Predicting the properties of random inbreds and second cycle hybrids using progeny families and hierarchical selfs of a four parent cross

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Progeny families (first selfs) and hierarchically extracted “second selfs” produced by consecutive selfing of random individuals of a four parent cross, are compared theoretically and experimentally for their accuracy and efficiency in providing estimates of predictors of the properties of random inbred lines and second cycle hybrids extractable from the same cross. In theory, both sources provide biased estimates of the predictors of means and variances both in the absence and presence of complications like epistasis, linkage dis-equilibrium and genotype $\times$ environmental interactions. While some of these biases are trivial and can be ignored for all practical purposes, those incurred by the predictors of the overall mean of random inbred lines ($\bar{D}c_1S_\infty$) are often significant and they can be corrected by estimating $\bar{D}c_1S_\infty$ as $2\bar{D}c_1S1 - \bar{D}c_1$ and $\frac{4}{3}\bar{D}c_1S2 - \frac{1}{3}\bar{D}c_1$, instead of as $\bar{D}c_1S1$ and $\bar{D}c_1S2$, respectively.

Both the “first selfs” and “second selfs” are therefore suitable sources of predictors of random inbred lines only if the above adjustments are incorporated into the predictions procedure. Predictors of second cycle hybrids, on the other hand, provide reliable and accurate predictions without adjustments.

INTRODUCTION

Pooni and Jinks (1985) have described procedures for predicting the properties of random inbred lines and second cycle hybrids that can be extracted from a double cross produced by intermating four pure breeding varieties. These procedures provide useful information on the inbreeding and outcrossing potentials of double crosses and could be used to discriminate between the more and the less productive crosses. However, a large number of generations, many of them only distantly related to the double cross itself are required for making these predictions. Consequently the breeding demands are high and particularly laborious when the material under investigation produces only one or few seeds per cross pollination. This could impose stringent restrictions on the experimental size and decrease the reliability of the results. Under these circumstances it is appropriate to ask

whether simpler and smaller breeding programmes, especially the ones that rely heavily on self-pollination can be acceptable alternative sources of the predictors.

In the present paper we explore the suitability of the progeny families (“first selfs”) and hierarchically extracted “second selfs” of a double cross as alternative sources of predictors. They will be compared theoretically and experimentally for their accuracy and efficiency in providing estimates of parameters that allow us to make predictions with an acceptable level of precision. Theoretically, we shall obtain the expectations of the predictor parameters and determine their biases under simple and complicated genetic situations. In practice, experimental data from two *Nicotiana rustica* experiments, one raised in 1981 and the other in 1983, will be analysed to make predictions and test their validity under field conditions.

RANDOM INBREDS, SECOND CYCLE HYBRIDS AND SOURCES OF PREDICTORS

The breeding programme for producing a double cross and extracting random inbred lines and second cycle hybrids from it is presented in a diagrammatic form in table 1. The double cross (Dc_1) is obtained by crossing two unrelated single crosses (F_1 's) which in turn are produced by crossing two pairs of pure breeding varieties, P_1 with P_2 and P_3 with P_4 . A random sample of n individuals is taken from the double cross and self-pollinated to produce the "first selfs" (Dc_1S_1). Each of these n progeny families (Dc_1S_1) is then advanced to the next generation (Dc_1S_2) by self-pollinating a single individual chosen at random. This procedure of random sampling and self-pollination is repeated until homozygosity is achieved.

Inbreeding by the method of single seed descent yields n inbred lines (Dc_1S_∞) from n individuals of the double cross. Because the number of families remain the same from the beginning to the end of the inbreeding programme, large samples of individuals can be taken from the double cross to take advantage of unrestricted recombination in the early generations and eliminate the risks of genetic drift and gene correlations. Pairwise intermating of these inbred lines should also lead to a better representative sample of

second cycle hybrids that can be extracted from the double cross.

One of the two sources that we shall use for predicting the properties of the random inbreds is the progeny families that are obtained by self-pollinating a random sample of individuals from the double cross generation. From the analysis of these "first selfs" we can estimate the overall mean ($\bar{Dc}_1S_1$) and the within ($\sigma_{wgDc_1S_1}^2$) and between family ($\sigma_{bDc_1S_1}^2$) components of genetic variation. To predict from "first selfs" alone we must therefore assume that $\bar{Dc}_1S_1 \approx \bar{Dc}_1S_\infty$, the overall mean of the random inbreds and that $\sigma_{bDc_1S_1}^2 + 2\sigma_{wgDc_1S_1}^2 \approx \sigma_{bDc_1S_\infty}^2$, the additive genetic variance of the inbred lines.

The second source that we shall use for making predictions is the hierarchical selfs (second selfs) that are extracted from the progeny families (first selfs) described earlier. From each of the "first selfs" a minimum of two plants must be selfed to construct a hierarchical structure amongst the second selfs (Dc_1S_2). Analysis of these hierarchical families provides estimates of their overall mean ($\bar{Dc}_1S_2$) and two between ($\sigma_{b1Dc_1S_2}^2$ and $\sigma_{b2Dc_1S_2}^2$) and a within family ($\sigma_{wgDc_1S_2}^2$) components of genetic variance. To make predictions from the "second selfs" alone we must assume that $\bar{Dc}_1S_2 \approx \bar{Dc}_1S_\infty$ and $\sigma_{b1Dc_1S_2}^2 + \sigma_{b2Dc_1S_2}^2 + 2\sigma_{wgDc_1S_2}^2 \approx \sigma_{bDc_1S_\infty}^2$.

Table 1 Method of single seed descent and the breeding of first selfs, second selfs, random inbred lines and second cycle hybrids from a double cross that is produced by crossing four pure breeding varieties

Table 2 Expected means of the parental, F_1 , double cross, first selfs and second selfs generations and random samples of inbred lines and second cycle hybrids on the additive dominance model of Pooni and Jinks (1985)

Source	Additive genetic components								Dominance components						
	m'	d_1	d_2	d_3	d_4	d_5	d_6	d_7	h_1	h_2	h_3	h_4	h_5	h_6	h_7
P_1	1	-1	1	1	1	1	1	1							
P_2	1	1	-1	1	1	1	-1	-1							
P_3	1	1	1	-1	1	-1	1	-1							
P_4	1	1	1	1	-1	-1	-1	1							
$F_1(P_1 \times P_2)$	1	0	0	1	1	1	0	0	1	1	0	0	0	1	1
$F_1(P_3 \times P_4)$	1	1	1	0	0	-1	0	0	0	0	1	1	0	1	1
Double cross (Dc_1)	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	1	$\frac{1}{2}$	$\frac{1}{2}$
First selfs (Dc_1S_1)	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	0	0	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$
Second selfs (Dc_1S_2)	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	0	0	0	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$
Random inbreds (Dc_1S_∞)	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	0	0	0	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$
Second cycle hybrids (SCH)*	1	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	0	0	0	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$

* Approximated when number of genes segregating in the double cross are small.

Table 3 Contributions of the genetic and environmental parameters (defined by Pooni and Jinks 1985) to the within variances of parental varieties, F_1 's and the double cross generation and the components of variance of the first selfs, second selfs, random inbreds and second cycle hybrids.

Source	Environmental component E	Additive genetic components						
		Vd_1	Vd_2	Vd_3	Vd_4	Vd_5	Vd_6	Vd_7
$P_1, P_2, P_3, P_4, F_1(P_1 \times P_2), F_1(P_3 \times P_4)$	1							
Double cross $\sigma_{wDc_1}^2$	1	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0	$\frac{1}{2}$	$\frac{1}{2}$
First selfs (i) between fams ($\sigma_{wDc_1S_1}^2$)	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0	$\frac{1}{2}$	$\frac{1}{2}$
(ii) within fams ($\sigma_{wDc_1S_1}^2$)	1	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$
Second selfs (i) between hierarchical groups ($\sigma_{bDc_1S_2}^2$)	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0	$\frac{1}{2}$	$\frac{1}{2}$
(ii) between fams/groups ($\sigma_{bDc_1S_2}^2$)	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$
(iii) within families ($\sigma_{wDc_1S_2}^2$)	1	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$
Random inbreds (i) between inbred fams ($\sigma_{bDc_1S_\infty}^2$)	0	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	1	1	1
(ii) within inbred fams ($\sigma_{wDc_1S_\infty}^2$)	1	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$
Second cycle hybrids (i) between SCH fams (σ_{wSCH}^2)*	0	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
(ii) within SCH fams (σ_{wSCH}^2)	1	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{3}{8}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$

Dominance components							Covariance components						
Vh_1	Vh_2	Vh_3	Vh_4	Vh_5	Vh_6	Vh_7	Wdh_1	Wdh_2	Wdh_3	Wdh_4	Wdh_5	Wdh_6	Wdh_7
$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	0	$\frac{1}{4}$	$\frac{1}{4}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	0	0	0
$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$	0	$\frac{1}{16}$	$\frac{1}{16}$	$-\frac{1}{4}$	$-\frac{1}{4}$	$-\frac{1}{4}$	$-\frac{1}{4}$	0	0	0
$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{8}$							
$\frac{1}{64}$	$\frac{1}{64}$	$\frac{1}{64}$	$\frac{1}{64}$	0	$\frac{1}{64}$	$\frac{1}{64}$	$-\frac{1}{8}$	$-\frac{1}{8}$	$-\frac{1}{8}$	$-\frac{1}{8}$	0	0	0
$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{32}$							
$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{16}$							
$\frac{15}{64}$	$\frac{15}{64}$	$\frac{15}{64}$	$\frac{15}{64}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$-\frac{3}{8}$	$-\frac{3}{8}$	$-\frac{3}{8}$	$-\frac{3}{8}$	0	0	0

Neither source is expected to provide estimates of the overall mean ($\bar{SCH}$) and genetic variance (σ_{bSCH}^2) of the second cycle hybrids (SCH) on its own. Thus we shall estimate the mean SCH as $\frac{1}{2}\bar{Dc}_1S1 + \frac{1}{4}\bar{F}_1(1 \times 2) + \frac{1}{4}\bar{F}_1(3 \times 4)$ or $\frac{1}{2}\bar{Dc}_1S1 + \frac{1}{4}\bar{Dc}_1 + \frac{1}{8}\bar{F}_1(1 \times 2) + \frac{1}{8}\bar{F}_1(3 \times 4)$ and the genetic variance σ_{bSCH}^2 as $\frac{1}{2}\sigma_{wgDc_1}^2 + \sigma_{wgDc_1S1}^2$. Here Dc_1 , $F_1(1 \times 2)$ and $F_1(3 \times 4)$ are the means of the double cross and its constituent single crosses and $\sigma_{wgDc_1}^2$ and $\sigma_{wgDc_1S1}^2$ are the genetic components of the within family variances of the double cross and "first selfs", respectively.

Theoretical expectations of the means and components of variances of the two source generations, the double cross and its constituent single crosses and the parental varieties are given alongside those of the random samples of inbreds and second cycle hybrids in tables 2 and 3. All of these expectations with the exception of the two sources have already been derived by Pooni and Jinks (1985) for a simple additive, dominance and additive environmental model. They are listed in these tables for comparison.

Further, there are 24 different ways of producing a double cross from four pure breeding varieties. For example, we can choose to cross $F_1(1 \times 2)$ with $F_1(3 \times 4)$ or $F_1(1 \times 3)$ with $F_1(2 \times 4)$ or $F_1(1 \times 4)$ with $F_1(2 \times 3)$ to produce the double cross. Moreover we may choose the first or the second F_1 , or one of their two reciprocals to be the female parent. The same choices are also available for selecting the male parent. While, however, these choices can lead to a significant difference in the mean performances of the double crosses, the properties of the random inbreds and second cycle hybrids that can be extracted from them are not expected to differ.

THEORETICAL BIASES OF THE PREDICTORS

Random inbreds

It is clear from table 2 that both sources provide biased estimates of $\bar{Dc}_1S_{\infty}$. The estimate from the "first selfs" deviates from $\bar{Dc}_1S_{\infty}$ by $\frac{1}{2}h'$ where $h' (= \frac{1}{2}h_1 + \frac{1}{2}h_2 + \frac{1}{2}h_3 + \frac{1}{2}h_4 + h_5 + \frac{1}{2}h_6 + \frac{1}{2}h_7)$ is a linear dominance component equal to the difference between the double cross mean ($\bar{Dc}_1$) and $\bar{Dc}_1S_{\infty}$ on a simple additive and dominance model. The "second selfs" on the other hand provide an estimate of $\bar{Dc}_1S_{\infty}$ which is biased by $\frac{1}{4}h'$. In the presence of non-allelic interactions these biases change from $\frac{1}{2}h'$ and $\frac{1}{4}h'$ to $\frac{1}{2}h' + \frac{1}{4}j' + \frac{1}{4}l'$ and $\frac{1}{4}h' + \frac{1}{8}j' + \frac{1}{16}l'$ where j' and l' are the additive $\times$ dominance and dominance $\times$ dominance interac-

tion components associated with the mean of the double cross generation. The situation can be further complicated by the presence of genotype $\times$ environmental interactions which could reduce or increase the biases by opposing or reinforcing the directional effects of $\frac{1}{2}h'$ and $\frac{1}{4}h'$ or $\frac{1}{2}h' + \frac{1}{4}j' + \frac{1}{4}l'$ and $\frac{1}{4}h' + \frac{1}{8}j' + \frac{1}{16}l'$ when epistasis is significant. The overall effects of these biases on the predictions will however be determined by their direction and magnitude which may differ with the pairing of the parental varieties in the single crosses.

The predictors of $\sigma_{bDc_1S_{\infty}}^2$, like those of $\bar{Dc}_1S_{\infty}$, are also biased. Again the estimate from "first selfs" is biased approximately twice as much as that from the "second selfs" and these biases fall into two categories. A major proportion of the bias is due to the dominance component of variance which is equal to $\frac{1}{4}(Vh_1 + Vh_2 + Vh_3 + Vh_4 + 2Vh_5 + Vh_6 + Vh_7)$ (see Pooni and Jinks, 1985, for definitions of Vh_i etc.) for "first selfs" and $\frac{1}{8}(Vh_1 + Vh_2 + Vh_3 + Vh_4 + 2Vh_5 + Vh_6 + Vh_7)$ for "second selfs". It is obvious that whenever dominance variation is significant both estimates of $\sigma_{bDc_1S_{\infty}}^2$ will be inflated, that from the "first selfs" more so than that from the "second selfs". The second cause of bias is the covariance component between the additive and dominance effects of those loci which have unequal gene frequencies. Its magnitude, $-\frac{1}{4}(Wdh_1 + Wdh_2 + Wdh_3 + Wdh_4)$ and $-\frac{1}{8}(Wdh_1 + Wdh_2 + Wdh_3 + Wdh_4)$ respectively, however is expected to be relatively small unless there is a systematic preponderance of either plus or minus alleles over the whole range of loci.

Similarly, neither source is expected to provide true estimates of $\bar{Dc}_1S_{\infty}$ and $\sigma_{bDc_1S_{\infty}}^2$ in the presence of epistasis, a linkage disequilibrium and genotype $\times$ environmental interactions. However, excluding the biases described above, they are expected to provide close approximations of $\bar{Dc}_1S_{\infty}$ and $\sigma_{bDc_1S_{\infty}}^2$.

The first and second degree statistics that are available from the double cross and the source generations also provide weighted least squares estimates of $\bar{Dc}_1S_{\infty}$ and $\sigma_{bDc_1S_{\infty}}^2$ for comparison (see Mather and Jinks 1982 for procedures). We can estimate $\bar{Dc}_1S_{\infty}$ which has the expectation $m' + \frac{1}{2}d_1 + \frac{1}{2}d_2 + \frac{1}{2}d_3 + \frac{1}{2}d_4 = m''$ by fitting an m'' and h' model to the means of various generations when they have the following expectations:

$$\bar{Dc}_1 = m'' + h'$$

$$\bar{Dc}_1S1 = m'' + \frac{1}{2}h'$$

and

$$\bar{Dc}_1S2 = m'' + \frac{1}{4}h'.$$

The model provides a comprehensive test for the presence of epistasis as a χ^2 of goodness of fit. The $\chi^2_{(1)}$ takes a significant value only when non-allelic interactions are present. Further tests of epistasis can be conducted by applying the scaling tests $2\bar{D}c_1S1 - \bar{D}c_1 - \frac{1}{4}(\bar{P}_1 + \bar{P}_2 + \bar{P}_3 + \bar{P}_4) = 0$ and $2\bar{D}c_1S2 - \bar{D}c_1S1 - \frac{1}{4}(\bar{P}_1 + \bar{P}_2 + \bar{P}_3 + \bar{P}_4) = 0$, respectively. Significant h' in the absence of epistasis also determines the magnitude and direction of bias that is associated with the predictors of $\bar{D}c_1S_{\infty}$.

We require six parameters, i.e., two additive genetic components, $D' (=Vd_1 + Vd_2 + Vd_3 + Vd_4 + Vd_6 + Vd_7)$ and $D'' (=Vd_1 + Vd_2 + Vd_3 + Vd_4 + 2Vd_5 + Vd_6 + Vd_7)$; two dominance components $H' (=Vh_1 + Vh_2 + Vh_3 + Vh_4 + Vh_6 + Vh_7)$ and $H'' (=Vh_1 + Vh_2 + Vh_3 + Vh_4 + 2Vh_5 + Vh_6 + Vh_7)$; one covariance component $F' (= -Wdh_1 - Wdh_2 - Wdh_3 - Wdh_4)$ and an environmental component E ; to describe the genetical and environmental differences between various components of variances. On this model each component has the following expectation:

Source	Component	Model
Double cross	$\sigma_{wDc_1}^2$	$\frac{1}{4}D' + \frac{1}{4}H' + \frac{1}{2}F' + E$
	$\sigma_{bDc_1S1}^2$	$\frac{1}{4}D' + \frac{1}{16}H' + \frac{1}{4}F'$
First selfs	$\sigma_{b1Dc_1S1}^2$	$\frac{1}{4}D'' + \frac{1}{8}H'' + E$
	$\sigma_{wDc_1S2}^2$	$\frac{1}{4}D' + \frac{1}{64}H' + \frac{1}{8}F'$
Second selfs	$\sigma_{b2Dc_1S2}^2$	$\frac{1}{4}D'' + \frac{1}{32}H''$
	$\sigma_{wDc_1S2}^2$	$\frac{1}{8}D'' + \frac{1}{16}H'' + E$
Parents and F_1 's	σ_w^2	E

Again the model provides a test of significance for each parameter and the goodness of fit of the model is tested as $\chi^2_{(1)}$. Component $\sigma_{bDc_1S_{\infty}}^2$ is estimated by combining D' and D'' as $\frac{1}{4}D' + \frac{1}{2}D''$.

Second cycle hybrids

Predictor estimates of the overall mean ($\overline{SCH}$) and the generation variance (σ_{bSCH}^2) of second cycle hybrids contain comparatively smaller biases. The two estimates of $\overline{SCH}$ that are obtained from $\frac{1}{2}\bar{D}c_1S1 + \frac{1}{4}\bar{F}_1(1 \times 2) + \frac{1}{4}\bar{F}_1(3 \times 4)$ and $\frac{1}{2}\bar{D}c_1S1 + \frac{1}{4}\bar{D}c_1 + \frac{1}{8}\bar{F}_1(1 \times 2) + \frac{1}{8}\bar{F}_1(3 \times 4)$ differ theoretically from $\overline{SCH}$ by $\frac{1}{8}(h_6 + h_7)$ and $\frac{1}{8}(2h_5 + h_6 + h_7)$, respectively. The distortions due to these biases are therefore expected to be negligible unless the dominance components h_5 , h_6 and h_7 have unusually large magnitudes and take the same sign. The biases incurred by the estimate of the variance component, $\frac{1}{64}(Vh_1 + Vh_2 + Vh_3 + Vh_4) - \frac{1}{8}(Wdh_1 + Wdh_2 + Wdh_3 + Wdh_4)$, are minor and can be ignored.

EXPERIMENTS AND RESULTS

Two experiments, one for making predictions and the other for testing the validity of predictions under field conditions were conducted during 1981 and 1983, respectively. The material in the prediction experiment consisted of four *Nicotiana rustica* varieties V_1 , V_5 , V_2 and V_{12} , single crosses $F_1(1 \times 5)$ and $F_1(2 \times 12)$ and their reciprocals, $F_1(1 \times 5) \times F_1(2 \times 12)$ and $F_1(2 \times 12) \times F_1(1 \times 5)$ reciprocals of the double cross and the "first" and "second" selfed generations extracted from the double cross by hierarchical selfing. Sixteen families of a full diallel produced from the parental varieties by pairwise crossing and selfing, four reciprocals $F_1(1 \times 5) \times F_1(2 \times 12)$, $F_1(2 \times 12) \times F_1(1 \times 5)$, $F_1(5 \times 1) \times F_1(12 \times 2)$ and $F_1(12 \times 2) \times F_1(5 \times 1)$ of the double cross generation and 120 Dc_1S6 inbred lines produced from the double cross by the method of single seed descent constituted the experimental material for the assessment experiment. The inbred lines grown in the assessment experiment and the "first selfs" and "second selfs" raised in the prediction experiment were extracted from equal numbers of plants taken at random from the reciprocals $F_1(1 \times 5) \times F_1(2 \times 12)$ and $F_1(2 \times 12) \times F_1(1 \times 5)$ of the double cross. Further details of the structure and dimensions of these experiments are given in table 4.

The material in both experiments was individually randomised in single blocks. Individual plants were scored for height at 4 and 6 weeks (H_4 and H_6) after transplanting in the field, flowering time from an arbitrary date (FT), height at the time of flowering (HFT), length (LL) and width (LW) of the largest leaf blade and final plant height (FH) at the end of the season. All characters were recorded in centimetres (to the nearest $\frac{1}{2}$ cm) except flowering time which was recorded in days. The data were processed through the University's Multics Computer to obtain the means and variances of all generations and carry out analyses of variance wherever appropriate.

The overall means of various generations obtained by averaging over reciprocals whenever reciprocal differences were significant, are given for the seven characters in table 5. To make predictions from the "first selfs" and "second selfs" alone we must assume that their means provide appropriate estimates of $\bar{D}c_1S_{\infty}$, the overall mean of the random inbreds that can be extracted from the double cross. To establish, however, the direction and magnitude of biases incurred by each of these estimates and to test the adequacy of a simple additived dominance model, we obtain weighted

Table 4 Structure and dimensions of the prediction and assessment experiments conducted during 1981 and 1983, respectively

Material	Details	No. of families	Family size	Total plants
(a) <i>Prediction Experiment (1981)</i>				
1. Parents	V_1, V_5, V_2 and V_{12}	4	30	120
2. Single crosses	$F_1(1 \times 5), F_1(5 \times 1), F_1(2 \times 12)$ and $F_1(12 \times 2)$	4	30	120
3. Double crosses	$F_1(1 \times 5) \times F_1(2 \times 12)$ and $F_1(2 \times 12) \times F_1(1 \times 5)$	2	20	40
4. First selfs	120 Double cross plants selfed (Dc_1S1)	120	10	1200
5. Second selfs	2 plants selfed from each of 120 first selfs (Dc_1S2)	240	10	2400
(b) <i>Assessment experiment (1983)</i>				
1. Parents and single crosses	Full diallel of V_1, V_5, V_2 and V_{12}	16	20	240
2. Double crosses	$F_1(1 \times 5) \times F_1(2 \times 12), F_1(2 \times 12) \times F_1(1 \times 5), F_1(5 \times 1) \times F_1(12 \times 2)$ and $F_1(12 \times 2) \times F_1(5 \times 1)$	4	25	100
3. Random inbreds	120 Dc_1S6 families	120	10	1200

least squares estimates of parameters m'' and h' and apply the scaling tests described in the previous section. The predictor estimates of SCH, the overall mean of the second cycle hybrids are obtained for each character by substituting the values of the relevant means in the formulae $\frac{1}{2}\bar{D}c_1S1 + \frac{1}{4}\bar{F}_1(1 \times 5) + \frac{1}{4}\bar{F}_1(2 \times 12)$ and $\frac{1}{2}\bar{D}c_1S1 + \frac{1}{4}\bar{D}c_1 + \frac{1}{8}\bar{F}_1(1 \times 5) + \frac{1}{8}\bar{F}_1(2 \times 12)$. These predictors and the tests of epistasis are tabulated in table 7.

The within family variances of the parental, F_1

and double cross generations and the mean squares of various items in the analyses of variance of the "first selfs" and "second selfs", are given in table 6. For the "first selfs" the significance of each between family mean square was tested against the corresponding within variance. For the "second selfs" between second selfs within first selfs mean squares were tested against the within family variances and between first selfs mean squares against the between second selfs within first selfs mean

Table 5 Mean performances of the parental F_1 , double cross, first selfs and second selfs for seven characters (prediction experiment, 1981)

Generation	n	Characters						
		H_4	H_6	FT	HFT	LL	LW	FH
V_1	29	11.60 +0.42	45.27 ±1.15	38.10 ±0.42	59.95 +1.38	21.65 ±0.49	20.55 ±0.53	114.67 ±1.22
V_5	30	10.70 ±0.53	49.42 ±1.82	39.20 ±0.61	70.67 ±2.21	21.02 ±0.48	20.15 ±0.48	132.10 ±2.23
$F_1(1 \times 5)$	60	12.62 ±0.41	52.83 ±1.36	38.32 ±0.39	71.39 ±1.71	23.03 ±0.35	22.14 ±0.48	141.07 ±1.34
V_2	30	5.29 +0.33	18.53 ±2.01	57.63 ±0.73	73.31 +2.67	21.77 ±0.57	16.47 ±0.51	97.87 +1.42
V_{12}	30	14.93 ±0.63	63.93 +2.02	41.43 ±0.39	96.28 ±1.82	24.39 ±0.55	19.92 ±0.50	148.37 ±1.41
$F_1(2 \times 12)$	60	11.47 ±0.44	51.78 ±1.27	46.45 ±0.40	118.47 ±1.82	27.89 ±0.28	25.67 ±0.35	186.09 +1.28
Double cross (1×5) $\times$ (2×12)	40	10.96 ±0.51	47.14 ±1.81	42.65 ±0.89	84.50 +3.28	24.73 ±0.57	22.41 ±0.62	150.95 +3.53
First selfs	1185	11.31 ±0.25	45.51 +0.92	42.84 ±0.45	80.46 ±1.31	23.22 ±0.17	20.53 ±0.17	142.00 ±1.31
Second selfs	2359	10.83 ±0.28	43.41 ±0.95	43.33 ±0.49	78.69 +1.08	22.45 ±0.18	19.39 ±0.18	135.68 +1.27
$\frac{1}{4}(V_1 + V_5 + V_2 + V_{12})$	119	10.63 ±0.25	44.29 ±1.02	44.09 ±0.28	75.05 ±1.04	22.21 ±0.26	19.27 ±0.25	123.13 ±0.81

Table 6 Within family variances of the parental, single cross and double cross generations and the analyses of variance (mean squares) of the first and second selfs for the seven characters

Generation	df	Characters						
		H ₄	H ₆	FT	HFT	LL	LW	FH
(a) <i>Parents and F₁'s</i>								
V ₁	28	5.35	39.44	5.38	57.32	7.26	8.39	44.84
V ₅	29	8.55	99.05	11.34	146.77	7.05	6.90	149.31
F ₁ (1 × 5)	58	10.07	110.41	9.32	175.58	7.18	13.80	107.94
V ₂	29	3.27	121.61	15.81	213.61	9.62	7.91	60.35
V ₁₂	29	12.09	121.87	4.54	98.89	9.08	7.59	59.24
F ₁ (2 × 12)	58	11.79	96.18	9.31	198.89	4.76	7.48	98.68
E	235	8.52	98.09	9.28	148.51	7.49	8.68	86.73
(b) <i>Double cross generation</i>								
Double cross	39	10.26	130.96	31.39	431.10	12.95	15.19	499.14
(c) <i>First selfs of the double cross generation</i>								
Between families	119*	76.38	1006.25	237.79	2044.59	33.22	33.16	2042.55
Within families	1065	14.12	141.26	26.03	271.19	12.42	13.11	372.85
(d) <i>Second selfs of the double cross generation</i>								
Between first selfs groups	119*	190.05	2143.99	558.48	4171.41	73.97	73.43	3825.37
Between second selfs/first selfs groups	120*	56.96	712.83	221.99	999.22	30.84	32.40	2070.59
Within second selfs families	2119	12.74	127.71	23.89	325.59	9.83	10.52	289.33

* Each mean square is highly significant against its appropriate error.

Table 7 Estimates of the predictors of the overall mean and genetic standard deviation of the first cycle random inbreds and second cycle hybrids and tests for the presence of non-allelic interactions

Source	Characters						
	H ₄	H ₆	FT	HFT	LL	LW	FH
(a) <i>Overall mean of random inbreds ($\bar{D}c_1S_{\infty}$)</i>							
First selfs	11.31	45.51	42.84	80.46	23.22	20.53	142.00
Second selfs	10.83	43.41	43.33	78.69	22.45	19.39	135.68
W.L.S.	11.08	42.40	43.01	79.95	21.69	18.36	130.61
(b) <i>Genetic s.d. of random inbreds ($\sigma_{bDc_1S_{\infty}}$)</i>							
First selfs	4.18	13.15	7.39	20.56	3.65	3.30	18.10
Second selfs	4.42	13.76	6.92	24.09	2.99	2.82	25.90
W.L.S.	4.05	13.77	7.63	17.59	2.81	2.77	19.12
(c) <i>Overall mean of second cycle hybrids ($\overline{SCH}$)</i>							
Single crosses and first selfs	11.68	48.91	42.61	87.70	24.34	22.22	152.79
Single crosses, double cross and first selfs	11.41	47.62	42.68	85.09	24.16	21.84	149.63
(d) <i>Genetic s.d. of second cycle hybrids (σ_{SCH})</i>							
Double cross and first selfs	2.54	7.72	5.27	16.25	2.89	2.77	16.92
(e) <i>Tests of epistasis</i>							
W.L.S. $\chi^2_{(1)}$	1.49 ^{NS}	0.46 ^{NS}	0.18 ^{NS}	0.93 ^{NS}	0.00 ^{NS}	0.21 ^{NS}	0.39 ^{NS}
$2\bar{D}c_1S1 - \bar{D}c_1 - \frac{1}{4}(P_1 + P_2 + P_3 + P_4)$	1.03 ^{NS}	-0.41 ^{NS}	-1.06 ^{NS}	1.37 ^{NS}	-0.50 ^{NS}	-0.62 ^{NS}	9.92*
$2\bar{D}c_1S2 - \bar{D}c_1S1 - \frac{1}{4}(P_1 + P_2 + P_3 + P_4)$	±0.76	±2.78	±1.28	±4.33	±0.71	±0.75	±4.48
	-0.28 ^{NS}	-2.98 ^{NS}	-0.27 ^{NS}	1.87 ^{NS}	-0.53 ^{NS}	-1.02*	6.23*
	±0.67	±2.35	±1.11	±3.14	±0.47	±0.47	±2.98

squares whenever the latter were significant. Every one of these tests was significant at 1 per cent level of significance. These mean squares provide estimates of the between and within family components of variances that we shall use for obtaining the predictor estimates of the variances of inbred lines and second cycle hybrids. The weighted least squares estimate of $\sigma_{bDc_1S_{\infty}}^2$ of a random sample of inbreds is obtained by fitting a D' , D'' , H' , H'' , F' and E model to these mean squares and to the variances of the double cross and parental and F_1 generations. The predictors of variances are presented as standard deviations $\sigma_{bDc_1S_{\infty}}$ and σ_{bSCH} with the predictors of the overall means of random inbreds and second cycle inbreds in table 7.

We shall use these predictors of $\bar{Dc}_1S_{\infty}$, $\overline{SCH}$, $\sigma_{bDc_1S_{\infty}}^2$ and σ_{bSCH}^2 to predict the transgressive properties of random inbreds and second cycle hybrids. The standards we expect them to transgress are the largest ($St_{largest}$) and the smallest ($St_{smallest}$) scoring genotypes amongst the four parental varieties and two single crosses (see table 4). Following Jinks and Pooni (1976) and Toledo, Pooni and Jinks (1984a; 1984b) we obtain the probabilities of the transgressive segregants in the upper and lower tails of each distribution by integrating the standard normal density functions

from

$$\frac{\bar{St}_{largest} - \bar{Dc}_1S_{\infty}}{\sigma_{bDc_1S_{\infty}}}$$

to $+\infty$ and from $-\infty$ to

$$\frac{\bar{St}_{smallest} - \bar{Dc}_1S_{\infty}}{\sigma_{bDc_1S_{\infty}}};$$

and from

$$\frac{\bar{St}_{largest} - \overline{SCH}}{\sigma_{bSCH}},$$

to $+\infty$ and from $-\infty$ to

$$\frac{\bar{St}_{smallest} - \overline{SCH}}{\sigma_{bSCH}}$$

respectively. These integrals were calculated for each character separately and then multiplied by 120, the number of random inbreds available from the double cross, to convert them into expected numbers. These are given in table 8.

The corresponding observed numbers however are available only for random samples of inbreds because random samples of second cycle hybrids were not included in the assessment experiment

Table 8 Predicted and observed numbers (out of 120) of random inbreds and predicted numbers of second cycle hybrids that score $\geq St_{largest}$ or $\leq St_{smallest}$

Population	Source	Character						
		H ₄	H ₆	FT	HFT	LL	LW	FH
(a) $\geq \bar{S}t_{largest}$								
First cycle random inbreds	First selfs	23	10 (9) [†]	3	4	12 (5)	7 (2)	1 (0)
	Second selfs	21	8 (7)	2	6	4 (2)	2 (1)	3 (2)
	W.L.S.*	21	7	3	2	2	1	0
	Observed	21	3	5	2	6	2	0
Second cycle hybrids	First selfs and F ₁ 's	12	3	0	3	13	13	3
	First selfs, double cross and F ₁ 's	10	2	0	2	12	10	2
(b) $\leq \bar{S}t_{smallest}$								
First cycle random inbreds	First selfs	9	3 (4)	31	19	33 (51)	13 (31)	1 (3)
	Second selfs	13	4 (5)	27	26	38 (49)	18 (30)	9 (12)
	W.L.S.	9	5	31	15	49	30	5
	Observed	9	2	26	28	26	35	5
Second cycle hybrids	First selfs and F ₁ 's	1	0	23	5	15	2	0
	First selfs, double cross and F ₁ 's	1	0	23	7	17	3	0

* W.L.S. = weighted least squares.

[†] Estimated from adjusted estimates of $\bar{Dc}_1S_{\infty}$ (see conclusions).

of 1983. These observed numbers were obtained by counting the inbreds that scored either $\geq \bar{S}t_{\text{largest}}$ or $\leq \bar{S}t_{\text{smallest}}$. The standards used in both the prediction and assessment experiments were the same except for height at flowering time where V_1 was replaced by V_5 as S_{smallest} in the assessment experiment. The observed numbers are listed alongside the predicted numbers in table 8.

CONCLUSIONS

Estimates of the predictors of the properties of random inbreds and second cycle hybrids that can be extracted from a four parent cross can be compared for their reliability on two criteria. The first and most obvious criterion is whether the theoretical expectations of the predicted and observed statistics are the same. On this criterion both sources are unreliable even in the absence of complications like non-allelic interactions, linkage disequilibrium and genotype $\times$ environment interactions. Predictors estimated from the "first selfs" are however expected to differ more from the observed statistics than those from the "second selfs" because the magnitudes of their biases are twice as large (see section on "Theoretical biases of the predictors" and tables 2 and 3). On the other hand, the two sources differ only marginally if we consider the second criteria, namely, the sampling errors of the estimates of the predictors. While those from "second selfs" are always expected to have the larger sampling errors when the experimental size is the same, their relative magnitudes can be easily manipulated by altering the structure of the experiment. Consequently the relative reliabilities of the two sources are expected to differ from experiment to experiment.

Predictors of the overall mean and genetic variance of the second cycle hybrids also differ theoretically from the corresponding observed statistics. These differences, however, are exceptionally small and often involve no more than small fractions of the dominance components of means and variances. Furthermore, these predictors are subjected to much smaller sampling errors than those of random inbred lines (see sections on "Theoretical biases of predictors" and tables 2 and 3).

The experimental results in table 7 are in general agreement with these theoretical conclusions.

For characters H_6 , LL, LW and FH the estimates of the predictors of $\bar{D}c_1S_\infty$ obtained from "first selfs", "second selfs" and weighted least squares analysis always rank 1, 2 and 3 following the contribution of the principal bias, the dominance component h' , which is positive and highly significant for these characters. For the remaining characters this ranking does not occur because neither the dominance nor epistatic component of the means was significant. Similar patterns expected between the estimates of the predictors of the variances, however, are masked by their larger standard errors. Nonetheless, it is clear that the predictors of $\sigma_{bDc_1S_\infty}$ are highly consistent over all the sources for H4, H6 and FT but less so for HFT, LL, LW and FH. The largest discrepancies between the estimates are for HFT and FH and in both cases it is the estimates from "second selfs" that are unreliable. Estimates of $\bar{S}CH$ on the other hand are highly consistent for all the characters.

The predictions of the numbers of random inbred lines that transgress the standards like the predictors themselves are biased in the direction of the dominance whenever it is significant (see table 8). Therefore, neither "first selfs" nor "second selfs" can be used alone to make predictions that can be considered reliable. However, the reliability of either source is improved appreciably if we substitute $2\bar{D}c_1S_1 - \bar{D}c_1$ and $\frac{4}{3}\bar{D}c_1S_2 - \frac{1}{3}\bar{D}c_1$ for $\bar{D}c_1S_1$ and $\bar{D}c_1S_2$ as predictors of $\bar{D}c_1S_\infty$ (see predictions given in brackets in table 8).

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