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Localization of genes controlling radioresistance in *Drosophila melanogaster*

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In previous tests an increased resistance to acute irradiation was established in a population which, during a great number of generations was irradiated at an early larval stage. The purpose of the present investigation was to localize genes, controlling radioresistance in this population to definite chromosomes. Chromosome substitution lines were used in which control was exercised over X and Y chromosomes, as well as the two big autosome pairs. The analyses have shown that resistance is dependent on a polygenic system.

Differences as regards radiosensitivity tested by different criteria have been noticed among lines in several animal species (BARTLETT and BELL 1962; EHLING 1964). However, the development of differences in radiosensitivity in lines with a common origin has seldom been possible to observe. When carrying out experiments with two populations of *Drosophila melanogaster*, which had been started from an inbred pair of flies, EICHE (1973 a) showed, that progenies from these populations have different radiosensitivity. Thanks to the possibility offered by the *Drosophila* technique, studies on the genetic background of these differencies can be carried out.

In the present paper an account is made of the methods used to localize genes controlling radioresistance. To achieve this aim chromosome substitution lines were established by means of marked inversions. These lines had different combinations of chromosomes from the two populations. Subsequently larvae from these lines were irradiated and differences in eclosion between the lines were studied. In this way a criterion of the impact of individual chromosomes with regard to the sensitivity to irradiation was acquired.

Material and methods

1. The background of the populations

Material used in this investigation originates from a wild type stock, Karsnäs. By means of marked inversions several lines from this stock were isogenized and propagated by brother × sister mating. Two populations, K and R, were started from one of these lines after the flies had been massmated during the course of some generations. Larvae from the R population aged for 20 ± 4 hours were exposed to irradiation with 1120 R in each generation except generations 13–25. The X-ray treatment was performed in cups, each cup containing exactly 100 individuals. The K population was not irradiated, but in all other respects it was treated in exactly the same way as population R. The size of each population amounted to 500–550 pairs, that is, it was made up of all flies from twelve cups in each generation. The populations were kept in an incubator at 25 ± 1°C. Unirradiated branches from both populations in generation 123, called K 123 and R 123, respectively, were used to establish the lines described below.

2. Substitution of chromosomes

Substitutions of chromosomes were made in accordance with the current established methods with all chromosomes but the small fourth pair under control. The marked inversion stocks used in the crossing procedure had the following constitutions:

(1) y ac sc pn w rb cm ct sn^3 ras^4 v m g f car/FM6, y^{31d} sc^8 dm B.
(2) In(2L + 2R)Cy, al^2 Cy lt^3 sp^2/In(2LR)Pm, dp b Pm^1; In (3LR)DC × F, ru h D ca/In(3R), Sb.

Since the possibility that the Y chromosome might influence the response to irradiation could hardly be excluded, steps were taken to render certain that the Y chromosome in each individual line originated from the same population as the X chromosome. All combinations of chromosomes from the R and K populations were established including lines with only R and K chromosomes. The origin of chromosomes has been designated by R and K. Below all the substituted lines are enumerated (the first letter designates the X and Y chromosomes, the second letter stands for the second chromosome pair and the third one for the third pair): RRR, KRR, RRK, RKR, KRK, KKR, RKK and KKK. Several chromosomes from the two populations made the basis for each line in order that the lines should reflect the variation present in the population.

3. Treatment of the substituted lines

The two lines which had been branched off from the main populations and the eight substituted lines were cultivated in 200 ml flasks. At the age of 4–6 days the flies were transferred from the flasks to population boxes, one for each line, and the boxes were coded. The number of flies per box was ca 400 pairs. There were openings in the cages on which Petri dishes could be placed for egg-laying. The dishes contained agar substance blackened with charcoal, brushed over with a slight film of yeast suspension. Each egg-laying period lasted three and a half hours per day over four days. Nineteen and a half hours after the start of the egg-laying larvae were cleared off from the Petri dishes. Collection of larvae started three hours later and had a duration of slightly less than one hour. Exactly 100 larvae were placed in each cup containing agar-yeast medium. Three hundred larvae per line were collected from each egg-laying period at the very most. The cups were irradiated either immediately after collection, series 2 ± 2, or after 18 hours, series 20 ± 2. In series 2 ± 2 the larvae were irradiated with 0, 1300 and 1500 R, but in series 20 ± 2 the dosage was 0, 2000, 2500 and 2800 R. Each dose was applied only to one cup per line from each period of egg-laying with a maximum of four per week. There were five repeats of the experiment, one repeat per week, during five successive weeks. Altogether 101 900 larvae were collected. Dosage 0, 1300, 1500 and 2500 was applied during all the five weeks, while dose 2000 R was given only during the first three weeks and dose 2800 R during the last two weeks.

All work and X-ray treatment were performed at room temperature, otherwise the flies were maintained in an incubator at $25 \pm 1°C$. The X-ray treatment was performed in a Scandia Intensiv apparatus operated at 170 kV, 15 mA and with an inherent filtration corresponding to 2 mm Al at a dose rate of 90 R/min. In each test the first count was carried out on the 9th day and the second on the 17th day after the larvae had been collected. A method, described by YATES (1955), which combines data from several four-fould tables was used in the statistical analyses.

Results

The pooled material from each dose and each line is shown in Table 1. It is of interest to note that, in comparison with other lines, R123 and RRR had a high rate of eclosion after irradiation while K 123 and KKK had a low rate. It is also worth mentioning that two of the lines, namely RRR and KRR, have a considerably lower rate of eclosion at dose 0 R than the other lines. Irradiation with 2000 R resulted in low mortality in all lines. In many cases this dose had almost a sublethal effect. Only doses which had been the cause of obvious mortality in all lines, namely 1300, 1500, 2500 and 2800 R have been included in the statistical analysis. Since the purpose of the investigation in the first place was to study the effects of irradiation after the substitution of certain chromosomes, each line was compared individually first with RRR and then with KKK. Material from each dose and each week was used

Table 1. Per cent eclosion (± S. E.) at different doses

Line		Series 2 ± 2 hours		Series 20 ± 2 hours		
	0 R	1300 R	1500 R	2000 R	2500 R	2800 R
R 123	93.78 ± 0.53	53.67 ± 2.53	18.33 ± 2.96	89.36 ± 1.51	72.30 ± 2.24	51.63 ± 4.84
RRR	84.97 ± 0.66	50.64 ± 3.78	21.00 ± 3.00	78.90 ± 1.47	63.06 ± 2.15	49.00 ± 4.85
KRR	88.53 ± 0.77	46.21 ± 3.23	12.11 ± 1.91	85.64 ± 1.42	60.63 ± 2.90	35.00 ± 3.44
RRK	95.27 ± 0.47	47.31 ± 3.57	8.11 ± 1.33	91.73 ± 1.14	65.05 ± 3.06	33.88 ± 5.31
RKR	95.06 ± 0.40	39.60 ± 3.25	10.00 ± 1.63	82.18 ± 2.29	52.05 ± 2.44	23.38 ± 2.06
KRK	93.76 ± 0.69	36.50 ± 3.42	7.00 ± 1.16	90.44 ± 1.49	50.44 ± 3.88	25.57 ± 4.67
KKR	92.60 ± 0.47	26.36 ± 4.13	2.73 ± 0.70	83.00 ± 1.85	46.45 ± 3.18	12.75 ± 2.48
RKK	93.64 ± 0.52	47.47 ± 2.74	5.81 ± 1.17	85.64 ± 1.56	58.16 ± 3.71	26.38 ± 5.39
KKK	93.17 ± 0.55	31.15 ± 3.49	3.93 ± 1.43	85.22 ± 2.46	41.85 ± 3.01	12.75 ± 3.52
K 123	93.88 ± 0.62	25.82 ± 3.83	4.00 ± 1.25	80.88 ± 2.03	37.71 ± 3.43	18.25 ± 3.32

Table 2. Combination of results from several 2 × 2 tables with t-values and level of significance

Lines compared	t-value	Level of significance
RRR > KRR	5.69	***
RRR > RRK	4.43	***
RRR > RKR	13.88	***
RRR > KRK	14.70	***
RRR > KKR	22.07	***
RRR > RKK	8.88	***
RRR > KKK	23.35	***
KRR > KKK	18.21	***
RRK > KKK	19.80	***
RKR > KKK	10.18	***
KRK > KKK	8.59	***
KKR > KKK	1.15	NS
RKK > KKK	14.93	***

in the statistical analysis as a separate unit. The analyses are based on seventeen partial comparisons, as three of the doses were applied during the course of five weeks and one dose during two weeks. Already a sign test revealed that considerable differences existed between the different lines. In all cases RRR was superior and KKK inferior to the other established lines. In most cases the difference was significant.

Due to differencies in eclosion at 0 R the combination of data from 2 × 2 tables was made both on the basis of original values and with each week's material adjusted to eclosion at 0 R. A summary of the results from the statistical treatment, based on the original values, is shown in Table 2. It should be stressed that in all cases RRR had a greater radioresistance whereas KKK was inferior. In all cases but one, the differences were on a statistically highly significant level. When the same type of analyses were made with values adjusted to the eclosion at 0 R, identical results were obtained, with the exception of the absolute size of the t-values.

Discussion

The rate of eclosion in the unirradiated controls was rather equal for all lines, except two. One of the divergent lines, where the eclosion rate was the lowest, contained all the three big chromosomes, and the other had the two biggest chromosomes from line R. The fact that these particular two lines had the greatest loss of individuals between the larval and the adult stage might certainly be a coincidence. However, it is much more probable that in spite of all the efforts made to maintain as broad a basis as possible at substitutions, chromosomes with considerably reduced viability were overrepresented. A rather frequent occurrence of chromosomes containing detrimentals has been reported in population R in a test on genetic load (Eiche 1973 b) and also in an earlier publication on somatic resistance to X-ray treatment (Eiche 1973 a), a decreased eclosion in population R was found.

Establishing of lines, where all big chromosomes originate from the same population, K or R respectively, was made with the view of confirming the efficiency of the substitutions. A comparison between the substituted RRR line

and the original population R (R 123) displays considerable similarity although in the control the RRR line shows a lower frequency of eclosion than the other lines. Adjusted for the lower control level the RRR is even slightly superior to population R. In a similar manner KKK compared with population K (K 123) shows a striking similarity. Hence, there seems to be reasonable ground for assuming that the newly established eight lines reflect fairly well the genetic constitution of the populations.

A closer examination of the results obtained from individual comparisons reveals that each substitution of a chromosome from population R for a chromosome from population K leads to an increase of radiosensitivity. In the same way, when a chromosome from population K is substituted for a chromosome from population R there is a reduction in radiosensitivity, except when the third chromosome is involved. Thus, it might be concluded, that the third chromosome exercises the least influence on radioresistance. Nevertheless, it is hardly possible to disregard sampling effects and technical deficiencies, when establishing the individual lines. The circumstance that a conspicuous decrease in survival after irradiation is obtained at reverse substitution, yields certain support to such assumptions. In all circumstances the analyses show that we are concerned with a polygenic system where all chromosomes have a bearing on the trait which is being studied. Except for the assertion that the third chromosome seems to have the least importance, it would be hazardous to estimate the contribution of each individual chromosome to sensitivity or resistance, respectively. There are grounds for pointing out the conspicuous similarities in the pooled material among the lines as regards the relative patterns of sensitivity at different doses. Consequently, the irradiation has had a similar effect both on the newly hatched larvae and on the somewhat older ones, and it seems that those factors which influence radioresistance had not changed during this period of time.

NÖTHEL (1972) studied a selected branch of a population of *Drosophila melanogaster*, irradiated during more than 250 generations. In the newly selected population he found an increased radioresistance when treating stage-7 oocytes. He mentions that in this respect part of the genetic information is located in chromosome 2. OGAKI and NAKASHIMA-TANAKA (1966) reported that high radioresistance in adult flies in two Japanese wildtype strains had been traced to chromosome three and that the gene(s) which determined it, were located in the right end. It is obvious that apparently deviating results can be obtained when tackling this complex problem. However, the results obtained do not need to be contradictory taking into consideration the fact that tests were made on different biological levels. Besides, it should also be kept in mind that various mechanisms may be concealed behind the broad term "radioresistance".

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