RADIATION-INDUCED DOMINANT LETHALITY IN HAPLOID AND DIPLOID SPERM OF THE WASP MORMONIELLA

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IN yeast, more dominant lethality is induced in diploid than in haploid vegetative cells (OWEN and MORTIMER 1956), and this is consistent with the observed increase in X-ray sensitivity of cells of higher ploidy in this organism (MORTIMER 1958). To study dominant lethality with respect to ploidy in a higher organism, the parasitic wasp Mormoniella was selected. Stocks are available which produce normal haploid males with haploid sperm as well as fertile diploid males with 100 percent diploid sperm. The males can be irradiated and mated with normal females for an unambiguous analysis of dominant lethality in haploid and diploid nuclei.

MATERIALS AND METHODS

Genetic stocks similar to those used in this experiment have been described previously (WHITING 1960). The stocks used in these experiments were generously provided by P. W. WHITING. The eye color markers vermilion (*vm*) and oyster (*oy*) were used to identify the stocks. These two markers are complementing alleles of the *R* locus, and recombination has never been observed to occur between them. Triploid females of genetic constitution *vm/oy/oy* and diploid females of constitution *oy/oy* were obtained. The diploid females when bred unmated produce only haploid *oy* males. The triploid females produce both haploid and diploid males as well as many inviable aneuploids. The identifiable male offspring are *vm* (haploid) and *vm/oy* (diploid), and these were the stocks used in this experiment. The remaining offspring are oyster-eyed and either haploid or diploid. The triploid is re-formed from the cross *vm/oy* males × *oy/oy* females. The diploid males were spontaneous in origin. If the male parent is irradiated before mating, dominant lethality will be expressed as a reduction in hatchability, a reduced number of female offspring, and a decrease in the ratio of females to total offspring. In this report, the change in sex ratio was used as the principal detector of dominant lethality. Reduction in number of female offspring is reliable but varies because total number of offspring per female varies. Hatchability was used to confirm the estimates of induced dominant lethality obtained by the sex ratio method. Unfortunately, it was difficult to score both hatchability and adult survival from the same embryos, as has been done with the

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wasp Habrobracon (ATWOOD, VON BORSTEL and WHITING 1956; VON BORSTEL and REKEMEYER 1959).

The proportion of eggs fertilized, f, can be computed from the sex ratio; f is equal to the frequency of female offspring, g, when all sperm are viable at zero dose of radiation. Assuming that f remains unchanged following irradiation of sperm up to 10 kr (cf. WHITING and VON BORSTEL 1954, and HEIDENTHAL 1945, for sperm inactivation effects at higher doses), then g will respond to the dose according to the relation

$$g = [f(1 - D')]/(1 - fD') \qquad (1)$$

where D' is the proportion of sperm containing a dominant lethal event. Thus the frequency of dominant lethality can be estimated at any dose by

$$D' = (1 - g/g_0)/(1 - g) \qquad (2)$$

where g_0 is the proportion of female offspring at 0 dose.

With hatchability experiments (cf. ATWOOD *et al.* 1956), the viable proportion of fertilized eggs, V_f, is given by

$$V_f = [m - V_u\,(1 - f)]/f \qquad (3)$$

where V_u is the viable proportion of unfertilized eggs and m is the hatchability of eggs from mated females. Fertilized eggs that hatch are those without a dominant embryo lethal, D; therefore

$$V_f = 1 - D. \qquad (4)$$

If it is assumed that induced postembryonic dominant lethality is negligible then

$$f = 1 - p/V'_u \qquad (5)$$

where p is the ratio of surviving adult males from mated females to total eggs from mated females, estimated by applying the adult sex ratio to the fraction that hatches, and V'_u is the proportion of unfertilized eggs surviving to the adult stage, here taken to be 1.0 (VON BORSTEL and REKEMEYER 1959). In the hatchability part of Series IV, D was estimated from equations (3) and (4); f was taken from equation (5) at each dose.

One- to two-day-old males were irradiated with X rays and mated with 2-day-old females. The males were removed after 3 hours and the females set with host pupae of the blowfly Sarcophaga. Each day for 3 to 4 days, the females were transferred to fresh pupae. In the initial experiments, four females per dose point were set in single tubes. This was changed to setting females singly when it was learned that some females did not mate and consequently produced only males. With the single-set method, the offspring of these females were not included in the calculations. Ten to 12 days after setting the females, the emerged offspring were counted and classified according to sex. Hatchability was scored by transferring freshly layed eggs from the host pupae to agar and examining 24 hours later for emergence of the larvae from the egg membranes.

RESULTS AND DISCUSSION

In Table 1 are presented the results of four separate experiments (I, II, III, and IV). The number of females bred, the numbers of female and male offspring,

TABLE 1

Numbers of progeny and hatchability for females crossed to haploid and diploid males irradiated at different doses

Male parent	Dose krad	I				II				III				IV					
		Numbers of				Numbers of				Numbers of				Numbers of					
		P_1♀♀	F_1♀♀	F_1♂♂	*D′*	P_1♀♀	F_1♀♀	F_1♂♂	*D′*	P_1♀♀	F_1♀♀	F_1♂♂	*D′*	P_1♀♀	F_1♀♀	F_1♂♂	*D′*	*m*	*D*
haploid	0	1	163	23	0	4	511	221	0	4	194	132	0	6	762	209	0	88/92	0.048
(*vm*)	0.5	1	122	22	0.22	3	239	117	0.117	.	. .	. .	. . .	.	. .	. .	. . .	. . . . .	. . . .
	1.0	.	. .	. .	. . .	2	153	93	0.29	1	56	30	. . .	.	. .	. .	. . .	. . . . .	. . . .
	2.0	.	. .	. .	. . .	3	282	135	0.097	2	54	83	0.56	5	244	255	0.737	24/37	0.510
	3.0	3	65	39	0.76	3	124	178	0.699	.	. .	. .	. . .	.	. .	. .	. . .	. . . . .	. . . .
	4.0	.	. .	. .	. . .	.	. .	. .	. . .	4	32	162	0.866	5	77	414	0.949	27/78	0.911
	6.0	.	. .	. .	. . .	.	. .	. .	. . .	3	2	155	0.991	.	. .	. .	. . .	. . . . .	. . . .
diploid	0	5	335	93	0	3	131	151	0	4	174	116	0	2	177	88	0	. . . . .	. . . .
(*vm/oy*)	0.5	1	24	2	. . .	2	90	73	. . .	3	103	105	0.346	.	. .	. .	. . .	. . . . .	. . . .
	1.0	.	. .	. .	. . .	2	73	132	0.36	3	61	141	0.712	5	168	347	0.760	43/82	0.719
	2.0	.	. .	. .	. . .	4	31	365	0.847	2	22	61	0.76	6	81	386	0.896	51/141	0.897
	3.0	4	6	37	0.95	5	4	240	0.981	.	. .	. .	. . .	.	. .	. .	. . .	. . . . .	. . . .

and embryo hatchability after irradiation of both haploid and diploid males are presented. The frequencies of dominant lethality estimated from the sex ratio, D', and estimated from hatchability, D, are also included. The hatchability of unfertilized eggs, V_u, was 95/98. The frequencies of sperm free of dominant lethals, $1 - D'$ and $1 - D$, are presented as a function of dose in Figure 1. A higher frequency of dominant lethality in diploid than in haploid sperm is revealed. As with the comparable experiments in yeast, approximately one half the dose is required to induce the same frequency of lethals in diploid as compared to haploid sperm.

That a reduction in fertilization frequency (sperm inactivation) rather than dominant lethality is the cause for the major part of the reduction in sex ratio appears not to be the case. The average number of offspring per female, which can be calculated from the data in Table 1, and the hatchability of the eggs both decrease; the decrease is accompanied by a shift in the sex ratio. Neither the average number of offspring per female nor hatchability should change if only fertilization efficiency were affected. The agreement of D with D' in Series IV of

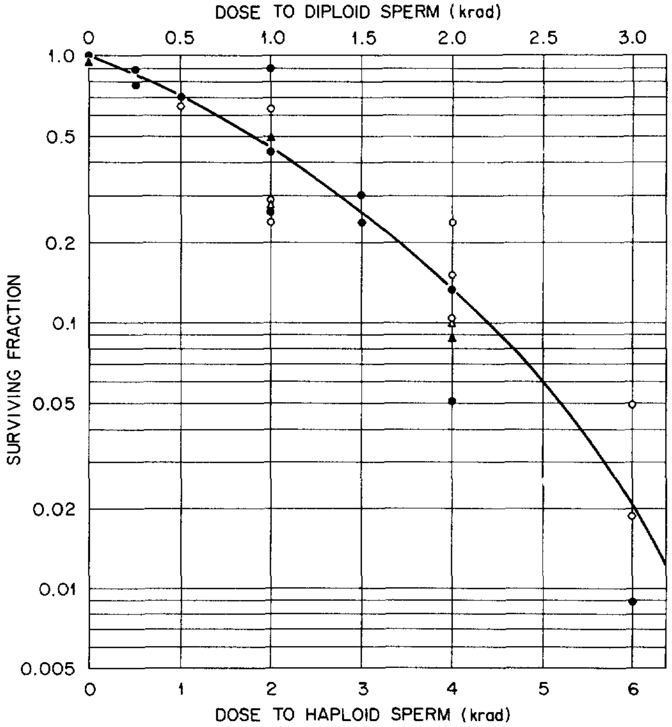

FIGURE 1.—Dose-survival curve plotted as a function of ploidy × dose for X-irradiated haploid and diploid sperm of the wasp Mormoniella. The plot on the ordinate is $1 - D'$ where D' is dominant lethality as measured by sex ratio shift (● haploid, ○ diploid), and $1 - D$ where D is dominant lethality deduced from hatchability of fertilized eggs (▲ haploid, △ diploid).

Table 1 indicates that most of the shift in sex ratio can be attributed to dominant lethality.

Doubling the chromosome number effectively doubles the target size. It is known that at any one dose the number of chromosome aberrations in a nucleus is doubled when the chromosome number is doubled (CONGER and JOHNSTON 1956). It therefore is plausible that the basis of dominant lethality in these experiments is chromosome breakage (cf. VON BORSTEL 1960).

SUMMARY

A doubling of the chromosome complement in Mormoniella sperm from haploid to diploid doubles the sensitivity to radiation when dominant lethality is the criterion.

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