we can exclude that stock *yw* carries any recessive or dominant genetic factor for the production of melanotic tumours in the three major chromosome pairs.

(C) *yw;CyL/+;Sb Mé/H*. This combination has been synthesized using *yw* and *+/+;CyL/Pm;Sb Mé/H*, therefore the wild 2nd chromosome derives from *yw*. Out of a total of 1129 individuals (resulting from forty-seven single pair cultures) only three cases of tumour carriers (0·2%) were found. All three tumours were abdominal and two of them particularly small. These results are sufficient to establish that *yw* combined with *CyL* and one of its original second chromosomes gives origin to a practically tumour-free phenotype. Actually the extremely low frequency of tumours (0·2%, none of them of *Frd* type) does not exceed that which

may be found in any stock; it is well known, in fact, that tumours are often a response to the most different injurious agents operating during development; hence, the erratic presence of extremely rare tumours does not allow one to infer the existence of *tu* genes in the genotype.

(D) *yw;CyL/Pm;Sb Mé/H*. Out of 1152 individuals (obtained from twenty-two single pair cultures) three flies showed a small tumour, two of them abdominal, and one (particularly small) in the head. Owing to the phenotypic similarity of the last case to an extremely weak expression of *Frd*, the progeny of its sibs was further scored: in 101 individuals no trace of melanization was found. Therefore, since the occurrence of tumours (as stated above) can be due to environmental factors, we can conclude that there is no evidence of a genetically controlled production of tumours in the *yw;CyL/Pm;Sb Mé/H* stock. This conclusion, obviously, extends to the 4th pair, which however carries no markers. The presence of a dominant in this pair, which is the most important point (see later), can be excluded; the presence of a recessive in heterozygous stage is less important; nonetheless it is unlikely because of the high inbreeding of the stock.

2. RESULTS

Frd, as already mentioned, is controlled by one gene located in the 2nd chromosome. In the present investigation, a more careful analysis has been devised in order to determine the position of the locus and to detect the existence of modifiers. Information on both these questions is needed in connexion with what we shall report later on.

(i) *Localization of* Frd.

Crosses have been made with *brown* (*bw*, 104·5) and *speck* (*sp*, 107·0). The backcross starting from all markers in *cis* (*Frdbwsp*/+ + +) gave the following crossover percentages: *Frd–bw* 2·11 ± 0·38; *bw–sp* 2·33 ± 0·40; *Frd–sp* 4·45 ± 0·55 (1369 flies scored). The backcross in *trans* (+ *bwsp*/*Frd* + +) confirmed these results, with only a slight change in crossover frequencies: *Frd–bw* 1·49 ± 0·28; *bw–sp* 1·43 ± 0·27; *Frd–sp* 2·87 ± 0·39 (1811 flies scored). We conclude that *Frd* is located to the left of *bw*, at around 102. The difference between the *cis* and the *trans* condition can be easily explained as depending on the different origin of the stocks used, and therefore can be attributed to differences in the genetic background.

The second question (i.e. the existence of modifiers) can be answered negatively; in fact, all crosses made with *Frd*, having the scope of bringing *Frd* into different genetic backgrounds, gave an absolutely constant *Frd* phenotype.*

(ii) *Weak* Frd

While the findings described above prove that the *Frd* phenotype is controlled by a single localizable unit, one occasional observation drew our attention. From

* This part of the investigation has taken advantage from the collaboration of Dr B. Nicoletti, Inst. Genetics, The University, Rome, to whom we express our gratitude.

twenty-three single pair crosses ♂*Frd/CyL* × ♀+/+, in addition to 1762 *CyL* and to 1703 *Frd*, 6 individuals arose showing *CyL* together with a weak expression of *Frd*: this manifestation was limited to one or a few tumours in the head and in the abdomen. Five of these flies, when mated to normal individuals, gave rise to an offspring of 1017 individuals, including 516 *CyL*, 498 + +, 2 *CyLweakFrd* and 1 + + *weak Frd* (0·3 %). Thus *Frd* seemed to have become weaker in both expressivity and penetrance, and to have become transmissible independently of the original second chromosome.

Table 1. *Occurrence of* weak Frd *in backcrosses of* yw;CyL/Frd × yw;+/+

Frd/CyL parent	Numbers of progeny			% [*Frd*] (in *CyL*)	Cultures with [*Frd*]
	CyL	*Frd*	*CyL*[*Frd*]		
♀	396	422	0	0·0	0/15
♂	993	1037	18	1·8	13/26

Table 2. *Transmission of* [Frd]: yw;CyL/+ + [Frd] × yw;+/+

[*Frd*] parent	Numbers of progeny				% [*Frd*]		Cultures with [*Frd*]
	CyL	+	*CyL*[*Frd*]	+[*Frd*]	In *CyL*	In +	
♀	106	64	3+5*	3	7·0	4·5	6/6
♂	56	34	1	0	1·7	0·0	1/2

* Exaggerated [*Frd*] flies.

These first accidental observations required confirmation. We considered it desirable to construct a genotype which could reproduce the phenomenon observed at a higher intensity and with good repeatability. We also thought that a major chromosome, other than the second one, might affect or control the transmission of *weak Freckled*. We then prepared (as a first attempt) a combination of *Frd/CyL* with different first chromosomes, i.e. *Muller-5* and two chromosomes marked one with *vermilion* and the other with *yellow white*, all three non-carriers of factors for melanotic tumours. The results obtained with *Muller-5* proved negative, because *weak Frd* failed to appear altogether. With *v*, *weak Frd* appeared very seldom and the investigation was discontinued. We can therefore omit, for brevity's sake, the data proving that *Muller-5* and *v* are themselves tumourless. With *yw* (for tumourlessness of *yw*, see Materials) we had a better yield although never higher than 5–6 % and frequently only 1–2 % (see Table 1); henceforward we shall deal only with the *yw*;*Frd/CyL* combination, indicating *weak Frd* in square brackets as [*Frd*]. In every cross, [*Frd*] appeared in both sexes with similar frequency; therefore classification according to sex has been omitted in all tables.

Let us now comment on the data shown in Table 1. Backcrosses of the type *yw*;*CyL/Frd* × *yw*;+ +/+ + produced a number of *CyL*[*Frd*], which appeared uniformly in nearly all cultures. In this particular series of crosses no *CyL*[*Frd*] was produced from the backcross ♀*yw*;*CyL/Frd* × ♂*yw*;+ +/+ +. On the other hand,

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other experiments did not substantiate that [*Frd*] is produced only through male meiosis. The *CyL*[*Frd*] individuals thus obtained were mated to *yw*; + + individuals (see Table 2). [*Frd*] reappeared with *CyL* and with + +, thus showing that the factor involved is not linked to the second chromosome. There is also no evidence of its being sex-linked, because [*Frd*] individuals are, with similar frequency, males and females.

Another phenomenon, which unfortunately was never observed again, was the appearance of individuals showing a normal *Frd* phenotype; some of them even showed an exaggerated phenotype, with morphological abnormalities, which proved lethal: in fact, all these individuals died soon after having emerged and could not be used for further crosses.

[*Frd*] individuals were obtained each time the *yw*; *CyL*/*Frd* × *yw*; + / + backcross was repeated, with a frequency generally oscillating between 1 and 5%.

The two available genotypes, namely *yw*; *CyL*/ + + [*Frd*] and *yw*; + + / + + [*Frd*] (for the moment we put the symbol for *weak Frd* as independent from the second chromosome, without going deeper into the problem of its localization), have been used to establish mass cultures, which, for more than thirty generations distributed over 3 years, have kept and still keep the manifestation of [*Frd*]. Hence [*Frd*] must be due to some self-perpetuating factor, not linked with the 1st or the 2nd chromosome.

Before attacking the problem of the localization of [*Frd*], we think it necessary to describe its phenotype more accurately. We have already stated that its most common trait consists in the presence of a few black masses, especially located in the head, thorax and legs; thus its appearance is exactly that of a low expression of *Frd*. Besides, we must mention that, in a minority of cases, also the pericardial cells are dark. However, such [*Frd*] individuals, submitted to histological analysis, showed that fat cells are not involved in melanization: very small melanotic granula are distributed in cells of different type (epithelial cells, for instance): blood cells are not involved. Therefore, in the specimens studied, [*Frd*] phenotype was not identical, but similar, to *Frd*, and did not show any resemblance with the melanization of blood cells, which is typical of the usual tumorous stocks, and appears also in a low percentage of *Frd* flies. Another aspect connected with [*Frd*] is a strong melanization of some larvae and pupae. Individuals full of black masses (including melanization of the pericardial cells) appear frequently, although irregularly, in [*Frd*] lines, and die before completing metamorphosis. Early melanization and late lethality indicate a peculiar condition, never observed either in *Frd* or in other melanotic stocks.

(iii) *Localization of* [Frd]

According to the evidence shown in section ii, [*Frd*] appears to be due to a self-perpetuating entity, which is certainly not linked with the 1st or 2nd chromosome, although both the *X* and the 3rd pair exert a significant influence in determining its manifestation. Now we must demonstrate whether [*Frd*] is linked with the 3rd or the 4th chromosome.

The replacement of the 3rd unmarked chromosome pair, originally present in *Frd*, *CyL* or *yw* stocks, has already been considered: the absence of *weak Frd* flies, when C_3GSb replaces the former chromosome, might prove that [*Frd*] is located in this chromosome. On the other hand, as already stated, the replacement of the original 3rd chromosome with *Sb Mé/H* leaves [*Frd*] unchanged, proving already that [*Frd*] is not linked with the 3rd chromosome. However, in order to test every possible chromosomal linkage, the following experiment has been devised: first

Table 3. *Demonstration of absence of linkage between* [Frd] *and any one chromosome*

Mating 1. Both parents $yw; \frac{CyL}{+\,+}[Frd]; \frac{+}{+}$

Markers	Non[*Frd*]	[*Frd*]	% [*Frd*]	Cultures with [*Frd*]
CyL	994	23	2·3	13/17
+	384	4	1·0	

Mating 2. $\delta\ yw; \frac{CyL}{Pm}[Frd]; \frac{Sb\ Mé\ (H)}{+} \times yw; \frac{CyL}{Pm}; \frac{Sb\ Mé}{H}$ ♀

Markers	Non[*Frd*]	[*Frd*]	% [*Frd*]	Cultures with [*Frd*]
$\frac{Sb\ Mé}{H}$	762	6	0·8	10/34
$\frac{Sb\ Mé}{+}$	851	2	0·2	
$\frac{H}{+}$	828	3	0·4	

Mating 3. Both parents $yw; \frac{CyL}{Pm}[Frd]; \frac{Sb\ Mé}{H}$

Non[*Frd*]	[*Frd*]	% [*Frd*]	Cultures with [*Frd*]
2153	63	2·8	23/26

Mating 4: control. Both parents $yw; \frac{CyL}{Pm}; \frac{Sb\ Mé}{H}; \frac{+}{+}$

Non-tumours	Tumours (not of *Frd* type)	% *tu*	Cultures with *tu*
1149	3	0·3	3/20

we constructed the stock *yw*;*CyL/Pm*;*Sb Mé/H*;+/+; then we crossed it with *yw*;*CyL/Frd* and removed *Frd* through appropriate crosses with *yw*;*CyL/Pm*; *Sb Mé/H*;+/+. It turned out that [*Frd*] was always present (Table 3). We may only notice that in crosses under (2) of the same Table the yield of [*Frd*] was exceptionally low; it could be interpreted as an effect of the 3rd chromosome, similar to that exerted by the 1st chromosome (see above).

Since the crosses needed to remove *Frd* must have involved, together with the other chromosomes, also the 4th pair, derived from the tumourless *yw*;*CyL/Pm*;

Sb Mé/H; + / + stock, and none the less the frequency of [*Frd*] did not change, we must conclude that [*Frd*] is also independent from the 4th pair, and that therefore it definitely has to be considered as an extrachromosomal element.

(iv) *Crosses* [Frd] × [Frd] *and* [Frd] × non[Frd]

Since *Frd* is lethal in double dose, in order to analyse more completely the manifestation of [*Frd*], we crossed *weak Freckled inter se* and with normal individuals derived from stocks where *Frd* had never been present.

Table 4. [Frd] *frequency in* [Frd] × [Frd] *and* [Frd] × *non*[Frd] *crosses*

Mating	Numbers of progeny [*Frd*]	Numbers of progeny +	% [*Frd*]	Mean no. of progeny per culture	Cultures with [*Frd*]
[*Frd*] × [*Frd*]	30	1089	2·7	46·6	13/24
[*Frd*] × wild stock	60	1697	3·4	58·6	26/30
Normal × normal*	44	870	4·6	28·9	20/25

* Phenotypically normal individuals from [*Frd*] stock.

The results are shown in Table 4. Notice that, in all cases, the first chromosome was always *yw*, and that the third chromosome was unmarked. No differential larval or pupal death between the two types of cross was observed. Since in both crosses the yield in [*Frd*] was fairly high, the conclusions are clear: [*Frd*] × [*Frd*] fails to give rise to lethal zygotes, although the offspring per culture, on the average, is slightly lower than the one obtained from the cross [*Frd*] × + ; on the other hand, the frequency of [*Frd*] among the offspring is even higher, although not significantly, when [*Frd*] is supplied by only one of the parents. This both marks a difference between *Frd* and [*Frd*], as far as lethality is concerned, and also indicates a sort of dominance of [*Frd*]; this sort of dominance is a further element in favour of the extrachromosomal location of [*Frd*], especially in connexion with a possible location in the 4th chromosome.

Genotypical selection and inbreeding for [*Frd*] have been continued for seven generations: at the end of the experiment the frequency of [*Frd*] was 5·7%; this does not differ significantly from the original frequency (4·9).

Finally we tried to eliminate [*Frd*] from a pedigree, selecting phenotypically normal individuals for further crosses; this operation was unsuccessful. After seven generations of genotypic selection, the percentage of [*Frd*] was still 4·6%. Since a non[*Frd*] individual is nearly always able to transmit [*Frd*] to its offspring, we must conclude that the extrachromosomal entity is endowed with low penetrance. The concepts of dominance and penetrance, however, are not properly applicable to extrachromosomal entities. By such words, one wants simply to indicate that the [*Frd*] phenotype appears also when only one of the parents carried the [*Frd*] factor, and that its presence does not always manifest itself in the phenotype.

3. DISCUSSION

The findings described above point to the existence of a new phenomenon, which must be discussed: after the removal of *Frd* (a 2nd chromosome gene, located at about 102), a weaker, similar but not identical phenotype remains, which is transmitted at a low percentage but indefinitely, and seems to be controlled by a factor (indicated as [*Frd*]) not linked with any chromosome.

Let us now sum up the proofs of the extrachromosomal location of [*Frd*]. The most important one consists in the persistence of [*Frd*], irrespective of the different chromosome replacements, as shown in Tables 1–3. Further evidence is provided by Table 4, as well as by the unsuccessful attempts to eliminate [*Frd*] from a pedigree, using standard selection techniques.

If we consider [*Frd*] as extrachromosomal, we may try to complete its description as far as its localization and nature are concerned. In fact an extrachromosomal localization can be visualized as (*a*) cytoplasmic or (*b*) non cytoplasmic, i.e. externally or internally attached to the nuclear or endonuclear membrane, embedded in the nuclear sap or lying in the chromosomes, even though remaining unlinked to their nucleotide sequence. The operational distinction between (*a*) and (*b*) is provided by a matroclinous transmission, which indicates a localization outside the nucleus. All our data fail to show any trace of matroclinous transmission, and, moreover, prove that both gametes transmit [*Frd*] with the same efficiency. In one case (see Table 1) we even had *weak Frd* individuals only through the *weak Frd* males; then, the factor must be located within or in the vicinity of the nucleus, i.e. in the part of the male cell which remains in the mature sperm and enters the egg upon fertilization. We have no evidence from which we can localize [*Frd*] more precisely.

On the other hand, three possibilities, based on a chromosomal localization, can be easily ruled out:

(i) [*Frd*] resulting from the presence in the *Frd* stock of a second dominant gene of lesser penetrance and expressivity, but of similar phenotype, not located in the second chromosome, which becomes unmasked whenever *Frd* is removed. This is obviously in disagreement with our findings, which proved that chromosome replacements did not make [*Frd*] disappear. The same reasons lead us to reject the hypothesis of a recessive gene endowed with the same properties, revealed by the removal of *Frd*; a recessive gene requires, for its manifestation, the presence of an identical factor in the other chromosome, it should thus be present also in the stocks used in the different crosses where [*Frd*] appeared. On the contrary, we demonstrated that the chromosomes derived from stocks other than *Frd* do not carry any recessive allele for tumours of *Frd* type. On the other hand, [*Frd*] behaves as dominant-like.

(ii) [*Frd*] results from recombination between *Frd* and its *non Frd* homologue. This is contradicted by the obvious lack of linkage between [*Frd*] and the second chromosome.

(iii) For the same reasons recurrent mutation as a cause for [*Frd*] must be

rejected, because we should have to postulate an exceptionally high mutation frequency. Moreover, mutability should depend on the previous presence of *Frd* in the genotype.

The only acceptable explanation, therefore, must take into consideration the extrachromosomal location of [*Frd*].

The elements, which can be gathered from the experimental data in order to define the [*Frd*] factor are two: its extrachromosomal but not cytoplasmic location, and its indefinite perpetuation.

We must now face the problem of the relationship between [*Frd*] and *Frd*, which shows, in our opinion, several possibilities:

(i) [Frd] *is unrelated to* Frd. This means that in the *Frd* stock extrachromosomal entities (plasmagenes) exist, producing a weaker phenotype similar to *Frd*, normally masked by the presence of *Frd* and the effect of which can be detected only when *Frd* is removed. Since *Frd* was obtained (Ehrlich, 1963) through an X-ray treatment, we may imagine [*Frd*] pre-existing in the stock before the treatment. This hypothesis cannot be proved or disproved. Good evidence in favour might consist in finding [*Frd*] already existing in the stock from which *Frd* derived; unfortunately the stock could not be obtained. However, this hypothesis postulates the presence by chance, in the same stock, of two independent genetical factors, having a similar phenotype. Such a coincidence seems rather unlikely.

(ii) [Frd] *is related to* Frd. This alternative can be visualized in four ways:

(*a*) both [*Frd*] and *Frd* are caused by an extrachromosomal entity, the presence and the frequency of which is controlled by the genotype. The 2nd chromosome, at about 102, provides the best conditions for the most intense presence of the extrachromosomal entity producing *Frd*; the 1st and the 3rd chromosome condition the extrachromosomal entity to a lower diffusion, therefore to a different phenotype, thus giving rise to [*Frd*]. This hypothesis resembles the well-known Killer phenomenon in ciliates. In principle, there are no fundamental objections to it, but two observations make it appear unlikely. First, there is no evidence of killer-like particles, even at ultrastructural level (the virus-like particles described by Bairati, 1964, are too inconstant). Secondly, when *Frd* was kept for several generations in such conditions as to give it an extremely reduced expression the sudden change in external conditions failed to provide evidence of the existence of an entity beside the *Frd*-gene. These negative observations, on the other hand, are not sufficient to disprove the hypothesis.

(*b*) The locus at nearly 102 of the 2nd chromosome is actually responsible for the *Freckled* phenotype, including early lethality in double dose, but, at the same time, is endowed with the ability to pass on its information to self-perpetuating particles (presumably of RNA), capable of indefinite transmission, which, being less efficient in producing melanization, are responsible for the weaker phenotype [*Frd*]. This explanation takes, as a model, the most recent re-elaboration of the metagon theory by Beale & McPhail (1967), where a function initiated by a gene is maintained after its removal. To this hypothesis there are no serious objections;

unfortunately, the theory itself is far from being definitely established, and this makes any further speculation difficult.

(*c*) *Frd* and [*Frd*] are respectively the integrated and the non-integrated state of an episome. Since we have no evidence of [*Frd*] becoming integrated, i.e. [*Frd*] acquiring the characters of *Frd*, we cannot seriously consider this explanation in its classical form (which (see Barigozzi, 1963) seemed acceptable for other tumorous stocks); however *Frd* might be thought to be an episome capable of leaving its chromosomal locus, but unable to re-integrate when extrachromosomal.

(*d*) [*Frd*] is caused by something similar to paramutation in Maize: paramutation, however, is strictly chromosomal and not permanent. Therefore this explanation must be ruled out.

Of the five possible explanations of the origin and transmission of [*Frd*], the last one is definitely unacceptable, while the other four show a certain degree of likelihood. Even though a choice between them cannot be made here, the preference falls on the third one, for the moment, i.e. the one based on the metagon model.

In any case, we cannot avoid attributing to the *Frd* stock a dual system of transmission for two similar phenotypes, the genotype of one of which is unlinked to chromosomes, thus presenting a very complicated case which ranks among the rare examples of extra-chromosomal activity in the genetics of *Drosophila*.

SUMMARY

Freckled (*Frd*) is a dominant mutant of *D. melanogaster*, lethal in double dose, which manifests itself by melanization in the adult stage, while nothing can be noticed in the larva and/or in the pupa. Its localization has been determined at nearly 102 of the second chromosome.

Among the offspring from backcrosses involving *Frd*, some individuals that do not carry the *Frd* gene exhibit a weak *Frd* manifestation in the adult stage, or a stronger one, mostly lethal, in the larval or pupal stage. Weak *Frd*, indicated as [*Frd*], is transmitted indefinitely without any recognizable linkage with the chromosomes, although its frequency is influenced by the 1st and, probably, by the 3rd chromosome. No matroclinous transmission has been found. It is concluded that [*Frd*] is probably controlled by an extrachromosomal entity, not located in the cytoplasm. The possible relationships between *Frd* and [*Frd*] are discussed.

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