POLYMORPHISM IN NATURAL POPULATIONS FOR GENES CONTROLLING RADIORESISTANCE IN *DROSOPHILA*¹

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THE genetics of radioresistance and radiosensitivity is little known in higher organisms, except for isolated reports in *D. melanogaster* (OGAKI and NAKASHIMA-TANAKA 1966) and mice (GRAHN 1958). However, in several microorganisms loci for radioresistance and radiosensitivity are known e.g. in *E. coli* (GREENBERG 1964 a,b) and in *Pseudomonas aeruginosa* (LEE and HOLLOWAY 1965). In this paper we propose to investigate more generally the genetics of radioresistance and radiosensitivity in *D. melanogaster*.

The procedures to be used follow some recent work of PARSONS and HOSGOOD (1967), HOSGOOD and PARSONS (1967a; 1968) and PARSONS (1968), who described experiments where a number of strains of *D. melanogaster* derived from single inseminated females collected from the same population in the wild, led to discrete strains for several quantitative traits, namely scutellar and sternopleural chaeta number, mating speed, duration of copulation, and the ability to survive extreme temperatures. The differences between strains were genetic, arising from differences between the founder females, as was confirmed in some cases by diallel crosses. The genetic heterogeneity between the founder females derived from the same population implies that the population must be polymorphic for genes (or polygenes) determining the traits (PARSONS, HOSGOOD and LEE 1967). The polymorphism for scutellar chaeta number has been exploited by HOSGOOD and PARSONS (1967b) and HOSGOOD, MACBEAN and PARSONS (1968), who found extremely rapid responses when directional selection for high chaeta number was based on those strains derived from single inseminated females which had a high chaeta number. In the various papers cited above (see also LEE and PARSONS 1968), it is argued that polymorphism for polygenes is ubiquitous for quantitative traits, and in this paper we extend our observations to a trait of a different kind, namely radioresistance and radiosensitivity to Co⁶⁰γ-rays.

METHOD

The experiments were based on eighteen strains set up from single inseminated females collected at Leslie Manor near Camperdown, Victoria. Three strains were collected in December 1963, and 15 in December 1965. The strains were kept in half-pint bottles at 25°C and were transferred every 3 weeks to set up the next generation. In all experiments, flies of different

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sexes were irradiated separately. Two doses of Co^{60} γ -rays, namely 90,000 and 110,000 rads were used. The intensity of irradiation was about 480,000 rads per hr. Irradiations were carried out at the Australian Atomic Energy Commission Research Establishment, Lucas Heights, N.S.W.

RESULTS

The percentage mortalities at 3 days following exposure are given in Table 1 for the 18 strains, with an analysis of variance of the data after applying the angular transformation. The angular transformation is necessary to remove the dependence of the variance on the mean, which occurs for percentages. The major source of variation is, as expected, the dose effect. There is a significant difference between sexes due to the greater susceptibility of males to irradiation as is usually observed (see OGAKI and NAKASHIMA-TANAKA 1966). Of particular interest for this paper is the significant strains effect, showing that in agreement with the

TABLE 1

Percentage mortalities of 50 flies at 2 days following irradiation with $\text{Co}^{60}\gamma$

Strain	Dose			
	90,000r		110,000r	
	♀	♂	♀	♂
1	2	0	54	100
2	4	58	100	100
3	0	0	6	44
20	4	8	46	72
21	2	12	36	48
22	0	0	22	100
23	22	46	60	100
24	8	16	60	100
25	0	2	34	72
26	8	12	8	38
27	18	24	24	66
28	0	10	10	68
29	0	2	0	30
30	10	2	4	72
31	4	12	56	64
32	0	2	94	88
33	0	0	72	74
34	2	2	72	80

Analysis of variance (after applying the angular transformation)

Source of variation	df	m.s.	F
Strains	17	608.2	3.90**
Sexes	1	4082.6	26.18***
Doses	1	27368.3	175.50***
Strains $\times$ doses	17	354.8	2.28*
Strains $\times$ sexes	17	80.9	0.52
Sexes $\times$ doses	1	1299.1	8.33*
Error	17	155.9	...

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 2

a. Percentage mortalities of flies 10 hours following irradiation in the 4 × 4 diallel cross from 4 of the 18 strains listed in Table 1
Each entry in the table is based on 25 flies

Strain of male parent		Mortality in females				Mortality in males			
		3	29	23	2	3	29	23	2
90,000 rads									
Strain of female parent	3	0	0	0	4	12	8	4	44
	29	0	0	8	4	12	28	40	40
	23	8	16	12	44	20	52	68	72
	2	8	4	44	24	28	32	92	88
110,000 rads									
Strain of female parent	3	12	16	44	32	44	20	60	60
	29	28	28	40	88	44	44	76	72
	23	36	68	92	96	60	88	88	92
	2	16	84	92	88	36	96	100	100

b. Analysis of variance (after applying the angular transformation)

Dose		90,000 rads		110,000 rads	
Source of variation	df	m.s.	F	m.s.	F
General combining ability	3	2010.7	53.2***	3115.4	65.5***
Specific combining ability	6	163.7	4.3**	208.5	4.4**
Reciprocal effect	6	62.2	1.6	93.8	2.0
Sexes	1	4425.1	117.1***	801.5	16.9***
Error	15	37.8	...	47.5	...

** P < 0.01, *** P < 0.001.

c. General combining abilities (percent)

Strain	90,000 rads	110,000 rads	
3	—17.0	—8.9	} radio-resistant
29	— 5.2	—0.8	
23	6.5	2.9	} radio-sensitive
2	15.1	4.8	

evidence cited in the INTRODUCTION, the strains derived from different founder females are heterogeneous, so that following the argument given in the INTRODUCTION, the population can be argued to be polymorphic for genes determining radioresistance or radiosensitivity.

Two of the most resistant strains were 3 and 29 and two of the most sensitive were 2 and 23. This then led to the second experiment, which was a complete 4 × 4 diallel cross between these 4 strains. It was set up to find out more about the nature of the genetic differences between the strains. The percentage mortalities at 10 hrs following irradiation are given in Table 2a, and the analyses of variance (after applying the angular transformation) following the model of GRIFFING (1956) in Table 2b. This analysis gave large significant general combining abilities for both doses, indicating additive differences between strains for percentage mortalities. There was a large sexes component for both doses reflecting the usual higher radiosensitivity of males compared with females. In com-

parison, the specific combining ability was found to be extremely small although it was significant. This indicates a small degree of non-additivity between the four strains.

In Table 2c, the actual general combining abilities are given. There is remarkably good agreement between doses, such that going from the most radioresistant strain to the most radiosensitive, the sequence is the same, namely 3, 29, 23 and 2. There is also a good correspondence between Tables 1 and 2, since 3 and 29 were chosen as radioresistant and 23 and 2 as radiosensitive strains. If one looks at the actual percentage mortalities as given in Table 2a, crosses between strains 29 and 3 give results similar to the strains 29 and 3 alone, which shows that there are likely to be similar genes for radioresistance in these two strains. The same situation appears for the two radiosensitive strains 23 and 2. Strain 3, which has the highest radioresistance of all strains, shows some dominance over the radiosensitive strains 23 and 2. This would seem to fit the observation of OGAKI and NAKASHIMA-TANAKA (1966), who found a locus determining radioresistance in the third chromosome, which was dominant to radiosensitivity.

In order to look further at the genetic architecture of radioresistance and radiosensitivity, two of the four strains 29 and 2 were taken for further analysis, the former being radioresistant and the latter radiosensitive. Strain 29 was not as resistant as Strain 3 in the diallel cross. However, the selection of the two strains was made prior to setting up the diallel cross, and was based on the results in Table 1. The diallel cross was, however, completed before the experiments now to be described, because the breeding procedures in the diallel cross were much simpler. In any case the diallel cross showed that the genetic architectures of the two radioresistant and two radiosensitive strains were rather similar.

TABLE 3

Matings between substitution lines (after KEARSEY and KOJIMA, 1967)

Females	Males							
	AAA	AAB	ABA	ABB	BAA	BAB	BBA	BBB
AAA	AAA				HAA (A)	HAH (A)	HHA (A)	
AAB	AAH	AAB		AHB				
ABA	AHA		ABA				HBA (A)	
ABB	AHH		ABH	ABB	HHH (A)			
BAA					BAA	BAH		BHH
BAB		HAB (B)				BAB		BHB
BBA					BHA		BBA	BBH
BBB		HHB (B)	HBH (B)	HBB (B)				BBB

The letters in parentheses denote the sources of the Y chromosome. H denotes a chromosomal heterozygote between A and B, while the letters A and B without parentheses denote chromosomal homozygotes.

The procedure used followed that described in a recent paper by KEARSEY and KOJIMA (1967) in studies on the genetic architecture of body weight and egg hatchability in *D. melanogaster*, using the 3 major chromosomes jointly extracted from a wide range of materials. We designate strain 2 by A, and strain 29 by B. Neglecting the Y and fourth chromosomes, eight true breeding combinations of the three major chromosomes from the two base strains can be synthesized, using techniques involving marked multiple inversion stocks. The inversion stock used was Basc; *Cy/Pm ds^{33k}*; *H/Sb*. Using this stock, and following the crossing procedures outlined by KEARSEY and KOJIMA (1967), eight true breeding substitution lines for the A and B strains can be obtained namely AAA, AAB, ABA, ABB, BAA, BAB, BBA and BBB, where the sequence of the letters corresponds to the source of the X, 2 and 3 chromosomes.

By crossing these 8 substitution lines in the manner outlined in Table 3, all the possible 27 female and 18 male homozygous and heterozygous chromosome combinations can be obtained. For each combination 25 flies per sex were tested at 90,000 and 110,000 rads and percentage mortalities obtained.

Analyses of variance of the female and male percentage mortalities 30 hrs after irradiation (after carrying out the angular transformation) are given in Table 4 at 110,000 rads. In this particular experiment the 90,000 rads data were non-informative as the death rates were too low to be discriminatory, so we will merely

TABLE 4

Analysis of variance of the percentage mortalities 30 hours after irradiation at 110,000 rads (after applying the angular transformation)

Source of variation	Chromosome	Females			Males		
		df	MS	F	df	MS	F
additive (a)	X	1	276.1	7.27*	1	526.0	16.59*
	2	1	13442.5	353.75***	1	1449.8	45.72**
	3	1	2385.1	62.77***	1	1943.1	61.28**
dominance (d)	X	1	9.9	0.26	1	83.1	2.62
	2	1	426.2	11.21*	1	83.1	2.62
	3	1	54.0	1.42	1	813.2	25.64**
a × a	X×2	1	159.9	4.21	1	716.1	22.58**
	X×3	1	22.4	0.59	1	26.7	0.84
	2×3	1	858.5	22.59**	1	579.7	18.28*
d × d	X×2	1	268.8	7.08*	1	...	...
	X×3	1	25.2	0.66	1	...	...
	2×3	1	338.1	8.90*	1	269.5	8.50*
a × d	X×2	1	7.8	0.21	1	400.7	12.64*
	2×X	1	7.7	0.20	1	...	...
	X×3	1	130.0	3.42	1	18.4	0.58
	3×X	1	5.6	0.15	1	...	...
	2×3	1	205.0	5.39*	1	191.0	6.02
	3×2	1	729.9	19.21**	1	173.3	5.47
Error		8	38.0	...	4	31.7	...

* P < 0.05, ** P < 0.01, *** P < 0.001.

present the data for 110,000 rads. The cause of this is probably due to undetected variations in dosimetry between experiments. The error component in the analyses of variance was obtained from the pooled triple interaction components. Apart from the triple interaction component, the sum of squares was partitioned into individual 1 df components representing the additive and dominance effects of each of the three chromosomes, and the various interactions between these various main effects.

The analysis shows clearly that the additive effects make the most significant contribution, while dominance and interchromosomal interactions are much smaller. The male and female data agree well. There are major additive effects associated with chromosomes 2 and 3, while there is a very minor effect associated with the *X* chromosome. The dominance effects are small, and the only effects of any magnitude are for chromosomes 2 and 3, which is reasonable, since these are the chromosomes giving the additive effects. This also agrees with the combining ability analysis in Table 2, where the general combining ability, representing additive gene effects, was found to be much larger than the specific combining ability, representing dominance effects.

The various interaction terms are all small compared with the additive effects of chromosomes 2 and 3, although as might be expected interactions involving these two chromosomes tend on the whole to be greater than the remaining interactions which involve the *X* chromosome.

DISCUSSION

It is clear that the genetic differences in radioresistance and radiosensitivity between the two strains under analysis are located in chromosomes 2 and 3, with little difference associated with the *X* chromosome. This therefore is a more complex situation than that found by OGAKI and NAKASHIMA-TANAKA (1966) who found one or more genes for radioresistance on chromosome 3. It is quite likely that other pairs of strains may reveal somewhat different situations, and work is in progress on this problem at the present time. Even so, the genetic architecture of radioresistance and radiosensitivity seems to be made up mainly of chromosomes with additive effects and additive $\times$ additive interactions.

Various workers have argued that there is a correspondence between the genetic architecture of a trait and the type of selection to which the trait has been exposed in the past. (BRESE and MATHER 1960, MATHER 1966). Thus directional selection will lead to a quantitative trait showing directional dominance and a duplicate-type gene interaction, as occurs for traits selected for uniformly high fitness such as viability (BRESE and MATHER 1960) and hatchability (KEARSEY and KOJIMA 1967). Stabilizing selection however, leads to little dominance and weak interactions. Such dominance and interactions that do occur tend to be ambidirectional. The genetic architecture of radioresistance thus corresponds more to traits subject to stabilizing than to directional selection, indicating that it is likely that there is an intermediate optimum level of radioresistance in a population, although it is difficult to explain why this should be so without knowing more

about the physiological and biochemical differences between strains having high and low levels of radioresistance.

The strains under analysis are all derived from the same population, being derived initially from single inseminated females. Since they differ genetically, it then directly follows that the Leslie Manor population is polymorphic for genes controlling radioresistance and radiosensitivity to Co^{60} γ -rays. This result is not surprising in view of the number of quantitative traits for which we now have evidence for polymorphism for polygenes (see INTRODUCTION). It is in agreement with LEWONTIN and HUBBY (1966) and LEWONTIN (1967) who argue for a high level of polymorphism in outbred species based on information in *Drosophila* and man. This means that the techniques developed and elaborated by Hosgood and PARSONS (1967b) and LEE and PARSONS (1968) for selecting for quantitative traits and studying them based on exploiting the variability in natural populations can be applied to radioresistance and radiosensitivity.

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SUMMARY

Eighteen strains set up from single inseminated females of *D. melanogaster* derived from a wild population have been shown to differ in radioresistance and radiosensitivity as measured by percentage mortalities following irradiation with Co^{60} γ -rays. A 4×4 diallel cross between two of the resistant and two of the sensitive strains showed that the differences between these 4 strains were mainly due to additive genes. This was further explored for one resistant and one sensitive strain using chromosome assay techniques. It was shown that the main difference between these two strains was due to additive genes on the second and third chromosomes.—The variation between the 18 strains argues, as in a number of quantitative traits, for polymorphism for the genes controlling radioresistance and radiosensitivity in natural populations.

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