ANALYSIS OF INDUCED QUANTITATIVE VARIATION I. HIERARCHICAL AND TRIPLE TEST CROSS ANALYSIS

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SUMMARY

The triple test cross and a hierarchical inbreeding design are compared for their abilities to detect and analyse the quantitative variation induced in an F_2 population by γ radiation. Both designs detected an increase in the additive genetical variation for four contrasting characters in the cross of varieties 1 and 5 of *Nicotiana rustica* following irradiation of the F_2 seed, but it was smaller in the triple test cross than in the hierarchical design. Because, however, the latter is based on inbreeding it provided no information on non-additive genetical variation in contrast to the triple test cross that detected a significant increase in the dominance variation.

The differences between the estimates of the additive genetic variance from the two designs can be largely accounted for by the differing contributions of repulsion linkages and the confounded effects of the unequal gene frequencies and multiple allelism created by the induced mutations in the rank 1 variances of the hierarchical design. The dominance contribution of loci at which multiple allelism has been induced may also be a contributing factor. Irrespective of the cause, however, the evidence for increased additive genetic variation following irradiation is unambiguous and it should lead to greater variation among the pure breeding lines which are in the process of being extracted from the irradiated F_2 .

1. INTRODUCTION

THE hierarchical biometrical genetical analysis of Virk, *et al.* (1978) and the F_2 triple test cross analysis of Kearsey and Jinks (1968) are in theory the most reliable methods for detecting induced quantitative variation in the generations immediately following a mutagenic treatment. In this paper we compare their abilities to detect, measure and analyse the variation in an F_2 of the cross between pure breeding varieties 1 and 5 of *Nicotiana rustica* following irradiation of the F_2 seed.

2. THEORY

Virk *et al.* (1978) gave general expectations for the generations derived by selfing a pure breeding line following a mutagenic treatment. Where, as in the present experiment, an F_2 is treated it becomes equivalent to the M_1 generation of their model. The expectations, assuming an additive, dominance model, for the variances of the F_3 and F_4 generations obtained from an unirradiated control F_2 and from the RF_3 and RF_4 generations obtained from an irradiated F_2 are given in table 1. In Virk *et al.*, analysis the highest ranking variances, in this case V_{1RF_3} and V_{1RF_4} , are either omitted

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TABLE 1

*Generalised genetical expectations for the variances obtainable for the F_3 , RF_3 , F_4 and RF_4 generations (After Virk, Jinks and Pooni, 1978)**

Variance	D	H	Variance	D	H	D_1	H_1	F_1
$V1F3$	$\frac{1}{2}$	$\frac{1}{16}$	$V1RF3$	0	$\frac{1}{8}$	$\frac{1}{2}$	$-\frac{1}{16}$	$-\frac{1}{4}$
$V2F3$	$\frac{1}{4}$	$\frac{1}{8}$	$V2RF3$	$\frac{1}{4}$	$\frac{1}{8}$	0	0	0
$V1F4$	$\frac{1}{2}$	$\frac{1}{64}$	$V1RF4$	0	$\frac{1}{32}$	$\frac{1}{2}$	$-\frac{1}{64}$	$-\frac{1}{8}$
$V2F4$	$\frac{1}{4}$	$\frac{1}{32}$	$V2RF4$	$\frac{1}{4}$	$\frac{1}{32}$	0	0	0
$V3F4$	$\frac{1}{8}$	$\frac{1}{16}$	$V3RF4$	$\frac{1}{8}$	$\frac{1}{16}$	0	0	0

* The definitions of the symbols are those of Virk, Jinks and Pooni (1978).

because they are biased by the effects of unequal gene frequencies and multiple allelism produced by mutation or additional additive and dominance components (D_1 , H_1 , F_1) must be fitted to the variances to accommodate these effects.

The detection and analysis of induced mutations using the F_2 triple test cross design (Kearsey and Jinks, 1968) rests upon the detectable consequences of using inadequate testers (Kearsey and Jinks, 1968; Mather and Jinks, 1971; Virk and Jinks, 1977). For an F_2 population the parental, P_1 and P_2 , and F_1 families from which it arises are ideal testers because the F_2 population can only segregate for the allelic differences for which P_1 and P_2 differ, and for which the F_1 will be heterozygous. Following irradiation of the F_2 seed, P_1 , P_2 and F_1 can become inadequate testers in two ways both with different detectable consequences:

- (1) Allelic differences can arise at the k' loci at which P_1 and P_2 do not differ. We shall designate these as common loci.
- (2) New alleles, different from those in P_1 and P_2 can arise at the k loci at which there are already allelic differences between P_1 and P_2 . We shall designate these as non-common loci.

For induced genetic differences at the k' common loci the triple test cross estimate of $D = \sum_{i=1}^k d_i^2$ becomes

$$\sum_{i=1}^k d_i^2 + \sum_{j=1}^{k'} 4\mu_j(1-2\mu_j)(d_j \pm h_j)^2$$

where μ_j , d_j and h_j are the mutation rate, and the additive genetic and dominance effects generated by the mutation at the j th common locus respectively. The sign of the h_j term is positive if the increasing allele mutates to a decreasing allele and is negative if the decreasing allele mutates to an increasing allele. The magnitude of $H = \sum_{i=1}^k h_i^2$ is unchanged by allelic differences arising at the k' common loci, and the estimates of epistasis are also unaffected unless these new allelic differences interact with those at the non-common loci.

Induced new alleles at the k non-common loci may deflate or inflate D , H and the epistatic components depending upon whether the additive, dominance and epistatic effects generated by the new alleles are smaller or greater than those generated by the original alleles they replace. The new alleles will always produce a heterozygous combination when crossed to any of the three testers, whilst the original alleles would have produced

homozygous and heterozygous combinations in equal frequencies. Dominance effects and epistatic interactions involving heterozygous combinations may, therefore, be increased.

In general, therefore, mutations at common loci will affect the estimate of D , whilst mutations at non-common loci will also affect the estimates of H and the epistatic components. Unless there are significant differences in the probability of mutations occurring at different loci the relative contributions of mutations at the two types of loci will depend primarily upon the proportion of the loci controlling a character for which P_1 and P_2 have different alleles *i.e.*, $k : k + k'$.

Radiation can also affect the F_2 population by increasing the recombination rate (Nilan, 1965). It cannot, however, affect any statistic of rank 1 since they are influenced only by recombination events during gametogenesis in the F_1 which occurred before the treatment was applied (Mather and Jinks, 1971). The estimates of the genetical components from the triple test cross and V_{1RF_3} and V_{1RF_4} will therefore be unaffected but the rank 2 and rank 3 statistics derived from RF_3 and RF_4 generations (table 1) will be affected. Since the predominant linkage phase in the cross of varieties 1 and 5 is known to be repulsion (Jinks and Perkins, 1969; Perkins and Jinks, 1970) these statistics should be inflated by any increased recombination.

3. EXPERIMENTAL DESIGN

The experimental material was derived from a random sample of 50 seeds of the F_2 of a cross between varieties 1 and 5 of *Nicotiana rustica*. Twenty five F_2 seeds were exposed to 20 kr of gamma-radiation from a Co^{60} radiation source. Preliminary investigations had shown that this dose was high enough to induce mutational changes, whilst producing limited deleterious effects on growth. The remaining 25 unirradiated F_2 seeds were used to produce the control families.

The 50 F_2 plants were raised and examined. No obvious chimaeral segments were found in the irradiated material. All 50 plants were therefore used to set up the following crosses:

- (1) Backcrosses onto their parents (V_1 and V_5) and F_1 ($V_1 \times V_5$) produced 24 sets of triple test cross families (Kearsey and Jinks, 1968) from the irradiated F_2 (RL_1 , RL_2 , RL_3), and 25 sets from the control F_2 (L_1 , L_2 , L_3).
- (2) The F_2 plants were selfed to produce 25 F_3 families from the control and 25 RF_3 families from the irradiated plants. Two randomly chosen siblings of each F_3 and RF_3 family were in turn selfed to produce 50 F_4 and 50 RF_4 families. Twelve individuals of each of the L_1 , L_2 , L_3 , RL_1 , RL_2 , RL_3 , F_3 , RF_3 , F_4 and RF_4 families were raised in a completely randomised experiment during the summer of 1979. The F_3 and RF_3 families were a second random sample of siblings from the families which provided the parents of the F_4 and RF_4 families.

Germination was poor for the triple test cross families derived from two of the irradiated F_2 plants. Only the remaining 22 complete sets of triple test cross families were, therefore, analysed.

TABLE 2
Analyses of variance for the triple test crosses derived from control and irradiated F_2 's

Comparison	Item	df	LW		FT		HFT		FH	
			ms	P	ms	P	ms	P	ms	P
(a) Control ($\bar{L}_1 + \bar{L}_2 - 2\bar{L}_3$)	Overall epistasis	1	14.30	***	0.00	NS	31.18	NS	80.80	*
	Epistasis $\times$ sets	24	1.39	**	3.50	**	33.96	**	20.70	NS
	Within families	824	0.75		1.85		17.47		18.91	
	Additive	24	3.63	***	12.92	***	90.20	***	67.88	***
	Within families	824	0.75		1.71		17.58		17.42	
	Dominance	24	0.83	NS	2.45	*	21.83	NS	19.56	NS
	Within families	550	0.76		1.56		17.69		15.93	
(b) Irradiated ($\bar{L}_1 + \bar{L}_2 - 2\bar{L}_3$)	Overall epistasis	1	1.49	NS	4.24	NS	83.28	*	50.49	NS
	Epistasis $\times$ sets	21	1.66	**	3.66	*	22.78	NS	34.74	**
	Within families	755	0.87		2.03		17.01		18.74	
	Additive	21	3.74	***	16.04	***	95.69	***	119.67	***
	Within families	755	0.83		1.92		16.23		15.89	
	Dominance	21	2.20	***	10.36	***	17.69	NS	30.44	***
	Within families	515	0.79		1.81		15.45		13.04	

*** $P \leq 0.001$; ** $\leq P < 0.01$; * $0.01 < P \leq 0.05$; NS $P > 0.05$.

No seed was available for two F_4 families and one RF_4 family. These families have subsequently been regenerated to avoid gaps in the later generations.

Leaf width (in cm) was measured at the widest point of the blade of the largest leaf six weeks after planting. Flowering time was recorded as the number of days to flowering after the 1 July. Height at flowering time and the eventual final height of the plants were measured to the nearest 0.5 cm.

4. RESULTS

The two epistasis ($L_1 + L_2 - 2L_3$) items in the triple test cross analyses of the controls (table 2) indicate low levels for all four characters. Irradiation appears to have had little effect on the total epistasis present although there are differences in its distribution between the two items (overall epistasis and epistasis $\times$ sets).

The additive ($L_1 + L_2 + L_3$) item is highly significant in all of the analyses (table 2) and is consistently larger but not significantly so following irradiation. In the controls the dominance ($L_1 - L_2$) item is significant for flowering time only, but it shows a marked increase and is significant for all characters except height at flowering time following irradiation. Estimates of the additive and dominance genetical components derived in the usual way (Kearsey and Jinks, 1968; Perkins and Jinks, 1970) are presented in table 3.

TABLE 3

Estimates of the components of variation from triple test cross analyses of the control and irradiated material

Character	Control	Irradiated
Leaf width	$D = 7.67 \pm 2.80$ $H = \text{NS}^*$ $E_w = 8.06$	$D = 7.77 \pm 3.08$ $H = 5.65 \pm 2.72$ $E_w = 8.28$
Flowering time	$D = 29.90 \pm 9.95$ $H = 3.56 \pm 2.85$ $E_w = 16.31$	$D = 37.65 \pm 13.20$ $H = 34.19 \pm 12.80$ $E_w = 14.06$
Height at flowering	$D = 193.66 \pm 69.49$ $H = \text{NS}^*$ $E_w = 182.49$	$D = 211.90 \pm 78.78$ $H = \text{NS}^*$ $E_w = 167.12$
Final height	$D = 134.55 \pm 52.30$ $H = \text{NS}^*$ $E_w = 193.85$	$D = 276.74 \pm 98.51$ $H = 69.59 \pm 37.72$ $E_w = 147.41$

* See Table 2.

Hierarchical analyses of variance for the F_3 , F_4 , RF_3 and RF_4 are presented in table 4. All items in the analyses are, with one exception (V_{1RF_4} for leaf width), significant. The effect of irradiation has been to increase the magnitude of the higher ranking mean squares for all characters except leaf width.

The models based upon the expectations in table 1 were fitted to the five variances available for each character in each treatment using standard weighted least squares procedures. Because of their relatively low

TABLE 4
Hierarchical analyses of variance of the F_3 , F_4 and RF_3 and RF_4 generations

Item	df	LW		FT		HFT		FH	
		ms	P	ms	P	ms	P	ms	P
(a) Control									
Between F ₃ fams/F ₂ parents	23	43.15	***	155.40	***	816.22	***	1047.36	***
Within F ₃ fams	239	8.52		27.31		266.83		260.09	
Between F ₄ fam groups/F ₂ parents	22	82.83	***	451.26	**	2379.62	*	1881.84	**
Between F ₄ fams/F ₃ parents	23	19.57	**	146.81	***	1082.23	***	630.38	***
Within F ₄ fams	506	9.90		16.06		190.17		197.53	
(b) Irradiated									
Between RF ₃ fams/RF ₂ parents	24	31.00	***	237.55	***	1748.70	***	1981.21	***
Within RF ₃ fams	268	11.57		23.61		212.03		228.65	
Between RF ₄ fam groups/RF ₂ parents	24	66.04	NS	542.49	**	3437.74	*	3587.36	*
Between RF ₄ fams/RF ₃ parents	25	43.53	***	180.28	***	1544.46	***	1694.52	***
Within RF ₄ fams	537	9.56		14.81		156.90		210.68	

*** $P \leq 0.001$; ** $0.001 \leq P \leq 0.01$; * $0.01 < P \leq 0.05$.

coefficients, there was, as expected, little evidence of a contribution from the dominance components (H and H_1). In fact in all cases a satisfactory model could be found based solely on additive genetic and additive environmental components.

For the controls a simple D, E_w model was inadequate for flowering time and height at flowering time (table 5). For these two characters a model which allows for linkage by allowing D to take a different value in the rank 1, 2 and 3 statistics (D_1, D_2 and D_3 respectively) was, therefore, fitted and found to be satisfactory. Indeed this model could be reduced to D_1, D_2 ($\equiv D_3$) and E_w without becoming unsatisfactory.

For the irradiated material the simple D, E_w model was adequate for all characters except leaf width, which fitted the D_1, D_2 ($\equiv D_3$) and E_w model (table 5). The estimates of the components in these models are not independent, and the value that any component takes depends, to some extent, on the other components being estimated simultaneously. To facilitate comparisons between the control and irradiated material solutions for both the D, E_w and D_1, D_2, E_w models are, therefore, given for all characters in table 5.

In every case the estimate of D_2 ($\equiv D_3$) is larger than that of D_1 but, as expected, only significantly so where the D, E_w model is inadequate (FT and HFT for control and LW for irradiated material). For the controls this must be interpreted as repulsion linkage (Mather and Jinks, 1971) whilst for the irradiated material it can arise in the absence of linkage since D_1 , but not D_2 (D of table 1) is affected by the unequal gene frequencies and multiple allelism generated by the induced mutation. With only two exceptions (D_1 for LW and D_2 for FT) the estimates of D, D_1 and D_2 are larger for the irradiated than for the control material, but only significantly so for HFT and FH for D , HFT for D_1 and LW for D_2 . There is, therefore, evidence of a significant increase in the additive genetic variance following irradiation for all characters except flowering time.

5. CONCLUSIONS

Both the hierarchical inbreeding experiment and the triple test cross detect an increase in the heritable variation following the irradiation of F_2 seed. In the hierarchical design it appears simply as a marked increase in the additive genetic variance (D, D_1 or D_2) for all characters except flowering time. The contribution that non-additive genetic components make to the variation in material as inbred as F_3 and F_4 is usually too small to be detectable. Not surprisingly, therefore, this design throws no light on these components of the induced variation.

In the triple test cross the increase in the heritable variation is shown by the additive genetic component for flowering time and final height and the dominance component for these characters and for leaf width. There is, however, no marked increase in the epistasis for any character. The interpretation of these increases is not, however, straightforward. The increase in the dominance components reflects solely the result of mutation at the k loci at which the parental varieties (1 and 5) already differ. The dominance created by mutation at the other k' loci makes its contribution to the additive component only (section 2). It is, therefore, confounded with the genuine additive genetic effects generated by mutation at all $k + k'$

TABLE 5
Additive models fitted by weighted least squares to the statistics from the hierarchical analyses

Character		D	D_1	D_2	E_w	χ^2 (df)
LW	Control	4.6 ± 1.05	.	.	8.8 ± 0.53	3.87 (3)
	Irradiated	5.5 ± 1.18	5.5 ± 1.50	2.8 ± 1.72	9.0 ± 0.59	2.75 (2)
FT	Control	38.6 ± 6.72	2.8 ± 1.11	11.2 ± 3.58	9.3 ± 0.56	8.61 (3)
	Irradiated	47.5 ± 7.47	21.0 ± 6.41	.	8.4 ± 0.77	0.70 (2)
HFT	Control	182.5 ± 36.98	33.1 ± 8.67	62.6 ± 12.73	12.7 ± 1.43	10.17 (3)
	Irradiated	320.4 ± 54.66	92.5 ± 34.33	384.9 ± 105.72	8.0 ± 2.31	2.52 (2)
FH	Control	134.7 ± 29.10	223.7 ± 57.78	436.7 ± 104.15	9.5 ± 1.41	3.55 (3)
	Irradiated	316.3 ± 56.36	119.1 ± 36.10	174.0 ± 66.62	7.2 ± 2.06	0.53 (2)
		.	248.4 ± 66.27	380.1 ± 105.91	182.6 ± 12.71	9.11 (3)
		.	.	.	148.5 ± 19.72	1.72 (2)
		.	.	.	120.6 ± 12.19	3.86 (3)
		.	.	.	102.5 ± 18.07	0.63 (2)
		.	.	.	193.9 ± 12.37	3.71 (3)
		.	.	.	187.0 ± 15.75	3.23 (2)
		.	.	.	166.0 ± 14.18	4.16 (3)
		.	.	.	156.2 ± 14.92	2.83 (2)

loci. Furthermore, since the dominance contributions at the k' loci can take sign depending on the direction of mutation, it can either subtract from or add to the genuine additive contributions to the additive genetic component (section 2).

From the increase in the dominance components we can therefore conclude that some of the mutations were induced at loci at which the F_2 was already segregating thus creating three or more alleles at these loci and significantly increasing the dominance but not the epistatic variation. Apart from final height the increases in the additive components are more modest, but as we have already seen they may not be reflecting the true increases in the additive genetic variation produced by mutation.

The estimates of D from the hierarchical and the triple test cross experiments for the control material are in excellent agreement for all characters (tables 3 and 5). For the irradiated material the estimates from the hierarchical experiment are larger for all except leaf width. The smaller estimates from the triple test cross are, of course, what we would expect if they were being reduced by the cancelling effect of dominance created by mutation at the k' loci. This is not, however, the only possible explanation. For example, there are differences in the linkage biases of the D 's obtained from the two sources which would make the estimates from the triple test cross the smaller in the presence of repulsion linkages (section 2).

In the control and irradiated material the estimates of D_1 from the hierarchical experiment are smaller than those of D_2 as expected in the presence of repulsion linkages (table 5). In addition, the estimates of D from the triple test cross, which are expected to have the same linkage biases as those of D_1 , are generally closer to the latter than to estimates of D_2 (tables 3 and 5). For the irradiated material, however, D_1 is also biased by the effects of multiple allelism and unequal gene frequencies created by the induced mutations. We might, therefore, expect the agreement between the estimates of D and D_1 to be closer for the control than for the irradiated material which is clearly not the case for HFT but appears to be so for LW and FH (tables 3 and 5).

There are therefore some unresolved questions but the main finding, that we can detect an increase in the heritable variation in an F_2 following irradiation by both of the crossing programmes, is unambiguous. One prediction that follows from this, namely, that a wider range of pure breeding lines can be extracted from the F_2 following irradiation is currently being confirmed.

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