PSEUDOTUMORS AND FITNESS IN *DROSOPHILA MELANOGASTER* AND *DROSOPHILA SIMULANS*

A. DI PASQUALE AND S. KOREF-SANTIBAÑEZ

Istituto di Genetica, Università di Milano, Milano, Italy

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MELANOTIC tumors ("pseudotumors") in *Drosophila* have been found to be very widespread in almost all laboratory stocks of *D. melanogaster* (BURDETTE and OLIVIER 1952; BARIGOZZI and DI PASQUALE 1956) and in several natural populations of different species (BRNCIC and KOREF 1953; MAKINO and KANEHISA 1954; DI PASQUALE and KOREF-SANTIBAÑEZ 1959).

Although this trait is very variable in its expression, and most probably in its genesis, the following are some of the characteristics common to all "pseudotumors": (a) In *D. melanogaster* their presence is due fundamentally to main genes localized on the second chromosome and to several modifiers on the others (BURDETTE and OLIVIER 1952; BARIGOZZI and DI PASQUALE 1956). (b) Their genetic determination is strongly heterogeneous, for in different stocks with a similar phenotype, nonallelic genes are involved (BARIGOZZI and DI PASQUALE 1956). (c) Their spontaneous incidence is low but may sometimes be increased by inbreeding (MAKINO and KANEHISA 1951). (d) Their incidence is modified by changes in the environment (HARNLY, FRIEDMAN and GLASSMAN 1951; HERSKOWITZ and BURDETTE 1951).

KOREF (1956) has postulated that these pseudotumors could be considered, owing to all these general characteristics, as *phenodeviant*s (LERNER 1953).

As a complement to the above hypothesis, DI PASQUALE and KOREF-SANTIBAÑEZ (1959) found that in natural populations of *D. melanogaster* and *D. simulans* pseudotumors were observed in high frequency in the individuals collected in nature. Moreover, in the descendants of single wild females, after 12 generations under laboratory conditions, without marked inbreeding or selection for the pseudotumoral trait, this trait was not eliminated. On the contrary, in certain cases the incidence of pseudotumors tended to increase. Another interesting fact was the marked genetic heterogeneity among the different lines, each of which originated from a single female, which resulted in very low tumor percentage following crossing of individuals of different tumor lines (DI PASQUALE and KOREF-SANTIBAÑEZ 1959). All this led the authors to postulate that these "pseudotumors" could in some way contribute to a higher fitness of their carriers which would therefore favor the maintenance of the trait in the populations.

The authors think that observations on the pseudotumors incidence in descendants from individuals captured in nature and maintained under different systems of breeding in order to allow them to reach different grades of homozy-

gosis may help to clarify the nature of the trait. In fact, if increasing in homozygosis causes a higher tumor incidence, this might support the hypothesis that pseudotumors are "phenodevians."

On the other hand, it seems interesting to inquire if in pseudotumor individuals or lines some of the most important components of fitness have higher values than in tumorless ones. In the affirmative case, one could conclude that the adaptive value of the tumoral genotype plays a role in maintaining the high incidence of the trait in the populations.

MATERIALS AND METHODS

Two main studies were here carried out in the two sibling species, *D. melanogaster* and *D. simulans*.

1. An analysis of pseudotumor incidence was made in several lines, each of which was derived from a single female captured in nature (at Aspra) and kept under different levels of inbreeding.
 - A. Four females were crossed with four males of the same generation—36 lines of *D. melanogaster* and 94 lines of *D. simulans* ("medium inbreeding").
 - B. Strict individual brother-sister mating was employed in 30 lines of *D. melanogaster* and 95 of *D. simulans* ("high inbreeding").
2. An investigation was made of some of the main components of fitness (BUZZATI-TRAVERSO 1953) such as fecundity, viability, sterility and rate of development. These elements were compared in the following types of stocks or lines.
 - A. Laboratory stocks with pseudotumors and laboratory stocks without pseudotumors. *D. melanogaster*: Varese (zero percent *tu*); *tu* B3 (100 percent *tu*); *D. simulans*: Aspra (zero percent *tu*); *tu* B1 (100 percent *tu*).
 - B. Lines originated from wild females, both tumoral and nontumoral.

D. melanogaster: line 52, maintained by medium inbreeding, with an increasing tumor incidence (from 7.6 percent in F_1 to 60.7 percent in F_{24}); line 14, maintained by medium inbreeding, with a low and stable pseudotumor incidence (less than one percent); line 19, highly inbred for three generations, with a low pseudotumor incidence, which frequently fell to zero percent; the same line 19, highly inbred for seven generations; line 8, highly inbred for three generations, with a low and stable pseudotumor incidence (1.2 percent), which is never reduced to zero; line 26, highly inbred for seven generations, with a low and stable incidence (5.7 percent).

D. simulans: line 37, mediumly inbred, with an increasing pseudotumor incidence (from 5.3 to 81.7 percent); line 8, mediumly inbred, with zero percent pseudotumors in all generations; line 68, highly inbred for three generations, with a low and stable pseudotumor incidence (5.0 percent); the same line 68, highly inbred for seven generations; line 110, highly inbred for three generations, with a very low pseudotumor incidence (less than one percent); line 48, highly inbred for three generations, with a stable pseudotumor incidence (20.0 percent); line 107, highly inbred for seven generations, with in-

creasing pseudotumor incidence (from 5.2 to 70.8 percent); line 115, highly inbred for seven generations, with a stable low incidence (less than one percent).

C. Within lines with high pseudotumor incidence, individuals carrying the trait were compared with individuals free from tumors. The following were the methods used for analyzing the above mentioned components of fitness. All experiments were made at a constant temperature of 24°C.

Fecundity: Daily egg production per female of 20 to 30 females of each stock or line was observed over a period of 20 days. For this purpose, 12-hour old males and females were aged separately for 24 hours and paired individually. Twenty-four hours later, in order to normalize oviposition, each couple was placed in an empty vial (3 × 8 cm), into which was introduced a plastic spoon with the usual *Drosophila* food medium, blackened with carbon, and touched with a suspension of fresh yeast. The spoons were renewed every 24 hours, and the eggs laid by each female were counted under a binocular microscope.

Viability: The analysis was based on the estimation of the percentage of adults emerged from a known number of eggs. The eggs obtained in the study of fecundity were used. Twenty-five eggs were put into each vial (five eggs per female), and emerged adults were counted. With this method, samples were obtained from females of equal age, and viability was controlled in relation to the age of female.

Sterility: One female was placed into each culture vial together with two males. Vials in which larvae appeared were considered fertile, and where no development was apparent the flies were transferred every four days to new cultures. If after 20 days no development occurred, the females were considered sterile.

Rate of development: Males and females collected within a period of 12 hours were allowed to age separately for 24 hours, mated, and left together for 48 hours. Groups of 20 pairs were placed in one fourth liter milk bottles, whose mouth was covered with a watch glass containing darkened *Drosophila* food painted with a suspension of fresh yeast. The flies were allowed to oviposit on this food over periods of four hours, after which the eggs were collected and placed 50 per 12 × 3 cm food vial. Two egg samples were taken each day (eggs laid between 9 A.M. and 1 P.M., and between 1 P.M. and 5 P.M.), over a period of six days. Each group of 20 couples was replicated once. The emergence of imagoes was scored every four hours (4–8–12 A.M., 4–8–12 P.M.); since the first controls showed very few flies emerged at 4 A.M. or 12 P.M., these counts were eliminated. The rate of development, expressed in hours, is therefore $\pm$ two hours from the period of egg laying to the emergence of adults.

RESULTS

Pseudotumor incidence in lines with medium and high inbreeding: In 66 lines of *D. melanogaster* and 189 of *D. simulans*, the pseudotumor incidence shows a varied behavior in the course of the generations under laboratory conditions (Table 1). Fluctuations of less than 20 percent in absolute incidence have not

TABLE 1

Classification of lines under different degrees of inbreeding, as respects behavior of pseudotumoral incidence under laboratory conditions

		Stable	Increasing	Decreasing	Irregular	Generations	No. lines	No. individuals
<i>D. mel.</i>	Medium inbreeding	32	1	1	2	14	36	45203
		88,9%	2,8%	2,8%	5,6%			
	High inbreeding	30	..	..	..	8	30	16532
		100%						
<i>D. sim.</i>	Medium inbreeding	74	8	1	11	14	94	108223
		78,7%	8,5%	1,1%	11,7%			
	High inbreeding	87	2	..	6	8	95	51185
		91,6%	2,1%	..	6,3%			

been considered as being meaningful. This was true for the majority of the lines, studied in some cases for eight generations (if the line did not become extinct before then); these lines are considered "stable." A small number of lines increased their tumor incidence by an absolute amount of over 20 percent; these are classified as "increasing." A few others showed a decrease of their pseudotumor incidence of more than 20 percent; these are the "decreasing" lines. Finally, a few lines, considered as "irregular," showed oscillations of more than 20 percent, but reached, at the end of the study, incidences which do not differ substantially from those in the first generation (Figure 1).

In both species, the behavior is qualitatively very similar, and although there seem to exist quantitative differences (a greater number of irregular and increasing lines in *D. simulans*), this difference is difficult to prove statistically.

It is also difficult to make a statistical comparison of lines maintained by medium and high inbreeding. Nevertheless, Table 1 shows that in *D. simulans*, although inbreeding seems to decrease the number of lines that show increasing or irregular pseudotumor incidence, the stability of most lines is not affected.

In order to know more about the behavior and structure of some stable and unstable lines, the genotypes of two lines of *D. melanogaster* were analyzed. One of these was an "increasing" line (52), and the other a "stable" one (69). An analysis of isogenic lines for certain chromosomal combinations (obtained by means of substitution of chromosomes containing balanced lethals) indicated that: (a) in both lines the trait is recessive; (b) the main factor or factors ("tu") responsible for the pseudotumoral trait are apparently limited to the second chromosome, while modifiers are localized on the other chromosomes, with the third chromosome being especially active in line 52.

FIGURE 1.—Behavior of the four types of individual lines: stable, increasing, decreasing, irregular. Ordinate: percent of tumors. Abscissa: generations.

Analysis of the components of fitness (Tables 2 and 3)

Fecundity: The mean number of eggs per day per female, and the mean total of eggs per female were recorded. Both these factors are characteristic for each line of each species, although in general *D. melanogaster* had a higher oviposition rate than *D. simulans*. In most cases egg laying remained constant throughout the 20 days. Only in two lines, one of each species (26 of *D. melanogaster* and *tu B1* of *D. simulans*) was there a tendency towards decrease with age.

In order to find differences in fecundity between tumoral and nontumoral stocks or lines, the data were compared by means of an analysis of variance. Thus, the total number of eggs per fly (20 flies per line) were compared with the corresponding values of 20 flies of the stock used as a control.

TABLE 2
Elements of fitness in D. melanogaster

Line	Percent <i>tu</i>	Average no. daily eggs/female	Fecundity Average total eggs/female	No. females	Percent reaching adulthood	Viability No. eggs	Sterility Percent females	No. females	Rate of development Mean no. hours to emergence	No. adults
Varese	0.0	45.3±3.11	828.2	20	87.0±1.23	745	0.0	10	292.9±0.81	231
<i>tu</i> B3	100.0	48.4±2.74	890.7	20	85.8±1.27	760	0.0	10	302.5±1.09	181
52 (1957)	60.7	30.8±5.25	542.7	15	{ 48.7±3.06	{ 267	{ 50.0	{ 8	{ 292.0±1.34	{ 95
14 (1957)	60.7	46.4±3.31	795.1	15	{ 54.9±2.35	{ 450	{ 14.3	{ 7	{ 303.1±2.04	{ 75
8 (1958) F ₃	0.0	38.6±3.37	668.9	30	55.3±1.30	1457	26.7	30	299.6±1.36	85
19 (1958) F ₃	0.0	38.9±3.79	710.7	20	70.7±1.21	1408	10.0	20	301.4±2.30	30
19 (1958) F ₃	1.2	36.9±1.61	658.3	20	73.9±1.53	820	10.0	10		
19 (1958) F ₇	0.0	35.8±3.90	601.1	19	66.2±1.92	610	0.0	10	268.1±1.01	171
19 (1958) F ₇	0.0	40.5±4.15	752.1	20	70.6±1.22	1396	0.0	20	271.2±0.41	729
26 (1958) F ₇	5.7	23.8±1.85	316.5	20	16.3±1.33	773	45.0	20	267.3±0.70	229

* wt = with tumor.
† tl = tumorless.

In *D. melanogaster*, all the lines and the *tu* B3 stock were compared with the nontumor bearing Varese stock. In line 52, females with tumors were taken separately from nontumoral females. The results of this analysis show no difference in egg laying between the flies from the tumor and the nontumor lines. In line 52, females free from tumors tended to lay more eggs than those with tumors. The low egg production of line 26 in the seventh generation of inbreeding may be attributed to this last fact.

In *D. simulans*, all lines were compared to the nontumor stock, Aspra. In line 37 and 107, egg production by tumor and nontumor females was considered separately. The Aspra stock had a significantly higher egg production than all lines excepting line 68 at the third and the seventh generations of inbreeding. Therefore, another comparison was made between the tumor stock, *tu* B1, and all others. This analysis showed no special tendency of egg production which could be related to the tumor trait. Within the tumor-bearing lines, there was no difference between tumor and nontumor females. Inbreeding does not seem to have affected oviposition in the line 68, although it may be responsible for its reduction in line 107.

Viability: Tables 2 and 3 show the percentage frequency of viability over the total number of eggs considered. Here also the percentage of individuals which reach adult age is characteristic for each stock or line. The possible relationship between age and fertility was calculated by the method of Skory for χ^2 ; the comparison of the total fertility of all lines was also carried out by the χ^2 method.¹

In *D. melanogaster*, aging does not seem to influence the viability. Only in F_7 of lines 19 and 26 was there a significant decrease of individuals which emerged from the pupa, the older the parents. This may be attributed to inbreeding.

The comparison with the Varese stock showed no difference with respect to *tu* B3 (both are also the stocks with the highest viability). All other stocks, especially lines 26 and 52, were less viable. In line 52, the viability was lower when both parents were either tumor or nontumor bearing, and higher when one of the parents carried the trait.

In *D. simulans* only three lines, *tu* B1, 110 F_3 and 68 F_7 , showed a decrease of the viability with age. The latter line had a very low total viability. Considering the tumoral stocks, only *tu* B1 showed any decrease in viability in relation to the Aspra stock. In all others, the number of adults emerged was slightly greater. Each of the lines with high tumoral incidence behaved differently. In lines 37 and 107, viability was lower when both parents have tumors than when both, or one, did not show the trait.

Summarizing, there is no clear tendency for a correlation of the viability with the tumoral trait. Only when the parents have no tumors does it seem to be slightly higher.

¹ Formula $\chi^2: (ad - bc - \frac{n}{2})^2 \times n / (a + b)(a + c)(b + d)(c + d)$ where a, b, c, d , are the frequencies of the four classes considered, and in this case a and c correspond to the adults of two lines, while b and d correspond to the individuals which failed to reach the adult stage; n is $a + b + c + d$.

Sterility: The results are of limited value, as the number of individuals and of lines studied was low.

In *D. melanogaster* there was no difference between the nontumor (Varese) and the tumor (*tu* B3) stock. In line 52, especially when both parents were either tumor or nontumor bearing, the sterility was high. Line 26, at the seventh generation of inbreeding, was also more sterile.

In *D. simulans*, although *tu* B1 is often sterile, there was no relation between this factor and tumor bearing, for in line 37 the highest sterility was found when both parents were either tumor bearing or nontumor bearing. In line 107, on the other hand, the least fertile were the following combinations: both parents tumor bearing, or female without tumors $\times$ male with tumors.

Rate of development: Rate of development is expressed as the mean hours which individuals of each line take from oviposition to imago emergence. The data of the different lines were compared by "Student's *t*." As in the aforementioned components of fitness, this one also shows important individual differences in each line.

In *D. melanogaster*, mean development took from 257.3 to 303.1 hours. Lines captured more recently in nature took less time, while inbreeding did not seem to exert any influence (line 19 in F_3 : 268.1 hours, in F_7 : 271.2 hours).

The comparison of the rates of development of all lines with the nontumor stock (Varese) shows that *tu* B3 and line 52 took significantly longer (302.9 and 299.6 hours, respectively). Within line 52, development was shorter in descendants parents with tumors (292.0 hours) than in those of nontumor flies (303.1 hours).

In *D. simulans* the mean development time ranged from 248.6 hours to 302.4 hours, the most rapid being also from the more recently captured flies. The comparison with the nontumoral stock (Aspra), shows that *tu* B1 had a shorter development (288.9 hours); the other two lines with intermediate times (line 37: 286.0 hours; line 107: 262.0 hours). Within line 37, development was more rapid in descendants of parents with tumors (285.0 hours) than in those without tumors (300.3 hours). Within line 107, on the other hand, descendants of nontumor flies took less time (261.7 hours) than did those with tumor (265.4 hours).

DISCUSSION AND CONCLUSION

The study of pseudotumors in 66 lines of *D. melanogaster* and 189 lines of *D. simulans*, originated from females captured in nature and maintained for several generations without selection for the tumor trait, shows that different lines have their own particular behavior with respect to pseudotumor incidence. This may mean that the genetic factors responsible for the trait may be different (although in lines 52 and 69 of *D. melanogaster* main genes were found to be recessive and localized in the second chromosome). It may also confirm (KOREF 1955) that the degree of penetrance and expressivity of the *tu* genes depends not only on the same *tu* genes but also on the rest of genotype. Therefore, the natural populations from which the original females were obtained must have been large and genetically very heterogeneous.

As to inbreeding, this does not seem to affect the tumor incidence in the lines here studied. Thus one of the most important elements to support the phenodeviant nature of pseudotumors is lacking. On the other hand, the fact that inbreeding maintains the irregular behavior of pseudotumor incidence in *D. simulans* agrees with the results obtained by BARIGOZZI (1960) in isogenic lines of *D. melanogaster*.

As to the components of fitness, which could clarify the possible adaptative value of the tumoral phenotype, none of those examined gave clear results. Although in line 52 fecundity was decreased, all four components have similar values in the line 110 of *D. simulans* (zero percent *tu*) and in line 37 of the same species (70 percent *tu*). In any case, no general tendency was found to have a higher fitness when pseudotumors are present. It must be concluded that, from the data obtained here, there are not elements which permit to attribute a higher fitness to the individuals carrying pseudotumors.

It is to be noted that changes of fitness related to inbreeding have been found in some lines. But, these do not seem to also involve changes in pseudotumor incidence; therefore they are probably due to homozygosis of genes not linked to or recombining with *tu* genes.

Therefore, at this point of our study, the high incidence of the tumoral trait and its irregular unpredictable behavior cannot be explained by the orthodox mechanisms for fitness which are already known to operate in Mendelian populations.

This negative conclusion is consistent with the postulate of BARIGOZZI (1959, 1960) that there may exist a certain type of genic instability which plays a role in the determination of the tumor phenotype. The present work does not, however, indicate whether this instability in tumor incidence is due to changes involving one or several different genes or their location.

SUMMARY

Pseudotumor incidence was studied in 66 lines of *D. melanogaster* and in 189 of *D. simulans* maintained under different levels of inbreeding for eight or 14 generations. Pseudotumor incidence was fairly constant in most lines. In some lines incidence changed significantly, sometimes increasing, decreasing, or being irregular.

A study of some of the components of fitness such as fecundity, viability, sterility and rate of development did not demonstrate any effect of the presence of the pseudotumoral trait.

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