

COMPARING PREDICTIONS OF MEAN PERFORMANCE AND ENVIRONMENTAL SENSITIVITY OF RECOMBINANT INBRED LINES BASED UPON F_3 AND TRIPLE TEST CROSS FAMILIES

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SUMMARY

We have previously shown that statistics estimated from the F_1 , F_2 , B_1 and B_2 families of a cross between two inbred lines or from a triple test cross on the F_2 of such a cross can be successfully used to predict the properties of the pure breeding lines extractable from the cross by single seed descent. In this paper we extend the method to properties such as mean performance and environmental sensitivity that can be measured only by replicating each family or generation over two or more contrasting environments.

The optimal design for making predictions for such characters is the families of the F_2 triple test cross replicated over environments. The simplest design, which assumes that dominance and other non-additive sources of variation are not large, is F_3 families replicated over environments. These two designs have, therefore, been compared using the cross between varieties 2 and 12 of *Nicotiana rustica*, two sowing dates a month apart as the environmental treatment, and mean performance and environmental sensitivity in respect of flowering time and final height as the properties to be predicted. The predicted properties of the pure breeding lines that would be derived from this cross, both singly and jointly, are compared with those of a random sample of its F_7 families produced by single seed descent.

There is no difference between the predictions based on the triple test cross and F_3 families, and the observed properties in respect of final height do not differ from either. For flowering time the agreement is not so good, the most likely cause being the unreliability of estimates based upon only two environments. Should the number of environments be increased to overcome this possibility or to cover a more realistic set of environmental variables of importance to the growth of the crop it can only improve the attractiveness of the simpler F_3 design over the triple test cross.

1. INTRODUCTION

IN previous papers we have shown how the properties of the recombinant inbred lines that can be derived from a cross between a pair of lines can be predicted from the early generations of the cross (Jinks and Pooni, 1976; Pooni and Jinks, 1978; Pooni, Jinks and Cornish, 1977; Pooni, Jinks and Pooni, 1978). So far these predictions have been made, and their validity confirmed, for those properties which can be measured on individuals raised in a single environment. In this paper we shall extend the approach to predictions of properties such as mean performance and sensitivity to macroenvironmental differences that can be measured only by raising replicate, random samples of a family in two or more environments. The measurement of such properties on the segregants from a cross between a pair of lines must, therefore, be delayed until each F_2 segregant can be

represented by one or more progeny families each of which can be raised in two or more environments.

The best source of the genetical parameters required for predicting the properties of recombinant inbred lines is the F_2 triple test cross (Kearsey and Jinks, 1968; Jinks and Perkins, 1970; Pooni and Jinks, 1979; Pooni, Jinks and Jayasekara, 1978) and its use can be extended to mean performance and environmental sensitivity by replicating each of the L_{1i} , L_{2i} and L_{3i} families over two or more environments. This requires a large, complex experiment and the commonly used simpler alternatives such as F_2 and first backcross families of the cross cannot be used (Jinks and Pooni, 1976; Pooni and Jinks, 1979). In fact the simplest alternative that would provide progeny families of F_2 segregants for replication over environments is an F_3 . In this paper we therefore compare the predictions about mean performance and environmental sensitivity that can be made from an F_2 triple test cross with those from the F_3 of a cross between varieties 2 and 12 of *Nicotiana rustica*. The validity of the predictions is examined by comparing them with the mean performances and environmental sensitivities of a random sample of F_7 families from the same cross.

2. MATERIAL

The material used for making these predictions consisted of pure breeding varieties 2 and 12 of *N. rustica*, the F_1 , F_2 and first backcross (B_1 and B_2) families produced from an initial reciprocal cross between them; an F_3 generation consisting of families produced by self-pollination of a random sample of F_2 plants and an F_2 triple test cross set of L_{1i} , L_{2i} and L_{3i} families produced by crossing a random sample of the F_2 to varieties 2 and 12 and their F_1 . The structure and dimensions of the experiment are given in table 1. To increase the amount of information obtainable from the experiment

TABLE 1

Structure and dimensions of the experiment grown in 1969 to estimate the statistics required for making predictions

Family	Family size per block	No. of families per block per sowing	Total plants in 2 blocks \times 2 sowings
V_{12}	40	1	160
V_2	60	1	240
F_1	100	1	400
F_2	220	1	880
B_1	120	1	480
B_2	120	1	480
F_3	10	18	720
L_1	10	18	720
L_2	10	18	720
L_3	10	18	720
Total experiment size			5520

the total of 5520 plants was divided among the different generations in proportion to their expected variances (see Jinks and Perkins 1969; Perkins and Jinks, 1970). Reciprocal crosses were equally represented in all generations from the F_1 onwards. Every family was divided equally among each of two replicate blocks in each of two environmental treatments

produced by an early (April 23rd) and a late (May 21st) date of sowing. All 1380 plants in each block of each sowing were completely randomised at the time of sowing. This material was raised in 1969. Parts of this experiment have previously been described and analysed by Jinks and Perkins (1970) and Brumpton, Boughey and Jinks (1977).

The material used to test the validity of the predictions consisted of pure breeding varieties 2 and 12, their reciprocal F_1 crosses and 60 F_7 families derived from a random sample of 60 plants of the F_2 of this cross, 30 from each of the two reciprocals, by single seed descent. Each of the original F_2 plants is, therefore, represented by a randomly chosen F_7 family. Ten individually randomised plants of each family were raised within each of two treatments which again consisted of the early and the late dates of sowing (see Jinks, Jayasekara and Boughey, 1977 and Boughey and Jinks, 1978, for further details).

3. METHOD

The analyses will be based on two characters, flowering time and final height which were scored on all plants in the two experiments. Mean performance, measured as the mean of each family over blocks and sowings, and environmental sensitivity, as the square root of the variance component of family means over sowings, are used in all subsequent biometrical genetical analyses. The variance component is obtained from a hierarchical analysis of variance of each family separately in which the total variation for the family is partitioned into three items, between sowings, between blocks within sowings and between plants within blocks within sowings.

The analysis of the basic generations (Parents, F_1 , F_2 , B_1 and B_2) to obtain estimates of the components of means and of the F_2 triple test cross families to obtain the components of variances then follows the standard procedures (Mather and Jinks, 1971; Jinks and Pooni, 1976). From these we can obtain the estimates of m , the mean of all possible inbred lines, and \sqrt{D} , the standard error of the means of all possible inbred families, which are required to make predictions about the mean performances and environmental sensitivities of the recombinant inbred lines that can be extracted from the cross of varieties 2 and 12. To make joint predictions about mean performance and environmental sensitivity we can also estimate the additive genetic correlation between them (Pooni and Jinks, 1978).

From an analysis of the mean performances and environmental sensitivities of the F_3 families alone we can estimate the mean ($\bar{F}_3 = m + \frac{1}{4}[h] + \frac{1}{16}[l]$) and the within and between family components of variation from an appropriate analysis of variance ($\sigma_w^2 = \frac{1}{4}D + \frac{1}{8}H + E_w$ and $\sigma_b^2 = \frac{1}{2}D + \frac{1}{16}H$, respectively). To predict from F_3 information alone we must therefore make the approximations

$$\bar{F}_3 \approx m \quad (\text{assuming } \frac{1}{4}[h] + \frac{1}{16}[l] \text{ is small})$$

and

$$\sqrt{2\sigma_b^2} \approx \sqrt{D} \quad (\text{assuming } \frac{1}{8}H \text{ is small}).$$

Similarly, the between family component of the covariance between mean performance and environmental sensitivity can be used as an approximate estimate of the additive genetic covariance from which we can derive the additive genetic correlation.

4. RESULTS

We shall illustrate the general outcome of the predictions for individual characters by predicting the number of pure breeding families that are expected to have phenotypic means greater or less than those of the parental varieties 2 and 12 and, if there is heterosis, greater or less than their F_1 . The information required for making these predictions from the combined triple test cross and basic generations and from the F_3 families is summarised in table 2. The predictions derived from this information by the methods

TABLE 2

Estimates of the statistics from the 1969 experiment required for making predictions about the mean performances and environmental sensitivities in respect of flowering time and final height of the pure breeding families that would be derived from the cross between varieties 2 and 12

Statistic and source	Flowering time	Final height	
		Mean performance	
\bar{V}_{12}	25.32	146.46	
\bar{V}_2	46.98	107.42	
\bar{F}_1	26.59	167.13	
TTC			
m	33.93	135.56	
\sqrt{D}	8.49	25.87	
F_3			
m	34.07	137.16	
\sqrt{D}	10.69	25.27	
		Environmental Sensitivity	
\bar{V}_{12}	6.06	13.77	
\bar{V}_2	4.28	12.06	
\bar{F}_1	5.97	23.13	
TTC _m	3.39	18.85	
\sqrt{D}	3.49	6.58	
$F_{3,m}$	3.67	18.94	
\sqrt{D}	3.65	8.00	

described by Jinks and Pooni (1976) are given in table 3 along with the numbers of families with the corresponding phenotypic properties observed in the F_7 generation. Both predictions and observations are given as whole numbers summing to 59, the total number of F_7 families for which we have observed means (one of the 60 F_7 families was omitted because of insufficient viable seed).

In the experiment which provided the observed numbers (section 2), parental variety 2 atypically showed no significant response for flowering time to the difference between the two sowing dates; this leads to a zero estimate for its environmental sensitivity. This unusual result could have been a specific reaction of variety 2 to the environment in the season in which it was compared with the F_7 families. Equally it could have resulted from sampling error bearing in mind that the estimate is based on a comparison for a single degree of freedom. A further estimate of the environmental sensitivity of variety 2 to sowing date in the same season for one degree of freedom supports the latter explanation. This alternative

TABLE 3

The frequencies of pure breeding families falling into each of the phenotypic classes predicted from the triple test cross and F₃ families and the correspondingly observed frequencies in the F₇ generation

Phenotypic class	Flowering time			Final height		
	Predicted		Observed	Predicted		Observed
	TTC	F ₃	F ₇	TTC	F ₃	F ₇
	Mean Performance					
>V ₁₂	50	47	57	20	21	23
<V ₁₂	9	12	2	39	38	36
>V ₂	4	7	15	51	52	47
<V ₂	55	52	44	8	7	12
>F ₁				7	7	5
<F ₁				52	52	54
	Environmental Sensitivity					
>V ₁₂	13	15	6	46	44	45
<V ₁₂	46	44	53	13	15	14
>V ₂	24	26	24	50	47	48
<V ₂	35	33	35	9	12	11
>F ₁				15	18	24
<F ₁				44	41	35

estimate, which was typical for variety 2, was therefore used to determine the number of F₇ families with environmental sensitivities greater or less than that of variety 2.

Reference to table 3 shows that there are no significant differences (2 × 2 contingency χ^2 's for 1 degree of freedom) between the predictions based on the combined triple test cross and basic generations, and those based solely on F₃ families. Furthermore, the asymmetries observed in the distribution of the F₇ families on either side of the means of the two parental and F₁ families are invariably in the direction predicted. For final height the observed and predicted frequencies in table 3 are significantly different in only one out of twelve comparisons, namely, that between the observed frequencies for the environmental sensitivity of the F₁ and the predicted frequencies obtained from the triple test cross and basic generations. For flowering time, on the other hand, the observed and predicted frequencies differ significantly in four out of the eight comparisons. For mean performance only the F₃ prediction for variety 2 is satisfactory while for environmental sensitivity only the F₃ prediction for variety 12 is not.

Of particular interest is the extent to which new combinations of mean performance and environmental sensitivity will appear among the pure breeding lines that can be derived from the cross between varieties 2 and 12. Using the method described by Pooni and Jinks (1978) we can predict the joint distribution of these two properties among the pure breeding lines. In table 4 we summarise the outcome as the number of such lines which are expected to have combinations of mean performance and environmental sensitivity that fall outside of the range of those found in varieties 2 and 12 and their F₁, where it shows heterosis. The numbers of F₇ families falling into each of the phenotypic classes in respect of both properties are also tabulated for comparison. The small numbers of families falling into most of the phenotypic classes rule out reliable statistical comparisons but it is again clear that the two sets of predictions are in reasonably good agreement and

TABLE 4

The frequencies of pure breeding lines falling into the phenotypic classes specified by mean performance and environmental sensitivity simultaneously predicted from the triple test cross and F₃ families and the corresponding observed frequencies in the F₇ generation

Mean performance	Environmental sensitivity	Predicted		Observed F ₇
		TTC	F ₃	
		Flowering Time		
>V ₂	>V ₁₂	1	0	3
>V ₂	<V ₂	2	6	10
<V ₁₂	>V ₁₂	2	6	1
<V ₁₂	<V ₂	5	3	0
Within V ₂ to V ₁₂ range		49	44	45
		Final Height		
>V ₁₂	>V ₁₂	18	19	20
>V ₁₂	<V ₂	1	1	2
<V ₂	>V ₁₂	5	3	8
<V ₂	<V ₂	3	3	3
Within V ₂ to V ₁₂ range		32	33	26
>F ₁	>F ₁	3	4	3
>F ₁	<F ₁	3	3	2
<F ₁	>F ₁	13	13	21
<F ₁	<F ₁	40	39	33

that the predicted and observed distributions agree well for final height but not for flowering time. More important, however, is that we both predict and observe new combinations of mean performance and environmental sensitivity.

5. DISCUSSION

For final height the agreement between the observed and predicted univariate and bivariate distributions of mean performance and environmental sensitivity among the pure breeding lines derived from the cross of varieties 2 and 12 is as good as any previously reported for simpler phenotypic properties (Jinks and Pooni, 1976; Pooni and Jinks, 1978; Pooni, Jinks and Pooni, 1978). For flowering time the agreement is not so good. The predictions are in no case qualitatively misleading but predictions and observations differ significantly in half the comparisons. Furthermore, the number of significant differences would have been greater had we not replaced an atypically low estimate of the environmental sensitivity of variety 2 from the experiment in which the F₇ families were assessed by a more typical contemporary estimate. Unrepeatability of the estimate of the environmental sensitivity of variety 2 when based on two environments only is clearly a weakness of the current experiment. Unrepeatability seems a less likely explanation of the differences between prediction and observation for mean performance. However, a more likely explanation—genotype × environment interactions between the genotypes and the environments in which they were grown for making predictions and for testing them, respectively—appears to be ruled out because the parental varieties and the F₁, which are the only genotypes grown in both environments, do not interact significantly with them. In the absence of any other explanation we

must conclude that our estimates of mean performance and environmental sensitivity for flowering time are unreliable.

A major conclusion of our analyses is that the predictions based on the triple test cross and basic generations and those based on the F_3 families do not differ. The bias introduced into the predictions based on F_3 families by assuming $\frac{1}{8}H = 0$ and $\frac{1}{4}[h] + \frac{1}{16}[l] = 0$ (see section 3) are not therefore important in practice even though there are substantial effects due to dominance. From the triple test cross analyses and basic generations we can quantify these biases by estimating $\frac{1}{8}H$, $\frac{1}{4}[h]$ and $\frac{1}{16}[l]$. For example, for mean performance and environmental sensitivity and inflationary bias due to $\frac{1}{8}H \neq 0$ on the estimate of \sqrt{D} is 4.8 per cent and 1.4 per cent for final height, and 3.0 per cent and 0.03 per cent for flowering time, respectively. Similarly the corresponding bias due to $\frac{1}{4}[h] + \frac{1}{16}[l] \neq 0$ on the estimate of m expressed as a proportion of \sqrt{D} , which is the standard error of the means of the pure breeding families normally distributed around m , is 0.39 and 0.39 for final height and 0.28 and 0.06 for flowering time, respectively. Within biases of the magnitudes we have observed in these data we can use predictions based upon random samples of F_3 families with the same confidence as those based on a triple test cross.

If there is a weakness in the present experiment it is that mean performance and environmental sensitivity have been observed over two environments only. This clearly makes the estimates extremely sensitive to sampling error and two environments are unlikely to provide information on the response of genotypes to all of the environmental variables of interest. The need to replicate the material used for making predictions over more environments is likely to increase the practical advantages of using F_3 as opposed to triple test cross families raised from the same F_2 .

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