Retrospective selection and sources of superior inbreds amongst pedigree inbred families of *Nicotiana rustica*

H. S. Pooni and
J. L. Jinks

Department of Genetics, University of Birmingham,
P.O. Box 363, Birmingham B15 2TT, UK.

A single cycle of selection in the F_2 , F_3 , F_4 and F_7 generations using the overall means of the individual hierarchies as their predicted breeding values, has been applied retrospectively to seven metrical traits scored on 784 F_7 inbreds extracted from the cross of varieties 2 and 12 of *Nicotiana rustica* by the method of pedigree inbreeding. The hierarchical structure within 98 F_2 groups each of 8 F_7 families is further used to determine the distribution and source of the superior inbreds within each hierarchy. While the efficiency of selection improved steadily the more advanced the generations in which it was applied, superior recombinants originated more frequently in the early than in the late generations. The hierarchical method of inbreeding, however, favours the recovery of recombinants from the advanced generations and in the present case the two balance each other exactly. The improvement in response to selection in the advanced generations is completely associated with the relative reductions in error variances and is, therefore, not attributable to any genuine change in the genetic covariances.

The present investigation also confirms that single seed descent is the most cost effective method of producing inbreds from a cross until and unless there are some unforeseen advantages in operating selections in the initial stages of a breeding programme.

INTRODUCTION

There are no theoretical or practical reasons for preferring pedigree inbreeding to single seed descent for extracting random samples of recombinant inbred lines from a cross (Jinks and Pooni, 1981a, 1981b, 1982, 1984). Traditionally, however, pedigree inbreeding includes early generation assessment as a basis for selecting for an increase in the frequency of desirable genotypes.

Preliminary observations on characters of intermediate to low heritability suggest that the value of such selection is low (Brumpton, Boughey and Jinks, 1977; Jinks, Jayasekara and Boughey, 1977; Boughey and Jinks, 1978; Jinks and Pooni, 1981a). These observations have been repeated on a larger scale for the highly heterotic cross of varieties 2 and 12 of *Nicotiana rustica* using characters which display a wide range of heritabilities and amounts and kinds of genetical variation (Pooni, Jinks and Jayasekara, 1978).

MATERIAL AND METHOD

The material is 784 F_7 families obtained by

pedigree inbreeding from a random sample of 98 F_2 plants of the cross of varieties 2 and 12 of *Nicotiana rustica*. Each original F_2 plant is represented by a random sample of 8, hierarchically related, F_7 families (see Jinks and Pooni, 1984 for details). The data consist of seven characters scored on eight replicates of each of the 784 families.

In a previous paper (Jinks and Pooni, 1984) we presented the frequencies of potentially useful recombinant inbred lines among the 784 families defining these as having mean performances which fell outside of the parental range ($\geq \bar{P}_1$, $\leq \bar{P}_2$) or better than their heterotic F_1 . We shall first examine the distribution of the superior inbreds within and between the 98 F_2 groups of 8 F_7 families that make up the 784. Within each F_2 group we shall determine at which generation of the pedigree the superior families emerged.

Since we shall be identifying the superior recombinant inbred lines at the F_7 , we shall also examine how much earlier in the breeding programme we could have identified those lineages which have yielded the higher than average frequencies of superior inbred lines.

RESULTS

Distribution of hierarchies

Results of the preliminary classification of hierarchies according to their potential for producing 0 to 8 recombinant inbreds that show superior performance ($\geq \bar{P}_1$ or $\leq \bar{P}_2$) are given for each character in table 1. It is clear from these results that the hierarchies are well spread between the nine classes (0 to 8) with their frequencies showing a

Table 1 Classification of the 98 hierarchies of 8 inbred families according to the number of inbreds in each hierarchy which are superior ($\geq \bar{P}_1$ or $\leq \bar{P}_2$) for each character

Character	Category	Number of superior inbreds in a hierarchy								
		8	7	6	5	4	3	2	1	0
H4	$\geq \bar{P}_1$	3	1	7	2	5	9	12	18	41
	$\leq \bar{P}_2$	1	1	3	3	5	3	9	14	59
H6	$\geq \bar{P}_1$	0	1	0	2	2	3	9	8	73
	$\leq \bar{P}_2$	2	1	1	1	5	5	11	14	58
FT	$\geq \bar{P}_1$	4	3	9	3	7	9	12	12	39
	$\leq \bar{P}_2$	4	5	6	5	11	9	17	12	29
HFT	$\geq \bar{P}_1$	4	10	11	13	9	11	6	19	15
	$\leq \bar{P}_2$	3	6	4	7	6	9	19	18	26
LL	$\geq \bar{P}_1$	7	7	16	6	15	9	10	9	19
	$\leq \bar{P}_2$	18	8	11	7	15	8	14	7	10
LW	$\geq \bar{P}_1$	8	8	10	5	15	4	10	14	24
	$\leq \bar{P}_2$	8	5	9	8	12	6	14	15	21
FH	$\geq \bar{P}_1$	4	8	10	7	10	11	11	7	30
	$\leq \bar{P}_2$	1	4	2	2	6	13	12	12	46

negative correlation with the number of superior inbreds in each class. In other words potentially superior hierarchies tend to be relatively rarer than the less potent hierarchies. The number of hierarchies falling into each class and their totals over classes with 1 to 8 superior inbreds also vary from character to character. These differences, however, depend exclusively on the degree of gene association in the parents and the amount of additive genetic variability which is present in the original cross.

Systematic labelling of each of the eight lineages within an hierarchy allows the 98 hierarchies to be cross classified on the basis of the source of origin of the superior inbreds. For example, hierarchies with 5 superior inbreds can be divided into three distinct groups where the sources of origin of these inbreds are:

1. One F_3 ancestor (giving 4 superior inbreds) and one F_5 ancestor (giving 1 superior inbred);
2. Two F_4 ancestors (each giving 2 superior inbreds) and one F_5 ancestor (giving 1 superior inbred) and
3. One F_4 ancestor (giving 2 superior inbreds) and three F_5 ancestors (each giving 1 superior inbred).

Similar groupings have been produced for the other classes. The results are tabulated in table 2.

The relative importance of F_2 , F_3 , F_4 and F_5 segregation as diminishing sources of superior inbreds is quite apparent from table 2. For example

Table 2 Division of the 98 hierarchies of 8 inbred families (class 0 to 8) into groups according to the generation of origin (source) of the superior inbreds in each lineage for each character

Class	Combination Source	Character															
		$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$	$\geq \bar{P}_1$	$\leq \bar{P}_2$
8	F_2 alone	3	1	0	2	4	4	4	3	7	18	8	8	4	1		
7	$F_2 + F_5$	1	1	1	1	3	5	10	6	7	8	8	5	8	4		
6	$4+1+1$ $F_3 + F_4$	3	2	0	1	3	0	6	2	10	3	4	3	6	1		
	$F_3 + F_5$	0	0	0	0	5	2	4	1	2	7	4	3	3	1		
	$2+2+1+1$ $F_4 + F_5$	4	1	0	0	1	4	1	1	4	1	2	3	1	0		
5	$4+1$ $F_4 + F_5$	0	1	0	1	1	3	8	4	4	2	3	3	3	1		
	$2+2+1$ $F_4 + F_5$	2	0	2	0	0	2	3	3	1	4	0	5	2	1		
	$2+1+1+1$ $F_4 + F_5$	0	2	0	0	2	0	2	0	1	1	2	0	2	0		
4	4 F_3 alone	2	4	1	3	5	4	5	3	7	6	7	4	7	4		
	$2+2$ F_4 alone	0	1	0	1	1	1	0	0	2	2	5	2	0	1		
	$2+1+1$ $F_4 + F_5$	3	0	1	1	1	5	3	3	6	7	3	5	3	1		
	$1+1+1+1$ F_5 alone	0	0	0	0	0	1	1	0	0	0	0	1	0	0		
3	$2+1$ $F_4 + F_5$	7	2	3	4	8	74	7	9	8	6	4	5	9	10		
	$1+1+1$ F_5 alone	2	1	0	1	1	5	4	0	1	2	0	1	2	3		
2	2 F_4 alone	6	5	7	7	6	8	5	12	4	10	5	9	6	8		
	$1+1$ F_5 alone	6	4	2	4	6	9	1	7	6	4	5	5	5	4		
1	F_5 alone	18	14	8	14	12	12	19	18	9	7	14	15	7	12		
0		41	59	73	58	39	29	15	26	19	10	24	21	30	46		

the sources identified with segregation in the F_2 and F_3 generations occur with a higher frequency among the hierarchies than those identified in the F_4 and F_5 . While this is expected because of a predictable reduction in the level of heterozygosity in the successive generations of a selfing programme these differences are also enhanced by the number of inbreds attributable to each source and the probability with which different events occur. For instance a superior recombinant contributes 8 superior inbreds when its source is the F_2 generation. The same recombinant, however, contributes only 4 superior inbreds when its source is the F_3 generation and its contributions are further reduced to 2 and 1 superior inbreds in the F_4 and F_5 , respectively. On the other hand the chances of recovery of such a recombinant multiply two-fold in each successive generation because we are doubling the number of lines from 98 in F_2 to 196 in F_3 , 392 in F_4 and 784 in F_5 . Therefore, if we wish to isolate the effects of selfing in the F_2 , F_3 , F_4 and $F_5 + F_6$ generations we must consider only the frequencies that are attributable exclusively to F_2 alone in class 8, F_3 alone in class 4, F_4 alone in class 2 and F_5 alone in class 1. These frequencies, which are 0.0488, 0.0226, 0.0179 and 0.0163 respectively approximate to $\frac{1}{2}:\frac{1}{4}:\frac{1}{8}:\frac{1}{32}$, the proportions of the additive genetic variance that the F_2 , F_3 , F_4 , and $F_5 + F_6$ generations contribute to the total variance of the final inbreds. In fact the first pair of values (0.0488 and 0.0226) are relatively smaller than their expected values and those of the second pair (0.0179 and 0.0163) relatively larger. Again these differences are expected because the additive genetic variances (D_1 and D_2) attributable to the F_2 and F_3 generations are observed to be smaller than those attributable to later generations (D_3 and $D_4 + D_5$) for most of the characters due to the presence of repulsion linkages (Jinks and Pooni, 1984).

Two further categorisations of the 98 hierarchies one based on the overall means of the hierarchies and the other on the upper and lower limits of the range of inbred means in each hierarchy are tabulated in table 3. The first divides the hierarchies into three categories each corresponding with $\geq \bar{P}_1$, $\leq \bar{P}_2$ and the remainder. The second categorises hierarchies into six groups whose upper and lower limits are defined as $\geq \bar{P}_1 \geq \bar{P}_1, \geq \bar{P}_1 > \bar{P}_2, \leq \bar{P}_2 \leq \bar{P}_2, < \bar{P}_1, \leq \bar{P}_2, \geq \bar{P}_1 \leq \bar{P}_2$ and $< \bar{P}_1 > \bar{P}_2$, respectively. This allows the hierarchies to be divided into high scoring less variable, high scoring more variable, low scoring less variable, low scoring more variable, highly variable and intermediate categories. This categorisation is specifically used to cross

Table 3 Categories of the 98 hierarchies according to their overall means and according to the overall range displayed by the inbred means derived from each hierarchy for each character

Upper	Limits		Character						
	Lower		H4	H6	FT	HFT	LL	LW	FH
<i>(a) Overall means of the hierarchies</i>									
$\geq \bar{P}_1$	—		18	4	25	45	42	33	31
—	$\leq \bar{P}_2$		5	7	22	21	56	36	10
$< \bar{P}_1$	$> \bar{P}_2$		75	87	51	32	0	29	57
<i>(b) Range of inbred means in each hierarchy</i>									
$\geq \bar{P}_1$	$\geq \bar{P}_1$		3	0	4	4	7	8	4
$\geq \bar{P}_1$	$> \bar{P}_2$		45	22	25	22	3	22	38
$\leq \bar{P}_2$	$\leq \bar{P}_2$		1	2	4	3	18	8	1
$< \bar{P}_1$	$\leq \bar{P}_2$		29	35	35	12	1	15	25
$\leq \bar{P}_1$	$\leq \bar{P}_2$		9	3	30	57	69	54	26
$< \bar{P}_1$	$> \bar{P}_2$		11	36	0	0	0	1	4

classify hierarchies which fall under the $\geq \bar{P}_1 \leq \bar{P}_2$ group because previously they were counted twice, once in the $\geq \bar{P}_1$ and then in the $\leq \bar{P}_2$ class (see table 1).

The proportions of hierarchies that fall into the three categories on the basis of their overall means is similar to those for inbred lines derived from the same cross by single seed descent (see table 5 of Jinks and Pooni, 1984) except that for every character the relative numbers in the $\geq \bar{P}_1$ and $\leq \bar{P}_2$ categories are always less than those of the SSD lines. This is expected because the total genetic variance present amongst the hierarchy means ($\frac{1}{2}D_1 + \frac{1}{8}D_2 + \frac{1}{32}D_3 + \frac{1}{128}D_4 + \dots = \frac{43}{64}D$) is significantly smaller than that present amongst the random inbreds extracted by single seed descent ($\frac{1}{2}D_1 + \frac{1}{4}D_2 + \frac{1}{8}D_3 + \frac{1}{16}D_4 + \dots = D$).

There are many hierarchies with a small range coupled with extreme performance ($\geq \bar{P}_1 \geq \bar{P}_1$ and $\leq \bar{P}_2 \leq \bar{P}_2$) and only few with a high or low performance coupled with a large range. The differences between the $\geq \bar{P}_1 \geq \bar{P}_1$ and $\geq \bar{P}_1 > \bar{P}_2$, and $\leq \bar{P}_2 \leq \bar{P}_2$ and $< \bar{P}_1 \leq \bar{P}_2$ categories depend directly on the proportion of the additive genetic difference between the parents ($\bar{P}_1$ and $\bar{P}_2$) that results in a net difference between their means, that is, the degree of gene association in the parents. Consequently the numbers of hierarchies falling into each of the six categories in table 3(b) should be highly correlated with the degree of gene association in the absence of complications such as epistasis and genotype $\times$ environmental interactions. These correlations over characters take the values of -0.8278 , 0.5020^{NS} , -0.6016^{NS} , 0.8613 , -0.9406 and 0.8030 for categories 1 to 6, respectively (see table 3).

Selection amongst lineages

The 784 F_7 families used in the present experiment were produced during a short span of three years starting in the summer of 1978 when the 98 F_2 plants of the $V_2 \times V_{12}$ cross were raised in a field experiment and selfed. While the material was rapidly advanced at the rate of two generations per year the performances of the 98 F_3 and 196 F_4 families were assessed during the summer of 1980 when the two generations were raised in two separate experiments. We can, therefore, apply selection to the F_2 , F_3 and F_4 data retrospectively to find out if selection in these early generations increases the chances of retaining lineages with superior inbreds. Only one cycle of selection on individual traits has, however, been applied. Multi-cycle/multi-trait selection will be the subject of a further paper.

The selection criterion we apply is relatively simple. For each character we take the phenotypic scores of the 98 F_2 individuals and select the 5 highest and 5 lowest scoring phenotypes. We then trace the descendents of the selected phenotypes through to the F_7 generation and assess their average performance by obtaining the overall mean of the hierarchies of families to which they give rise. We apply the same selection criteria to the F_3 , F_4 and F_7 generations and make the results comparable by selecting only amongst the 98 hierarchy means. The results of these selections are presented in table 4.

Also given for comparison are the average performances of the 5 highest scoring and 5 lowest scoring inbred lines that are present amongst the

60 inbreds extracted from the $V_2 \times V_{12}$ cross by the method of single seed descent (Pooni, Jinks and Jayasekara, 1978).

Selection in the early generations of a pedigree breeding programme requires assessment of the relevant material under field conditions by conducting an appropriately designed experiment and scoring for all of the quantitative and qualitative characters under selection. This in turn makes extra demands on manpower, financial resources and facilities compared with inbreeding without selection. Assessments of the manpower requirements for the *Nicotiana rustica* experiment, which is the largest component of the cost, are listed in table 5. Also given in table 5 are the comparative requirements for producing seed by selfing the same number of families by the method of single seed descent.

CONCLUSIONS

The main conclusion we can draw from the first part of "Results" is that each cycle of recombination makes an important and an approximately equal contribution to the production of superior inbreds (see tables 1, 2 and 3). While superior recombinants in the F_2 and F_3 generations are essential for obtaining superior hierarchies that have a majority of inbreds (5 or more out of 8) with a superior performance defined as $\geq \bar{P}_1$ or $\leq \bar{P}_2$, the recombination in the F_4 and F_5 generations generates disproportionately large numbers of superior inbreds in spite of the predictable reduction in heterozygosity. This is because the

Table 4 Average F_7 performances of the selections made in the F_2 , F_3 , F_4 and F_7 generations and in the SSD inbred lines, overall mean of the 98 lineages and the h_n^2 values for each character

Method of Selection	H4	H6	FT	Characters HFT	LL	LW	FH
<i>(a) Selection for high mean</i>							
1. F_2 plants	23.78	59.77	35.90	—*	24.42	20.40	147.08
2. F_3 fam. means	21.52	62.01	42.26	85.36	23.81	19.91	159.97
3. F_4 hier. means	24.45	65.24	43.72	94.84	25.36	21.52	166.25
4. F_7 hier. means	25.09	67.85	43.72	99.52	26.59	22.47	170.06
5. SSD	24.04	68.33	42.97	122.62	27.54	23.54	173.15
<i>(b) Selection for low mean</i>							
1. F_2 plants	4.79	32.91	16.53	—	19.26	15.37	109.27
2. F_3 fam. means	8.26	31.49	15.39	44.12	19.81	15.92	99.65
3. F_4 hier. means	5.45	28.09	13.77	40.63	17.89	12.36	93.33
4. F_7 hier. means	4.51	22.14	13.21	40.34	17.04	11.44	90.66
5. SSD	4.10	20.41	13.44	40.79	15.49	10.59	86.19
Overall mean of 98 lineages	12.14	44.78	25.00	65.61	21.53	17.03	125.86
h_n^2	0.44	0.45	0.69	0.47	0.39	0.34	0.55

* measurements on HFT were not recorded in 1978.

Table 5 Costs of seed production and field assessment of *N. rustica*

Operation	Detail	Cost (man days)
<i>(a) Seed production (cost per 800 selfs)</i>		
1. Pre-sowing	Preparing labels, seeds, pots	3
2. Sowing	Sowing 1600 pots	3
3. Management	Watering, spraying, general maintenance	7
4. Seed production	Bagging, selfing, collecting capsules, thrashing, etc.	9
		22
<i>(b) Experiment (of 4000 plants and 4 months duration)</i>		
1. Pre-sowing	Label preparations (2), randomisation (5), seed packets (2), bench prep. (2)	11
2. Sowing	Soil sterilisation (1), soil mixing (1), pot filling (4), sowing (8)	14
3. Post-sowing	Transplanting (1), thinning (2), watering and general maintenance (3)	6
4. Cold-frame weather hardening	Transfer (2) and general maintenance (2)	4
5. Planting	Preparing field labels (1), marking field (3), planting (10)	14
6. Post-planting	Watering (2), hoeing and weed control (6), disposal (4)	12
7. Data recording	Average 4 hours/day for 60 days	30
8. Data processing	Punching cards (4), checking (2), preliminary analysis (2) prior to making selections	8
	Total	99

hierarchical system of inbreeding allows more chances of recovering recombinants in the more advanced than in the early generations. In the bifurcating system used in our experiment the chances of recovering recombinants doubles every generation. On the other hand, the frequency of recombinants is much higher in the early generations and in the present case these two opposing trends balance each other exactly. Consequently the proportions of superior inbreds arising from segregation in the various generations are approximately equal.

It is also clear from table 3 that nearly all of the 98 F_2 and 196 F_3 plants chosen for further selfing were heterozygous. Virtually none of the lineages have therefore attained complete homozygosity in the early generations. Conversely none of these F_2 and F_3 plants appears to be heterozygous at as many loci as the original F_1 because not a single hierarchy has inbred lines that display

variability comparable to that of the single seed descent lines extracted from the same cross. This may, however, in part be attributable to the sample of inbred lines per hierarchy being only 8 compared with a sample of 60 SSD inbreds.

A significant conclusion which we draw from the second part of "Results" is that selection is effective in every generation although its efficiency increases steadily the later the generation in which it is imposed. The efficiency, however, increases much more for characters with low than with high heritabilities. The present results therefore differ from those for the $V_1 \times V_5$ cross which has lower levels of heritability (Jinks and Pooni, 1981a). Further, the method of selection we have applied is particularly designed to show the influence of error variations on the efficiency of selection. The genetic covariance between the phenotypes in the selection generation and the overall mean of the corresponding lineages in the F_7 is constant and equal to $\approx \frac{1}{2}D$ and only their correlations vary because the genetic and environmental variances of the F_2 , F_3 and F_4 generations differ.

With qualitative characters materials can often be screened under glasshouse or small scale pot or field experiments. Rigorous selection is normally applied to quantitative characters, however, only after a large scale field assessment. The modest sized *N. rustica* experiment which we have had to conduct to apply one cycle of selection, including seed production (99+22 man days), costs almost 6 times the manpower that is required for advancing one generation of inbreeding by single seed descent (22 man days). Therefore, until and unless there are some clear advantages in imposing selections in the initial stages of a breeding programme such as better truncation levels for multiple traits, single seed descent is the most cost effective method for producing inbreds from a cross.

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