The predictive value of quantitative genetic parameters

De voorspellende waarde van schattingen van kwantitatief-genetische parameters voor de veredeling van zelfbevruchtende gewassen

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The predictive value of estimates of quantitative genetic parameters in breeding of autogamous crops

Proefschrift
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Stellingen

1. Bij de veredeling van zelfbevruchtende gewassen kan het selektiekriterium gebaseerd zijn op schattingen van kwantitatief-genetische parameters. Deze methode voldoet niet, indien de in het selektie-milieu aan genotypen gemeten fysieke grootheid niet in redelijke mate overeenkomt met dezelfde fysieke grootheid van deze genotypen in het doel-milieu.

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2. Bij een hoge tussen-lijnen erfelijkheidsgraad ligt de schatting van de genetische variantie van een F_3 gemiddeld dichter bij de waarheid dan op grond van een Williams-Tukey betrouwbaarheidsinterval te verwachten is.

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3. Een publikatie van een genetische kaart, die geen melding maakt van de statistische betrouwbaarheid van de positie van de loci, is onbetrouwbaar.

Helentjaris, TIG (1987) 3: 217-221; Young & Tanksley, TAG (1989) 77: 95-101

4. De grote interesse in de literatuur voor de kans op negatieve ANOVA-schattingen van variantiekomponenten leidt de aandacht af van het werkelijke probleem: de relatief grote mate van onnauwkeurigheid van schatters van variantiekomponenten.

Bridges & Knapp, TAG (1987) 74: 269-274; Tan & Wong, Biom.J. (1978) 20: 69-79; Verdooren, Biom.J. (1982) 24: 339-360

5. Bij de ontwikkeling van een praktisch toepasbaar model dient men de uiteindelijke bruikbaarheid van het model te toetsen aan realistische praktijkomstandigheden in plaats van aan ándere modelsystemen.

Jinks & Pooni, Heredity (1976) 36: 253-266, en Heredity (1980) 45: 305-312

6. Met behulp van over het genoom verspreide merkergenen verloopt de introgressie van een gen in een ras van een zelfbevruchtend gewas enkele malen doelmatiger dan met konventionele methoden.

7. Het in gebruik nemen van snellere methoden door plantenveredelingsbedrijven komt overeen met een wapenwedloop.

8. Een "QTL" (quantitative trait locus) is een hoofdgen (major gene).

Paterson et al, Nature (1988) 335: 721-726

9. Honden moeten wettelijk worden gelijk gesteld aan wapens.

Stellingen behorend bij het proefschrift "The predictive value of estimates of quantitative genetic parameters in breeding of autogamous crops" van Johan W. van Ooijen

Wageningen, 29 november 1989

Aan mijn ouders
Aan Anne-marie

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Contents

Voorwoord

Dit proefschrift is het eindprodukt van mijn promotie-onderzoek, uitgevoerd aan de vakgroepen erfelijkheidsleer en plantenveredeling. Het is een samenbundeling van drie artikelen die in wetenschappelijke tijdschriften gepubliceerd of in de pers zijn, en een hoofdstuk waarvan tezijnertijd nog één of twee artikelen van geschreven gaan worden. Dit geheel wordt voorafgegaan door een inleidend hoofdstuk, en afgesloten met een algemene diskussie.

U moet dit boekje niet zien als een produkt van slechts één persoon. Er zijn een groot aantal mensen betrokken geweest bij het onderzoek, zowel bij de praktische uitvoering van de proeven, als bij het uiteindelijke opschrijven van de resultaten in wetenschappelijke artikelen. Daarom wil ik op deze plaats de mensen nog eens noemen en bedanken voor hun bijdrage.

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Johan W. van Ooijen

1. General introduction

During the last four decades quantitative genetics theory has developed models in order to provide a scientific basis for the selection on quantitative characters in self fertilising crops. With the quantitative genetic models, among other possibilities, the genotypic variation can be described, and more importantly, the progeny of crosses between pure lines can be predicted. The prediction concerns the mean and variance of the F_∞-generation. Knowing the mean and variance, and assuming a normal distribution, the probability of obtaining superior segregants in the F_∞-progeny of a cross can be calculated. In a breeding programme the two parameters (the F_∞-mean and F_∞-variance) can be estimated in an early generation (e.g. the F_3) for all crosses. Subsequently, the probability to obtain segregants superior to a certain threshold level can be predicted for each cross. The breeder can select the most promising crosses, and concentrate in the subsequent breeding programme on the progeny of these crosses.

Though the theory has been available for some time now, the only current usage of the theory in practical plant breeding is describing the amount of genotypical variation, and choosing accordingly the appropriate selection method by some rule of thumb. Practical plant breeding does not apply the prediction procedure, because of serious doubt about its predictive value. The predictive value has only been established for traits with high heritability (c.f. Jinks & Pooni, 1976, 1980; Snape & Parker, 1986). The prediction procedure is prone to various types of errors, which possibly invalidate the procedure: 1) stochastic variation, 2) the genetic assumptions on which the theory is founded are incorrect, and 3) genotype-environment interaction, in particular intergenotypic competition. The present study intends to evaluate the prediction procedure by studying the effects of the individual sources of error. The study has employed field experiments, computer simulation, and mathematical statistics theory.

The estimation and prediction procedure, and the assumptions

In order to predict the probability of obtaining superior segregants in the F_∞-progeny of a cross, one needs to know the probability distribution of the quantitative character of this F_∞-progeny. It is generally assumed that a quantitative trait is determined by a large number of independently segregating genes with equal individual effects on the genotypic value. A second assumption

is that epistatic effects are absent, i.e. there is no interaction between the loci. If these assumptions are valid, then the F_∞-generation (when it is obtained without selection) has a normal probability distribution, which is fully determined by its mean and variance.

This mean and variance must be estimated using an early generation of the cross, so that the plant breeder can predict the F_∞-progeny as early as possible, and hence make an early decision on whether to select the cross for the succeeding breeding programme. A number of estimation methods, which have been developed, such as the North Carolina experiment III (Comstock & Robinson, 1952), the triple test cross design (Kearsey & Jinks, 1968), and the method using basic generations (F_1, F_2, B_1 and B_2) described by Jinks & Perkins (1970), require large numbers of test crosses to be evaluated. Since this is very labour intensive, it makes these methods very unattractive for application in practical breeding. The present study concentrates upon the procedure, which employs the F_3-generation. The F_3 is still an early generation, that can be obtained without further crossing, and it has the advantage over the F_2 of having more individuals to assess, and thus offers a greater precision for the estimation of the parameters. Another advantage is that the dominance component of the genotypic effects (if present) ([h] in the terminology of Mather & Jinks, 1971) in the F_3 is half the size of that in the F_2.

A breeding programme employing the F_3 has the following appearance. A number of crosses are made between pure breeding lines. The F_1's and F_2's are grown, and if necessary, selection between crosses is applied for qualitative traits only. The F_3's are grown in an appropriate statistical design, that enables the mean (m_{F3}) and the between and within line genotypic variance (V_{1F3} and V_{2F3} respectively) to be estimated for each F_3. An assumption, necessary with respect to certain confidence intervals of the estimates, is that the residual variances, i.e. both the genotypic and the environmental, are homoscedastic. This means that all F_3-lines should have equal residual variances. For a good comparability of the F_3's the design has to ensure, that there are no non-genetic systematic differences between the F_3's, and that the random differences are as small as possible. The estimated F_3-mean is taken as the prediction of the F_∞-mean:

$$\hat{m}_{F\infty} = \hat{m}_{F3}.$$

Under the above mentioned assumptions the F_∞-variance ($V_{F\infty}$) equals the additive component of genotypic variance (D), while V_{1F3} and V_{2F3} are different functions of both the additive and the dominance (H) component of the genotypic variance:

$$V_{1F3} = \frac{1}{2} \cdot D + \frac{1}{16} \cdot H, \quad \text{and}$$

$$V_{2F3} = \frac{1}{4} \cdot D + \frac{1}{8} \cdot H.$$

The unbiased estimator of D is taken as the predictor of the F_∞-variance:

$$\hat{\underline{V}}_{F\infty} = \hat{\underline{D}} = \frac{4}{3} \cdot (2 \cdot \hat{\underline{V}}_{1F3} - \hat{\underline{V}}_{2F3}).$$

The definition of superior segregants in the F_∞ depends on the breeding goal. A logical choice would be the lines superior to the level of the currently best cultivar, or, probably better, superior to the expected level of the cultivars at the time when the breeding programme has to produce the new cultivar. This level will be called the selection threshold level (T). Since we have a prediction for the mean and the variance of each F_∞-progeny, and we have defined a common threshold level, we can predict for each F_∞-progeny the probability of obtaining superior segregants (P_T). This prediction is based upon the assumption that the genotypic values of the F_∞ follow a normal distribution:

$$\hat{\underline{P}}_T = Pr(\hat{\underline{m}}_{F\infty} + (\sqrt{\hat{\underline{V}}_{F\infty}}) \cdot \underline{x} > T) \qquad (\underline{x} \text{ is a standard normal random variable}).$$

The crosses with the highest probabilities are selected for further line breeding. The numbers of evaluated and selected crosses depend on the capacity of the breeding programme; this is not subject of the present study. The justification for the use of a normal distribution of genotypic values rests on the assumption, that in a quantitative trait many genes with small individual effects are involved.

Error through stochastic variation

The prediction of the F_∞ is based on estimated parameters. The estimators are random variables. The stochastic variation is caused by genetic sampling and by environmental (residual) error. The latter includes the internal developmental differences that occur in plants. An F_3-population of finite size is a genetic sample (through the meiosis of the F_1 and the F_2) of all possible F_3-genotypes that are embedded in the F_1. Residual and genetic sampling error determine the accuracy of the estimators. Jinks & Pooni (1980) introduced an alternative method of estimating the additive genotypic variance, which showed an improved accuracy relative to the above mentioned estimator. The method performs a trade-off between bias and variance. Jinks & Pooni did not extend their conclusion on the accuracy of the estimator beyond their specific case of

two traits in tobacco.

The accuracy of both estimators can be improved by taking more F_3-lines, by increasing the number of plants per line, and/or by cultural practices for reducing environmental error. Chapter 2 presents for both estimators an optimization of the F_3-structure (i.e. the number of plants per line) given the F_3-size, such that each estimator has minimum mean square error. Subsequently, both estimators are compared, each under their optimum F_3-structure, for various combinations of heritability, dominance level and F_3-size.

Bias through invalidity of (genetic) assumptions of the theory

One of the important assumptions in the quantitative genetic theory on autogamous crops is that the studied quantitative trait is determined by a large number of independently segregating genes of small effect. This assumption enables the theory to utilize the normal distribution (because of the central limit theorem), which greatly simplifies further estimation and prediction procedures (c.f. Bulmer, 1985). However, careful study of some traits that were previously believed to be polygenic turned out to be oligogenic or even monogenic (Thompson & Thoday, 1974). It is very difficult, not to say virtually impossible, to obtain an accurate estimate of the number of genes, that are involved in the segregation of a quantitative trait, just by studying its phenotypic frequency distribution (Thoday & Thompson, 1976). This may have important consequences for the applicability of the theory. Simulation studies with data of a quantitative trait in *Arabidopsis thaliana*, which was known to be determined by two independently segregating genes, produced some interesting results regarding the precision of the estimate of D. This study is described and elaborated in chapter 3.

In this *Arabidopsis* study violations of the assumption of homoscedasticity were encountered. First, if a quantitative trait is determined by only two loci, then the various lines will differ in the genotypic within line variance, because some lines will segregate for both loci, some for one locus, and some will not segregate at all. So, in this case the requirement of homoscedasticity of residual genotypic effects cannot be satisfied through the very nature of genetic segregation in the generation following a cross between two pure lines. This effect will, of course, diminish when many loci are involved. The second violation of homoscedasticity in the *Arabidopsis* study was that the various genotypes had rather deviating environmental variances. Often an observed heterogeneity of variances can be cured by a suitable transformation of the

data. For some data, though, it may be hard to find a proper transformation. Chapter 3 describes the investigations on the robustness of the estimation of D to heterogeneity of variances.

The genotypic variance components of a breeding population are usually considered as parameters of the probability distribution from which the actual population was sampled. Consequently, statements about these parameters, such as confidence intervals, apply to this conceptual probability distribution. In the case of a cross between two pure lines this means that the confidence interval for the parameter D is a characteristic of the cross. D is the genotypic variance of the F_∞-generation to be obtained by subsequent selfing an infinite number of plants. The plant breeder, however, is not so much interested in parameters of this probability distribution, i.e. the potency of the cross, but rather in the potential future of the actual, and finite, F_3-population. The estimated value will on the average be closer to the true value of the actual sample than to the true value of the cross from which the actual F_3 was sampled. As a consequence the confidence interval for the parameter D (the method of Williams and Tukey, described by Boardman, 1974) will be conservative. There is no standard method for a confidence interval of D, that is correct for inference with respect to the actual F_3. The behaviour of the Williams-Tukey confidence interval on D, when the inference concerns the current F_3, is studied by means of computer simulation in chapter 3.

Bias caused by genotype-environment interaction

Normally, when a quantitative trait is investigated in an early breeding generation it is assumed (sometimes tacitly), that it corresponds to the same phenotypic trait in the commercial growing environment, which is the environment the breeding programme is aimed at. One of the characteristics of an early generation breeding method is, that, as a consequence of genetic segregation, each evaluated population consists of many different genotypes. For an agriculturally important trait like grain yield of wheat or barley, it is known that yield of a genotype measured in a mixed stand of many genotypes can deviate substantially from yield of the same genotype in a pure stand (monoculture) (Spitters, 1979, 1984). This phenomenon is called intergenotypic competition. Spitters (1984) concluded that competitive ability in spring wheat is uncorrelated to yield capacity in a pure stand. Yield assessed in an F_3 of wheat is subject to intergenotypic competition. So, in this case the trait measured in the early breeding generation does not correspond to the same phenotypic

trait in the commercial growing condition. As a consequence parameters like m and D are also affected by intergenotypic competition. To reduce the effects of intergenotypic competition, it is sometimes advised to grow at very wide stands (Fasoulas, 1977). But in that case the adverse effects of intergenotypic competition are replaced by the adverse effects of differential reactions of genotypes to wide stands (Spitters, 1979).

In this thesis the growing conditions of an F_3, with its mixture of many genotypes, are referred to as the "selection environment", whereas the commercial growing conditions are referred to as the "goal environment". Intergenotypic competition is a specific type of genotype-environment interaction. It is specific to the proposed early generation breeding system. Other types of genotype-environment interaction, such as genotype-location and genotype-season interaction, are not specific to this breeding system. On the contrary, any breeding system will have to cope with the problems that arise from these interactions. Chapter 4 and 5 present the research on the effects of intergenotypic competition on the estimation of the parameters m and D, respectively. The research was performed with spring wheat. The experiments were set up in such a way, that estimation of the parameters (m and D) in both the selection environment and in the goal environment was possible. For this purpose F_3's were simulated in a special way, called "pseudo-lines" method. In the "pseudo-lines" method Mendelian segregation is mimicked by using mixtures of true breeding genotypes (varieties and other accessions). On the one hand, simulated F_3's were grown according to the proposed procedure, imitating a practical breeding programme with realistic plot sizes, numbers of lines, etc.; this enabled estimation of parameters in the selection environment. On the other hand, large monoculture trials of the varieties, that were used for the simulation of the F_3's, enabled calculation of the same parameters in the goal environment.

Linkage and epistasis

It is most likely that the assumptions of absence of linkage and epistasis will be violated in many quantitative genetic traits. A number of studies (Weber, 1982; Kearsey, 1985) conclude that the influence of linkage is unimportant. When a trait is determined by many loci, it is very likely that these loci will be scattered over all chromosomes. Since chromosomes segregate independently, the loci will more or less behave as independent linkage blocks (corresponding to the chromosomes) with joint genotypic effects of the loci within the blocks.

The presence of epistasis can be tested with the so-called analysis of means (Mather & Jinks, 1971; Bulmer, 1985). Subsequently, epistatic variances can be included in the estimation and prediction procedure. Expressions have been derived that include only digenic interactions (Van der Veen, 1959), but there seems little reason why higher interactions should not be important if epistasis is present at all (Bulmer, 1985). However, the formulas become very complicated with many parameters, that have to be estimated. As a consequence, the experimental size necessary to obtain accurate estimates of the interaction parameters would be far beyond a manageable breeding programme.

Effects of linkage and epistasis are not the subject of a separate chapter, but they are discussed briefly in chapters 2, 4 and 5.

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2. Estimation of additive genotypic variance with the F_3 of autogamous crops

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Summary

The additive genotypic variance, D, estimated with the F_3 of autogamous crops can be taken as an estimate of genotypic variance of its F_∞-progeny. Two possible ways of estimating D are compared on the basis of their mean square error. For each of the two estimators the F_3-population design, i.e. the number of lines, the number of plants per line and the number of parent plants, is chosen such that for a given experimental capacity its mean square error is minimal. Subsequently the two estimators are compared for various combinations of F_∞-heritability, dominance level and experimental size. In by far the most cases the second estimator, $\underline{D}_2$, which takes twice the between F_3-line genotypic variance as its estimate, outperforms the first estimator, $\underline{D}_1$, which uses both the between and the within F_3-line genotypic variance. Further it is shown that, when it is necessary to work with plot totals because of low F_∞-heritability, the performance of $\underline{D}_1$ becomes very poor. With respect to the estimator of the dominance component of genotypic variance, H, its very large mean square error and its highly negative correlation with $\underline{D}_1$ are demonstrated.

INTRODUCTION

Quantitative genetic theory has developed models that enable the prediction of the F_∞-progeny (its genotypic mean and variance) of a cross between two pure-breeding lines (e.g. Mather & Jinks, 1971). With the predicted mean and variance, and with a normality assumption, the ability of the cross to produce superior inbred lines can be predicted (Jinks & Pooni, 1976). The necessary parameters have to be estimated in a time and labour extensive way in order to be applicable in a practical breeding programme. One of the few approaches that meet these requirements is the method employing the F_3-generation. This paper concentrates on the estimation of the F_∞-variance. In the absence of epistasis and linkage this F_∞-variance equals the additive genotypic variance D. We will assume that epistasis and linkage are absent, but in the discussion we will comment on these assumptions and try to relax these assumptions.

There are two straightforward methods to estimate the additive genotypic variance D from an F_3 of a cross between two inbred lines. One method is to estimate the genotypic between line variance (V_{1F3}) and the genotypic within line variance (V_{2F3}), and successively estimate D and H (H is the dominance component

of the genotypic variance). Since the genotypic variances are different linear combinations in D and H (e.g. Mather & Jinks, 1971):

$$V_{1F3} = \frac{1}{2}\cdot D + \frac{1}{16}\cdot H \quad \text{and} \quad V_{2F3} = \frac{1}{4}\cdot D + \frac{1}{8}\cdot H,$$

D and H can be estimated from the estimated V_{1F3} and V_{2F3} (defining estimators $\underline{D}_1$ and $\underline{H}_1$):

$$\underline{D}_1 \overset{\text{def.}}{=} \frac{4}{3}\cdot(2\cdot\hat{\underline{V}}_{1F3} - \hat{\underline{V}}_{2F3}), \quad \text{and} \tag{1}$$

$$\underline{H}_1 \overset{\text{def.}}{=} \frac{16}{3}\cdot(2\cdot\hat{\underline{V}}_{2F3} - \hat{\underline{V}}_{1F3}). \tag{2}$$

The second method is to estimate only V_{1F3}, and successively estimate D as follows (Jinks & Pooni, 1980) (defining estimator $\underline{D}_2$):

$$\underline{D}_2 \overset{\text{def.}}{=} 2\cdot\hat{\underline{V}}_{1F3}. \tag{3}$$

A disadvantage of $\underline{D}_2$ is, in contrast to $\underline{D}_1$, that it is biased if dominance variance is present (H>0):

$$E(\underline{D}_2) = E(2\cdot\hat{\underline{V}}_{1F3}) = D + H/8.$$

Another supposed disadvantage is that the dominance component H cannot be estimated. However, H describes genetic variation that cannot be exploited in autogamous crops, unless one is interested in making hybrid varieties (which we are not in the present study). An advantage of $\underline{D}_2$ is that there is no need to estimate the residual (environmental) variance by growing isogenous material (mostly the parents), for this may take up a fairly large proportion of the experimental field. $\underline{D}_2$ was introduced by Jinks & Pooni (1980), and they concluded that the $\underline{D}_2$-estimate could be used with the same confidence as the estimate from the (elaborate) triple test cross. However, they did not extend their conclusion beyond their case of two traits in tobacco. The purpose of this paper is to show that in many situations (i.e. combinations of heritability, dominance level and experimental size) $\underline{D}_2$ is a better estimator of D than $\underline{D}_1$, i.e. the mean square error of $\underline{D}_2$ is smaller than that of $\underline{D}_1$. We make the usual assumptions: 1) the quantitative trait is determined by a large number of independently segregating loci, and hence that the trait will have a normal distribution, 2) the residual error also has a normal distribution, 3) there is no epistasis. We define the F_∞-heritability: $h^2_{(F\infty)}=D/(D+E)$.

EXPERIMENTAL DESIGN BASED ON INDIVIDUAL PLANTS

Numerous experimental designs are possible. A standard design is a completely randomized design (a 1-way classification), in which each F_3-line is represented by the same number of plants and all plants of all lines are randomized. To estimate the residual error usually parent plants are added. The accompanying analysis of variance is given in Table 1.

Table 1. Analysis of variance of a completely randomized F_3.

MS	name	df	$E(\underline{MS})$
MSB	between lines	l-1	$E + V_{2F3} + n \cdot V_{1F3}$
MSW	within lines	$l \cdot (n-1)$	$E + V_{2F3}$
MSI	within parents	$2 \cdot (i-1)$	E

l - No. of lines; n - No. of plants per line;
i - No. of plants per parent; E - residual variance;
V_{1F3} - genotypic between line variance;
V_{2F3} - genotypic within line variance.

Mean square errors of the estimators

A measure for comparing estimators is the mean square error (*MSE*). It comprises both the variance and the bias of the estimator. We will derive the mean square error of both estimators ($\underline{D}_1$ and $\underline{D}_2$). The mean squares of Table 1 have chi-square-like distributions:

$$\underline{MS} \simeq \frac{E(\underline{MS})}{df} \cdot \underline{\chi}^2(df).$$

($\underline{\chi}^2(df)$ is a chi-square random variable with df degrees of freedom.)

Since $var(\underline{\chi}^2(df)) = 2 \cdot df$, the variance of the mean squares is:

$$var(\underline{MS}) = \frac{E^2(\underline{MS})}{df^2} \cdot 2 \cdot df = \frac{2 \cdot E^2(\underline{MS})}{df}. \qquad (4)$$

As a consequence of the experimental design the three mean squares are mutually stochastically independent. The estimators of V_{1F3} and V_{2F3} are:

$$\hat{\underline{V}}_{1F3} = (\underline{MSB} - \underline{MSW})/n, \quad \text{resp.} \quad \hat{\underline{V}}_{2F3} = \underline{MSW} - \underline{MSI}. \qquad (5)$$

Combining equations (1),(2) and (3) with (5) results in (simultaneously defining coefficients f_1 up to f_8):

$$\underline{D}_1 = \frac{8}{3\cdot n}\cdot\underline{MSB} + \frac{-8-4\cdot n}{3\cdot n}\cdot\underline{MSW} + \frac{4}{3}\cdot\underline{MSI} \quad \stackrel{def.}{=} \quad f_1\cdot\underline{MSB} + f_2\cdot\underline{MSW} + f_3\cdot\underline{MSI}, \qquad (6)$$

$$\underline{H}_1 = \frac{-16}{3\cdot n}\cdot\underline{MSB} + \frac{16+32\cdot n}{3\cdot n}\cdot\underline{MSW} + \frac{-32}{3}\cdot\underline{MSI} \quad \stackrel{def.}{=} \quad f_4\cdot\underline{MSB} + f_5\cdot\underline{MSW} + f_6\cdot\underline{MSI}, \qquad (7)$$

$$\underline{D}_2 = \frac{2}{n}\cdot\underline{MSB} + \frac{-2}{n}\cdot\underline{MSW} \quad \stackrel{def.}{=} \quad f_7\cdot\underline{MSB} + f_8\cdot\underline{MSW}. \qquad (8)$$

The variances of the estimators are:

$$var(\underline{D}_1) = f_1^2\cdot var(\underline{MSB}) + f_2^2\cdot var(\underline{MSW}) + f_3^2\cdot var(\underline{MSI}), \qquad (9)$$

$$var(\underline{H}_1) = f_4^2\cdot var(\underline{MSB}) + f_5^2\cdot var(\underline{MSW}) + f_6^2\cdot var(\underline{MSI}), \qquad (10)$$

$$var(\underline{D}_2) = f_7^2\cdot var(\underline{MSB}) + f_8^2\cdot var(\underline{MSW}). \qquad (11)$$

The covariance of $\underline{D}_1$ with $\underline{H}_1$ is:

$$cov(\underline{D}_1,\underline{H}_1) = f_1\cdot f_4\cdot var(\underline{MSB}) + f_2\cdot f_5\cdot var(\underline{MSW}) + f_3\cdot f_6\cdot var(\underline{MSI}). \qquad (12)$$

The (usual) definition of the mean square error of a (possibly biased) estimator $\underline{X}$ of a certain parameter θ is: $MSE(\underline{X})=E(\underline{X}-\theta)^2$. If the bias is δ, i.e. $E(\underline{X})=\theta+\delta$, then: $MSE(\underline{X})=var(\underline{X})+\delta^2$. Thus, the mean square errors of the three estimators are:

$$MSE(\underline{D}_1) = var(\underline{D}_1), \quad MSE(\underline{H}_1) = var(\underline{H}_1), \text{ and } \quad MSE(\underline{D}_2) = var(\underline{D}_2) + \frac{1}{64}\cdot H^2.$$

If there is no dominance variance, then $\underline{D}_2$ is unbiased and hence its mean square error is equal to its variance. Comparing equation (9) with (11) we can see that in this case the MSE of $\underline{D}_1$ will always be larger than the MSE of $\underline{D}_2$:

$$f_1^2 = \frac{64}{9\cdot n^2} = \frac{7.111}{n^2} > \frac{4}{n^2} = f_7^2, \quad \text{and}$$

$$f_2^2 = \left(\frac{-8-4\cdot n}{3\cdot n}\right)^2 = \left(\frac{-2.667}{n} - 1.333\right)^2 > \left(\frac{-2}{n}\right)^2 = f_8^2.$$

and additionally the variance of MSI contributes to the variance of $\underline{D}_1$. Furthermore, the experimental size needed for $\underline{D}_1$ in this comparison is larger because of the need to estimate the residual variance. Therefore, we conclude that in the absence of dominance it is always better to use $\underline{D}_2$. Of course it is realized that one never knows beforehand the presence or level of the dominance variance (which also applies to epistasis and linkage). Thus subsequently only situations in which dominance is present need to be studied.

We define the scale independent parameter, the coefficient of error (*CE*) of estimator $\underline{X}$ of Θ: $CE(\underline{X})=\sqrt{(MSE(\underline{X}))}/\Theta$. For an unbiased estimator the coefficient of error equals the coefficient of variation.

Optimum allocation of the experimental size

Equations (9) and (11) show that, at a given experimental size k, the variance of $\underline{D}_1$ and $\underline{D}_2$ depends on the design of the F_3-population and, additionally for $\underline{D}_1$, on the proportion of the experimental size that is assigned to parent plants. In order to make a fair comparison between the two estimators we need to find the design, in which the number of lines (l), the number of plants per line (n), and the number of plants per parent (i) are optimal, i.e. the design in which l, n and i are chosen such that the *MSE*, and consequently the variance, of the estimator is minimal. In practice, of course, the maximum number of seeds produced per F_2-plant may be smaller than the optimum number of plants per line, in which case one will have to settle for a sub-optimal situation.

The variance of $\underline{D}_2$ can be minimized for a given F_3-population size $k=l\cdot n$ by substitution of l by k/n in an elaborated form (using equations (4) and (8)) of equation (11). The variance of $\underline{D}_2$ becomes a function in n (as far as the allocation of the experimental size is concerned), and using the first derivative of this function ($\delta var(\underline{D}_2)/\delta n$), the optimum number of plants per line for a given F_3-population size k, and given magnitudes of variance components (V_{1F3}, V_{2F3} and E) can be found:

$$n_{opt} = \frac{(1+k)\cdot(E+V_{2F3})+k\cdot V_{1F3}}{2\cdot(E+V_{2F3})+k\cdot V_{1F3}} \text{ , and hence } l_{opt} = k/n_{opt}.$$

Since n and l are integer numbers, we have to evaluate $var(\underline{D}_2)$ at the smaller and the larger integer numbers next to n_{opt}; consequently the product $l_{opt}\cdot n_{opt}$ may sometimes not be exactly equal to k. The constraints on account of the ANOVA are: $l\geq 2$ and $n\geq 2$. Fig. 1 presents the optimum number of plants for a few situations. It shows that n_{opt} depends chiefly on the F_∞-heritability and for medium to low F_∞-heritability also on the experimental size. There is very little influence of the dominance level.

The minimization of $var(\underline{D}_1)$ is somewhat less straightforward, because the experimental size is a function of three parameters: $k=l\cdot n+2\cdot i$. However, l and n appear only in the first part of (the elaborated form of) equation (9) $[f_1^2\cdot var(\underline{MSB})+f_2^2\cdot var(\underline{MSW})]$, and i appears only in the last part $[f_3^2\cdot var(\underline{MSI})]$. For a given number of F_3-plants $c=l\cdot n$ we can obtain the optimum number of plants

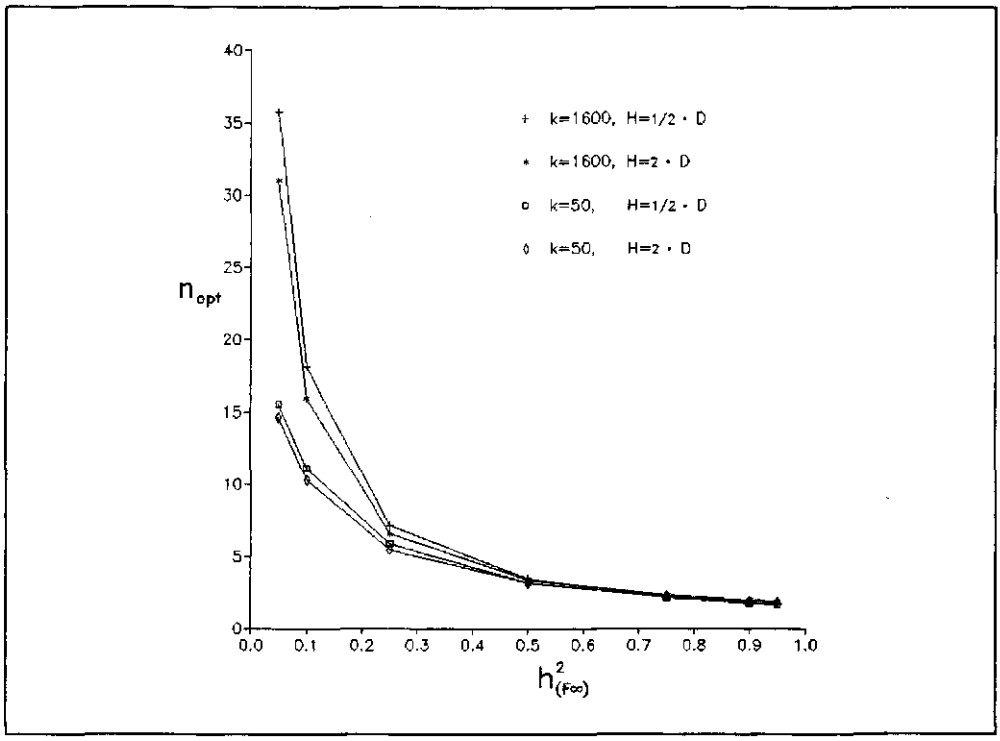

Figure 1. Optimum number of plants per line (n_{opt}) for $\underline{D}_2$ for various F_∞-heritabilities, two experimental sizes (k) and two dominance levels.

per line by minimizing this first part of equation (9) in a manner similar to the minimization of var($\underline{D}_2$). This results in:

$$n_{opt} = \frac{(2+3\cdot c)\cdot(E+V_{2F3})+2\cdot c\cdot V_{1F3}}{5\cdot(E+V_{2F3})+2\cdot c\cdot V_{1F3}}, \text{ and hence } \quad i_{opt} = c/n_{opt}.$$

Since i=(k-c)/2, we can now rewrite equation (9) by taking the minimum of the first part plus the second part, in which i is substituted by (k-c)/2. The resulting equation for var($\underline{D}_1$) depends solely on c (as far as the allocation of the experimental size is concerned):

$$\text{var}(\underline{D}_1) = \frac{64\cdot(E+V_{2F3}+c\cdot V_{1F3})\cdot(5\cdot(E+V_{2F3})+2\cdot V_{1F3})^2}{9\cdot((2+3\cdot c)\cdot(E+V_{2F3})+2\cdot c\cdot V_{1F3})\cdot(c-1)} +$$

$$\frac{96\cdot((4+c)\cdot(E+V_{2F3})+2\cdot c\cdot V_{1F3})^2\cdot(E+V_{2F3})}{9\cdot((2+3\cdot c)\cdot(E+V_{2F3})+2\cdot c\cdot V_{1F3})\cdot(c-1)\cdot c} + \frac{32\cdot E^2}{9\cdot(k-c-2)}.$$

The first derivative of this function in c, δvar($\underline{D}_1$)/δc, could not be solved

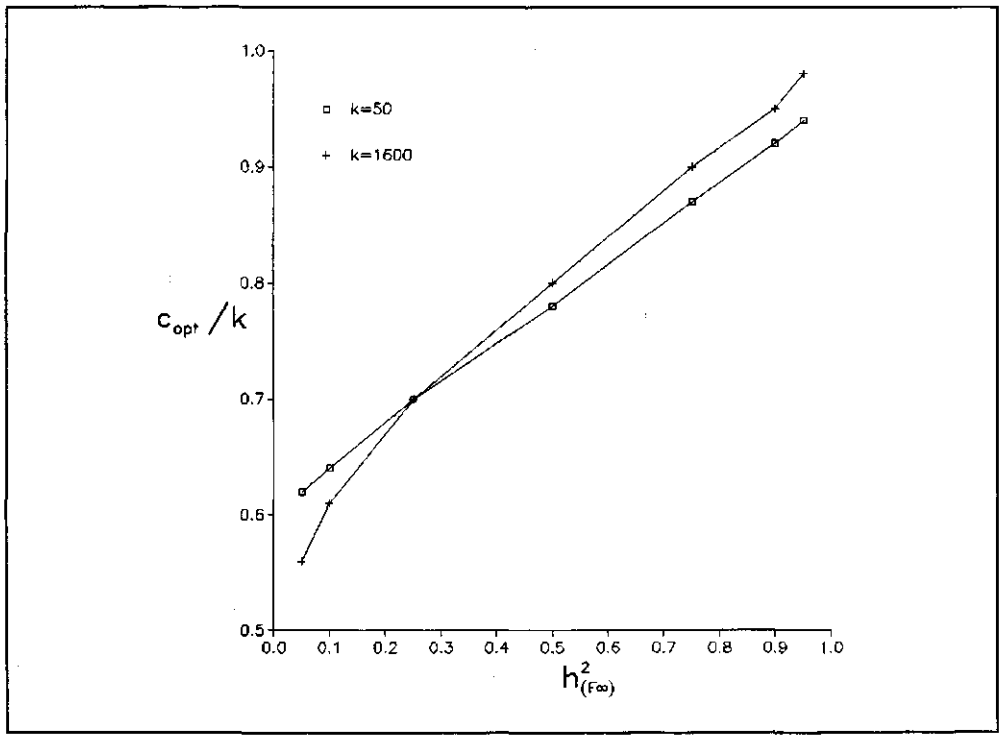

Figure 2. Optimum fraction of the total experimental size taken up by the F_3 (c_{opt}/k) for $\underline{D}_1$ for various F_∞-heritabilities, two experimental sizes (k) and dominance level H=½•D.

to find a solution for c. Therefore, the behavior of var($\underline{D}_1$) was studied numerically; it appeared that a unique minimum exists (at $c=c_{opt}$) for $1<c<k-2$. Fig. 2 shows the optimum fraction of the total experimental size taken up by the F_3 (c_{opt}/k). It depends mainly on the F_∞-heritability, it varies only slightly with the experimental size. For situations without dominance (H=0) up to a high dominance level (D=2•H) the fraction deviates, for the same value of k, not more than 0.02 from the fractions presented in Fig. 2 (with H=½•D). Since l,n and i are integer numbers, var($\underline{D}_1$) must be evaluated at the smaller and larger integer numbers next to $i_{opt}=(k-c_{opt})/2$ and next to n_{opt}. The constraints on account of the ANOVA are: $l\geq 2$, $n\geq 2$ and $i\geq 2$.

Comparing $\underline{D}_1$ with $\underline{D}_2$

Now that we have established ways to obtain optimum population designs for any situation (within the boundaries of the current experimental design), both for $\underline{D}_1$ and $\underline{D}_2$, we can compare the two estimators. Above it has already been stated

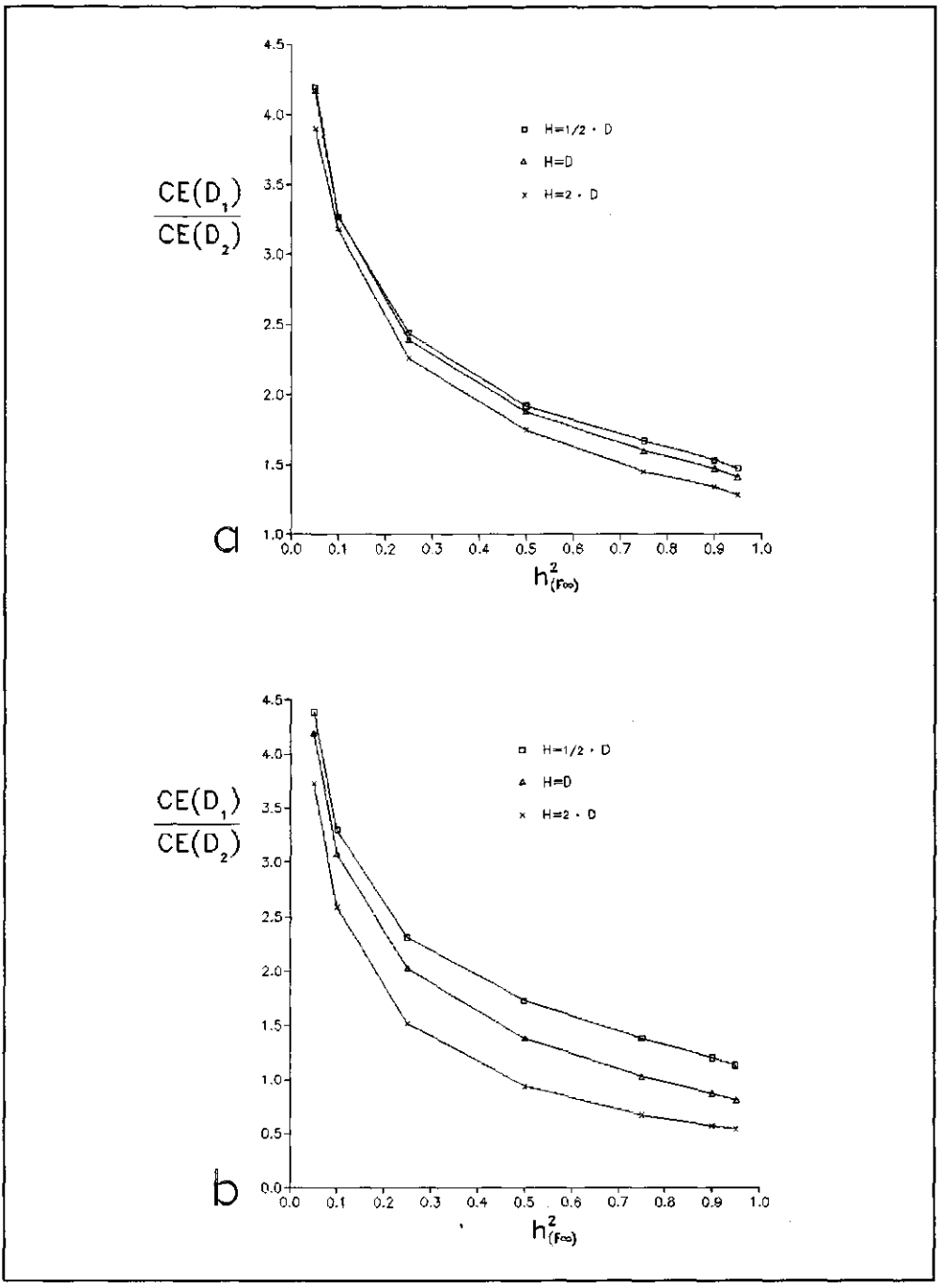

Figure 3a,b,c. Ratio of the coefficients of error of $\underline{D}_1$ and $\underline{D}_2$ **a** for various F_∞-heritabilities, three dominance levels and experimental size k=100; **b** for various F_∞-heritabilities, three dominance levels and experimental size k=1600; **c** for various experimental sizes (k), three dominance levels and F_∞-heritability of 0.75.

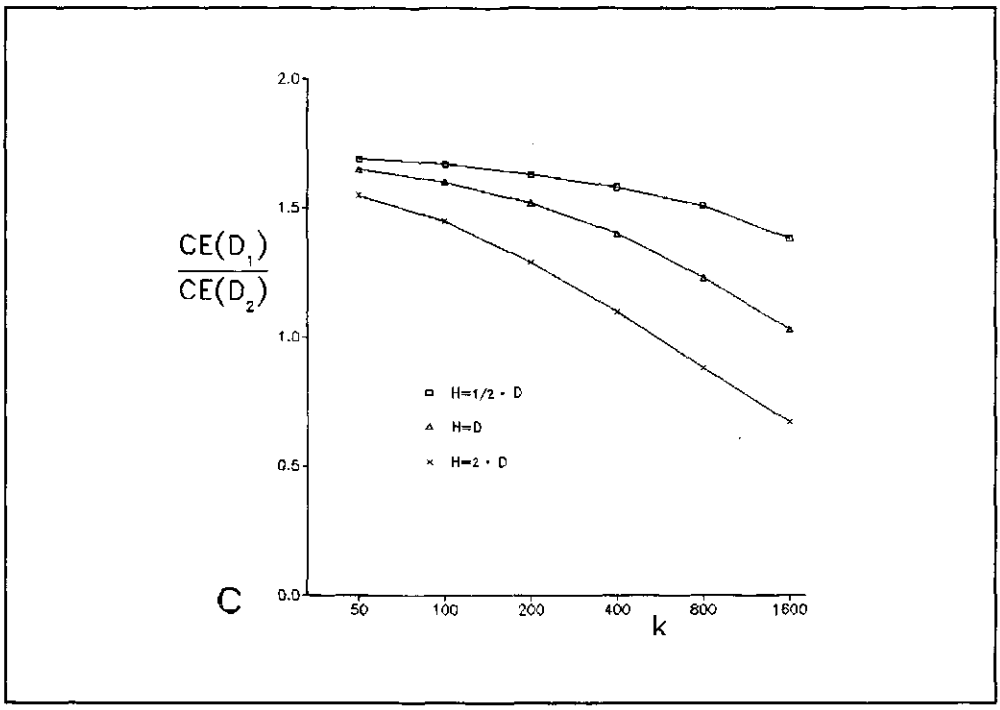

Figure 3c.

that for situations with no dominance (H=0) $\underline{D}_2$ is always better than $\underline{D}_1$. For situations with dominance the ratio of the *CE*'s of $\underline{D}_1$ and $\underline{D}_2$ depends as well on the ratio of the variances of the two estimators as on the dominance level. The variance of $\underline{D}_2$ is always smaller than that of $\underline{D}_1$. However, at very large experimental sizes and/or at high F_∞-heritabilities the difference between the variances of $\underline{D}_1$ and $\underline{D}_2$ will be small, and hence the *CE* of $\underline{D}_1$ will eventually become smaller than the *CE* of $\underline{D}_2$ because of the contribution of the dominance level to the *CE* of $\underline{D}_2$. We computed the *CE*'s of $\underline{D}_1$ and $\underline{D}_2$ for all combinations of seven F_∞-heritability values ($h^2_{(F\infty)}$=0.05 , 0.10, 0.25, 0.50, 0.75, 0.90, 0.95), six experimental sizes (k=50, 100, 200, 400, 800, 1600), and four dominance levels (H=0, ½·D, D, 2·D). The ratio $CE(\underline{D}_1)/CE(\underline{D}_2)$ varied from 0.54 (at $h^2_{(F\infty)}$=0.95, k=1600, H=2·D) up to as large as 4.59 (at $h^2_{(F\infty)}$=0.05, k=800, H=0). Fig. 3 shows that the relative performance of $\underline{D}_1$ increases with the F_∞-heritability level and the experimental size, but that $\underline{D}_1$ only outperforms $\underline{D}_2$ at a high dominance level combined with a large experimental size and a medium to high F_∞-heritability level. Of all the 168 studied combinations only 11 combinations showed a $\underline{D}_1$ outperforming $\underline{D}_2$, of which 9 were situations with

extreme overdominance (H=2·D).

$\underline{H}_1$

The optimum allocation of the experimental size with respect to $\underline{H}_1$ can be determined in a very similar way as applied to $\underline{D}_1$. This optimum is different from the optimum with respect to $\underline{D}_1$. This can already be seen at the optimum number of plants per line for a given F_3-population size (c=1·n):

$$n_{opt} = \frac{(1+3\cdot c)\cdot(E+V_{2F3})+c\cdot V_{1F3}}{4\cdot(E+V_{2F3})+c\cdot V_{1F3}}.$$

We determined the optima for $\underline{H}_1$ numerically for all previously mentioned 168 combinations of F_∞-heritability, experimental size and dominance level, and subsequently evaluated the mean square errors at these optima. The optimum number of parent plants and the optimum number of plants per line were higher than for $\underline{D}_1$. In effect this means that $\underline{H}_1$ needs a more accurate estimate of V_{2F3}. The *CE* is in many of these cases rather high, e.g. at a $h^2_{(F\infty)}$=0.25 for k=1600 with H=2·D *CE*=1.4 up to as large as *CE*=32.0 for k=50 with H=½·D.

For all 168 combinations, for which the allocation of the experimental size was optimized for $\underline{D}_1$ (!), we also computed the correlations of $\underline{H}_1$ with $\underline{D}_1$ (using equations (9), (10) and (12)). These were found to be highly negative: ranging from -0.83 to -0.95. Graphical demonstrations of these highly negative correlations can be found in Van Ooijen (1986) and in Shaw (1987).

EXPERIMENTAL DESIGN BASED ON PLOT TOTALS

Sometimes the F_∞-heritability of a certain trait is so low that the experimental size, necessary for an accurate estimate of D, expands too much to be able to score each individual plant. In that case the experimental design will be based on plot totals (or plot means). A corresponding standard design is also a completely randomized design, but now based on plot totals (or plot means). The accompanying analysis of variance is presented in Table 2.

Working with plot totals instead of with individual plants means loss of information on genotypic within line variance. As plot size increases there will be hardly any information left on genotypic within line variance. For example, the mean of 2 plots of 100 plants of the same line will hardly differ genotypically, instead most of the difference will be of environmental origin (residual variance). Thus V_{2F3} will become hard to estimate, its estimator will have a very large variance, and as a result the *CE* of $\underline{D}_1$ will increase. For

Table 2. Analysis of variance of a completely randomized design of plots of F_3-lines, based on plot totals.

MS	name	df	$E(MS)$
MSB	between lines	l-1	$n \cdot E_w + n^2 \cdot E_b + n \cdot V_{2F3} + p \cdot n^2 \cdot V_{1F3}$
MSW	within lines	l·(p-1)	$n \cdot E_w + n^2 \cdot E_b + n \cdot V_{2F3}$
MSI	within parents	2·(i-1)	$n \cdot E_w + n^2 \cdot E_b$

l - No. of lines; n - No. of plants per plot; p - No. of plots per line; i - No. of plots per parent; V_{1F3} - genotypic between line variance; V_{2F3} - genotypic within line variance; E_w - residual within plot variance; E_b - residual between plot variance.

example, doubling the experimental size by taking a plot of two plants instead of just one plant resulted in an increase (!) in the *CE* of $\underline{D}_1$ for all studied combinations (mentioned above) with an F_∞-heritability of up to 0.75; only the studied combinations with an F_∞-heritability of 0.90 or 0.95 showed a slight decrease in the *CE* of $\underline{D}_1$. (Rem.: for these calculations E was split into E_w and E_b by using the empirical law of H.F. Smith (1938) with a coefficient of heterogeneity b=0.5).

In contrast with this is the effect on $\underline{D}_2$. $\underline{D}_2$ does not need an estimate of V_{2F3}, it only depends on the accuracy of the estimator of V_{1F3}, which is even raised by increasing plot size. Theoretically there will, of course, be an optimum allocation of experimental size regarding plot size, number of plots and number of lines. However, for many crops plot size will primarily be dictated by agricultural practice, such as the number of seeds produced per plant and the capacity of the harvesting equipment. Therefore it is not attempted in this paper to determine a way of obtaining the optimum allocation of such an experiment.

DISCUSSION

It will be clear that estimator $\underline{D}_2$ is more accurate than $\underline{D}_1$ in many cases. When it is necessary to use plot totals because of low F_∞-heritability, the performance of $\underline{D}_1$ becomes very inaccurate. When working with individual plants the accuracy of $\underline{D}_1$ can only be better than that of $\underline{D}_2$ in combinations with a high dominance level, a higher F_∞-heritability, and/or a large experimental size. In practice there need be no doubt about $\underline{D}_2$ when H<D, $h^2_{(F\infty)}$<0.75, and n<400. For any situation it will be possible to approximate the mean square errors of both

estimators once the variance components have been (roughly) estimated in a pilot experiment. The experimenter can decide on which estimator to use, thereby also considering the available experimental capacity and the desired accuracy.

The results may be extended to other experimental designs, such as a complete block design. These designs mostly aim at reducing the residual error. Therefore, we expect to find similar results. Of course it would be best to consider the mean square errors of both estimators for any specific desired design.

Linkage and epistasis may bias both estimators. Depending on the magnitude of the linkage and epistasis parameters, $\underline{D}_2$ may even have a somewhat larger bias than $\underline{D}_1$. A number of studies (Weber, 1982; Kearsey, 1985) conclude that the influence of linkage is unimportant, when we are regarding D as the F_∞-variance and not as the "true" additive variance (Pooni & Jinks, 1986). The latter can be interpreted as the theoretical F_∞-variance that would be obtained if linked loci were segregating independently. Because from the breeder's point of view the F_∞-variance rather than the true additive variance is relevant, the present paper focuses on D as the F_∞-variance. Therefore, linkage is not likely to invalidate the main results. The influence of epistasis depends on the relative magnitude of its parameters. Pooni & Jinks (1979) describe methods to obtain estimates of these parameters. However, and this applies also to the paper of Jinks & Pooni (1982), in which methods are introduced that try to correct for linkage, 1) these methods are always too elaborate to include in a practical breeding programme (c.f. Van der Veen, 1959), and 2) the more parameters have to be estimated, the less accurate the estimates usually become.

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3. Statistical aspects of estimation and prediction of additive genotypic variance in the offspring of crosses between pure breeding lines

Co-author: P. Stam

Summary

Genotypic additive variance (D), with respect to a certain quantitative trait, estimated in the F_3 of autogamous crops can be used to predict the probability to obtain superior recombinant inbreds in the offspring of the cross between two pure breeding lines. Confidence intervals for the estimated genotypic variance are based on the assumption, that genotypic and environmental effects have a normal probability distribution, and on the assumption of homoscedasticity of residual variances. Normality of genotypic effects is in turn based on the assumption, that the quantitative trait is determined by a large number of independently segregating genes with equal and infinitesimal effect. This paper investigates the behaviour of the confidence interval (method of Williams, 1962, and Tukey, 1951) on the genotypic variance when only a limited number of genes determine the quantitative trait. The paper also investigates the robustness of the confidence interval to heteroscedasticity of residual effects.

The confidence interval of the method of Williams and Tukey is inference about the genotypic variance that is enclosed in the original cross between the pure breeding lines. The breeder, however, is not so much interested in the potency of the original cross, but more in the potential future of the actual F_3-population, because he will want to continue the breeding programme with this material. Since there is no standard method for a confidence interval when the inference is about the current F_3, one might still apply the Williams-Tukey confidence interval. The behaviour of this confidence interval in this situation is studied.

1. INTRODUCTION

Estimates of the additive genotypic component of phenotypic variance in a population with respect to a quantitative character are indicative for the future success of directional selection in that population. In outbreeding species the heritability (the genotypic part of the total variance) can be used to predict future selection response. In self fertilizing species the genotypic variance, which is generated by crossing two pure breeding lines, changes with generations (F_2, F_3, etc.) and the purpose of estimation is slightly different. Usually, the genotypic variance of the F_∞-generation ($V_{F\infty}$ or D) is taken as an indication of the potential genotypic progress enabled by such a cross; the

larger D is, the larger the probability of obtaining transgressive recombinants in future generations. Estimates of D can be obtained in several ways from early generations. One of the most efficient ways (in terms of experimental effort and accurateness) is to use the estimated variance between F_3-lines (Jinks & Pooni, 1980; Van Ooijen, 1989).

Confidence intervals for the estimated variance component are mostly based on the (usual) assumptions about the distribution of genotypic and environmental effects, i.e. normality and homoscedasticity. A normal distribution of genotypic effects is in turn based on the assumption of the polygenic nature of quantitative characters, i.e. a large number of genes with small individual effects. In quantitative genetics theory this assumption of normally distributed joint effects of the genes plays an important role (see e.g. Bulmer, 1985). Though the theory assumes that many genes are involved in quantitative characters, the actual number of genes contributing to the genotypic variation is generally unknown and very hard to determine (Thoday & Thompson, 1976). Recently, a renewed interest in the possible oligogenic basis of quantitative genetic variation has arisen from studies in which molecular genetic markers have been used to detect possible quantitative trait loci (QTL) (Soller & Beckman, 1988; Helentjaris, 1987; Paterson et al, 1988). In cereals partial resistance to fungal diseases, a character of quantitative nature, seems to be governed by a few major genes (Parlevliet, 1978; Broers & Jacobs, 1989). For this reason the present paper investigates in some detail the confidence intervals of D-estimates under the assumptions of a limited number of genes being involved in a quantitative character.

In addition to this, the influence of heteroscedasticity (i.e. heterogeneous within line variances) on the confidence intervals was studied. The most commonly observed form of heterogeneity of variances is of the type "constant coefficient of variation". When this is due to the multiplicative nature of the character (such as sizes and weights of organs and developmental times) this can, of course, be "dealt with" by a suitable transformation of the data. However, apart from environmental influences, heterogeneity of variances also results from the very nature of the genetic segregation in the generations following a cross of pure lines. When a limited number of major genes are segregating, the within line genotypic variance in an F_3-generation may vary considerably, not necessarily leading to constant coefficients of variation. Therefore, the robustness of confidence intervals (based on the usual assumptions) to violations of these assumptions was investigated briefly.

The genotypic variance components in a breeding population are usually considered as parameters of the probability distribution from which the actual population has been sampled. Consequently, statements about these parameters, such as confidence intervals of estimates, apply to this conceptual probability distribution. The hypothetical nature of this probability distribution is evident in a breeding programme: the breeder's interest is in the potential future of the actual population rather than in the genotypic variance of the imaginary population from which the actual population was sampled. Referring to the case of a cross between pure lines, the parameter D (additive genotypic variance) is a characteristic of that cross; it is the genotypic variance that would be observed in the F_∞-generation to be obtained by subsequent selfing of an infinite number of plants. From the breeder's point of view this parameter is less relevant than the genotypic variance which is to be expected in future generations derived from the plant material from which the estimate was obtained. In order to deal with this problem we introduce, in addition to the parameter D in the usual sense, a sample dependent parameter, D_S, which is the F_∞-variance which would be observed upon selfing of the sample population. The discrepancy between D and D_S is entirely due to genetic sampling. D is the F_∞-variance which corresponds to (exact) gene frequencies, $p=q=\frac{1}{2}$, per locus, whereas D_S depends on the actual gene frequencies in the sample population. $\underline{D}_{F2}$ and $\underline{D}_{F3}$ will refer to (sample) generations F_2 and F_3 respectively. Since estimates of D are most efficiently obtained from F_3 data, $\underline{D}_{F3}$ is the parameter which is of interest when the estimate ($\hat{D}$) is used in the prediction of the potential future of the actual population. For these reasons we have studied the behaviour of the mean square errors $E(\hat{\underline{D}}-D)^2$ and $E(\hat{\underline{D}}-\underline{D}_{F3})^2$, and of the confidence interval, formulated for inference on $\hat{\underline{D}}$ with respect to D, but now applied to D_{F3}.

2. BEHAVIOUR OF THE D-ESTIMATOR IN A SIMULATED EXPERIMENT

As a first approach to study the behaviour of the D-estimator, a classical experimental setup was simulated using data collected on flowering time of *Arabidopsis thaliana*. Two true breeding lines were differing for two independently segregating genes for flowering time (f_b- and f_y-locus, Koornneef et al, 1983). The nine possible genotypes at this pair of loci had been obtained by crossing and line breeding. Of each genotype 20 plots of 6 plants had been grown in the greenhouse. The data collected with this oversized experiment were taken as the "true" values of the genotypes. Table 1 shows the estimates of the

population mean, the within plot variance and the between plot variance for each of the genotypes. It is clear that the heterogeneity of variances is not of the type "constant coefficient of variation".

Table 1. Estimates of mean (m), within plot residual variance (E_w), and between plot residual variance (E_b) of the nine genotypes of a cross between two pure lines of *Arabidopsis thaliana* differing for two independent genes (loci f_b and f_y) with respect to flowering time. m is in days, and E_w and E_b are in days^2.

	m			E_w			E_b		
	YY	Yy	yy	YY	Yy	yy	YY	Yy	yy
BB	23.4	23.1	32.6	1.80	0.49	8.53	0.40	0.25	6.42
Bb	24.6	24.7	34.3	2.17	0.89	21.77	0.52	0.45	0.81
bb	41.8	42.0	50.3	2.50	7.64	7.00	0.85	1.50	0 *)

*) The ANOVA estimate was -0.41.

2.1 Methods

The (computer) simulated experiment consisted of a number of random F_3-lines, each derived from individual F_2-plants, grown in individual plots in a balanced completely randomized design. In the simulation random sampling of genotypes, which applies both for sampling of F_2-parents and for sampling of F_3-genotypes from the sampled F_2-parents, was done according to the Mendelian ratio's of two unlinked loci. The genotypic values of Table 1 were used. Within plot residual deviations were sampled for each individual from a normal distribution with zero mean and variance depending on the genotype from Table 1 (E_w). This implies heteroscedasticity for the residual plant effects. The between plot residual deviates were sampled as follows: for all plants within a plot one single standard normal random deviate was sampled, and for each individual plant translated in an individual between plot deviation by multiplying this deviate with the residual between plot standard deviation depending on the genotype of the plant (the square root of E_b from Table 1). This implies heteroscedasticity for the residual plot effects. The phenotypic value of a plant was the sum of its genotypic value, its within plot residual deviate, and its between plot residual deviate.

From the simulated F_3 the parameter D was estimated using the ANOVA of Table 2. D was estimated as twice the between F_3-line variance, i.e.

Table 2. Analysis of variance of a nested design of an F_3.

source	MS	df	$E(MS)$
lines	MSL	l-1	$V_{2F3} + E_w + n \cdot E_b + p \cdot n \cdot V_{1F3}$
plots within lines	MSP	l·(p-1)	$V_{2F3} + E_w + n \cdot E_b$
within plots	MSR	l·p·(n-1)	$V_{2F3} + E_w$

E_w - within plot residual variance; l - number of lines;
E_b - between plot residual variance; p - number of plots per line;
V_{1F3} - between line genotypic variance; n - number of plants per plot;
V_{2F3} - within line genotypic variance.

$\hat{D}=2 \cdot \hat{V}_{1F3}=2 \cdot (MSL-MSP)/(p \cdot n)$ (parameters defined in Table 2). (Though this estimator is biased when dominance and/or epistasis are present, it is generally to be preferred to unbiased estimators because of its small mean square error; see Van Ooijen, 1989.) For each simulated F_3 an approximate confidence interval of D was calculated using the method of Williams-Tukey. Boardman (1974) has shown that the methods of Williams (1962) and Tukey (1951) are equivalent; it is based upon normality and homoscedasticity of all random effects. He has also shown that this method is one of the best available. Confidence intervals using this method will hereafter be referred to as WT-confidence intervals. The lower and upper WT-confidence bounds for D are (confidence coefficient = 1-α):

$$\text{WT-lower} = 2 \cdot MSP \cdot \frac{MSL/MSP - F(r_1,r_2,1-\alpha/2)}{p \cdot n \cdot F(r_1,\infty,1-\alpha/2)},$$

$$\text{WT-upper} = 2 \cdot MSP \cdot \frac{MSL/MSP - 1/F(r_2,r_1,1-\alpha/2)}{p \cdot n / F(\infty,r_1,1-\alpha/2)},$$

in which $F(a,b,1-\alpha)$ is the right α-point of the F-distribution ($Pr\{F(a,b) \leq F(a,b,1-\alpha)\}=1-\alpha$), $r_1=l-1$, $r_2=l \cdot (p-1)$; l, p, n, MSL and MSP are defined in Table 2. The confidence coefficient used in all simulations was 0.95 (α=0.05). (Rem.: since $\hat{D}=2 \cdot \hat{V}_{1F3}$ the confidence bounds for D are obtained by multiplying those for V_{1F3} by 2.)

Since the true genotypic values are known, the expected value of the estimator $\hat{D}$, including the bias from dominance and epistasis, can be calculated. Subsequently, the realized confidence, which is the frequency with which a calculated confidence interval includes the true value, also referred to as coverage, can be determined from a large number of simulated F_3's. For each

situation we simulated 1000 F_3's, hence for a 95% confidence interval one expects 950 cases in which the true D is comprised in the calculated interval. Additionally, the variance of $\hat{D}$ was estimated over the simulated F_3's; the expectation of this variance was calculated assuming a normal and homoscedastic distribution of all effects (and hence a chi-square type distribution of the mean squares: $MS \sim E(MS) \cdot \chi^2_{df}/df$); this expected variance will be labeled $var(\hat{D}|normality)$ (or $var(\hat{D}|norm.)$).

In order to study the effect of unequal vs. equal E_w and E_b over the genotypes (hetero- vs. homoscedasticity), a set of simulations was performed with equal (average) E_w and E_b. In another set of simulations the (relative) magnitude of the residual variances was increased. The parameter used to describe the relative magnitude of the genotypic vs. the residual effects is the between line heritability : $h^2(bl)=(V_{2F3}+p \cdot n \cdot V_{1F3})/(V_{2F3}+E_w+n \cdot E_b+p \cdot n \cdot V_{1F3})$ (parameters from Table 2).

2.2 Results

The results are presented in Table 3. A first remark is that the between line heritability of the studied character (flowering time) is very high. For five experimental designs (E_w and E_b unmodified from Table 1, the five upper left cases of Table 3) we found a coverage of the WT-confidence interval of D above the 95% level (which means that the interval is conservative). Accordingly, the variance of $\hat{D}$, estimated from 1000 replicate runs, was smaller than $var(\hat{D}|normality)$ for all five cases. We realize that estimating variances over 1000 replicate runs can be inaccurate. Therefore we performed for all situations two extra sets of 1000 replicate runs. These simulations showed results (data presented in the addendum of this chapter) very similar to those presented in Table 3.

Possible causes for the effects on the WT-confidence interval and the variance of $\hat{D}$ are: 1) non-normality of the genotypic effects, 2) heteroscedasticity of the genotypic effects, and 3) heteroscedasticity of the residual effects. To identify the main cause another set of simulations were performed, but now with equal, i.e. homoscedastic, E_w and E_b. The new E_w was the weighted (according to the genotype frequencies) mean of the individual E_w-values, and the new E_b was the square of the weighted mean of the square roots of the individual E_b-values. (Using the homoscedastic E_w and E_b, calculated this way, results in mean squares with the same average over replicate runs as the mean squares in the heteroscedastic cases.) The results with equal residual

Table 3. Results of simulations of F_3 of Arabidopsis. 2 loci; 6 plants per plot; varying numbers of lines (lines) and numbers of plots per line (plots); variances of $\hat{\underline{D}}$ in $(days^2)^2$.

	heteroscedastic E_w and E_b					homoscedastic E_w and E_b				
lines	25	50	100	25	25	25	50	100	25	25
plots	2	2	2	4	8	2	2	2	4	8
E_w and E_b unmodified from Table 1:										
h^2 (bl)	0.985	0.985	0.985	0.992	0.996	0.985	0.985	0.985	0.992	0.996
% coverage	98.3	98.7	99.1	97.2	98.2	98.2	98.7	98.9	97.5	97.4
$\hat{var}(\hat{\underline{D}})$	839	412	199	828	737	912	419	198	813	753
var($\hat{\underline{D}}$|norm.)	1251	613	303	1168	1129	1251	613	303	1168	1129
E_w and E_b 100 x the values of Table 1:										
h^2 (bl)	0.396	0.396	0.396	0.561	0.716	0.395	0.395	0.395	0.560	0.716
% coverage	94.8	94.4	94.5	97.0	97.4	96.3	96.1	96.4	98.2	96.7
$\hat{var}(\hat{\underline{D}})$	15836	7888	4103	4278	1941	10321	5190	2699	3436	1992
var($\hat{\underline{D}}$|norm.)	10618	5230	2596	3897	2211	10628	5235	2599	3900	2212

variances for both the coverage and the variance of $\hat{\underline{D}}$ are similar to those with unequal residual variances (Table 3, upper right part). This indicates that the heteroscedasticity of the residual effects is not the main cause of the raised coverage of the WT-confidence interval and the lowered variance of $\hat{\underline{D}}$.

Since the heritability in the previous simulations is rather high, the influence of heteroscedasticity of the residual effects was also studied with lower heritability. The simulations with equal and unequal E_w and E_b were carried out with 100 times increased values of E_w and E_b. Their results are in the lower part of Table 3. Here the coverages of the confidence interval of D are closer to the desired 95% level, both for homo- and heteroscedastic E_w and E_b, and especially for the cases with a lower between line heritability (i.e. cases with 2 plots per line).

Referring to Table 3 it is seen that in the case of a low heritability, heteroscedasticity of E_w and E_b influences the discrepancy between estimated variance of $\hat{\underline{D}}$ and var($\hat{\underline{D}}$|normality). Homoscedasticity of E_w and E_b causes the estimated variance to be much closer to var($\hat{\underline{D}}$|normality). We can look at the components of the variance of $\hat{\underline{D}}$ in Table 4. This table presents the estimated variances of the mean squares together with their expected values based upon

Table 4. Continuation of results of simulations of Table 3; estimated variances of the mean squares (from Table 2) and their expected values based upon normality assumptions (var(MS|norm.)); variances in $(days^2)^2/100$, except for the variances of MSP and MSR in the upper part of the table, that are in $(days^2)^2$.

	heteroscedastic E_w and E_b					homoscedastic E_w and E_b				
lines	25	50	100	25	25	25	50	100	25	25
plots	2	2	2	4	8	2	2	2	4	8
E_w and E_b unmodified from Table 1:										
$\hat{var}$(MSL)	284	140	67	1162	4183	315	141	67	1145	4288
var(MSL|norm.)	449	220	109	1681	6502	449	220	109	1681	6502
$\hat{var}$(MSP)	279	151	76	117	71	266	160	73	114	70
var(MSP|norm.)	178	89	44	59	25	178	89	44	59	25
$\hat{var}$(MSR)	55	29	15	51	42	51	27	14	47	42
var(MSR|norm.)	14	7	4	7	4	14	7	4	7	4
E_w and E_b 100 x the values of Table 1:										
$\hat{var}$(MSL)	3412	1633	900	5357	10445	2534	1354	669	4784	11315
var(MSL|norm.)	2782	1363	674	5265	12589	2784	1364	675	5269	12594
$\hat{var}$(MSP)	2545	1421	677	1122	804	1074	532	259	365	150
var(MSP|norm.)	1041	520	260	347	149	1042	521	261	347	149
$\hat{var}$(MSR)	132	69	32	108	87	30	15	7	16	7
var(MSR|norm.)	31	15	8	15	8	31	15	8	15	8

normality assumptions (and homoscedasticity). In the heteroscedastic cases the estimated variances of the mean squares are larger than their expected values, except for the variance of MSL for the case with 8 plots per line. This is in contrast to the homoscedastic cases, where the estimated variances are all approximately equal to their expected values, except for the variance of MSL for the cases with 4 or 8 plots per line. It is clear that heteroscedasticity of the residual variances rises the variances of the mean squares and hence the variance of $\hat{D}$. In both the hetero- and the homoscedastic case with 8 plots per line the estimated variance of MSL is smaller than the expected value. One expects that heteroscedasticity of the genotypic (within line) effects has a similar variance increasing effect on the mean squares as heteroscedasticity of the residual effects. This cannot be detected in the homoscedastic cases with low heritability, but in the cases (both hetero- and homoscedastic) with high heritability the estimated variances of MSP and MSR were larger than their

expected values (Table 4). But even in those cases the estimated variance of MSL was smaller than the expected value. The only remaining invalid assumption responsible for this smaller variance of MSL is non-normality of the genotypic effects. Therefore, we can state that non-normality of genotypic effects most likely reduces the variance of MSL. In one of the heteroscedastic cases (25 lines, 8 plots/line) the between line heritability is of such a high level (0.716, Table 3), that the variance increasing effect of heteroscedasticity of the residual variances is counteracted so much by the variance reducing effect of the non-normality of the genotypic effects, that the variance of $\hat{D}$ has become smaller than its expected value.

2.3 Non-normality of the genotypic effects

In order to further investigate the influence of non-normality of the genotypic effects, caused by the oligogenic nature of the quantitative trait, we simulated F_3's for various small numbers of loci, analogous to the simulation described in section 2.1, i.e. genotypes were sampled at random according to the Mendelian segregation ratio's. Dominance, linkage, and epistasis were absent. The value of the additive genotypic effect per locus (d) was equal for all loci and was chosen depending on the number of loci such that the true value of D was always equal to 1 (it can be shown that in the absence of epistasis and linkage D equals the sum of d^2 for each locus, i.e. $D=\Sigma d^2$, e.g. Mather & Jinks, 1977). Residual effects were normally distributed with equal variances for all genotypes. The F_3's consisted of 25 lines, 2 plots per line and 6 plants per plot. The simulations were performed for three levels of the residual variances. For each level var($\hat{D}$|normality) of was calculated. For each situation the coverage of the WT-confidence interval was determined and the variance of $\hat{D}$ estimated over 1000 replicate runs. (Rem.: as stated before, we repeated the 1000 replicate runs two more times, of which the results showed the same trends; see addendum of this chapter.)

The results are presented in Table 5. The effects of non-normality decrease with increasing number of loci and with decreasing heritability. The ratio of the estimated variance of $\hat{D}$ and var($\hat{D}$|normality) rises above 0.90 when more than eight loci are involved at high heritability, when more than two loci are involved at intermediate heritability, and even starting at one locus at low heritability. The coverage of the WT-confidence interval only deviates substantially from the desired 95% level at one or two loci combined with high or intermediate heritability. (Rem.: Since the WT-confidence interval is not an

Table 5. Results of simulations of F_3's with 25 lines, 2 plots per line, 6 plants per plot, at varying number of loci, and at 3 levels of E_w and E_b; 1000 replications per situation; E_w and E_b in days^2.

loci	1	2	4	5	8	10	14	16
$E_w=0.25^2$, $E_b=0.10^2$, $h^2(bl)=0.98$:								
% coverage	99.2	97.6	96.8	96.0	96.3	96.5	96.7	96.7
$\hat{var}(\hat{D})/var(\hat{D}\mid norm.)$	0.51	0.77	0.86	0.84	0.90	0.96	0.95	0.93
$E_w=1.5^2$, $E_b=0.5^2$, $h^2(bl)=0.63$:								
% coverage	98.6	97.6	96.3	97.8	97.1	96.8	95.9	97.6
$\hat{var}(\hat{D})/var(\hat{D}\mid norm.)$	0.77	0.97	0.97	0.95	0.97	0.96	1.07	0.97
$E_w=2.5^2$, $E_b=1.0^2$, $h^2(bl)=0.34$:								
% coverage	97.1	96.8	97.2	96.7	96.9	96.7	96.6	97.0
$\hat{var}(\hat{D})/var(\hat{D}\mid norm.)$	0.92	0.98	0.92	0.92	0.97	0.89	1.01	0.97

exact confidence interval, the approximate confidence was determined for the case in which all effects (genotypic and residual) do have a normal distribution, using a comparable computer simulation (100,000 replications). For the above used F_3-size and -design and for the same three levels of E_w and E_b the coverage of the 95% WT-confidence interval was approximately 96.5%).

3. DISCRETENESS OF THE DISTRIBUTION OF GENOTYPIC VALUES

The results from the previous section indicate that the D-estimator of oligogenic quantitative characters has a smaller variance than would be expected based on normality assumptions. This resulted in some cases in a higher realized confidence of the WT-confidence intervals. Therefore we will investigate the variance of the estimator of D when the number of genes is limited, and compare it to its variance when normality is valid.

The estimator of D in an experiment as described in the previous section essentially estimates twice the genotypic variance between F_2-individuals. Since the estimator of the F_2-variance is much simpler than the D-estimator for the F_3, we will concentrate on properties of the estimator of the F_2-variance. We will use a general formula for the variance of the usual variance estimator in terms of cumulants, and subsequently use the cumulants of the presupposed distributions to compare the variances for oligogenic and polygenic (i.e. *infinite number of independent genes with small equal effects*) characters.

Let $\underline{x}_1,\ldots,\underline{x}_N$ be N identically distributed sample values. The variance of this distribution is estimated by:

$$\underline{V}_x = \hat{\text{var}}(\underline{x}) = \frac{\Sigma\, \underline{x}^2 - (\Sigma\, \underline{x})^2/N}{N-1}.$$

The variance of $\underline{V}$ can be expressed in terms of the cumulants of $\underline{x}$. Let κ_i be the i^{th} cumulant. Then:

$$\text{var}(\underline{V}_x) = \frac{2\cdot\kappa_2^2(\underline{x})}{N-1} + \frac{\kappa_4(\underline{x})}{N} \quad \text{(e.g. Kendall \& Stuart, 1958, Chapter 12).}$$

If the phenotype $\underline{p}$ is the sum of two independent variables (genotypic value $\underline{g}$ and environmental/residual error $\underline{e}$), then the cumulant of this sum of independent variables is the sum of their cumulants (e.g. Cramér, 1946, Chapter 15). Thus,

$$\text{if:}\quad \underline{p} = \underline{g} + \underline{e}, \quad \text{then:}\quad \kappa_i(\underline{p}) = \kappa_i(\underline{g}) + \kappa_i(\underline{e}).$$

Accordingly, the variance of $\underline{V}_p$ can be written as:

$$\text{var}(\underline{V}_p) = \frac{2\cdot\kappa_2^2(\underline{g})}{N-1} + \frac{\kappa_4(\underline{g})}{N} + \frac{2\cdot\kappa_2^2(\underline{e})}{N-1} + \frac{\kappa_4(\underline{e})}{N} + \frac{4\cdot\kappa_2(\underline{g})\cdot\kappa_2(\underline{e})}{N-1}. \qquad (1)$$

If we assume independently segregating loci with equal effects without epistasis, then the distribution of $\underline{g}$ is binomial. With L segregating loci and no dominance or epistasis the distribution of $\underline{g}$ is $\underline{g}\simeq B(2\cdot L,\frac{1}{2})$; with (unidirectional) dominance the distribution of $\underline{g}$ is $\underline{g}\simeq B(L,\frac{1}{4})$ (or equivalently: $\underline{g}\simeq B(L,3/4)$). ($B(n,p)$ refers to a binomial distribution with parameters n (number of trials) and p (probability of success).) We further assume that the residual error $\underline{e}$ is normally distributed with mean zero and variance σ^2_e ($\underline{e}\simeq N(0,\sigma^2_e)$). When comparing the alternatives, i.e. a normal vs. a binomial distribution of genotypic values, we will assume that the normal distribution has mean and variance equal to mean and variance of the corresponding binomial distribution. Thus, using expression (1) and the cumulants of the distributions of $\underline{g}$ and $\underline{e}$, we are in a position to compare the variances of $\underline{V}_p$ under the two alternative assumptions. Abramowitz & Stegun (1970, 26.1.20) give a recurrent equation for the cumulants of the binomial distribution $B(n,p)$:

$$\kappa_{i+1} = p\cdot(1-p)\cdot\delta\kappa_i/\delta p \quad (\text{for } i\geq 1), \text{ while } \kappa_1 = n\cdot p.$$

From this equation the second and fourth cumulants can be derived:

$$\kappa_2 = n\cdot p\cdot(1-p),$$

$\kappa_4 = n\cdot(p-7\cdot p^2+12\cdot p^3-6\cdot p^4)$.

For the normal distribution one has (Abramowitz & Stegun, 1970, 26.1.26):

$\kappa_1 = \mu$, $\kappa_2 = \sigma^2$, and $\kappa_4 = 0$.

As an example of a comparison consider the case of L loci, no dominance:

$\underline{g} \sim B(2\cdot L,\frac{1}{2})$, $\kappa_1(\underline{g}) = L$, $\kappa_2(\underline{g}) = \frac{1}{2}\cdot L$, and $\kappa_4(\underline{g}) = -\frac{1}{4}\cdot L$,

then under the alternative assumption one has a normal distribution with mean L and variance $\frac{1}{2}\cdot L$:

$\underline{g} \sim N(L,\frac{1}{2}\cdot L)$, $\kappa_1(\underline{g}) = L$, $\kappa_2(\underline{g}) = \frac{1}{2}\cdot L$, and $\kappa_4(\underline{g}) = 0$;

using these cumulants, the cumulants of a normally distributed $\underline{e}$, and expression (1), the variance of $\underline{V}_p$ can be calculated for the alternative assumptions. In the calculations the relative magnitude of the environmental variance (σ_e^2) is expressed in terms of the heritability (h^2), i.e.:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2}, \quad \text{so} \quad \sigma^2_e = \frac{1 - h^2}{h^2}\cdot\sigma^2_g,$$

$$\text{or equivalently} \quad \kappa_2(\underline{e}) = \frac{1 - h^2}{h^2}\cdot\kappa_2(\underline{g}).$$

The results of the calculations are summarized in Fig.1, where the variances of $\underline{V}_p$ under the alternative assumptions are expressed as a ratio. Since the results expressed in this way do not critically depend on the sample size N (as can be seen from (1)), only a single sample size (N=40) is represented in Fig.1.

It is seen that assuming a normal distribution of genotypic effects in case that the true distribution is binomial, may lead to considerable overestimation of var($\underline{V}_p$), especially when heritability is high. As a result the WT-confidence interval (which assumes a normal distribution) will be very conservative. Of course, the discrepancy decreases with an increasing number of loci and with a decrease of heritability (both changes result in a more normal appearance of the distribution of the phenotype $\underline{p}$). When more than five loci of equal effects are involved the difference is always less than approximately 7%.

These results are in accordance with the observations of the previous section, where the same trend was observed in the simulations. With only a few genes segregating, the observed variance of $\hat{\underline{D}}$ was less than the variance calculated from the data assuming a normal distribution of phenotypes and correspondingly the coverage of the Williams-Tukey confidence interval was

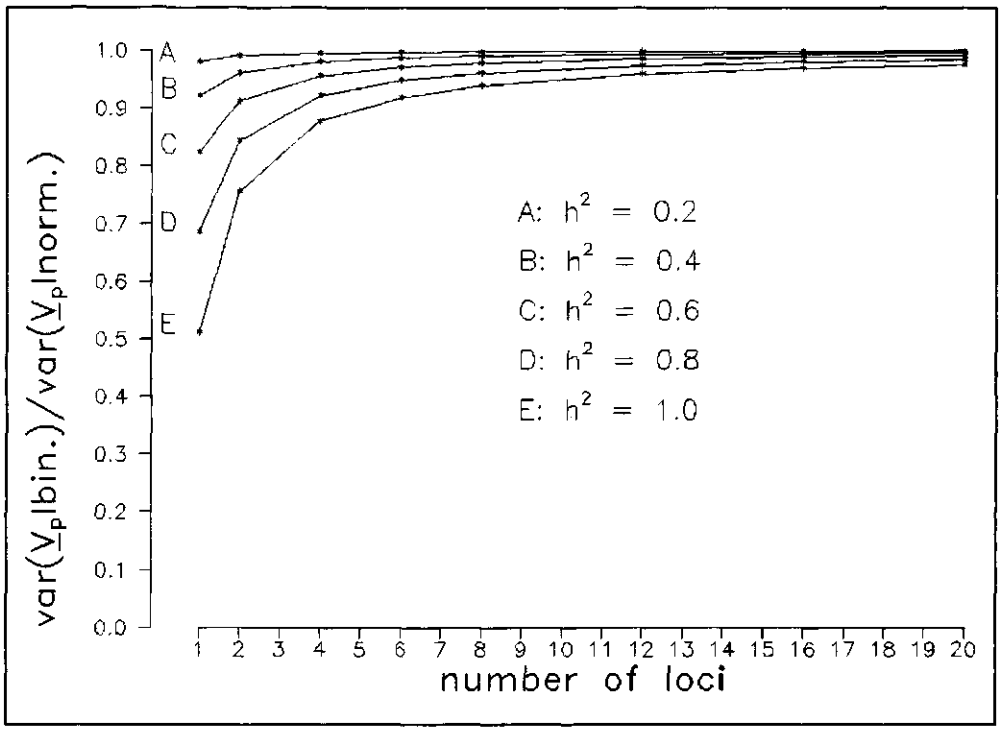

Figure 1. Ratio of the variance of the phenotypic variance between F_2-individuals under the assumption of a binomial distribution, var($\underline{V}_p$|bin.), and under the assumption of a normal distribution of the genotypic values, var($\underline{V}_p$|norm.). Dominance, epistasis and linkage are absent; the sample size N is 40.

higher than expected. For example, in Table 5, we found for the case of two loci and h^2(bl)=0.98 a value of 0.77 for the ratio of the observed and the expected variance of $\hat{\underline{D}}$. Since the estimator $\hat{\underline{D}}$ essentially estimates twice the genotypic variance between F_2-individuals, $\underline{V}_g$, the heritability used for the F_2 corresponds to the between line heritability of the F_3. The ratio of $\underline{V}_p$'s under the alternative assumptions in an F_3 corresponds to the ratio of $\underline{MSL}$'s under the alternative assumptions. Since heritability is very high (0.98), the variance of $\underline{MSP}$ is very small compared to the variance of $\underline{MSL}$, therefore this ratio is very close to the 0.77: $\hat{var}(\underline{MSL})/var(\underline{MSL}|norm.)=0.75$ (data not shown). For a binomial distribution with two loci we have: L=2, $\kappa_2(\underline{g})=1$, and $\kappa_4(\underline{g})=-0.5$, while for the corresponding normal distribution we have: $\kappa_2(\underline{g})=1$, and $\kappa_4(\underline{g})=0$; with h^2=0.98 we get: $\kappa_2(\underline{e})=0.02$. This results in a value of 0.76 for the ratio of the variances of $\underline{V}_p$, which is close to the observed 0.75.

4. GENETIC SAMPLING AND PREDICTION

As mentioned in the introduction, estimation of D in an early generation of a breeding programme primarily serves a practical goal, i.e. the prediction of the genotypic variance which is to be expected in future generations. Since the latter depends on the actual genetic composition of the population from which the estimate is obtained (mostly the F_3), such a predictive estimate should refer to the actual sample population rather than to the probability distribution from which the sample was taken (mostly determined by the F_1). For this reason we introduce the sample dependent value of D. The sample dependent value D_S is the expected value of the variance of the F_∞-generation ($V_{F\infty}$) that can be derived by (continued) selfing the sample population: $D_S=E(\underline{V}_{F\infty}|\text{sample})$. We will consider the following sample dependent D-values:

F_1-generation: D_{F1} (is a constant);

in the F_1 allele frequencies are exactly equal ($\frac{1}{2}$), so D_{F1} is the parameter D in the usual sense;

F_2 and F_3-generation: $\underline{D}_{F2}$ and $\underline{D}_{F3}$ respectively;

$\underline{D}_{F2}$ and $\underline{D}_{F3}$ may differ from D_{F1} as the result of genetic sampling, i.e. deviations from $p=q=\frac{1}{2}$ in the actual population; given a certain F_2 or F_3 the respective parameters $\underline{D}_{F2}$ and $\underline{D}_{F3}$ are fixed constants (D_{F2} and D_{F3}), rather than random variables. ($D_{F2}=(\underline{D}_{F2}|F_2)$ and $D_{F3}=(\underline{D}_{F3}|F_3)$).

Obviously, the distinct D-types correspond to fixed or random genotypic effects. When $\hat{\underline{D}}$ is an estimator of D_{F1}, then the genotypic effects have to be treated as random. But when $\hat{\underline{D}}$ is an estimator of D_{F3}, then both the genotypic between and within line effects have to be treated as fixed. D_{F3} is a quadratic function of both the fixed genotypic between and the within line effects. These effects are only temporarily fixed: the next generation is determined by genetic segregation and hence genetic sampling of the F_3-genotypes. Since there are no standard methods for determining a confidence interval on this parameter D_{F3}, one might simply neglect the fact that one has to do with fixed effects, and apply the Williams-Tukey method.

In the following it is assumed that the estimate $\hat{\underline{D}}$ is obtained from an F_3-generation in the way described in section 2. In order to obtain an impression as to which degree $\hat{\underline{D}}$ is a better estimator of $E(\underline{V}_{F\infty}|\text{sample})$ than might be expected by considering it as an estimator of D_{F1}, we calculated the following quantities in a number of simulations:

- the mean square error $MSE_1=\Sigma(\hat{\underline{D}}-D_{F1})^2/n$ (n=number of simulations), which

reflects the error with respect to the probability distribution from which the sample was taken; it is also an estimator of the variance of $\hat{\underline{D}}$ (slightly biased);

- the mean square error $MSE_3=\Sigma(\hat{\underline{D}}-\underline{D}_{F3})^2/n$, which reflects the deviation due partially to environmental error in the F_3-generation, and partially to the realized distribution of genotypes over the lines;
- the correlation $corr(\hat{\underline{D}},\underline{D}_{F3})$, which is the correlation between the estimator and the goal parameter of the actual sample population;
- and the correlation $corr(\underline{D}_{F3},\underline{D}_{F2})$, which shows the amount of genetic sampling that occurs in advancing from F_2 to F_3.

As stated above, $\Sigma(\hat{\underline{D}}-\underline{D}_{F3})^2/n$ rather than $\Sigma(\hat{\underline{D}}-D_{F1})^2/n$ describes the predictive performance of $\hat{\underline{D}}$.

4.1 Method

The simulations were analogous to those described in section 2.3. The F_3-size and the number of loci could be varied. All loci had equal genotypic additive effects d, such that D_{F1} equals 1, independent of the number of loci. Linkage, dominance and epistasis were absent. All genotypes had equal residual variances E_w and E_b. Of each situation we determined the above mentioned parameters over 1000 replicate runs. (Rem.: as stated before, we repeated the 1000 replicate runs two more times, of which the results showed the same trends; see addendum of this chapter.)

4.2 Results

First we will look at the effects of different population structures and sizes, of which the results of the simulations are presented in Table 6. In these simulations the heritability was of the same high level as found for the *Arabidopsis* flowering time. MSE_1 is in all cases reasonably close to $var(\hat{\underline{D}}|normality)$, meaning that the influence of the discreteness of the distribution of genotypic values (determined by four loci) is limited. The correlation between $\underline{D}_{F3}$ and $\underline{D}_{F2}$ is in all situations high to very high. The smallest correlation coefficient was found, when each F_2-plant is sampled as only 2 F_3-plants (Table 6, case H) : 0.78, while already at 6 F_3-plants (Table 6, case J) the correlation is higher than 0.90. This means that the F_3 is a genetically very close sample of the F_2.

Since the ratio MSE_3/MSE_1 is around 0.40 in all cases (except maybe case H),

Table 6. Results of simulations of F_3's at different population sizes and structures, with 4 loci determining the genotypic effects; E_w=0.25^2 days^2; E_b=0.10^2 days^2; 1000 replications per situation; MSE_1 and var($\hat{\underline{D}}$|norm.) in (days^2)^2.

case:	A	B	C	D	E	F	G	H	I	J
lines	25	50	100	25	25	25	25	25	25	25
plots	2	2	2	4	8	2	2	2	2	2
plants	6	6	6	6	6	12	24	1	2	3
h^2 (bl)	0.981	0.981	0.981	0.990	0.995	0.985	0.988	0.945	0.965	0.972
var($\hat{\underline{D}}$|norm.)	0.094	0.046	0.023	0.089	0.086	0.090	0.087	0.154	0.116	0.104
MSE_1	0.082	0.043	0.022	0.078	0.078	0.075	0.077	0.136	0.095	0.094
MSE_3/MSE_1	0.40	0.40	0.39	0.40	0.40	0.41	0.39	0.50	0.45	0.43
corr($\hat{\underline{D}}$,$\underline{D}_{F3}$)	0.90	0.91	0.91	0.91	0.92	0.90	0.91	0.81	0.85	0.88
corr($\underline{D}_{F3}$,$\underline{D}_{F2}$)	0.95	0.95	0.96	0.98	0.99	0.97	0.99	0.78	0.87	0.91
% coverage D_{F1}	96.5	97.0	96.3	96.8	96.5	97.2	96.3	97.4	98.2	96.7
% coverage $\underline{D}_{F3}$	99.7	99.8	100.0	99.9	99.8	99.9	99.9	99.9	99.9	99.9

this indicates that this ratio does not depend much on the population size, although it varies with the between line heritability (which depends on the population structure), which is smallest in case H. It demonstrates our point that $\hat{\underline{D}}$ is much closer to $\underline{D}_{F3}$, the sample dependent parameter, than to D_{F1}, the parameter of the population that the F_3 was sampled from. Likewise, this affects the coverage of the 95% WT-confidence intervals. The coverage with respect to D_{F1} is around 96.5% (which is close to the expected coverage, see section 2), whereas the coverage with respect to $\underline{D}_{F3}$ is around 99.9%. Thus, the WT-confidence interval with respect to $\underline{D}_{F3}$ is in these cases very conservative. Because the heritability in these simulations is very high, the correlation of $\hat{\underline{D}}$ with $\underline{D}_{F3}$ is also quite high (± 0.90, Table 6).

In order to get an impression of the influence of the heritability level and the number of loci, we performed some simulations with a fixed population structure of 25 lines, 2 plots per line, and 6 plants per plot, at varying numbers of loci and at 3 levels of residual variance. The results are given in Table 7.

The effects of the discreteness of the genotypic effects, which are described in section 2.3 for the variance of $\hat{\underline{D}}$ and the coverage of the WT-confidence interval, are similar to the effects here for the corresponding parameters MSE_1 and the coverage of the WT-confidence interval with respect to D_{F1}.

Table 7. Results of simulations of F_3's with 25 lines, 2 plots per line, 6 plants per plot, at varying numbers of loci and at three heritability levels; 1000 replications per situation; E_w and E_b in days^2; MSE's and var($\hat{\underline{D}}$|norm.) in (days^2)^2.

loci	1	2	4	8	16
E_w=0.25^2, E_b=0.10^2, $h^2(bl)$=0.98, var($\hat{\underline{D}}$|norm.)=0.094:					
MSE_1	0.045	0.069	0.087	0.089	0.092
MSE_3	0.044	0.038	0.036	0.033	0.031
MSE_3/MSE_1	0.96	0.55	0.41	0.37	0.33
corr($\hat{\underline{D}}$,$\underline{D}_{F3}$)	0.19	0.78	0.90	0.94	0.95
corr($\underline{D}_{F3}$,$\underline{D}_{F2}$)	0.96	0.95	0.95	0.95	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.017	0.019	0.020
% coverage D_{F1}	99.7	97.8	96.2	97.1	95.9
% coverage $\underline{D}_{F3}$	99.7	99.7	99.9	100.0	99.8
E_w=1.5^2, E_b=0.5^2, $h^2(bl)$=0.63, var($\hat{\underline{D}}$|norm.)=0.267:					
MSE_1	0.20	0.23	0.25	0.27	0.26
MSE_3	0.20	0.20	0.20	0.21	0.20
MSE_3/MSE_1	1.00	0.86	0.82	0.78	0.79
corr($\hat{\underline{D}}$,$\underline{D}_{F3}$)	0.06	0.42	0.49	0.56	0.54
corr($\underline{D}_{F3}$,$\underline{D}_{F2}$)	0.96	0.95	0.95	0.95	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.010	0.016	0.018	0.018
% coverage D_{F1}	98.7	97.8	97.5	96.7	96.5
% coverage $\underline{D}_{F3}$	98.7	98.9	98.8	98.2	97.9
E_w=2.5^2, E_b=1.0^2, $h^2(bl)$=0.34, var($\hat{\underline{D}}$|norm.)=1.139:					
MSE_1	1.10	1.07	1.13	1.18	1.11
MSE_3	1.09	1.04	1.08	1.13	1.06
MSE_3/MSE_1	1.00	0.97	0.95	0.96	0.95
corr($\hat{\underline{D}}$,$\underline{D}_{F3}$)	0.06	0.18	0.26	0.23	0.25
corr($\underline{D}_{F3}$,$\underline{D}_{F2}$)	0.96	0.95	0.95	0.95	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.017	0.018	0.019
% coverage D_{F1}	96.2	96.7	95.8	96.3	96.2
% coverage $\underline{D}_{F3}$	96.2	97.0	96.1	96.3	96.2

The correlation between $\underline{D}_{F3}$ and $\underline{D}_{F2}$, which is purely genetic and thus not influenced by the heritability level, is in all cases about 0.95. This shows, that there is not much variance introduced by genetic sampling going from F_2 to F_3 (as was already evident from Table 6), and this appears to be independent of the number of loci.

The (estimated) variance of $\underline{D}_{F3}$ is very small when only one locus determines

the genotypic effects. Obviously, with the used F_3-size there will be hardly any variation possible in F_3 samples concerning the $\underline{D}_{F3}$-value.

MSE_3 is only visibly influenced by the number of loci at high heritability (h^2=0.98). As stated above, this mean square error reflects the estimation error due partially to environmental variance, and partially to the distribution of genotypes over the lines, i.e. $\underline{D}_{F3}$ is a function of both the genotypic between and within line variance, whereas $\hat{\underline{D}}$ in fact just estimates twice the genotypic between line variance. For example, two genetically different F_3's may have the same $\underline{D}_{F3}$-value, but different genotypic between line variances and hence different expected $\hat{\underline{D}}$-values (expectation over all environments). When more loci become involved, the average difference (reflected in MSE_3) between the expected $\hat{\underline{D}}$-value and twice the between line variance reduces, because with increasing numbers of loci the heterogeneity of genotypic within line effects decreases. This is seen to happen, even while the variance of $\hat{\underline{D}}$ (MSE_1) increases when more loci become involved, due to the normalization of genotypic between line effects. Since the (estimated) variance of $\hat{\underline{D}}$ increases and MSE_3 decreases at more loci the correlation $corr(\hat{\underline{D}},\underline{D}_{F3})$ can only but rise, as is evident from Table 7. Because of the extremely small variance of $\underline{D}_{F3}$ at one locus, this correlation at one locus is very low. The correlation between $\hat{\underline{D}}$ and $\underline{D}_{F3}$ is, apart from the number of loci, influenced by the heritability level. The $\hat{\underline{D}}$-estimation error, given an F_3, i.e. MSE_3, is only visibly affected by the numbers of loci at high heritability. Apparently, when heritability decreases, the error due to environmental variance becomes much more important than the error due to the distribution of genotypes over the lines. At the lowest tested heritability the MSE_3 even approaches MSE_1 (c.f. the quotient MSE_3/MSE_1 in Table 7). So, at low heritability $\hat{\underline{D}}$ is on the average about as close to $\underline{D}_{F3}$ as expected by considering it an estimator of D_{F1}. But at intermediate and high heritability $\underline{D}_{F3}$ is evidently closer, and thus describing the F_3-sample better.

At the three heritability levels the coverage of the WT-confidence interval with respect to $\underline{D}_{F3}$ is approximately the same for all tested numbers of loci. However, the coverage clearly depends on the heritability level. At the high heritability level the WT-confidence interval is rather conservative with respect to $\underline{D}_{F3}$, while at the low heritability level (h^2=0.34) the coverage is close to the [desired 95%] expected 96.5% level. At the intermediate heritability level the effect can still be detected.

5. DISCUSSION AND CONCLUSIONS

This paper presents results of research on some statistical aspects concerning the estimation of the genotypic additive variance in autogamous crops. These aspects are: 1) the possible invalidity of usual assumptions in quantitative genetic theory: a) the quantitative trait is determined by a limited number of genes, instead of by an infinite number of genes with equal and infinitesimal effect, b) there is heteroscedasticity of genotypic effects, and c) there can be heteroscedasticity of residual effects, as we found in our *Arabidopsis* experiment; and 2) the erroneous interpretation of an F_3-experiment: treating fixed effects as random when the statistical inference actually concerns the current F_3-sample, instead of the F_1 from which the current F_3 is realized. Although the research has not been extensive for all aspects, there are some interesting results.

From the simulation of an *Arabidopsis* experiment (section 2) already some aspects become clear. Heterogeneity of residual effects has a variance increasing impact on the estimator $\hat{\underline{D}}$. But this is only visible when the between line heritability is intermediate or low (i.e. when the residual effects are relatively large) and when the heterogeneity has a magnitude that is used in the simulations. Whether this magnitude of heterogeneity of residual variances at lower heritability is present in real experiments, remains a question, although we consider it to be quite great. In any case it should not be hard to detect in an experiment. Additionally, this type of heteroscedasticity is not very likely when more loci become involved in the determination of a trait; in that case the only likely type of heteroscedasticity of residual effects would seem to be of the "constant coefficient of variation" type, which can be cured by a data transformation.

Heteroscedasticity of residual effects has, though, a limited impact on the WT-confidence interval. The influence of heteroscedasticity of residual effects is that sometimes residual effects of predominantly large residual variances are sampled, sometimes of predominantly small variances, and sometimes of both. But in each particular case the residual variance part of the expectation of the MSP is the same as that of the MSL. This explains why the WT-confidence interval is relatively unaffected by heterogeneity of residual variances.

We could only detect influence of the heteroscedasticity of genotypic effects at very high heritability. It only increased the variances of MSP and MSR in the studied case with 2 loci (Table 4), while the variance of MSL is concurrently and stronger decreased as a result of non-normality; as a result the variance

of $\hat{\underline{D}}$ was decreased. The magnitude of the heterogeneity is too small to become important, because when more loci become involved the influence of non-normality decreases, the influence of heteroscedasticity will also decrease.

The non-normality of genotypic effects, caused by a limited number of loci determining the quantitative trait, may have an evident impact on the variance of $\hat{\underline{D}}$, and thus on the WT-confidence interval on D. Only when the number of loci determining the trait is small, combined with an intermediate to high between line heritability, is the deviation from normality large enough to have a significant influence. In such a case the variance of $\hat{\underline{D}}$ is smaller than expected, and the WT-confidence interval is rather conservative. A theoretical study on this subject in section 3 leads us to the conclusion that the effect of only a limited number of loci being involved can only become important when the number of loci is less than five, together with a higher heritability. Kelleher et al. (1958) have investigated the presence of non-normality (i.c. a significant fourth degree statistic) with respect to the precision of estimates of variance components on yield data in a large corn experiment. They did not indicate any possible cause of non-normality. They concluded, that the estimates of variance components of their corn yield data were unaffected by non-normality, to the level investigated. Yield is considered a trait of really polygenic nature, therefore their results meet the expectations, unless the non-normality is caused by environmental effects.

When the inference concerns the actual sample F_3, the (erroneously applied) Williams-Tukey procedure may lead to rather conservative intervals, i.e. the estimate is much closer to the true value than expected when observing the WT-confidence interval. This is especially manifest, when the between line heritability is high. But when the between line heritability is below 0.6 the procedure is reasonably accurate.

An interesting observation has been that the correlation between $\underline{D}_{F3}$ and $\underline{D}_{F2}$ is always very large. For practical breeding this means that the sample size of the F_2 is the bottleneck for the range of recombinant inbreds that can be expected in the offspring of a cross.

Summarizing, we may conclude 1) that heteroscedasticity of genetic and residual effects are not important; 2) that, if the quantitative trait is determined by a limited number of genes (say <5), the observed estimate of D is closer to the true value than expected with the WT-confidence interval, but this is only so when heritability is larger than say 0.5; 3) the erroneous use of the WT-confidence interval leads only to important deviations when between line

heritability is above 0.6.

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Table 3a. Results of first extra set of simulations described with Table 3.

	heteroscedastic E_w and E_b					homoscedastic E_w and E_b				
lines	25	50	100	25	25	25	50	100	25	25
plots	2	2	2	4	8	2	2	2	4	8
E_w and E_b unmodified from Table 1:										
h^2 (bl)	0.985	0.985	0.985	0.992	0.996	0.985	0.985	0.985	0.992	0.996
% coverage	98.3	98.8	99.4	98.3	98.5	98.2	99.1	99.4	97.8	97.6
$\hat{var}(\hat{D})$	904	377	195	738	756	847	392	195	828	778
var($\hat{D}$|norm.)	1251	613	303	1168	1129	1251	613	303	1168	1129
E_w and E_b 100 x the values of Table 1:										
h^2 (bl)	0.396	0.396	0.396	0.561	0.716	0.395	0.395	0.395	0.560	0.716
% coverage	93.7	93.9	94.8	95.3	97.4	97.4	96.9	97.2	96.5	97.4
$\hat{var}(\hat{D})$	17528	8881	4031	4591	1902	9807	5143	2472	3821	1864
var($\hat{D}$|norm.)	10618	5230	2596	3897	2211	10628	5235	2599	3900	2212

Table 4a. Results of first extra set of simulations described with Table 4.

	heteroscedastic E_w and E_b					homoscedastic E_w and E_b				
lines	25	50	100	25	25	25	50	100	25	25
plots	2	2	2	4	8	2	2	2	4	8
E_w and E_b unmodified from Table 1:										
$\hat{var}$(MSL)	309	127	66	1031	4294	290	132	66	1163	4426
var(MSL|norm.)	449	220	109	1681	6502	449	220	109	1681	6502
$\hat{var}$(MSP)	283	148	71	119	67	277	145	70	117	72
var(MSP|norm.)	178	89	44	59	25	178	89	44	59	25
$\hat{var}$(MSR)	57	26	14	46	43	54	26	13	47	42
var(MSR|norm.)	14	7	4	7	4	14	7	4	7	4
E_w and E_b 100 x the values of Table 1:										
$\hat{var}$(MSL)	4198	1957	899	5617	10061	2634	1331	602	5129	10548
var(MSL|norm.)	2782	1363	674	5265	12589	2784	1364	675	5269	12594
$\hat{var}$(MSP)	2420	1367	655	1221	743	1068	526	272	355	152
var(MSP|norm.)	1041	520	260	347	149	1042	521	261	347	149
$\hat{var}$(MSR)	120	72	33	104	83	29	14	7	15	7
var(MSR|norm.)	31	15	8	15	8	31	15	8	15	8

Table 3b. Results of second extra set of simulations described with Table 3.

	heteroscedastic E_w and E_b					homoscedastic E_w and E_b				
lines	25	50	100	25	25	25	50	100	25	25
plots	2	2	2	4	8	2	2	2	4	8
E_w and E_b unmodified from Table 1:										
h^2 (bl)	0.985	0.985	0.985	0.992	0.996	0.985	0.985	0.985	0.992	0.996
% coverage	98.8	98.2	99.3	98.3	97.5	97.9	98.0	99.7	98.6	98.2
$\hat{var}(\hat{\underline{D}})$	825	431	189	750	771	869	423	184	748	745
var($\hat{\underline{D}}$|norm.)	1251	613	303	1168	1129	1251	613	303	1168	1129
E_w and E_b 100 x the values of Table 1:										
h^2 (bl)	0.396	0.396	0.396	0.561	0.716	0.395	0.395	0.395	0.560	0.716
% coverage	93.9	94.8	94.3	95.5	98.2	96.0	96.4	97.0	95.8	97.4
$\hat{var}(\hat{\underline{D}})$	17197	8248	3955	4370	1915	11066	5183	2750	3796	1772
var($\hat{\underline{D}}$|norm.)	10618	5230	2596	3897	2211	10628	5235	2599	3900	2212

Table 4b. Results of second extra set of simulations described with Table 4.

	heteroscedastic E_w and E_b					homoscedastic E_w and E_b				
lines	25	50	100	25	25	25	50	100	25	25
plots	2	2	2	4	8	2	2	2	4	8
E_w and E_b unmodified from Table 1:										
$\hat{var}(\underline{MSL})$	279	146	63	1048	4381	295	144	63	1052	4245
var($\underline{MSL}$|norm.)	449	220	109	1681	6502	449	220	109	1681	6502
$\hat{var}(\underline{MSP})$	306	147	75	119	74	299	147	72	115	65
var($\underline{MSP}$|norm.)	178	89	44	59	25	178	89	44	59	25
$\hat{var}(\underline{MSR})$	55	27	14	47	46	52	26	13	46	39
var($\underline{MSR}$|norm.)	14	7	4	7	4	14	7	4	7	4
E_w and E_b 100 x the values of Table 1:										
$\hat{var}(\underline{MSL})$	3958	1860	868	5365	10445	2759	1315	697	5055	9978
var($\underline{MSL}$|norm.)	2782	1363	674	5265	12589	2784	1364	675	5269	12594
$\hat{var}(\underline{MSP})$	2616	1404	699	1209	748	1044	469	276	348	157
var($\underline{MSP}$|norm.)	1041	520	260	347	149	1042	521	261	347	149
$\hat{var}(\underline{MSR})$	133	60	34	105	76	29	15	7	15	7
var($\underline{MSR}$|norm.)	31	15	8	15	8	31	15	8	15	8

Table 5a. Results of first extra set of simulations described with Table 5.

loci	1	2	4	5	8	10	14	16
$E_w=0.25^2$, $E_b=0.10^2$, $h^2(bl)=0.98$:								
% coverage	99.1	98.0	96.4	97.1	97.4	96.9	96.7	95.9
$\hat{var}(\hat{\underline{D}})/var(\hat{\underline{D}}\mid norm.)$	0.50	0.76	0.91	0.87	0.88	0.91	0.86	0.96
$E_w=1.5^2$, $E_b=0.5^2$, $h^2(bl)=0.63$:								
% coverage	98.2	97.0	95.2	97.6	97.2	97.5	97.4	97.5
$\hat{var}(\hat{\underline{D}})/var(\hat{\underline{D}}\mid norm.)$	0.85	0.93	1.06	1.02	0.96	0.98	0.94	0.99
$E_w=2.5^2$, $E_b=1.0^2$, $h^2(bl)=0.34$:								
% coverage	96.9	97.3	97.6	95.8	96.7	95.4	96.6	95.6
$\hat{var}(\hat{\underline{D}})/var(\hat{\underline{D}}\mid norm.)$	0.94	0.92	0.94	1.01	0.99	1.01	0.95	1.01

Table 6a. Results of first extra set of simulations described with Table 6.

case:	A	B	C	D	E	F	G	H	I	J
lines	25	50	100	25	25	25	25	25	25	25
plots	2	2	2	4	8	2	2	2	2	2
plants	6	6	6	6	6	12	24	1	2	3
h^2 (bl)	0.981	0.981	0.981	0.990	0.995	0.985	0.988	0.945	0.965	0.972
$var(\hat{\underline{D}}\mid norm.)$	0.094	0.046	0.023	0.089	0.086	0.090	0.087	0.154	0.116	0.104
MSE_1	0.081	0.042	0.021	0.073	0.080	0.088	0.078	0.130	0.107	0.097
MSE_3/MSE_1	0.39	0.40	0.39	0.41	0.39	0.41	0.40	0.53	0.46	0.41
$corr(\hat{\underline{D}},\underline{D}_{F3})$	0.90	0.90	0.90	0.90	0.92	0.91	0.91	0.79	0.86	0.88
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.95	0.95	0.96	0.97	0.99	0.98	0.99	0.77	0.87	0.92
% coverage D_{F1}	97.1	97.0	97.2	97.0	96.0	95.8	95.7	97.4	96.4	96.4
% coverage $\underline{D}_{F3}$	100.0	100.0	100.0	99.7	99.9	99.6	99.9	99.7	99.7	100.0

Table 5b. Results of second extra set of simulations described with Table 5.

loci	1	2	4	5	8	10	14	16
$E_w=0.25^2$, $E_b=0.10^2$, $h^2(bl)=0.98$:								
% coverage	99.1	97.8	97.3	95.7	96.3	95.7	96.9	96.0
$\hat{var}(\underline{\hat{D}})/var(\underline{\hat{D}}\mid norm.)$	0.52	0.76	0.80	0.94	0.94	1.00	0.94	0.99
$E_w=1.5^2$, $E_b=0.5^2$, $h^2(bl)=0.63$:								
% coverage	98.6	97.8	97.1	96.7	96.2	97.3	96.3	96.7
$\hat{var}(\underline{\hat{D}})/var(\underline{\hat{D}}\mid norm.)$	0.75	0.85	0.93	1.04	0.98	0.99	1.00	1.03
$E_w=2.5^2$, $E_b=1.0^2$, $h^2(bl)=0.34$:								
% coverage	96.6	96.3	96.3	95.9	97.0	96.7	96.8	96.9
$\hat{var}(\underline{\hat{D}})/var(\underline{\hat{D}}\mid norm.)$	0.94	0.94	1.02	1.00	0.96	0.97	0.97	0.87

Table 6b. Results of second extra set of simulations described with Table 6.

case:	A	B	C	D	E	F	G	H	I	J
lines	25	50	100	25	25	25	25	25	25	25
plots	2	2	2	4	8	2	2	2	2	2
plants	6	6	6	6	6	12	24	1	2	3
h^2 (bl)	0.981	0.981	0.981	0.990	0.995	0.985	0.988	0.945	0.965	0.972
$var(\underline{\hat{D}}\mid norm.)$	0.094	0.046	0.023	0.089	0.086	0.090	0.087	0.154	0.116	0.104
MSE_1	0.081	0.040	0.020	0.077	0.074	0.074	0.075	0.130	0.109	0.095
MSE_3/MSE_1	0.40	0.40	0.39	0.39	0.39	0.41	0.39	0.52	0.45	0.41
$corr(\underline{\hat{D}},\underline{D}_{F3})$	0.90	0.89	0.90	0.91	0.91	0.90	0.91	0.80	0.86	0.89
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.95	0.95	0.95	0.98	0.99	0.97	0.99	0.79	0.87	0.91
% coverage D_{F1}	97.4	96.9	97.7	96.7	96.4	97.2	96.3	97.7	97.2	96.6
% coverage $\underline{D}_{F3}$	99.8	99.9	99.9	99.6	99.6	99.9	100.0	99.8	99.6	99.7

Table 7a. Results of first extra set of simulations described with Table 7.

loci	1	2	4	8	16
$E_w=0.25^2$, $E_b=0.10^2$, $h^2(bl)=0.98$, $var(\hat{\underline{D}}\mid norm.)=0.094$:					
MSE_1	0.046	0.072	0.086	0.087	0.090
MSE_3	0.044	0.039	0.035	0.032	0.030
MSE_3/MSE_1	0.96	0.55	0.41	0.37	0.33
$corr(\hat{\underline{D}},\underline{D}_{F3})$	0.21	0.79	0.90	0.93	0.95
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.96	0.95	0.95	0.94	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.016	0.018	0.020
% coverage D_{F1}	99.6	98.2	97.3	96.7	96.2
% coverage $\underline{D}_{F3}$	99.5	99.8	100.0	100.0	99.8
$E_w=1.5^2$, $E_b=0.5^2$, $h^2(bl)=0.63$, $var(\hat{\underline{D}}\mid norm.)=0.267$:					
MSE_1	0.20	0.25	0.27	0.29	0.27
MSE_3	0.20	0.22	0.21	0.22	0.21
MSE_3/MSE_1	0.99	0.87	0.78	0.77	0.77
$corr(\hat{\underline{D}},\underline{D}_{F3})$	0.07	0.41	0.56	0.57	0.56
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.95	0.95	0.95	0.96	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.016	0.019	0.020
% coverage D_{F1}	98.0	97.5	96.6	96.1	96.7
% coverage $\underline{D}_{F3}$	97.9	98.5	98.3	97.5	98.2
$E_w=2.5^2$, $E_b=1.0^2$, $h^2(bl)=0.34$, $var(\hat{\underline{D}}\mid norm.)=1.139$:					
MSE_1	1.16	1.05	1.12	1.21	1.19
MSE_3	1.16	1.01	1.07	1.14	1.12
MSE_3/MSE_1	1.00	0.97	0.95	0.94	0.94
$corr(\hat{\underline{D}},\underline{D}_{F3})$	-0.00	0.19	0.27	0.29	0.28
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.96	0.95	0.95	0.95	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.016	0.020	0.018
% coverage D_{F1}	96.3	97.1	96.6	95.5	95.5
% coverage $\underline{D}_{F3}$	96.1	97.1	96.9	96.1	95.6

Table 7b. Results of second extra set of simulations described with Table 7.

loci	1	2	4	8	16
$E_w=0.25^2$, $E_b=0.10^2$, $h^2(b1)=0.98$, $var(\hat{\underline{D}}\mid norm.)=0.094$:					
MSE_1	0.048	0.074	0.081	0.089	0.087
MSE_3	0.047	0.040	0.032	0.032	0.029
MSE_3/MSE_1	0.99	0.53	0.41	0.36	0.33
$corr(\hat{\underline{D}},\underline{D}_{F3})$	0.15	0.80	0.90	0.93	0.95
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.96	0.96	0.96	0.95	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.012	0.016	0.019	0.020
% coverage D_{F1}	99.3	98.0	97.0	96.7	96.5
% coverage $\underline{D}_{F3}$	99.4	99.7	100.0	99.9	99.8
$E_w=1.5^2$, $E_b=0.5^2$, $h^2(b1)=0.63$, $var(\hat{\underline{D}}\mid norm.)=0.267$:					
MSE_1	0.23	0.23	0.27	0.27	0.28
MSE_3	0.23	0.19	0.22	0.22	0.22
MSE_3/MSE_1	1.00	0.85	0.82	0.79	0.77
$corr(\hat{\underline{D}},\underline{D}_{F3})$	0.10	0.44	0.50	0.54	0.56
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.96	0.95	0.96	0.95	0.96
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.017	0.017	0.020
% coverage D_{F1}	98.0	97.6	96.5	96.6	96.1
% coverage $\underline{D}_{F3}$	98.1	98.0	98.0	98.0	98.1
$E_w=2.5^2$, $E_b=1.0^2$, $h^2(b1)=0.34$, $var(\hat{\underline{D}}\mid norm.)=1.139$:					
MSE_1	1.13	1.13	1.09	1.20	1.17
MSE_3	1.13	1.09	1.04	1.14	1.10
MSE_3/MSE_1	1.00	0.97	0.95	0.95	0.94
$corr(\hat{\underline{D}},\underline{D}_{F3})$	0.01	0.21	0.26	0.25	0.29
$corr(\underline{D}_{F3},\underline{D}_{F2})$	0.96	0.95	0.96	0.95	0.95
$\hat{var}(\underline{D}_{F3})$	0.001	0.011	0.018	0.019	0.020
% coverage D_{F1}	96.6	96.4	96.9	96.1	96.1
% coverage $\underline{D}_{F3}$	96.6	96.3	96.8	96.5	96.9

4. Bias caused by intergenotypic competition: 1. F_∞-mean

This chapter is in press for Euphytica.

Summary

Quantitative genetic theory for autogamous crops enables the estimation of the parameters m (F_∞-mean) and D (F_∞-variance) in the F_3-generation. With these estimated m and D a prediction can be made of the probability of finding superior inbred lines in the F_∞-offspring of the cross. The accuracy of this procedure is influenced by the correctness of the genetic model, by the magnitude of the error variance, and by the bias caused by intergenotypic competition, which is present in the environment in which the F_3 is grown and absent in the monoculture environment at which the selection is aimed, especially in a cereal crop like wheat. The influence of the intergenotypic competition was investigated by a special method of simulating segregating populations. In this method genotypes in the offspring of a cross are represented by varieties and segregating populations are composed by mixing the appropriate varieties according to the proper segregation frequencies. Growing the simulated population enables the estimation of m and D in the normal selection environment, while simultaneously growing the varieties in a large monoculture trial enables the calculation of "true" values of m and D in the monoculture environment. Hence, a comparison is possible.

The first of this set of two papers presents the investigations on the influence of intergenotypic competition on the estimation of the parameter m. The correlation coefficient of the estimates from the selection environment with the calculated "true" values from the monoculture environment was small. Sometimes the selection environment estimation significantly underestimated the (true) monoculture value of m, sometimes it significantly overestimated the monoculture value. On the average the smaller true values of m were underestimated and the larger ones overestimated.

INTRODUCTION

Quantitative genetic theory provides models for the prediction of the progeny of crosses between two pure-breeding lines with respect to a certain quantitatively inherited character (see Mather & Jinks, 1971,1977). The theory enables the estimation of quantitative genetic parameters, i.e. m (F_∞-mean: mean of all possible pure-breeding lines derived from the cross) and D (F_∞-variance: genotypic variance of all possible pure-breeding lines). With these estimates the ability of a cross to produce superior recombinant inbred lines can be predicted. In order to be useful in a practical breeding programme the estimation procedure should be time and labor extensive. This requires that the

estimates can be obtained in early generations without many (test) crosses to be made. The North Carolina Experiment III (Comstock & Robinson, 1952), the triple test cross design (Kearsey & Jinks, 1968) and the method using basic generations (F_1,F_2,B_1 and B_2) described by Jinks & Perkins (1970) require large numbers of test crosses to be evaluated, making these methods very unattractive for use in practical breeding programmes. One of the few remaining methods, that do not suffer from this disadvantage, is the estimation of the relevant quantitative genetic parameters using F_3-lines. This method, which is described by Jinks & Pooni (1980), comprises of estimating m as the mean of the F_3-offspring and estimating D as twice the genotypic between F_3-line variance.

Assuming a normal distribution of the genotypic values of all possible F_∞-lines derived by inbreeding a cross between two pure-breeding lines, only two parameters, i.e. m and D, need be estimated in order to predict the distribution of the F_∞-offspring. Given a certain threshold value T, e.g. the value of the best currently available variety, the probability, P_T, of finding in the F_∞-offspring of a cross a recombinant inbred line superior to the threshold value can be predicted. Estimating m and D for a number of crosses allows thus for the ranking of these crosses based upon their (predicted) probability of finding superior inbred lines. Hence, selection of the potentially better crosses will be possible, enabling the breeding programme to concentrate on these crosses.

Several factors may influence the accuracy of the predictions based on this theoretical model. First, the underlying genetic model may be erroneous. In its simplest form the model assumes absence of dominance, linkage and epistasis. Dominance, linkage and epistasis will, in general, result in biasedness of the estimators (see Mather & Jinks, 1971). Second, the error variance of the estimates may be considerable. Especially estimators of variance components have large error variances (compared to estimators of means). Third, genotype x environment interactions may seriously affect the estimates, especially in a cereal crop like wheat. Ideally, the growing conditions of the F_3-lines (from which the estimates are derived) should be as similar as possible to the commercial growing conditions. (In this paper the growing conditions of an F_3-generation are referred to as the "selection environment", whereas the commercial growing conditions are referred to as the "goal environment".) The main differences between selection environment and goal environment in a wheat breeding programme are:

- presence of intergenotypic competition in the selection environment, which is

absent in the goal environment. An F_3-generation is segregating both within and between lines, causing intergenotypic competition to be present within and between plots. It is well known that genetic variation among genotypes in competitive ability may result in large errors when yield in the selection environment (mixed stand) is extrapolated to the goal environment (pure stand). See e.g. Spitters (1979,1984) for a detailed account on the effects of intergenotypic competition on yield.

- difference due to plot type. Though sowing density in the selection environment can be taken the same as in the goal environment, the experimental layout of an F_3-trial, with empty space at the front and rear ends of the plots, will result in a growing condition different from the goal environment.

The aim of this set of two papers is to investigate the effects of these genotype x environment interactions on the predictive value of the estimated parameters m and D. The first paper concentrates on m; in the second the influence on D will be evaluated.

MATERIALS AND METHODS

Simulation of segregating populations - pseudo-lines

In the investigations presented here a special method of simulating segregating populations of a self fertilizing crop is used. The basic idea of the approach is that mixtures of varieties (or pure-breeding lines) can be used to simulate a segregating generation by letting each genotype in the offspring of a cross be represented by a variety. A simulated segregating line is called a pseudo-line, a simulated F_3 is called a pseudo-F_3. In this research segregation of two unlinked loci was simulated. The cross can be symbolized by: AAbb x aaBB. This cross has transgressive segregants. After assignment of varieties to genotypes, the composition of pseudo-F_3-lines is determined. For example, the pseudo-F_3-line, which is to mimic the F_3-line derived from the homozygous F_2-genotype AABB, will consist of 1/1 of the variety representing the genotype AABB. The pseudo-F_3-line, which is to mimic the F_3-line derived from the F_2-genotype aaBb, will consist of 1/4 of the variety representing genotype aaBB, 1/2 of the variety representing genotype aaBb, and 1/4 of the variety representing genotype aabb. The pseudo-F_3-line, which is to mimic the F_3-line derived from the F_2-genotype AaBb, will consist of respectively 1/16,2/16,1/16, 2/16,4/16,2/16, 1/16,2/16,1/16 of the varieties representing the genotypes AABB,AaBB,aaBB, AABb,AaBb,aaBb, AAbb,Aabb,aabb. Et cetera. Segregation ratio's

between and within pseudo-F_3-lines were chosen according to the expected Mendelian ratio's (no genetic sampling error).

A pseudo-F_3 is grown and analyzed as if it were a real F_3. Means and variance components are estimated in the usual way (except for the between line effect which has become a fixed effect). Simultaneously, the varieties are grown in large plots, representing the goal environment. The yields obtained from these plots can be taken as the true genotypic values (within the limits of experimental error) of the components of each pseudo-F_3. The advantage of this approach will be obvious: since the "true" genotypic value of each genotype in the goal environment is known, the "true" parameter values (m and D) of a pseudo-F_3 can be calculated (predicted) and can be compared with the estimates obtained directly in the selection environment.

General

In this paper we refer to four experiments:

a. the pseudo-F_3 experiment, in which simulated F_3's were grown as real F_3's, enabling estimation of m and D in the selection environment;
b. the monoculture experiment, in which the varieties, that were used in the simulation of the F_3's, were grown in pure stand in a large yield trial, enabling, for each simulated F_3, calculation of m and D in the goal environment;
c. the competition experiment, in which most of the varieties used in the simulation of the F_3's were grown in mixed stand, enabling, for each simulated F_3, calculation of m and D in a competition environment, which was expected to be comparable to the selection environment;
d. the experiment with real F_3's and related F_9-lines, which will give an impression of the values of m and D with real crosses.

The pseudo-F_3 experiment and the monoculture experiment were both carried out at two locations: APM (ir.A.P.Minderhoudhoeve, Agric.Univ.Wag., Swifterbant) and IVP (Dept.of Plant Breeding, Agric.Univ.Wag., Wageningen). The APM location has better growing conditions, which in general lead to higher yields. The competition experiment was performed only at APM. The experiment with real F_3's was carried out only at IVP. The character under investigation was grain yield of spring wheat, which is the most important quantitatively inherited character of spring wheat. Sowing was done in the first week of April 1987. Harvesting was done in September 1987. Dry matter content was determined, enabling correction of all yield data to 100% dry matter yield. All yield data were converted to

kilograms dry matter per hectare ($kg \cdot ha^{-1}$, 0% moisture). Harvesting, threshing, drying and weighing were done replicate-wise.

Material

23 (commercial) varieties or pure-breeding lines (hereafter referred to as varieties) of spring wheat and two varieties of spring barley were used. Spring barley was included for its known competitive ability (Estramil & Van Balen, 1983) in order to ensure inclusion of strong competitors in some of the pseudo-F_3's. The two barley varieties were chosen on the basis of their late maturity date, enabling harvest at the same time as spring wheat. The varieties of spring wheat were ranked for their yield capacity, based upon a number of yield trials in the 2 previous years. This rank cannot be considered to be more than an indication of the yield capacity. The 25 varieties are listed and numbered in Table 1.

Table 1. List of used varieties. The 23 spring wheat varieties are ordered according to their approximate yielding capacity. The two spring barley varieties are marked with (*).

No.	Name	No.	Name	No.	Name
1	Prinqual	9	Spartacus	17	vdH 1132
2	Axona	10	Melchior	18	vdH 1166-76-2
3	Sicco	11	Adonis	19	G 74010
4	Wembley	12	Bastion	20	TK 2832 2
5	Sunnan	13	G 8005	21	TK 2832 3
6	Kokart	14	vdH 3132	22	ZESC 1963-6
7	Heros	15	Darima	23	Minaret
8	Ralle	16	Stratos	24	Dauphne (*)
				25	Minerva (*)

Pseudo-F_3 experiment

15 crosses (pseudo-cross A to O) were created using the 25 varieties. They were composed in such a way that the pseudo-crosses would have clearly distinct values of the parameters m and D. For this purpose use was made of the estimated yield capacities of the varieties. Table 2 shows the intended levels of the parameter values. Table 3 shows the composition of the pseudo-F_3's.

The composition of pseudo-lines was based on numbers of seeds. 1000 grain weights were determined of all varieties. Using these 1000 grain weights the numbers of seeds were determined by weighing. The components of the pseudo-lines were mixed according to the expected segregation frequencies. Each F_3 consisted of 48 lines. The frequencies of lines also exactly mimicked the expected segregation frequencies, i.e. 3 (=1/16) lines derived from each F_2-genotype AABB, aaBB, AAbb and aabb, 6 (=2/16) lines derived from each

F_2-genotype AaBB, Aabb, AABb and aaBb, and 12 (=4/16) lines derived from F_2-genotype AaBb.

Each pseudo-F_3 was grown in a randomized block design with two replicates (=blocks). Each line was grown in one 3-row plot per block. Actually, an F_2-plant of spring wheat will produce just enough seed for growing its F_3-line in two of these 3-row plots. So this is the largest practically applicable design with respect to the size of the F_3-line. Of each of the 'pseudo-parents' of the pseudo-F_3, i.e. genotypes AAbb and aaBB, six 3-row plots were added to each replicate, in order to enable estimation of the mean value of the parents (the mid-parent value), and the residual variance. The mid-parent value is another estimator of the F_∞-mean. Because the means of all F_3's have to be comparable, the replicates of the F_3's were grown as superplots in a superimposed randomized block design, i.e. there were two superblocks, each containing one replicate of each F_3.

Table 2. Intended levels of parameter values of m and D of all pseudo-F_3's. Crosses marked with (*) contain spring barley.

Cross	m	D	Cross	m	D
A	low	low	J	medium	high
B	low	low	K	medium	high
C	low	low	L	medium	high
D	medium	low	M (*)	medium	high
E	medium	low	N (*)	medium	high
F	medium	low	O (*)	medium	high
G	high	low			
H	high	low			
I	high	low			

3-row plots were sown with a 6-row 'Seedmatic' plot seeder manufactured by the firm Walter and Wintersteiger (two neighboring plots simultaneous). Sowing density was 240 seeds m^{-2}. Plot length was 2.0 and 1.8 m at APM and IVP

Table 3. Composition of pseudo-F_3's. The numbers correspond with the varieties in Table 1.

Genotype	Pseudo-F_3														
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
AABB	9	10	11	13	14	15	21	22	23	21	22	23	24	24	24
AaBB	8	9	10	12	13	14	20	21	22	20	21	22	20	21	22
aaBB	7	8	9	11	12	13	19	20	21	19	20	21	19	20	21
AABb	6	7	8	10	11	12	18	19	20	10	11	12	10	11	12
AaBb	5	6	7	9	10	11	17	18	19	9	10	11	25	25	25
aaBb	4	5	6	8	9	10	16	17	18	8	9	10	8	9	10
AAbb	3	4	5	7	8	9	15	16	17	3	4	5	3	4	5
Aabb	2	3	4	6	7	8	14	15	16	2	3	4	2	3	4
aabb	1	2	3	5	6	7	13	14	15	1	2	3	1	2	3

respectively. Row distance was 20.8 cm. Plots were harvested with a Mitsubishi row binding machine. Afterwards the harvested plots were threshed with a combine.

Monoculture experiment

The 25 varieties were grown in a randomized block design with 12 and 9 replicates at locations APM and IVP respectively. The plots were sown with a 12-row sower (make Øyord). Sowing density was 240 seeds m^{-2}. Plot length was 8.0 and 7.0 m at resp. APM and IVP. Row distance was 12.5 cm. The experiment was combine-harvested.

Competition experiment

In order to demonstrate differences in competitive ability, especially of barley, 1-row plots were grown of 18 of the 25 varieties in a randomized block design with 24 replicates at the APM location. These were the varieties 1 to 5, 7 to 12, and 19 to 25. The plots were sown with the 6-row 'Seedmatic' plot seeder. Sowing density was 240 seeds m^{-2}. Plot length was 2.0 m. Row distance was 20.8 cm. Plots were harvested with a Mitsubishi row binding machine. Afterwards the harvested plots were threshed with a combine.

Analysis of pseudo-F_3's

The statistical model applied to analyze the pseudo-F_3 experiment is: (random effects are underlined)

$$\underline{y}_{ijk} = \mu + m_{F3i} + \underline{r}_j + \underline{s}_{(ij)} + g_{k(i)} + \underline{e}_{(ijk)},$$

μ	-	overall mean,	
m_{F3i} fixed, $\Sigma_i\, m_{F3i} = 0$	-	mean of pseudo-F_3 i	(i=1..15),
$\underline{r}_j \simeq N(0,\sigma_r^2)$	-	effect of replicate j	(j=1..2),
$\underline{s}_{(ij)} \simeq N(0,\sigma_{eS}^2)$	-	superplot effect,	
$g_{k(i)}$ fixed, $\Sigma_k\, g_{k(i)} = 0$	-	mean of line k of pseudo-F_3 i	(k=1..48),
$\underline{e}_{(ijk)} \simeq N(0,\sigma_{ep}^2)$	-	residual (plot) effect.	

The statistical model applied to the parents of the F_3's is:

$$\underline{y}_{ijkl} = \mu + m_{Pi} + \underline{r}_j + \underline{s}_{(ij)} + p_{k(i)} + \underline{e}_{l(ijk)},$$

μ	-	overall mean,
m_{Pi} fixed, $\Sigma_i\ m_{Pi} = 0$	-	mean of the parents of pseudo-F_3 i (i=1..15),
$\underline{r}_j \simeq N(0,\sigma_r^2)$	-	effect of replicate j (j=1..2),
$\underline{s}_{(ij)} \simeq N(0,\sigma_{eS}^2)$	-	superplot effect,
$p_{k(i)}$ fixed, $\Sigma_k\ p_{k(i)} = 0$	-	mean of parent k of pseudo-F_3 i (k=1..2),
$\underline{e}_{l(ijk)} \simeq N(0,\sigma_{eP}^2)$	-	residual (plot) effect (l=1..6).

The F_∞-mean m of each pseudo-F_3 was estimated as the average yield over all its 96 plots: $\mathbf{m}_{F3ps}$ (= estimator of $\mu+m_{F3i}$). The mid-parent value was estimated as the average over all 24 plots of the parents: $\mathbf{m}_{Pps}$ (= estimator of $\mu+m_{Pi}$). The variance of these estimators is resp.:

$$\text{var } \bar{\underline{y}}_{i..} = \sigma_r^2/2 + \sigma_{eS}^2/2 + \sigma_{eP}^2/(2\cdot 48),$$

$$\text{var } \bar{\underline{y}}_{i...} = \sigma_r^2/2 + \sigma_{eS}^2/2 + \sigma_{eP}^2/(2\cdot 2\cdot 6).$$

These variances are estimated using the analysis of variance of Table 4 and a similar ANOVA of the parents.

Table 4. Analysis of variance of pseudo-F_3 experiment.
m_{F3i} - mean of pseudo-F_3 i (fixed effect);
$g_{k(i)}$ - mean of line k of pseudo-F_3 i (fixed effect);
σ_r^2 - between replicate variance;
σ_{eS}^2 - between superplot residual variance;
σ_{eP}^2 - between plot residual variance;
c - No. of pseudo-F_3's = 15; l - No. of lines per pseudo-F_3 = 48;
r - No. of replicates = 2.

Source of variation	Mean square	Degrees of freedom	Expected mean square
between F_3's	MSBF	c-1	$\sigma_{eP}^2 + l\cdot\sigma_{eS}^2 + r\cdot l\cdot \sum_{i=1..c} m_{F3i}^2/(c-1)$
between replicates	MSBR	r-1	$\sigma_{eP}^2 + l\cdot\sigma_{eS}^2 + c\cdot l\cdot\sigma_r^2$
residual	MSR	$(c-1)\cdot(r-1)$	$\sigma_{eP}^2 + l\cdot\sigma_{eS}^2$
residual within F_3's	MSWF	$c\cdot(l-1)\cdot(r-1)$	σ_{eP}^2
for each F_3 i=1..c:			
between lines	$MSBL_i$	l-1	$\sigma_{eP}^2 + r\cdot \sum_{k=1..l} g_{k(i)}^2/(l-1)$

Prediction of pseudo-F_3's using monoculture data

The monoculture experiments were analyzed as a randomized block experiment. Applied model:

$$\underline{y}_{ij} = \mu + \alpha_i + \underline{b}_j + \underline{e}_{(ij)} \qquad i=1..25;\ j=1..B,\ B=12 \text{ at APM, } B=9 \text{ at IVP;}$$

α_i fixed, $\Sigma_i\, \alpha_i = 0$ - variety effect,
$\underline{b}_j \simeq N(0,\sigma_B^2)$ - replicate effect,
$\underline{e}_{(ij)} \simeq N(0,\sigma^2)$ - residual plot effect.

The mean variety yields were estimated. Error variance of the mean variety yield was estimated by:

$$\hat{\text{var}}\ \bar{\underline{y}}_{i.} = \hat{\sigma}^2/B + \hat{\sigma}_B^2/B = \hat{\sigma}_v^2 = s_v^2$$

Replicates are treated as random effects, because the yields in the monoculture experiment have to be comparable with yields in the F_3-experiment on an adjacent part of the experimental field.

Since the pseudo-F_3's simulate the segregation of two independent loci, the genotypic values of the nine genotypes can be described in terms of the parameters for a two-locus model (Mather & Jinks, 1971, p 83; 1977, p 100). This model is given in Table 5. The nine parameters of the model uniquely define the nine genotypic values. Thus, the monoculture yields of the constituent genotypes, which can be taken as estimates of the genotypic values, provide estimates of the nine parameters for each pseudo-F_3. In turn, these estimates can be used to predict the parameters m and D, which are also estimated directly from the pseudo-F_3's. The F_∞-mean m of the goal environment (monoculture), unbiased by intergenotypic competition, was predicted by:

$$m_{F\infty mo} = \sum_{i=1,3,7,9} \underline{g}_i\ /4\ , \quad \text{with variance} \quad \text{var}\ m_{F\infty mo} = \sigma_v^2/4.$$

The F_3-mean of the goal environment could be predicted using some of the (estimated) 9 parameters:

$$m_{F3mo} = m + h_a/4 + h_b/4 + 1/16.$$

m_{F3mo} could also be predicted directly in terms of $\underline{g}_i$, since the pseudo-F_3's were

Table 5. Model of Mather and Jinks (1977) describing the genotypic values of the nine possible genotypes of a digenic cross.

Genotype 2nd locus	Genotype 1st locus AA	Aa	aa
BB	$g_1 = m + d_a + d_b + i$	$g_2 = m + h_a + d_b + j_{ba}$	$g_3 = m - d_a + d_b - i$
Bb	$g_4 = m + d_a + h_b + j_{ab}$	$g_5 = m + h_a + h_b + l$	$g_6 = m - d_a + h_b - j_{ab}$
bb	$g_7 = m + d_a - d_b - i$	$g_8 = m + h_a - d_b - j_{ba}$	$g_9 = m - d_a - d_b + i$

Parameter	Effect
g_i	genotypic value of genotype i
m	F_∞-mean, mean of g_1, g_3, g_7 and g_9
d_a	additive effect of 1st locus
d_b	additive effect of 2nd locus
h_a	dominance effect of 1st locus
h_b	dominance effect of 2nd locus
i	effect of homozygote x homozygote interaction
j_{ab}	effect of homozygote x heterozygote interaction
j_{ba}	effect of heterozygote x homozygote interaction
l	effect of heterozygote x heterozygote interaction

composed exactly according to the Mendelian segregation frequencies:

$$m_{F3mo} = [\ 9 \cdot \sum_{i=1,3,7,9} \underline{g}_i + 6 \cdot \sum_{i=2,4,6,8} \underline{g}_i + 4 \cdot \underline{g}_5\ ]/64.$$

This expression enables the derivation of the variance of m_{F3mo}:

$$\text{var } m_{F3mo} = 121 \cdot \sigma_v^2/1024.$$

The ultimate comparison to be made is that between m_{F3ps} and $m_{F\infty mo}$. However, this comparison involves the effects of dominance, epistasis and intergenotypic competition. These effects can be separately studied by comparing m_{F3mo} to m_{F3ps}, which comparison studies the effect of intergenotypic competition, and by

comparing $m_{F\infty mo}$ to m_{F3mo}, which comparison studies the confounded effects of dominance and epistasis.

A short description of all used estimation and prediction parameters is given in Table 6. Since intergenotypic competition (which is absent in monoculture) may affect the genotypic values, it is to be expected that the parameter values predicted from the monoculture yields will show a poor fit with the direct estimates from the pseudo-F_3's.

Table 6. Description of estimation and prediction parameters. Predictions are done with data either from the monoculture or from the competition experiment.

m_{Pps}	mid-parent value, average of the 24 plots of the "pseudo-parents"
m_{F3ps}	F_3-mean, average of the 96 plots of the pseudo-F_3
m_{F3mo}	F_3-mean, prediction of $m+h_a/4+h_b/4+1/16$
$m_{F\infty mo}$	F_∞-mean, prediction of m

Prediction of pseudo-F_3's using competition data

The competition experiment was analyzed similar to the monoculture experiments. There is only a difference in the number of varieties and in the number of replicates. However, not all varieties, that were used in the composition of pseudo-F_3's, were present in the competition experiment. Therefore, not all pseudo-F_3's could be predicted completely. In order to still be able to make predictions about all pseudo-F_3's, the yields of the varieties not present in the competition experiment were traced back in the pseudo-F_3 experiment from all 3-row plots, that consisted of only one variety (pseudo-lines from F_2-genotypes g_1, g_3, g_7, and g_9 (Table 5), and also the pseudo-parents). Variety 18 was present only in segregating lines in the pseudo-F_3 experiment. For this variety a 3-row plot yield was predicted by linear regression of 3-row plot yields on monoculture yields. With the average yields of the varieties in the competition experiment, together with these traced yields, predictions could be made exactly like with the monoculture data. This resulted in six completely predictable crosses (J,K,L,M,N,O), three crosses with one dependent variety yield (A,B,C), three crosses with two to three (D,E,F), and three crosses with four to six dependent variety yields (G,H,I). Of course, the predictions based on a number of dependent yield data should tend to show a better fit. In contrast to the

prediction from monoculture data, it is expected to find a better fit of the prediction to the directly estimated parameters, because both the yields in the competition experiment and the yields in the pseudo-F_3 experiment are realized under conditions of intergenotypic competition.

Additional experiment with real F_3's and related F_9-lines

In order to obtain an impression of the parameter values that are to be expected in real F_3's and later generations of spring wheat, some material descending from three crosses made at IVP in 1981 was analyzed. These crosses involved a breeding line, TK 2832, as one parent, and each of the commercial varieties Stratos, Heros and Minaret as the other parent. From each of the three crosses 24 random F_7-lines were obtained by normal line breeding and single seed descent for two generations. During the winter 1985/86 the F_7-lines were multiplied in the greenhouse and in the normal 1986 growing season the F_8-lines were multiplied in the field in order to obtain sufficient seed for sowing a large 1987 yield trial. The TK 2832 line was expected to be pure-breeding. However, protein analysis of the seed showed, that the crosses had been made with two sub-lines, i.e. TK 2832 2 and TK 2832 3. Of the cross with Stratos 12 F_9-lines had been derived from a cross with sub-line 2, and 12 from a cross with sub-line 3. The same was the case for the cross with Heros. All 24 lines of the cross with Minaret were from TK 2832 3. In 1985 the five crosses of Table 7 (F_3 column) were made. The F_1-generation was grown in the greenhouse during winter 1985/86. In the 1986 season the F_2's were grown in the field at wide stand (40 x 40 cm^2) in order to obtain enough seed of the F_3-lines.

Table 7. Real crosses and related F_9-lines.

Cross	F_3	Cross	F_9
V	TK 2832 2 x Stratos	V&W	TK 2832 2/3 x Stratos
W	TK 2832 3 x Stratos		
X	TK 2832 2 x Heros	X&Y	TK 2832 2/3 x Heros
Y	TK 2832 3 x Heros		
Z	TK 2832 3 x Minaret	Z	TK 2832 3 x Minaret

In 1987 the real F_3's were grown at the IVP location in an experiment identical to the pseudo-F_3's, except for the larger number of lines per F_3, which was 172. The statistical model is the same as for the pseudo-F_3's except

for the genotypic between line effect, which is now a random effect: $\underline{g}_{k(i)} = N(0,\sigma_{gB}^2)$, in which σ_{gB}^2 is the genotypic between line variance. In the ANOVA of Table 4 the expected mean square of $MSBL_i$ becomes: $\sigma_{eP}^2 + r \cdot \sigma_{gB}^2$. The mean of each F_3 is the best available estimator of m, which is of course determined in the selection environment.

On an adjacent part of the experimental field the related F_9-lines were grown in three randomized block experiments with each ten blocks (per cross a separate experiment). The experimental conditions were exactly the same as for the previously described monoculture experiment. These experiments were analyzed as a normal randomized block experiment. The F_9-generation can be considered as an approximate F_∞. The mean of each F_9 is therefore an estimate of m in the goal environment.

RESULTS AND DISCUSSION

General

The variety yields in the monoculture and competition experiment, and also the variety yields of the 3-row plots traced back in the pseudo-F_3 experiment are presented in Table 8. The mean squares of the pseudo-F_3 experiment (except for

Table 8. Variety yields in $kg \cdot ha^{-1}$, 0% moisture. No. are the varieties, see Table 1. APM and IVP are monoculture experiments, comp is the competition experiment, and 3-row is the yield in 3-row plots traced back in the pseudo-F_3 experiment at APM. s.e. - standard error. *) This is the average s.e., it varies from 65 up to 226; the 3-row plot values are averages based on 6 to 72 plots with an average of 31 plots.

No.	APM	IVP	comp	3-row	No.	APM	IVP	comp	3-row
1	3990	3724	5885	5102	14	5118	4065		6345
2	4842	3895	4772	5561	15	4798	4326		6364
3	4752	3883	6943	6137	16	5493	4424		7025
4	4460	3679	4000	4930	17	5225	4552		6422
5	4948	4057	6539	6067	18	5287	4067		
6	4468	3895		5362	19	5344	4291	5371	6384
7	5062	4236	7914	6696	20	4511	4981	4850	5024
8	4982	4193	6200	6611	21	4877	4824	5300	5629
9	4809	4243	5725	6082	22	5054	4118	4815	6267
10	4749	4017	5699	5592	23	4534	3844	6794	5645
11	4727	3966	7108	5716	24	4699	3522	9205	6832
12	4754	4016	4794	5697	25	3971	3438	8617	
13	4919	3638		6626	s.e.	87	74	197	119*)
					mean	4815	4076	6141	6005

the $MSBL_i$'s) are given in Table 9. The estimations and predictions of the F_∞-mean are listed in Table 10. The yield level of the pseudo-F_3 experiment is much higher than that of the monoculture experiment. This is caused by the difference in effective plot area, i.e. the 3-row plots of the pseudo-F_3 have a relatively large amount of adjacent path area. Front and rear neighboring plots were separated by a path of 0.5 m. In order to be able to compare the experiments the yield data of the pseudo-F_3 experiment, and also of the competition experiment, were multiplied by 4816/6033 at APM and by 4118/4958 at IVP (c.f. Table 10). All subsequent analyses are based on these transformed yield data. There will probably be a genotype x environment interaction regarding these different plot types. In the analysis this interaction will be confounded with the interaction caused by intergenotypic competition. This plot type interaction is expected to be of minor importance, because Kramer et al. (1982) observed a correlation coefficient of 0.89 of this plot type (here 6-row plots, that are less biased by between plot competition than the present 3-row plots) with monoculture plot type with respect to grain yield (Kramer et al., Fig.6, p 556).

Table 9. Mean squares of pseudo-F_3 experiment. $(kg \cdot ha^{-1})^2$, 0% moisture.

Source of variation	APM		IVP	
	F_3	parents	F_3	parents
between crosses	5,243,323	6,193,690	14,949,118	4,647,823
between replicates	14,388,828	697,025	533,174	20
residual (superplot)	5,248,937	2,117,342	10,426,552	3,201,112
residual (plot)	307,794	329,284	192,438	200,457
missing plots (no.)	1	0	15	2

Mid-parent

The estimation of the F_∞-mean by using the average yield of the parent plots is not satisfactory. Correlations of m_{Pps} with the other F_∞-mean parameters are given in Table 11. The correlation between this mid-parent value, m_{Pps}, and the estimated F_3-mean, m_{F3ps}, is poor at APM, while at IVP it is quite high. Clearly, there is a large genotype x location interaction. The correlation of m_{Pps} to $m_{F\infty mo}$ is not better than that of m_{F3ps}, the mean of the whole F_3, to $m_{F\infty mo}$. The standard error of m_{Pps} is larger than that of m_{F3ps} (Table 10), which is of course caused by the smaller number of parent plots compared to F_3 plots. Increasing the number of parent plots would mean, that a breeding programme would contain a

Table 10. F_∞-mean. Estimates with pseudo-F_3 and predictions with monoculture and competition experiment. kg·ha^{-1}, 0% moisture. s.e. - standard error per cross.

Cross	APM						IVP			
	pseudo-F_3		monoculture		competition		pseudo-F_3		monoculture	
	m_{Pps}	m_{F3ps}	m_{F3mo}	$m_{F\infty mo}$	m_{F3mo}	$m_{F\infty mo}$	m_{Pps}	m_{F3ps}	m_{F3mo}	$m_{F\infty mo}$
A	6943	6179	4685	4654	6037	6617	4600	4348	3984	4022
B	5815	6092	4791	4758	5785	5168	4647	4598	4002	3946
C	6014	5899	4771	4809	6189	6579	5159	4810	4015	4037
D	6046	5800	4842	4914	6389	7047	5544	5339	4012	3974
E	6184	6117	4844	4831	6115	5675	4590	4541	4033	4042
F	6447	6279	4888	4897	6349	6657	5406	5445	4087	4111
G	6624	6509	5044	4985	6060	5915	4937	5008	4330	4270
H	6022	6092	5066	5044	5854	5759	5728	5432	4414	4397
I	6099	6266	4974	4859	6022	6220	6043	5574	4385	4386
J	5835	5752	4757	4741	5680	5875	5076	5185	4219	4181
K	4970	5813	4747	4717	5300	4609	5333	5244	4182	4168
L	5760	5606	4766	4778	5851	6394	5310	4973	4068	4152
M	5833	5983	4679	4696	6410	6851	4945	4727	3985	3855
N	5073	6019	4648	4628	6100	5707	4583	4497	4062	4019
O	5803	6088	4742	4819	6284	6997	4995	4650	3989	4072
s.e.	290	247	30	44	68	99	353	319	25	37
mean	5965	6033	4816	4809	6028	6138	5126	4958	4118	4109

large amount of parent material, which is undesirable. Therefore, the use of the mid-parent is not to be recommended.

F_3-mean

A more interesting comparison is that between m_{F3ps}, the most accurate estimator *of the* F_∞*-mean in the* F_3, *and* $m_{F\infty mo}$, *the predictor of the* F_∞*-mean in the goal* environment. There are three factors involved in this comparison. First and second, there are the confounded effects of dominance and epistasis, i.e. the F_3-mean m_{F3} is $m+h_a/4+h_b/4+l/16$, while the F_∞-mean is m. Third, there is the effect of intergenotypic competition, by which cause the genotypic values in the F_3-environment can be different from the genotypic values in monoculture. The effects of dominance and epistasis are not important, although present. The correlation between $m_{F\infty mo}$ and m_{F3mo} is high, at APM as well as at IVP (Table 11). The effect of intergenotypic competition is much more important. The correlation between m_{F3ps} and m_{F3mo} is low, at both APM and IVP (Table 11). This effect is

Table 11. Correlation coefficients between estimates and predictions of the F_∞-mean.

	APM			IVP			competition	
	m_{F3ps}	m_{F3mo}	$m_{F\infty mo}$	m_{F3ps}	m_{F3mo}	$m_{F\infty mo}$	m_{F3mo}	$m_{F\infty mo}$
m_{Pps}	0.57	0.41	0.39	0.92	0.61	0.65	0.43	0.48
m_{F3ps}		0.52	0.37		0.68	0.66	0.30	0.02
m_{F3mo}			0.92			0.93		0.84

shown in Fig.1. Ideally, the points in this figure should lie on the line $y = x$, which is surely not the case here. The values at APM are larger than at IVP, because of the better growing conditions at APM.

An important phenomenon is the fact that the regression lines have a regression coefficient much smaller than 1 (0.36 at APM; 0.32 at IVP), meaning, that the range of m_{F3} in the selection environment is larger than in the goal environment. This phenomenon can be explained with the proportional competition model of Spitters (1984):

$$Y_{i,mix} = b_i \cdot Y_{i,mono},$$

in which $Y_{i,mix}$ is the yield of genotype i in a mixed stand, $Y_{i,mono}$ is the yield of genotype i in monoculture, and b_i is the proportionality factor measuring the competitive ability of the genotype in the mixture. Now, if in a certain pseudo-F_3 there happens to be a negative correlation between the yield and the proportionality factor of the constituent genotypes, then the average of this pseudo-F_3 will be lower than its average predicted from monoculture yields without taking account of the b_i's. Similarly, if the correlation between the yield and the competitive ability of the genotypes happens to be positive in a certain pseudo-F_3, then the average of this pseudo-F_3 will be higher than its average predicted from monoculture yields. If both types of pseudo-F_3's, as well as intermediate types, are present, then it will result in a larger range of m_{F3} in the selection environment.

In Fig.1 the standard errors of each point in both the x- and the y-direction are represented in the top and at the right hand side by error bars. Taking into account these standard errors, it is clear from Fig.1a, that m_{F3ps} sometimes significantly underestimates m_{F3} (e.g. at APM cross L, and at IVP cross A), and that m_{F3ps} sometimes significantly overestimates m_{F3} (e.g. at APM cross G, and at

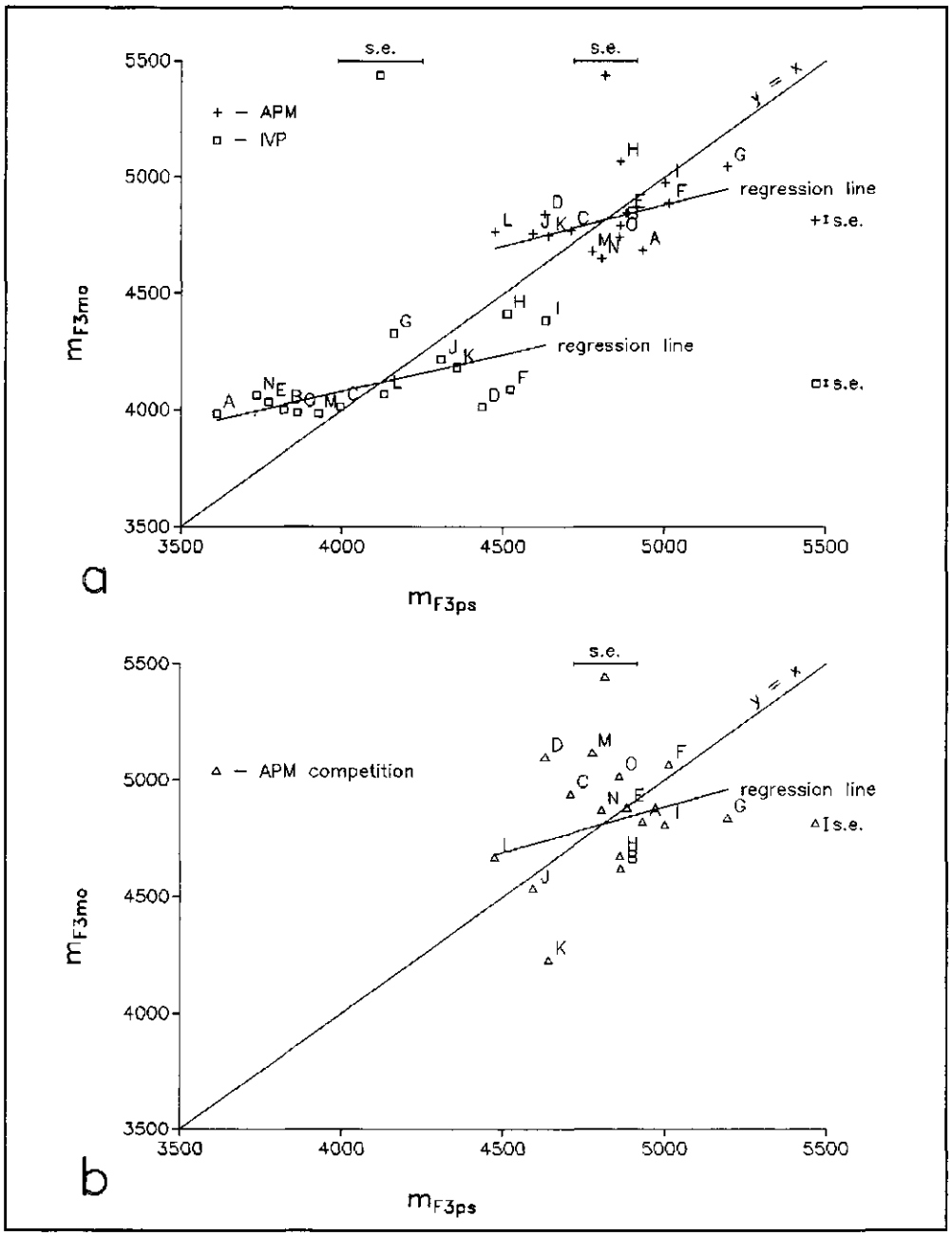

Figure 1a,b. Scatterdiagram of the prediction of the F_3-mean, m_{F3mo}, against the estimation of the F_3-mean in the selection environment, m_{F3ps}, for the pseudo-crosses A to O. **a** The prediction is done for the monoculture environment at APM and IVP. **b** The prediction is done for the competition environment at APM. Standard errors (s.e.) as well in the x- as in the y-direction are represented by error bars at the top resp. the right hand side of the diagrams. Regression lines are obtained by linear regression of m_{F3mo} on m_{F3ps}. kg·ha^{-1}, 0% moisture.

IVP cross F). Generally speaking, the larger values of m_{F3ps} are overestimated, and the smaller values are underestimated.

In contrast to what was expected, the correlations of the pseudo-F_3 estimates with the predictions using competition data were low, even lower than the correlations to the predictions based on monoculture data (Table 11). Fig.1b demonstrates significant differences between estimates and predictions. The cause of this becomes clear with Fig.2. This figure gives a representation of the genotype x environment interaction effects caused by intergenotypic competition. The coefficient of correlation of monoculture yield to 3-row plot yield is 0.77, whereas to 1-row plot yield of the competition experiment -0.19. The coefficient of correlation of 3-row to 1-row plot yield was found to be 0.64. The degree to which genotypes influence one another is of course relative to their distance in the field. In the pseudo-F_3 experiment the pseudo-lines derived from homozygous F_2-genotypes consist of only one variety, and therefore experience a relatively minor degree of intergenotypic competition, i.e. only

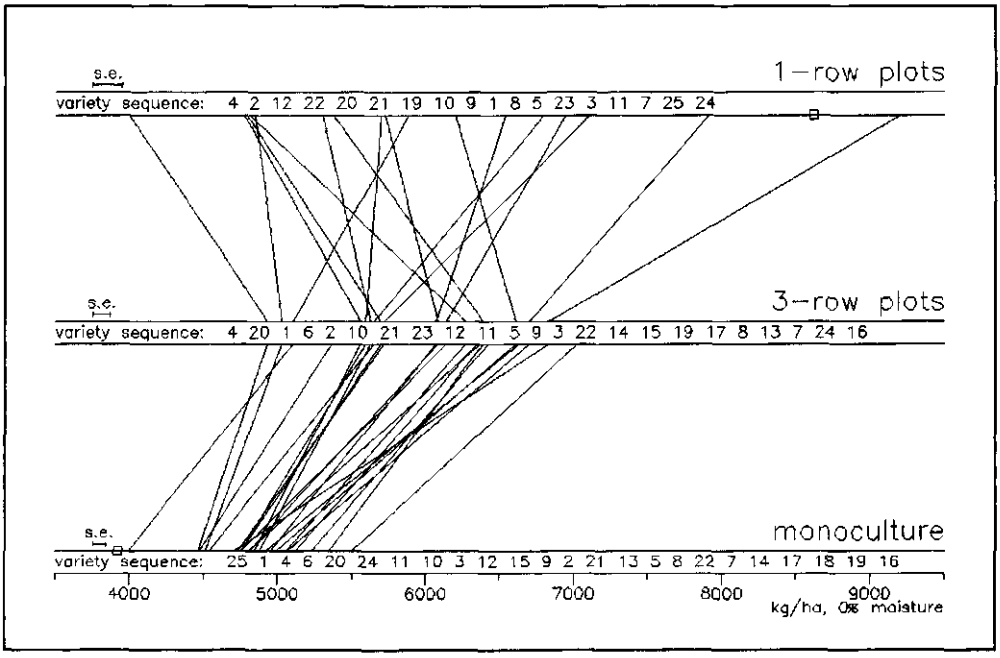

Figure 2. Variety yields at different plot types at APM. The 1-row plot yields are obtained with the competition experiment (24 replicates of 0.4 m^2 per variety), the 3-row plot yields are obtained by tracing the varieties in the pseudo-F_3 experiment (6 up to 72 replicates of 1.25 m^2 per variety), and the monoculture yields are obtained with the monoculture experiment (12 replicates of 12 m^2 per variety). The standard errors (s.e.) are represented by an error bar per plot type. Variety 25 could not be traced back in the 3-row plot type; therefore it is only represented by a square in the 1-row plots and monoculture bars.

between plots. But other pseudo-lines consist of more than one variety and therefore also endure within row intergenotypic competition. Therefore, the cause of the poor correlation between the predictions based on competition data and the pseudo-F_3 estimates is the difference in the degree of intergenotypic competition in both experiments.

Another important phenomenon is the difference of the parameter values at the different locations. The values of the correlation coefficient between APM and IVP for the F_∞-parameters are given in Table 12. This is another demonstration of the large effect of genotype x location interaction.

Table 12. Correlation coefficients of estimates and predictions between the experimental locations APM and IVP.

m_{Pps}	m_{F3ps}	m_{F3mo}	$m_{F\infty mo}$
-0.02	-0.02	0.78	0.59

Real F_3's and related F_9-lines

The results of the experiment with real F_3's and related F_9-lines are presented in Table 13. The yield level of the F_3's is higher than that of the F_9's. This is similar to yields of the pseudo-F_3 experiment compared with the monoculture experiment. It is caused by the difference in plot type. If the yield level of the F_3's is transformed by multiplication with 4453/5163 to the yield level of the F_9 monoculture plots, then a comparison is possible. The same picture emerges as with the pseudo-F_3's: the larger mean is overestimated and the smaller mean is underestimated, the regression of F_9-mean on F_3-mean has a coefficient smaller than 1.

Table 13. F_∞-mean estimation with real F_3's and related F_9's. *) - this F_3-mean is the F_3-mean multiplied with 4453/5163. kg·ha^{-1}, 0% moisture. s.e. - standard error.

Cross	F_3-mean	F_3-mean*)	Cross	F_9-mean	(s.e.)
V	6059	5226	V&W	4580	(87)
W	5847	5043			
X	4641	4003	X&Y	4431	(61)
Y	4905	4231			
Z	4364	3764	Z	4348	(76)
mean	5163	4453		4453	
s.e.	229	198			

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5. Bias caused by intergenotypic competition: 2. F_∞-variance

This chapter is in press for Euphytica.

Summary

Quantitative genetic theory for autogamous crops enables the estimation of the parameters m (F_∞-mean) and D (F_∞-variance) in the F_3-generation. With these estimated m and D a prediction can be made of the probability of finding superior inbred lines in the F_∞-offspring of the cross. The accuracy of this procedure is influenced by the correctness of the genetic model, by the magnitude of the error variance, and by the bias caused by intergenotypic competition, which is present in the environment in which the F_3 is grown and absent in the monoculture environment at which the selection is aimed, especially in a cereal crop like wheat. The influence of the latter was investigated by a special method of simulating segregating populations. In this method genotypes in the offspring of a cross are represented by varieties and segregating populations are composed by mixing the appropriate varieties according to the proper segregation frequencies. Growing the simulated population enables the estimation of m and D in the normal selection environment, while simultaneously growing the varieties in a large monoculture trial enables the calculation of "true" values of m and D in the monoculture environment. Hence, a comparison is possible.

The second of this set of two papers presents the investigations on the influence of intergenotypic competition on the estimation of the parameter D. The correlation between the estimates from the selection environment and the calculated "true" values from the monoculture environment was low. On the average the true values were overestimated, however in a few cases the monoculture values were significantly underestimated. Using the estimated m's and D's to rank crosses may lead to rather erroneous results.

INTRODUCTION

The first paper (Van Ooijen, 1989) describes a method of cross prediction, which is applicable in a practical breeding programme of autogamous crops. The method comprises of estimating the mean and variance of the F_∞-offspring of a cross (resp. m and D) with its F_3-population, followed by the prediction of P_T of the cross (under the assumption of normality of the F_∞), which is the probability of finding a superior recombinant inbred line in the F_∞-offspring (Fig.1). Estimating m and D for a number of crosses allows for the ranking of those crosses based upon their respective P_T's. Hence, selection of the potentially better crosses will then be possible, so that the breeding programme can concentrate on these crosses.

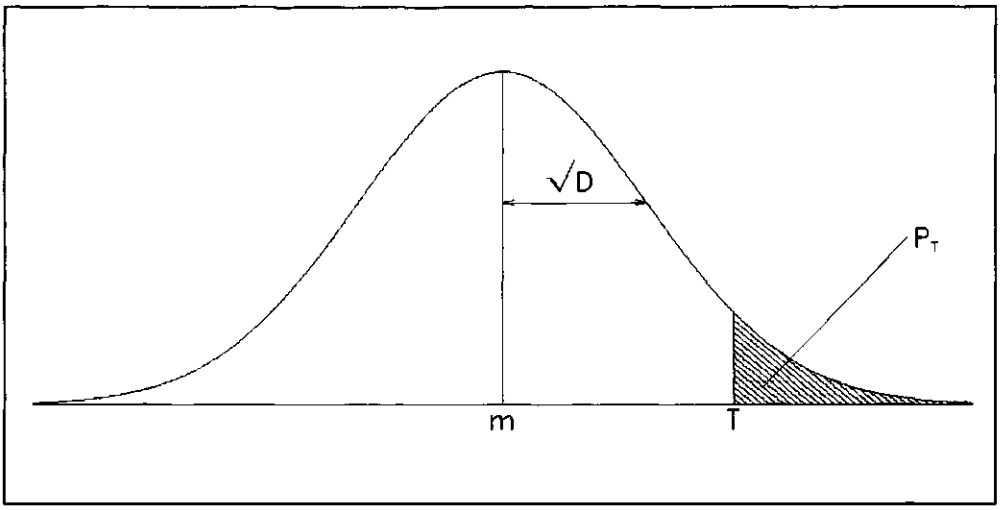

Figure 1. Distribution of the F_2-offspring of a cross between 2 pure-breeding lines of an autogamous crop with concern to the genotypic value of a quantitative trait. m - genotypic mean; D - genotypic variance; T - threshold value; P_T - probability of finding a recombinant inbred line superior to T.

As has been stated in the first paper, the predictive value of the method is determined by a number of factors. First, the underlying genetic model may be incorrect. The model assumes absence of dominance, linkage and epistasis; when present these phenomena will in general result in biased estimators. Second, the magnitude of the error variance of the used estimators may be too large to obtain accurate estimates. Estimators of variance components are known to have relatively large error variances. Third, genotype x environment interactions may seriously affect the genotypic values, and hence the estimators of derived parameters. In an F_3 selection environment (i.e. the growing conditions of the F_3-generation) neighboring plants are genetically different, whereas in the goal environment (i.e. the commercial growing conditions, mostly monoculture) the neighboring plants are genetically identical. From Spitters (1979,1984) it is known, that intergenotypic competition can influence the expression of characters like grain yield in cereals. Since the parameters m and D are estimated in the selection environment, the estimates may be biased with respect to the goal environment. This has indeed been found by Caligari & Powell (1986). Further, biased estimates of m and D will lead to biased predictions of P_T.

This paper presents the investigations on the bias caused by intergenotypic competition (which is in fact a special type of genotype x environment interaction) on the estimation of the quantitative genetic parameter D, the

genotypic variance of the F_∞-offspring of a cross between two pure-breeding lines of spring wheat.

MATERIALS AND METHODS

General

The experiment has been extensively described in the first paper (Van Ooijen, 1989). Here only a brief description is given. Segregating F_3-populations were simulated by a method called pseudo-lines method. The principle of this approach is, that mixtures of varieties (or pure-breeding lines) are used to simulate a segregating generation by letting each genotype in the offspring of a cross be represented by a variety. In this study the varieties were mixed according to the expected segregation frequencies of an F_3-generation; two independently segregating loci were simulated. A simulated F_3-generation is called a pseudo-F_3. On the one hand growing and analyzing pseudo-F_3's as if they were real F_3's enables estimation of quantitative genetic parameters in the selection environment, while on the other hand growing the used varieties in the goal environment (monoculture) enables estimation of the same parameters in the goal environment. This way a possible bias caused by the selection environment on the parameters can be investigated. The character under investigation was grain yield.

15 pseudo-F_3's were composed with a set of 23 available spring wheat and two spring barley varieties. Each pseudo-F_3 was made up of 48 pseudo-lines. Each pseudo-F_3 was grown in a randomized block design with two blocks (=replicates). Each line was grown in a 3-row plot ($\pm$ 1.2 m^2) per block. The blocks of the pseudo-F_3's were arranged as superplots in a superimposed randomized block design in order to be able to compare the F_3-means. The 25 composing varieties were grown in a monoculture experiment. It had a randomized block design with large plots ($\pm$ 11 m^2). The experiment as a whole was performed at two locations, APM and IVP. At APM the monoculture experiment consisted of 12 replicates, at IVP of 9 replicates. In order to get yield data of the varieties in an environment with intergenotypic competition, a competition experiment was designed. 18 varieties were grown in a randomized block experiment of 1-row plots ($\pm$ 0.4 m^2) with 24 replicates at APM.

At the IVP location there was an additional experiment with real spring wheat F_3's and their related F_9-lines. This experiment was to give an impression of the parameter values in real crosses. The real F_3's were grown in exactly the same

way as the pseudo-F_3's except for the number of lines, which was 172. The three families of related F_9-lines were each grown in a randomized block design with 10 blocks, and further similar to the monoculture experiment.

Analysis of pseudo-F_3's

As described in the first paper (Van Ooijen, 1989) the yield level of the small plots (pseudo-F_3 exp.,competition exp., real F_3 exp.) was found to be higher than that of large plots (monoculture exp.). It was considered to be caused by a difference in effective plot area. In addition to the interaction caused by intergenotypic competition, there will be a confounded genotype x environment interaction effect due to the differential capability of genotypes to utilize the plot area, but this was considered to be of minor importance relative to the interaction due to intergenotypic competition. To enable further comparison of parameters the plot yields of pseudo-F_3 plots were multiplied by 4816/6033 at APM and by 4118/4958 at IVP (c.f. Van Ooijen, 1989, Table 10), resulting in the same yield level for both plot types. It should be realized that this transformation reduces (quadratically) the variances calculated for the pseudo-F_3's.

The statistical model applied to the analysis of the pseudo-F_3 experiment is: (random effects are underlined)

$$\underline{y}_{ijk} = \mu + m_{F3i} + \underline{r}_j + \underline{s}_{(ij)} + g_{k(i)} + \underline{e}_{(ijk)},$$

μ	-	overall mean,	
m_{F3i} fixed, $\Sigma_i\, m_{F3i} = 0$	-	mean of pseudo-F_3 i	(i=1..15),
$\underline{r}_j \simeq N(0,\sigma_r^2)$	-	effect of replicate j	(j=1..2),
$\underline{s}_{(ij)} \simeq N(0,\sigma_{eS}^2)$	-	superplot effect,	
$g_{k(i)}$ fixed, $\Sigma_k\, g_{k(i)} = 0$	-	mean of line k of pseudo-F_3 i	(k=1..48),
$\underline{e}_{(ijk)} \simeq N(0,\sigma_{eP}^2)$	-	residual (plot) effect.	

Table 1 presents the analysis of variance of the pseudo-F_3 experiment. The between line genotypic variance of each pseudo-F_3 σ_{gB}^2 was estimated by:

$$\hat{\sigma}_{gBi}^2 = ((l-1)/l)\cdot(MSBL_i - MSWF)/r.$$

The factor (l-1)/l is present, because the lines exactly represent the expected segregation frequencies. Therefore the statistical effect of lines can be considered as a fixed effect. The F_∞-variance D of each pseudo-F_3 was estimated

by D_{F3ps}:

$$D_{F3ps} = 2 \cdot \hat{\sigma}_{gBi}^2.$$

The variance of this estimator was estimated by (see appendix):

$$\hat{var}\ D_{F3ps} = [\frac{2\cdot(1-1)}{1\cdot r}]^2 \cdot [\frac{4}{1-1} \cdot MSBL_i \cdot MSWF - \frac{2\cdot c\cdot(r-1)-2}{c\cdot(1-1)\cdot(r-1)+2} \cdot MSWF^2].$$

(N.B.: i fixed)

If the genotypic mean and variance of the F_∞ are known (estimated in the F_3), and the distribution of the F_∞ concerning the genotypic value is assumed to be normal, then the probability P_T of finding a recombinant inbred line in the F_∞-offspring of an F_3 superior to a threshold value T can be estimated. The pseudo-F_3 estimators of genotypic F_∞-mean and F_∞-variance are m_{F3ps} resp. D_{F3ps}. Therefore, P_T of a pseudo-F_3 was estimated by P_{TF3ps}:

$$P_{TF3ps} = P(m_{F3ps} + \sqrt{D_{F3ps}} \cdot \underline{x} > T), \text{ in which } \underline{x} \simeq N(0,1),$$

and P() denotes a probability.

The threshold value T was chosen as the yield of the best yielding variety in

Table 1. Analysis of variance of pseudo-F_3 experiment.
m_{F3i} - mean of pseudo-F_3 i (fixed effect);
$g_{k(i)}$ - mean of line k of pseudo-F_3 i (fixed effect);
σ_r^2 - between replicate variance;
σ_{eS}^2 - between superplot residual variance;
σ_{eP}^2 - between plot residual variance;
c - No. of pseudo-F_3's = 15; l - No. of lines per pseudo-F_3 = 48;
r - No. of replicates = 2.

Source of variation	Mean square	Degrees of freedom	Expected mean square
between F_3's	MSBF	c-1	$\sigma_{eP}^2 + 1\cdot\sigma_{eS}^2 + r\cdot 1\cdot \sum_{i=1..c} m_{F3i}^2/(c-1)$
between replicates	MSBR	r-1	$\sigma_{eP}^2 + 1\cdot\sigma_{eS}^2 + c\cdot 1\cdot\sigma_r^2$
residual	MSR	(c-1)·(r-1)	$\sigma_{eP}^2 + 1\cdot\sigma_{eS}^2$
residual within F_3's	MSWF	c·(1-1)·(r-1)	σ_{eP}^2
for each F_3 i=1..c:			
between lines	$MSBL_i$	1-1	$\sigma_{eP}^2 + r\cdot \sum_{k=1..1} g_{k(i)}^2/(1-1)$

the monoculture experiment at each experimental location. These were the variety Stratos at APM and the pure-breeding line TK 2832 2 at IVP. The assumption of normality is of course not realistic in this particular case (a system with two loci is simulated), but, since in a real F_3 it remains the most reasonable assumption, normality is also used here.

Prediction of pseudo-F_3's using monoculture data

The monoculture experiments were analyzed as a normal randomized block experiment. Applied model:

$$\underline{y}_{ij} = \mu + \alpha_i + \underline{b}_j + \underline{e}_{(ij)} \qquad i=1..25;\ j=1..B,\ B=12 \text{ at APM, } B=9 \text{ at IVP;}$$

α_i fixed, $\Sigma\, \alpha_i = 0$ - variety effect,
$\underline{b}_j \simeq N(0,\sigma_B^2)$ - replicate effect,
$\underline{e}_{(ij)} \simeq N(0,\sigma^2)$ - residual plot effect.

The mean variety yields were estimated. The error variance of the mean variety yield was estimated by:

$$\hat{\text{var}}\ \bar{\underline{y}}_{i.} = \hat{\sigma}^2/B + \hat{\sigma}_B^2/B = \hat{\sigma}_v^2 = s_v^2$$

Replicates are treated as random effects, because the yields in the monoculture experiment have to be comparable with yields in the F_3-experiment on an adjacent part of the experimental field.

Since the pseudo-F_3's simulate the segregation of two independent loci, the genotypic values can be described in terms of the parameters of a two-locus model of Mather & Jinks (1971, p 83; 1977, p 100). This model is given in Table 5 of Van Ooijen (1989). The nine parameters of the model uniquely define the nine genotypic values. Since the genotypic values of each of the nine constituent genotypes of each pseudo-F_3 were estimated in the monoculture experiment, they can be used for estimating the nine parameters for each pseudo-F_3. In turn, these estimated parameters can be used for predicting the parameters m and D under monoculture growing conditions. Because m and D are also estimated directly in the selection environment from the pseudo-F_3's, a comparison is possible.

The prediction of the between F_3-line variance of the goal environment is (see Mather & Jinks, 1971, p 173; 1977, p 113; V_{IF3}):

$$\sigma_{gB}^2 = (d_a+j_{ab}/4)^2/2 + (d_b+j_{ba}/4)^2/2 + (h_a+1/4)^2/16 + (h_b+1/4)^2/16 + i^2/4 + j_{ab}^2/32 + j_{ba}^2/32 + l^2/256.$$

σ_{gB}^2 can be expressed directly in terms of g_i, since the pseudo-F3's were composed according to the Mendelian segregation frequencies:

$$\begin{aligned}\sigma_{gB}^2 = [\ & 243\cdot\sum_{i=1,3,7,9}\underline{g}_i^2 + 108\cdot\sum_{i=2,4,6,8}\underline{g}_i^2 + 48\cdot\underline{g}_5^2 \\ & - 90\cdot(\underline{g}_1\cdot\underline{g}_3 + \underline{g}_1\cdot\underline{g}_7 + \underline{g}_3\cdot\underline{g}_9 + \underline{g}_7\cdot\underline{g}_9) - 154\cdot(\underline{g}_1\cdot\underline{g}_9 + \underline{g}_3\cdot\underline{g}_7) \\ & - 40\cdot(\underline{g}_2\cdot\underline{g}_4 + \underline{g}_2\cdot\underline{g}_6 + \underline{g}_2\cdot\underline{g}_8 + \underline{g}_4\cdot\underline{g}_6 + \underline{g}_4\cdot\underline{g}_8 + \underline{g}_6\cdot\underline{g}_8) \\ & + 36\cdot(\underline{g}_1\cdot\underline{g}_2 + \underline{g}_1\cdot\underline{g}_4 + \underline{g}_3\cdot\underline{g}_2 + \underline{g}_3\cdot\underline{g}_6 + \underline{g}_7\cdot\underline{g}_4 + \underline{g}_7\cdot\underline{g}_8 + \underline{g}_9\cdot\underline{g}_6 + \underline{g}_9\cdot\underline{g}_8) \\ & - 92\cdot(\underline{g}_1\cdot\underline{g}_6 + \underline{g}_1\cdot\underline{g}_8 + \underline{g}_3\cdot\underline{g}_4 + \underline{g}_3\cdot\underline{g}_8 + \underline{g}_7\cdot\underline{g}_2 + \underline{g}_7\cdot\underline{g}_6 + \underline{g}_9\cdot\underline{g}_2 + \underline{g}_9\cdot\underline{g}_4) \\ & - 40\cdot\sum_{i=1,3,7,9}\underline{g}_i\cdot\underline{g}_5 + 16\cdot\sum_{i=2,4,6,8}\underline{g}_i\cdot\underline{g}_5\ ]/4096.\end{aligned}$$

Straightforward prediction of σ_{gB}^2 by using the above expression, would lead to an overpredicted σ_{gB}^2. Each $\underline{g}_i$ is not exactly known, but is estimated with the monoculture experiment. Each $\underline{g}_i$ in the above equation can be replaced by $\mu_i+\underline{e}_i$, in which μ_i is the expected monoculture yield of the variety and $\underline{e}_i$ the residual error (with variance σ_v^2) of the mean variety yield estimated with the monoculture experiment. Writing the above expression for σ_{gB}^2 as a function f of $\underline{g}_1$ to $\underline{g}_9$, it can be shown, that the expectation of $f(\underline{g}_1,..,\underline{g}_9) = f(\mu_1+\underline{e}_1,..,\mu_9+\underline{e}_9)$ is:

$$E(f(\mu_1+\underline{e}_1,..,\mu_9+\underline{e}_9)) = f(\mu_1,..,\mu_9) + 363\cdot\sigma_v^2/1024.$$

The unbiased predictor of the between F_3-line variance of the goal environment, $\sigma_{gB}^2{}_{mo}$, is obtained by correcting $f(\underline{g}_1,..,\underline{g}_9)$ with the estimated $363\cdot\sigma_v^2/1024$:

$$\sigma_{gB}^2{}_{mo} = f(\underline{g}_1,..,\underline{g}_9) - 363\cdot s_v^2/1024.$$

This leads us to the unbiased predictor of twice the between F_3-line variance, D_{F3mo}, which is:

$$D_{F3mo} = 2\cdot\sigma_{gB}^2{}_{mo}.$$

It should be realized that this D_{F3mo} is not the genotypic variance of the F_∞; it contains dominance and epistatic components, that are not present in the

genotypic F∞-variance. It is predicting the with the F_3-generation best available estimator of this variance (which is twice the genotypic between F_3-line variance) in the growing conditions of the goal environment. So, discrepancies between D_{F3ps} and D_{F3mo} can be attributed solely to differences in growing conditions, i.e. in degree of intergenotypic competition.

In terms of the model of Mather and Jinks, the genotypic variance of the F_∞, $\sigma_{gF\infty}^2$, is:

$$\sigma_{gF\infty}^2 = d_a^2 + d_b^2 + i^2.$$

$\sigma_{gF\infty}^2$ expressed directly in terms of g_i becomes:

$$\sigma_{gF\infty}^2 = 3 \cdot \sum_{i=1,3,7,9} \underline{g}_i^2/16 - (\underline{g}_1 \cdot \underline{g}_3 + \underline{g}_1 \cdot \underline{g}_7 + \underline{g}_1 \cdot \underline{g}_9 + \underline{g}_3 \cdot \underline{g}_7 + \underline{g}_3 \cdot \underline{g}_9 + \underline{g}_7 \cdot \underline{g}_9)/8.$$

Writing the above expression for $\sigma_{gF\infty}^2$ as a function h of the $\underline{g}_i$'s, then, similar to σ_{gB}^2, $h(\underline{g}_1,\underline{g}_3,\underline{g}_7,\underline{g}_9) = h(\mu_1+\underline{e}_1,\mu_3+\underline{e}_3,\mu_7+\underline{e}_7,\mu_9+\underline{e}_9)$ would overpredict $\sigma_{gF\infty}^2$:

$$E(h(\mu_1+\underline{e}_1,\mu_3+\underline{e}_3,\mu_7+\underline{e}_7,\mu_9+\underline{e}_9)) = h(\mu_1,\mu_3,\mu_7,\mu_9) + 3 \cdot \sigma_v^2/4.$$

Therefore, the unbiased predictor of the genotypic variance of the F_∞ in the goal environment, $D_{F\infty mo}$, is:

$$D_{F\infty mo} = h(\underline{g}_1,\underline{g}_3,\underline{g}_7,\underline{g}_9) - 3 \cdot s_v^2/4.$$

Comparing $D_{F\infty mo}$ to D_{F3ps} is the ultimate comparison to be made. However, it involves differences in effects of dominance, of epistasis and of intergenotypic competition. The influence of dominance and epistasis (their effects are confounded) can be studied by comparing D_{F3mo} to $D_{F\infty mo}$, while the influence of intergenotypic competition can be studied by comparing D_{F3mo} to D_{F3ps}.

The variances of the predictors D_{F3mo} and $D_{F\infty mo}$ were approximated using the so-called delta technique (Bulmer, 1985, pp 82-83). In both cases the bias corrections ($363 \cdot s_v^2/1024$ and $3 \cdot s_v^2/4$) were neglected, because they are of minor importance. This results in:

$$\hat{var}\ D_{F3mo} \approx 4 \cdot \hat{var}\ f(\underline{g}_1,..,\underline{g}_9) \approx 4 \cdot s_v^2 \cdot \sum_{i=1..9} \frac{\delta f}{\delta g_i}, \quad \text{and}$$

$$\hat{var}\ D_{F\infty mo} \approx \hat{var}\ h(\underline{g}_1,\underline{g}_3,\underline{g}_7,\underline{g}_9) \approx s_v^2 \cdot \sum_{i=1,3,7,9} \frac{\delta h}{\delta g_i}.$$

The probability P_T of finding a recombinant inbred line in the F_∞-offspring of a pseudo-F_3 superior to a threshold value T, which should be attained by an F_3 in the goal environment, was predicted by P_{TF3mo}:

$$P_{TF3mo} = P(m_{F3mo} + \sqrt{D_{F3mo}} \cdot \underline{x} > T), \text{ in which } \underline{x} \simeq N(0,1).$$

The real P_T in the goal environment was predicted by $P_{TF\infty mo}$:

$$P_{TF\infty mo} = P(m_{F\infty mo} + \sqrt{D_{F\infty mo}} \cdot \underline{x} > T), \quad (\underline{x} \simeq N(0,1)).$$

The threshold value T was the same as with P_{TF3ps}, the yield of the best yielding variety at each experimental location.

A short description of all used estimation and prediction parameters is given in Table 2. The expectation was to find a poor fit of the prediction to the directly estimated parameters, which would be caused by intergenotypic competition.

Table 2. Description of estimation and prediction parameters. Predictions are done with data either from the monoculture or from the competition experiment.

m_{F3ps}	F_3-mean, average of the 96 plots of the pseudo-F_3
m_{F3mo}	F_3-mean, prediction of $m+h_a/4+h_b/4+l/16$
$m_{F\infty mo}$	F_∞-mean, prediction of m
D_{F3ps}	twice the between F_3-line variance, estimated with the pseudo-F_3
D_{F3mo}	twice the between F_3-line variance, prediction
$D_{F\infty mo}$	F_∞-variance, prediction
P_T	probability of finding a recombinant inbred line superior to the threshold value T in the F_∞-offspring of a cross
P_{TF3ps}	P_T estimated with m_{F3ps} , D_{F3ps} and T
P_{TF3mo}	P_T predicted with m_{F3mo} , D_{F3mo} and T
$P_{TF\infty mo}$	P_T predicted with $m_{F\infty mo}$, $D_{F\infty mo}$ and T

Prediction of pseudo-F_3's using competition data

The competition experiment was analyzed similar to the monoculture experiments. Yield data of varieties not included in this experiment were obtained by tracing 3-row plots of these varieties in the pseudo-F_3 experiment. Variety 18 could not

be traced this way. The yield of this variety was predicted from the regression of monoculture on 3-row plot yields. The prediction of pseudo-F_3's was similar to prediction with monoculture data. Because the prediction was done with some dependent yield data of varieties not included in the competition experiment, the prediction should tend to show a better fit than when all data would have been independent. The prediction with competition data is a prediction in a competition environment. Because an F_3 is also grown in a competition environment, the expectation was to find a good fit of this prediction to the pseudo-F_3 estimation.

Analysis of the experiment with real F_3's and related F_9-lines

Similar to the pseudo-F_3 experiment the yield level of the F_3-plots was higher than that of the F_9 monoculture plots. Prior to further analysis the F_3-plot yields were multiplied by 4453/5163 (c.f. Van Ooijen, 1989 Table 13). The real F_3's were analyzed exactly like the pseudo-F_3's with the exception, that the between line effect was treated as a random effect: $\underline{g}_{k(i)} \sim N(0,\sigma_{gB}^2)$, in which σ_{gB}^2 is the genotypic between line variance.

The F_9-generation can be considered as an approximate F_∞-generation. The genotypic variance between the F_9-lines can be considered as the genotypic variance of the F_∞ in a monoculture environment.

RESULTS AND DISCUSSION

Genotypic variance of the F_∞

Table 3 presents the results concerning the genotypic variance of the F_∞. Negative estimates of D_{F3ps} were set to zero. The coefficients of correlation between the parameters are given in Table 4. Similar to the F_∞-mean the most interesting comparison is that between the best available estimator of the F_∞-variance, D_{F3ps}, and the prediction of the F_∞-variance of the goal environment, $D_{F\infty mo}$. This comparison involves three factors: dominance, epistasis (which are confounded), and intergenotypic competition. The two confounded factors have little effect. The correlation of the prediction of twice the genotypic between F_3-line variance in the goal environment, D_{F3mo}, with the prediction of the F_∞-variance in the goal environment, $D_{F\infty mo}$, is high, both at APM and at IVP (Table 4). The third factor, however, does have a large effect. The correlation of D_{F3ps} with D_{F3mo} is low, especially at APM (Table 4). This result is also presented in Fig.2. From this figure we can see, that in most crosses the

Table 3. Mean squares between lines (MSBL) and estimates and predictions of the genotypic standard deviation of the F_∞. Residual mean square within F_3's: MSWF(APM)=196152; MSWF(IVP)=132731. Mean squares in $(kg \cdot ha^{-1})^2$, 0% moisture; standard deviations in $kg \cdot ha^{-1}$, 0% moisture.

Cross	APM				competition		IVP			
	MSBL	$\sqrt{D}_{F3ps}$	$\sqrt{D}_{F3mo}$	$\sqrt{D}_{F\infty mo}$	$\sqrt{D}_{F3mo}$	$\sqrt{D}_{F\infty mo}$	MSBL	$\sqrt{D}_{F3ps}$	$\sqrt{D}_{F3mo}$	$\sqrt{D}_{F\infty mo}$
A	396677	443	328	393	692	691	247817	336	215	216
B	263556	257	148	176	647	662	222313	296	175	176
C	296097	313	114	40	519	404	215486	285	132	118
D	147716	0	101	94	576	414	165953	180	164	207
E	262469	255	180	234	631	486	240384	325	124	86
F	178412	0	87	75	600	621	117938	0	210	267
G	252823	236	230	198	559	449	339082	450	407	416
H	628091	650	294	342	684	752	268290	364	308	358
I	309784	334	286	235	509	423	248385	337	281	354
J	715546	713	348	480	456	506	257628	350	449	420
K	532403	574	165	232	540	247	382639	495	484	490
L	180263	0	116	138	528	499	242461	328	326	391
M	213877	132	357	474	971	1168	273670	371	331	275
N	608701	636	130	132	1311	1628	358558	470	521	567
O	309087	333	165	63	972	1118	279646	379	382	471

F_∞-variance is significantly overestimated, but that there are also some crosses, of which the F_∞-variance is significantly underestimated (e.g. M at APM, and F at IVP). It should be noted, that in spite of the reducing transformation of pseudo-F_3 yield data to monoculture yield level, there still is an overestimation. This can be explained as follows. In underestimated crosses the variance is reduced, because the higher yielding genotypes happen to have a small competitive ability and the lower yielding genotypes happen to have a large competitive ability. While in overestimated crosses there happens

Table 4. Correlation coefficients between estimates and predictions of the genotypic standard deviation of the F_∞.

	APM		IVP		competition	
	$\sqrt{D}_{F3mo}$	$\sqrt{D}_{F\infty mo}$	$\sqrt{D}_{F3mo}$	$\sqrt{D}_{F\infty mo}$	$\sqrt{D}_{F3mo}$	$\sqrt{D}_{F\infty mo}$
$\sqrt{D}_{F3ps}$	0.45	0.43	0.67	0.55	0.19	0.21
$\sqrt{D}_{F3mo}$		0.92		0.95		0.97

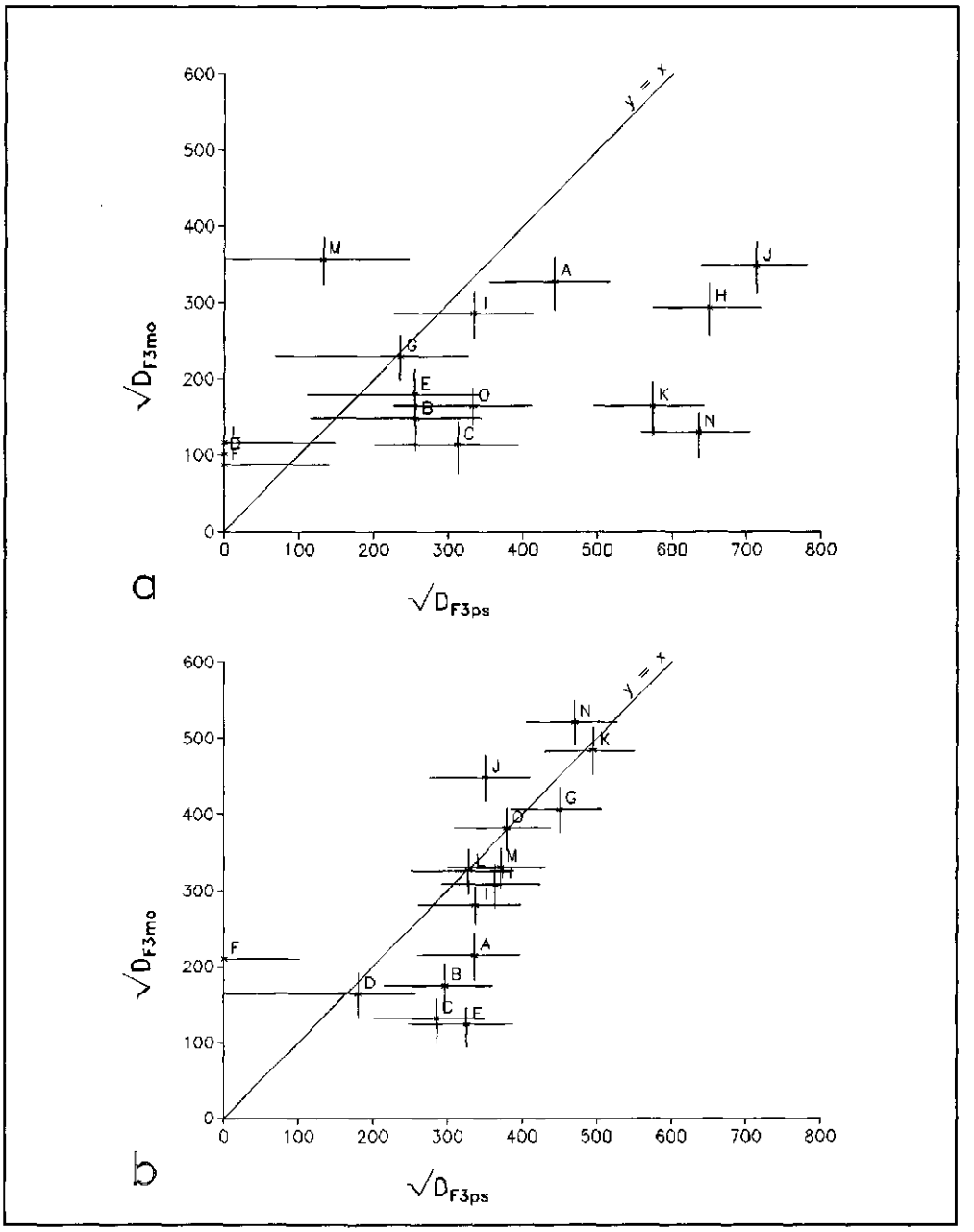

Figure 2a,b,c. Scatterdiagram of the prediction of the F_3-standard deviation, D_{F3mo}, against the estimation of the F_3-standard deviation in the selection environment, D_{F3ps}, for the pseudo-crosses A to O. **a** prediction for the monoculture environment at APM **b** prediction for the monoculture environment at IVP **c** prediction for the competition environment at APM. Error bars for each point in x- and y-direction represent the range from $\sqrt{(D - s.e.(D))}$ to $\sqrt{(D + s.e.(D))}$, s.e. - standard error. kg·ha^{-1}, 0% moisture.

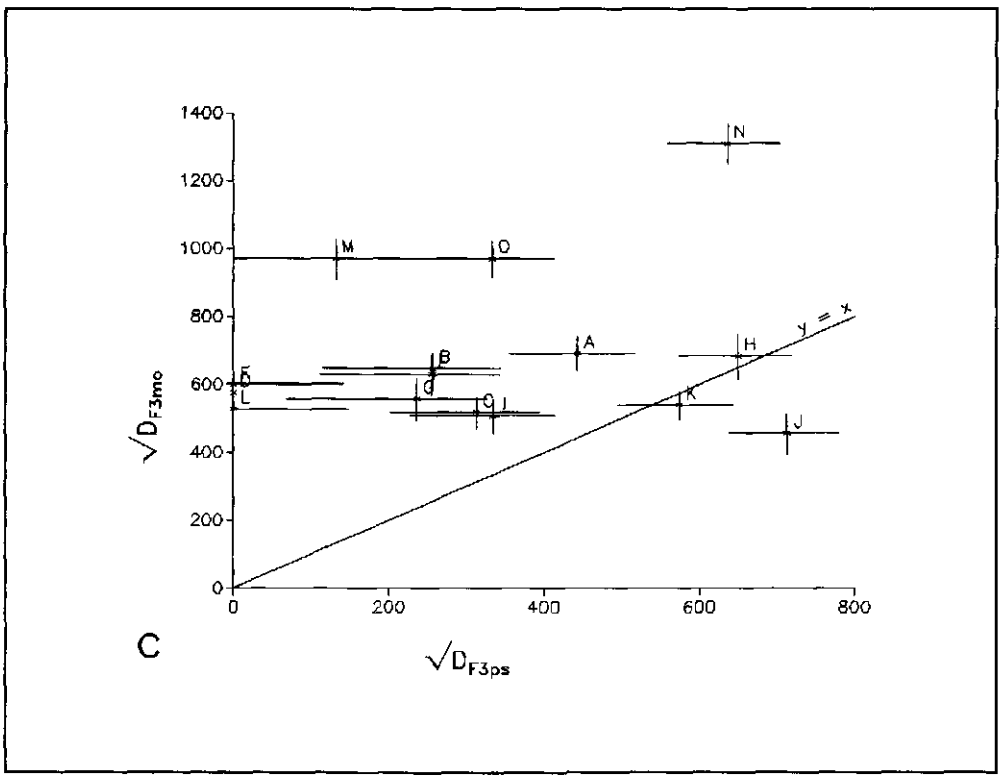

Figure 2c.

to be a positive, or even zero, correlation between genotype yield and competitive ability.

The prediction using the competition data resulted in a very low correlation of the prediction to the estimation parameters (Table 4). Similar to the F_∞-mean, this can be explained by the difference in the proportion of intergenotypic competition in both experiments. The effect of intergenotypic competition is relative to the distance between genotypes. A pseudo-F3 contains pseudo-lines from heterozygous F_2-parents; these lines even experience within row intergenotypic competition. But a pseudo-F_3 also contains pseudo-lines from homozygous F_2-parents, that experience only a minor degree of intergenotypic competition, i.e. competition between 3-row plots. The average level of intergenotypic competition in a pseudo-F_3 will therefore be lower than the level of intergenotypic competition experienced by genotypes in the competition experiment. The resulting overprediction is evident with Fig.2c, especially for the pseudo-F_3's with barley (crosses M,N, and O).

The phenomenon of a large genotype x location interaction is also evidently present in the F_∞-variance parameters (Table 8). The correlation between the same parameters at APM and IVP is low.

Real F_3's and related F_9-lines

The results of the real spring wheat crosses on the F_∞-variance are similar to the results of the pseudo-F_3's (Table 5). The levels of the parameters are the same as in the pseudo-F_3 experiment. The F_∞-variance in the monoculture environment is much smaller (significant) than the estimations in the F_3's. The fact, that two F_9's (V&W and X&Y) were actually a mixture of the offspring of two crosses, does not influence this result, since this fact should only cause the variance of these populations to be larger than the individual variances.

Table 5. F_∞-standard deviation estimation with real F_3's and prediction with related F_9's. $l=\sqrt{(D-s.e.(D))}$, $u=\sqrt{(D+s.e.(D))}$. $kg \cdot ha^{-1}$, 0% moisture.

Cross	l	u	$\sqrt{D_{F3}}$	Cross	l	u	$\sqrt{D_{F9}}$
V	427	526	479				
W	298	402	354	V&W	174	240	209
X	265	373	323				
Y	318	421	373	X&Y	154	214	187
Z	316	418	371	Z	213	291	255
mean			380				217

Consequences to the ranking of crosses

As mentioned before, estimating m and D for each cross in a breeding programme allows for the ranking of the crosses based upon their predicted probability, P_T, of finding superior inbred lines in the F_∞. The results on P_T are given in Table 6 and presented in Fig.3. We have seen, that the parameters m (Van Ooijen, 1989) and D are not accurately estimated. First, there is a minor effect of dominance and epistasis on both parameters. Second, but much more important, there is the effect of intergenotypic competition. It influences both parameters. The parameter m is sometimes overestimated and sometimes underestimated. This fact, that the estimated F_3-means have a larger range than the predicted F_3-means, causes a bias in the prediction of P_T. P_T will depend more on the F_3-mean and less on D_{F3ps} than when the F_3-mean would not have been

Table 6. Estimates and predictions of the probability of finding superior inbred lines in the F_∞. At APM (also for competition) T=5493, at IVP T=4981 kg·ha^{-1}, 0% moisture. E-notation example: "2.13 E-2" means: $2.13 \cdot 10^{-2} = 0.0213$.

Cross	APM			competition		IVP		
	P_{TF3ps}	P_{TF3mo}	$P_{TF\infty mo}$	P_{TF3mo}	$P_{TF\infty mo}$	P_{TF3ps}	P_{TF3mo}	$P_{TF\infty mo}$
A	1.03 E-1	6.88 E-3	1.64 E-2	1.65 E-1	3.80 E-1	2.28 E-5	1.77 E-6	4.51 E-6
B	7.12 E-3	1.05 E-6	1.48 E-5	8.81 E-2	1.94 E-2	4.33 E-5	1.11 E-8	2.05 E-9
C	6.18 E-3	1.2 E-10	7.9 E-66	1.43 E-1	2.75 E-1	2.70 E-4	1.3 E-13	6.3 E-16
D	0	5.8 E-11	3.7 E-10	2.48 E-1	6.26 E-1	1.19 E-3	1.73 E-9	5.74 E-7
E	8.37 E-3	1.56 E-4	2.33 E-3	1.66 E-1	2.39 E-2	9.96 E-5	1.1 E-14	4.8 E-28
F	0	1.8 E-12	9.7 E-16	2.39 E-1	3.87 E-1	0	1.04 E-5	5.60 E-4
G	1.04 E-1	2.55 E-2	5.15 E-3	1.21 E-1	4.30 E-2	3.39 E-2	5.49 E-2	4.37 E-2
H	1.67 E-1	7.32 E-2	9.46 E-2	1.15 E-1	1.17 E-1	9.83 E-2	3.28 E-2	5.14 E-2
I	7.08 E-2	3.48 E-2	3.49 E-3	8.92 E-2	1.06 E-1	1.48 E-1	1.70 E-2	4.64 E-2
J	1.03 E-1	1.72 E-2	5.86 E-2	1.77 E-2	5.63 E-2	2.69 E-2	4.48 E-2	2.84 E-2
K	6.86 E-2	3.08 E-6	4.12 E-4	9.72 E-3	1.1 E-13	1.03 E-1	4.94 E-2	4.85 E-2
L	0	1.8 E-10	1.10 E-7	5.98 E-2	2.18 E-1	4.74 E-3	2.55 E-3	1.70 E-2
M	2.92 E-8	1.13 E-2	4.63 E-2	3.49 E-1	4.92 E-1	2.23 E-3	1.31 E-3	2.12 E-5
N	1.40 E-1	4.0 E-11	2.8 E-11	3.17 E-1	2.82 E-1	4.01 E-3	3.89 E-2	4.49 E-2
O	2.87 E-2	2.67 E-6	5.3 E-27	3.12 E-1	5.33 E-1	1.58 E-3	4.70 E-3	2.68 E-2

Table 7. Spearman rank correlation coefficients between estimates and predictions of the probability P_T of finding superior inbred lines in the F_∞. At APM (also for competition) T=5493, at IVP T=4981 kg·ha^{-1}, 0% moisture.

	APM		IVP		competition	
	P_{TF3mo}	$P_{TF\infty mo}$	P_{TF3mo}	$P_{TF\infty mo}$	P_{TF3mo}	$P_{TF\infty mo}$
P_{TF3ps}	0.58	0.48	0.78	0.82	-0.26	-0.47
P_{TF3mo}		0.86		0.90		0.76

biased. But, on the other hand, D_{F3ps} is averagely overestimated, resulting in an overprediction of P_T. Since the function P_T is a complex kind of function, the effect of the inaccuracies in the estimation of the F_∞-mean and of the F_∞-variance is also complex. It has resulted in the low Spearman rank correlation between P_T of the selection environment and P_T of the monoculture environment at the APM location, while at IVP the correlation is moderately high (Table 7). P_T is chiefly overpredicted at APM, while at IVP there are many underpredictions (Fig.3). The effects of dominance and epistasis are present, but not as important as intergenotypic competition (Table 7).

The prediction of P_T using competition data is very poor, the rank

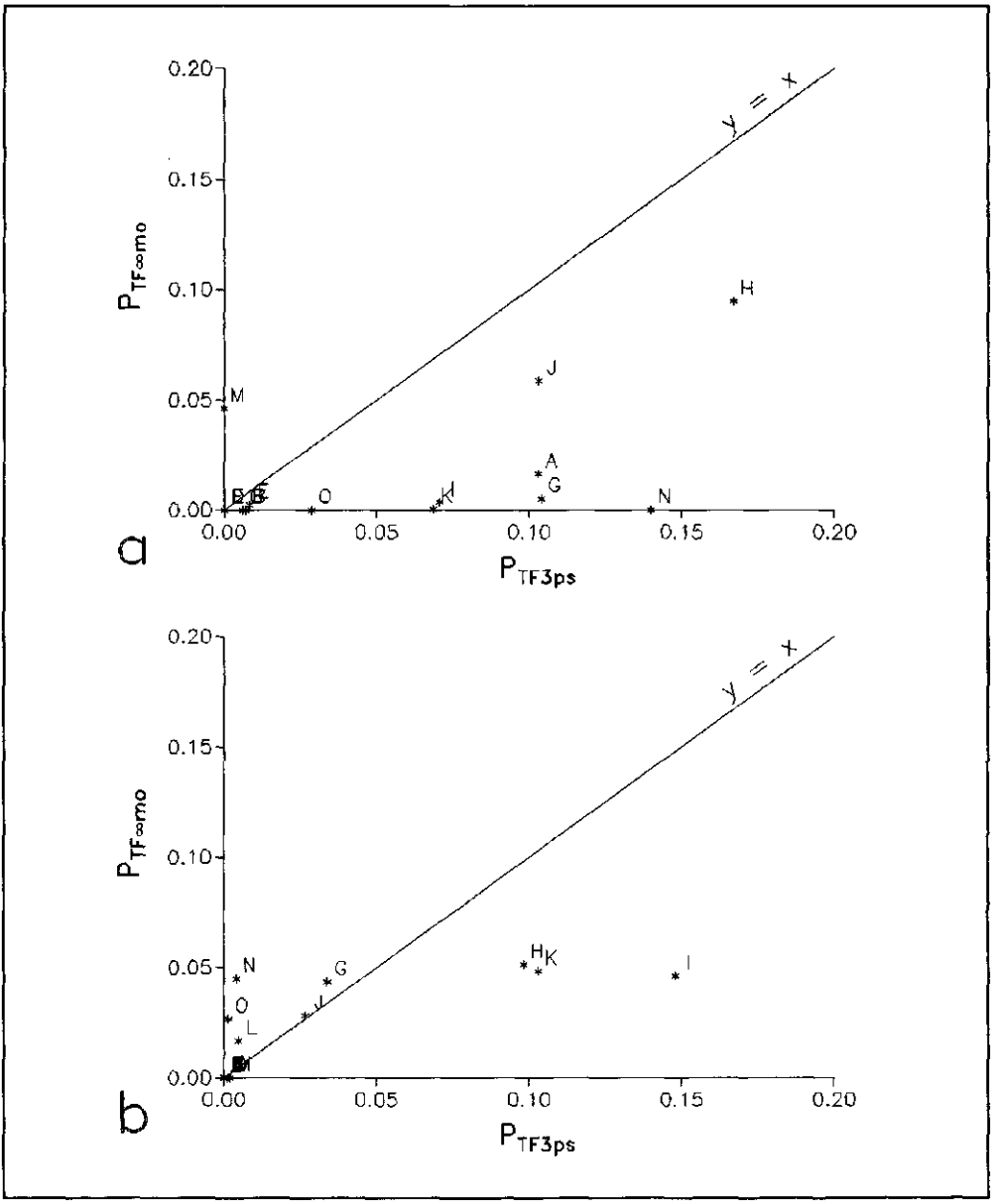

Figure 3a,b. Scatterdiagram of the cross prediction P_T in the monoculture environment, $P_{TF\infty mo}$, against the cross prediction in the selection environment, P_{TF3ps}, for the pseudo-crosses A to O. **a** APM results. **b** IVP results.

correlation is even negative (Table 7). This is due to the difference in the proportion of intergenotypic competition in the competition experiment compared to the pseudo-F_3 experiment.

The genotype x location interaction is also evident in P_T. The rank

Table 8. Linear (F_∞-standard deviation) and Spearman rank (P_T) correlation coefficients of estimates and predictions between the experimental locations APM and IVP.

F_∞-stand.dev.		P_T	
$\sqrt{D}_{F3ps}$	0.61	P_{TF3ps}	0.46
$\sqrt{D}_{F3mo}$	0.23	P_{TF3mo}	0.42
$\sqrt{D}_{F\infty mo}$	0.04	$P_{TF\infty mo}$	0.33

correlation coefficients between APM and IVP are small (Table 8).

GENERAL DISCUSSION AND CONCLUSION

The first article reports on the effects of intergenotypic competition on the estimation of the F_∞-mean with an F_3 of spring wheat. This second article reports on the effects of intergenotypic competition on the estimation of the F_∞-variance and the consequences for cross prediction. We were confronted with the problem of different yield levels of the 2 plot types used in the F_3 and the monoculture experiments. Simple transformation (by multiplication) enabled a comparison of pseudo-F_3-parameters to monoculture parameters. Although discrepancies were mostly ascribed to only intergenotypic competition, they also originate partially in differential reactions of the used genotypes to the plot type. Since the F_3-plot type is the largest possible in a practical breeding programme of spring wheat, it is not important, whether the differences between F_3-parameters and F_∞-parameters are caused by one or the other interaction. However, research of Kramer et al. (1982) and also the awkward results, obtained when predicting with yield data from the competition experiment, lead to the conclusion, that the discrepancies are mainly caused by intergenotypic competition.

This effect of a different yield level of the F_3-plot type also concerns the choice of the threshold value T. It is important to know the value T at the yield level, at which the probability P_T is calculated, since the P_T-function depends in a complex manner on T. A different T-value may result in a different rank of crosses.

In this experiment the pseudo-lines were composed exactly according to the segregation frequencies. This has led to less variable estimates of m_{F3ps} and D_{F3ps}, less than when we would have been dealing with real F_3's. In the case of real F_3's the between line effect will be a random instead of a fixed effect.

In our case it was calculated that this would lead to a variance of D_{F3} roughly 1.3 times the variance of D_{F3ps}. Increasing the number of lines per cross would solve this problem.

The genotypic within F_3-line variance has not been mentioned, so far, because of its unimportant influence. Its magnitude is of the same order as the genotypic between F_3-line variance ($\pm$ 300^2; c.f. Table 3). In an F_3-experiment like ours it would be confounded with the between plot residual variance ($\pm$ 300,000; c.f. Van Ooijen, 1989, Table 9). Since a plot consists of about 300 plants, roughly $1/300^{th}$ part of the genotypic within line variance ($300^2 \cdot 1/300 = 300$) is the part of genetic origin in the between plot residual variance, which is just about 0.1% (300/300,000). Therefore we can conclude, that the influence of genotypic within F_3-line variance on the between plot residual variance is not important. As a result the between plot residual variance was taken the same for all pseudo-F_3's.

Although it has not been of primary interest, genotype x location interaction has shown to be of considerable importance. Even if intergenotypic competition would not have been an important bias in the cross prediction, cross prediction would be valid on one location (environment) only. This conclusion agrees with the results and conclusion of Caligari et al. (1985), who found a very poor agreement between cross predictions done in two different growing seasons.

The results show, that the F_∞-mean is sometimes over- and sometimes underestimated in the F_3, that the F_∞-variance is mostly over- but also sometimes seriously underestimated in the F_3. The cause of this is intergenotypic competition. Dominance or epistasis have only a mild influence on the accuracy of the estimators. Caligari et al. (1985) compared F_∞-variance estimations in F_3, similar to D_{F3ps}, with F_∞-variance estimations using doubled haploids in barley. They found a better performance of the doubled haploid method, and ascribe the worse performance of the F_3-method to dominance. However, they did not consider intergenotypic competition (undoubtedly also present in their barley F_3's) as a possible bias, of which the present results show the greater importance than dominance and epistasis.

Using the estimated F_∞-means and -variances to rank crosses may lead to erroneous results. Altogether, the method of prediction of the progeny of crosses, although in principle practically applicable, is, for a crop like spring wheat with its inevitable amount of intergenotypic competition effects, a very inaccurate one.

Is it realistic to generalize the conclusions of a pseudo-lines experiment

to real F_3's? A quantitative genetic character will seldom be determined by just two loci, there will probably be linkage for a number of involved loci, and epistasis may also be present. In our experiment the epistatic effects were quite large (they can be calculated from Van Ooijen, 1989, Table 8), but they did only mildly influence the parameters. From a numerical investigation Kearsey (1985) concluded, that for a wide range of linkage and dominance values D_{F3} (i.e. twice the between F_3-line variance) is an adequate predictor of the F_∞-variance. Also, the results of the experiment with the real F_3's and their related F_9's show the same tendencies as the results with the pseudo-F_3 and monoculture experiment. So it appears reasonable to generalize the conclusions.

What are the limitations of an experiment with pseudo-lines? In principle more segregating loci can be simulated with this method. However, if the pseudo-lines are composed exactly according to the Mendelian segregation ratio's (no genetic sampling error, in order to get more accurate results), there will soon be the problem that not every possible pseudo-line can be included in the experiment (due to the limitations of the experimental size). This problem can be overcome by simulating the genetic sampling in the composition of the lines, thereby loosing some accuracy in the experimentation. An accompanying problem will be the size of the monoculture experiment, although this also depends on the experimental error of the character under investigation. Linkage and epistasis can also be incorporated in a pseudo-lines experiment. However, a prespecified type of epistasis requires precise knowledge of the genotypic values of the constituent genotypes. The primary aim of the experiments described in these papers was to investigate the effect of genotype x environment interaction with respect to the extrapolation of estimates of m and D from the selection environment to the goal environment. Studying just the effects of more loci, linkage, or epistasis does not require a pseudo-lines experiment.

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APPENDIX

Variance of a mean square, that has a fixed effect

Two-way crossed classification model:

$$\underline{y}_{ij} = \mu + \alpha_i + \underline{b}_j + \underline{e}_{(ij)} \qquad i=1..a; \quad j=1..b;$$

$$\alpha_i \text{ fixed; } \Sigma_i\, \alpha_i = 0; \quad \underline{b}_j \simeq N(0,\sigma_B^2); \quad \underline{e}_{(ij)} \simeq N(0,\sigma^2);$$

ANOVA

MS	df	E(MS)
MSA	a-1	$\sigma^2 + b \cdot \sum_{i=1..a} \alpha_i^2/(a-1)$
MSB	b-1	$\sigma^2 + a \cdot \sigma_B^2$
MSR	r	σ^2

(Rem.: r=(a-1)•(b-1))

The mean square MSR is distributed as a function of a central chi-square variable: $MSR \simeq (\sigma^2/r) \cdot \underline{\chi}^2(r)$, in which $\underline{\chi}^2(r)$ is a random variable having a central chi-square distribution with r degrees of freedom. The mean square MSA is a function of a noncentral chi-square variable (Hogg and Craig, 1978, sec 8.5): $MSA \simeq (\sigma^2/(a-1)) \cdot \underline{\chi}^2(n,\Theta)$, in which $\underline{\chi}^2(n,\Theta)$ is a random variable having a noncentral chi-square distribution with n=a-1 degrees of freedom, and

noncentrality parameter $\theta = b \cdot \Sigma\alpha_i^2/\sigma^2$. The expectation and variance of a noncentral chi-square variable are: $E(\underline{\chi}^2(n,\theta)) = n + \theta$, resp. $\text{var } \underline{\chi}^2(n,\theta) = 2 \cdot n + 4 \cdot \theta$. The variance of MSA can be derived:

$$\text{var MSA} = \frac{2 \cdot \sigma^2}{a-1} \cdot [2 \cdot (\sigma^2 + \frac{b \cdot \Sigma\alpha_i^2}{a-1}) - \sigma^2].$$

If this variance is to be estimated, substitution of the mean squares:

$$\hat{\text{var}}\ \text{MSA} = \frac{2 \cdot \text{MSR}}{a-1} \cdot [2 \cdot \text{MSA} - \text{MSR}],$$

leads to a biased estimate:

$$E(\hat{\text{var}}\ \text{MSA}) = \frac{2 \cdot \sigma^2}{a-1} \cdot [2 \cdot (\sigma^2 + \frac{b \cdot \Sigma\alpha_i^2}{a-1}) - \frac{r+2}{r} \cdot \sigma^2].$$

Therefore an unbiased estimator of the variance of MSA is:

$$\hat{\text{var}}\ \text{MSA} = \frac{2 \cdot \text{MSR}}{a-1} \cdot [2 \cdot \text{MSA} - \frac{r}{r+2} \cdot \text{MSR}].$$

Using this estimator we can derive an estimator of the variance of D_{F3ps} (N.B.: here, r is the number of replicates):

since $D_{F3ps} = \frac{2 \cdot (l-1)}{l \cdot r} \cdot (\text{MSBL}_i - \text{MSWF})$, and MSBL_i and MSWF are mutually independent, $\hat{\text{var}}\ D_{F3ps} = [\frac{2 \cdot (l-1)}{l \cdot r}]^2 \cdot [\hat{\text{var}}\ \text{MSBL}_i + \hat{\text{var}}\ \text{MSWF}]$.

Now, since MSWF is a function of a central chi-square variable:

$\text{MSWF} \simeq (\sigma_{eP}^2/(c \cdot (l-1) \cdot (r-1))) \cdot \underline{\chi}^2(c \cdot (l-1) \cdot (r-1))$, its variance can be estimated by: $\hat{\text{var}}\ \text{MSWF} = \frac{2 \cdot \text{MSWF}^2}{c \cdot (l-1) \cdot (r-1) + 2}$,

and according to the above derivation:

$$\hat{\text{var}}\ \text{MSBL}_i = \frac{2 \cdot \text{MSWF}}{l-1} \cdot [2 \cdot \text{MSBL}_i - \frac{c \cdot (l-1) \cdot (r-1)}{c \cdot (l-1) \cdot (r-1) + 2} \cdot \text{MSWF}],$$

which leads to:

$$\hat{\text{var}}\ D_{F3ps} = [\frac{2 \cdot (l-1)}{l \cdot r}]^2 \cdot [\frac{4}{l-1} \cdot \text{MSBL}_i \cdot \text{MSWF} - \frac{2 \cdot c \cdot (r-1) - 2}{c \cdot (l-1) \cdot (r-1) + 2} \cdot \text{MSWF}^2].$$

(Rem.: There should be a correction for the few missing plots, but this hardly influenced the values of $\hat{\text{var}}\ D_{F3ps}$, just up to 0.2%.)

6. General discussion

In the previous chapters a number of sources of error, that possibly invalidate the proposed prediction procedure (described in the general introduction) have been evaluated. Although not all sources of error have been studied to the same extent, we will try to reach a general conclusion about the predictive value of the procedure.

Alternative estimator

In chapter 2 the superiority of an alternative estimator of D ($\underline{D}_2$) under most circumstances of heritability, dominance level and experimental size, has been established. This estimator has been used in the subsequent chapters assuming it is the best, although the assumptions made in the comparison of the two estimators were not always valid. In chapter 3, for example, the influence of non-normality of genotypic effects is investigated. Also, the estimator is applied in situations with fixed genotypic effects. It has been tacitly assumed then, that also under these circumstances the estimator $\hat{\underline{D}}_2$ is outperforming $\hat{\underline{D}}_1$. Whether this assumption is valid cannot be answered without further research, but it is intuitively felt to be so.

Heteroscedasticity

In chapter 3 various statistical aspects of estimating D have been studied. Heteroscedasticity of residual variances of the magnitude used in the simulations, which is considered to be great, had an important variance increasing influence on the estimator of D, but only when the heritability was intermediate or low ($h^2(bl)<0.6$). The coverage of the WT-confidence interval, however, was hardly affected by heteroscedasticity of residual variances. The general conclusion with regard to heteroscedasticity of residual variances is, that, if there is no correlation between genotypic effects and residual variance the WT-confidence interval is robust against heteroscedasticity. If there is a positive correlation between genotypic effects and residual variance, then one has to do with heterogeneity of variances of the type "constant coefficient of variation", that can be corrected for by a data transformation.

Influence of heteroscedasticity of genotypic within line effects, that is present if the number of loci determining the trait is finite, could only be detected at very high heritability, in a case where two loci determined the

trait. In this case the (variance increasing) influence of heteroscedasticity on the mean square between lines was counteracted so much by the (variance decreasing) influence of non-normality of genotypic effects (which will be discussed further on), that the variance of the mean square was still smaller than its expected value based upon normality and homoscedasticity. As a result the variance of $\hat{D}$ was smaller than its expected value (var($\hat{D}$|normality)). When more loci determine the quantitative trait, on the one hand the heteroscedasticity of genotypic within line effects will reduce, and on the other hand the non-normality will gradually change into normality (as will be discussed further on). Therefore we conclude that the magnitude of the genotypic heteroscedasticity is too small compared to the size of the residual effects to have a noticeable influence.

Non-normality of genotypic effects

An aspect, that might have important consequences for the prediction procedure, is the possible non-normality of genotypic effects, caused by a limited number of loci determining the quantitative trait. The conclusion of chapter 3 was that the influence of non-normality can only become important when the number of loci is less than five, combined with an intermediate to high heritability ($h^2(bl)>0.5$). If there is an influence, then it can be considered positive: the estimated value of D is closer to the true value than may be expected by considering its confidence interval.

In this chapter we did not consider the cross prediction (P_T). This prediction assumes a normal probability distribution of the F_∞ (chapter 1). When only a limited number of genes are involved in the quantitative trait, then the distribution of the F_∞ deviates from normality. Hence, the prediction of P_T may have a significant error. For example, if only one gene is involved, then P_T is zero (if the threshold value T is equal to or larger than the best parent), since the (most) extreme genotype is already involved in the cross and hence there is zero probability to obtain a recombinant inbred better than this extreme. Therefore, this prediction will always be erroneous. Because the threshold value T will be somewhere in the tail of the distribution, it appears that the performance of the prediction of P_T largely depends on the shape of the tails of the probability distribution of the F_∞. The shape of these tails depend importantly on the numbers of genes involved in the cross. If all crosses in a breeding programme differ in approximately the same number of genes, the bias in this prediction will apply to all crosses, presumably leading to a correct

rank of the crosses. However, when there are just a few (major) genes involved in the quantitative trait (i.e. the gene pool the breeder extracts the crosses from has polymorphism at just a few loci), some crosses in a breeding programme will differ for only one gene, others for maybe five. In that case there will be a difference in the deviation from normality between some of the crosses, presumably leading to an incorrect rank of crosses. When there are more than 10 genes of approximately equal effect in the gene pool, the crosses will segregate for roughly the same number of genes. At the same time the deviation from normality will have nearly diminished, and hence the ranking of crosses will be approximately unbiased. These are just speculative remarks, that should be confirmed by thorough investigations. There has not been any research on the sensitivity of the cross prediction (P_T) to deviations from normality of the F_∞. Preliminary results of investigations on this subject by Van Oeveren (pers.comm.) show that, when four or five loci are involved, the rank of the crosses, calculated with m, D, and the normal distribution, may be different from the rank of the crosses based upon the true probability to produce superior segregants (there are many crosses with zero true probability). But even then, in most cases the best cross came out on top. When there is polymorphism at just two or three loci in the gene pool, the chance is there, that the best genotype is already in the gene pool, or will soon be produced by a cross in the breeding programme. In that particular case the cross prediction method will always give the breeder the idea that genetic progress can be made while it cannot. If the between line heritability is increased enough, the breeder should be able to detect segregation at just these few loci. The conclusion is, that, if the trait is really oligogenic (say less then 5 loci), there will be significant non-normality of the probability distribution of the genotypic effects, which on the one hand leads to a more accurate estimate of D if the between line heritability is larger than 0.5, but on the other hand may lead to an erroneous ranking of crosses. But if the number of loci is larger than 5, the cross prediction procedure is correct.

A problem, however, is that it is very difficult to determine the number of genes involved in a quantitative trait. This problem can possibly be overcome by new methods, that employ molecular genetic markers to discover linkage between markers and putative quantitative trait loci (e.g. Paterson et al, 1988). The subsequent breeding methods have a totally different approach, in which direct selection for quantitative traits is partially replaced by indirect selection via qualitative marker genes. Whether this approach will be generally

applicable is still an open question.

In chapter 5 the pseudo-crosses were segregating for two unlinked loci. In this case the use of the assumed normality of the F_∞ is incorrect. However, both sides of the made comparison, i.e. the estimation in the selection environment and in the goal environment, used this assumption. Therefore, the comparison will not be too much affected by this incorrectness.

Fixed versus random effects

Chapter 3 also considered the aspect of deliberately applying the Williams-Tukey confidence interval on D, when the inference concerns the actual F_3-population, and not the genotypic variance of the imaginary population from which the actual population was sampled, the latter being the parameter for which the WT-confidence interval is correct (random effects model), the former for which it is incorrect (fixed effects model). The breeder is, of course, mostly interested in the current F_3-population. He would lose a few years of his breeding programme, if he would have to go back to making the original cross again. The conclusion of chapter 3 is clear: if the between line heritability is below (roughly) 0.6 the (erroneously) applied WT-procedure for a confidence interval is reasonably accurate. If the heritability is higher, the estimate will on the average be closer to the parameter of interest, with a mean square error of up to 3 times smaller than the mean square error with respect to the alternative parameter, at a between line heritability of 0.98. This is of course a positive outcome.

Intergenotypic competition

In chapters 4 and 5 the research on the influence of intergenotypic competition in spring wheat on the estimation of the parameters m and D is presented. Through the "pseudo-lines" method we were able to make a comparison between the parameters under conditions of intergenotypic competition and the parameters under monoculture conditions. The experiments had been performed at two locations (APM and IVP). At APM the intergenotypic competition bias was larger than at IVP. The linear correlation coefficients between the parameters (m and D) under the alternative conditions were poor. These results were strengthened by comparable results obtained with a few real F_3's of spring wheat and their related F_9's. The general conclusion of these chapters was, that the prediction procedure is very inaccurate if the studied quantitative trait is influenced by

intergenotypic competition up to the level found in the spring wheat experiment.

As an example we may look at Figure 3 of chapter 5. If we decide to take the 5 best crosses out of the 15 using the F_3-prediction procedure, then at APM (Fig. 3a) we fail to include the very good cross M, and at IVP (Fig. 3b) we fail to include the very good cross N. If we increase selection intensity and take only the two best crosses, then at APM we obtain one good (even the best) and one poor cross, and at IVP we obtain two good crosses, although the best is not included.

Conclusion

There is no doubt that the proposed cross prediction procedure will perform fairly well in many situations, but there are a two restrictions. One of the restrictions has to do with the number of genes: if there are very few loci, less than five, then the ranking of the crosses may be biased. If the best genotype is already in the gene pool from which the breeder extracts his crosses, and he has not raised the between line heritability to a high level to detect this, then he will erroneously be informed by the procedure of possible genetic progress. The second restriction is that, if the trait is influenced by intergenotypic competition of the level encountered in the pseudo-lines experiment, the procedure will lead to erroneous results.

Heteroscedasticity of residual effects and genotypic within line effects is not likely to invalidate the procedure. Although one should of course be aware of it. Linkage, as mentioned in the general introduction, is according to a number of studies not likely to lead to important deviations. Fairly large epistatic effects were encountered in the pseudo-lines experiment, but they did only mildly influence the parameters.

There are some open questions, though. What precision need the estimates of m and D have, to come to a reasonably accurate ranking of crosses ? Once a pilot experiment has produced rough estimates of the variance components, and given the necessary precision one can easily calculate the required experimental size with the help of equations like those employed in chapter 2. If the between line heritability is high, this calculated experimental size can be regarded as a little bit oversized, because the parameter D of the fixed effects model is closer to its true value than that of the random effects model, on which the equations of chapter 2 are based. Similarly, if the quantitative trait is based on a small number of loci, and the between line heritability is high, the parameter D will on the average be closer to its true value.

Then there is the question of how many crosses must be evaluated and how many must be selected for further breeding. Probably there will be an optimum given the total experimental capacity.

There are also some disadvantages to the method. Many crosses will produce a number of inferior lines. These cannot be discarded, because each line of each F_3 must be evaluated in order to obtain unbiased estimates. This makes the method expensive. Current line breeding methods tend to discard many lines in F_3 or F_4 based on empiric judgement by the "breeder's eye", rather than by statistical procedures. A comparison is needed between the genetic progress and the costs of the proposed breeding method and current line breeding schemes, single seed descent methods, or methods employing doubled haploids.

Reference

Paterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln and S.D. Tanksley, 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721-726.

7. Abstract

Quantitative genetic theory provides models to predict the probability to obtain superior recombinant inbreds in the offspring of a cross between two pure breeding lines. The prediction procedure is prone to various types of error, which possibly invalidate the prediction procedure: 1) stochastic variation, 2) incorrectness of the genetic assumptions, on which the theory is founded, and 3) genotype-environment interaction, in particular intergenotypic competition. The predictive value of the procedure is evaluated by studying the effects of the individual sources of error.

Chapter 2 deals with stochastic variation; it establishes the superiority of an alternative estimator of the additive genotypic variance under most practical circumstances. Chapter 2 also presents a method to optimize the population design (number of lines, size of the lines) with respect to the accuracy of the estimator.

Chapter 3 investigates various violations of the assumptions, on which the theory is founded, such as non-normality of genotypic effects, heteroscedasticity, and fixed versus random effects.

Chapters 4 and 5 investigate the bias on the estimates of the F_∞-mean and -variance, respectively, caused by intergenotypic competition.

8. Samenvatting

De voorspellende waarde van schattingen van kwantitatief-genetische parameters voor de veredeling van zelfbevruchtende gewassen

De kwantitatieve genetica is een wetenschap die zich bezig houdt met het bestuderen van de genetica van kwantitatieve eigenschappen van planten en dieren. Dit zijn eigenschappen die zich laten beschrijven met schaal-grootheden, d.w.z. (kilo)grammen, (centi)meters, enzovoort. Ze staan in tegenstelling tot kwalitatieve eigenschappen, zoals bloemkleur en kleurenblindheid. Kwantitatieve eigenschappen hebben de nare bijkomstigheid dat ze nogal variabel zijn; de ene keer meet je dat een ras bijv. 5000 kg/ha opbrengt, en de andere keer, onder nagenoeg identieke omstandigheden, 5500 kg/ha.

De laatste tientallen jaren is de kwantitatieve genetica onder meer bezig geweest met het ontwikkelen van modellen, die een wetenschappelijke basis kunnen geven voor de veredeling van zelfbevruchtende gewassen. De modellen bieden de mogelijkheid om reeds in een vroeg stadium van een veredelingsprogramma (waarin verschillende kruisingen worden gemaakt) een voorspelling te geven van de kans op het vinden van een beter (beter ten opzichte van de huidige rassen) individu in de nakomelingschap van een kruising. Met deze informatie kan de veredelaar zich voor het vervolg van het veredelingsprogramma koncentreren op de nakomelingschappen van de goed voorspellende kruisingen, en kan hij de slechte kruisingen uit het veredelingsprogramma verwijderen. Indien deze procedure korrekt is, dan komt dit de efficiëntie van een veredelingsprogramma ten goede.

Er zijn echter een aantal foutenbronnen, die de voorspelprocedure nadelig kunnen beïnvloeden, te weten:

1) Toevalsvariatie. De voorspelling is gebaseerd op geschatte parameters. We weten dus nooit de exakte waarde van deze parameters, de ene keer schatten we ze wat groter, de andere keer wat kleiner.
2) Onjuistheid van de veronderstellingen die aan een model ten grondslag liggen. Het meest gangbare model veronderstelt dat de verschillende genen, die bij een eigenschap betrokken zijn, onafhankelijk van elkaar werken. In werkelijkheid is dit vaak niet het geval.

   Verder veronderstelt het model dat een kwantitatieve eigenschap wordt bepaald door een groot aantal genen, ieder met een klein effekt. Het kan

echter ook zijn dat een bepaalde eigenschap door maar een gering aantal genen wordt bepaald.

Bij het doen van een betrouwbaarheidsuitspraak van een geschatte parameter wordt verondersteld dat de zogenaamde restvarianties homogeen zijn, hoewel dit lang niet altijd het geval zal zijn.

Een ander probleem is dat de betrouwbaarheidsuitspraak in feite een uitspraak is over de oorspronkelijke kruising en alle mogelijke daaruit voortkomende nakomelingschappen, terwijl de veredelaar een uitspraak wil doen over die ene nakomelingschap van de kruising, die hij op dit moment in handen heeft.

3) Intergenotypische konkurrentie. De schattingen worden verricht aan kruisingsmateriaal. Dit materiaal is genetisch niet homogeen, in tegendeel: het splitst uit. Het uiteindelijke produkt van de veredeling, een ras, zal genetisch wel homogeen zijn. Nu kan het probleem zich voordoen, dat een eigenschap die aan planten in een genetisch heterogeen milieu gemeten wordt (dus ook aan kruisingsmateriaal), niet overeenkomt met diezelfde eigenschap gemeten in een genetisch homogeen milieu (een zogenaamde monokultuur). Bijvoorbeeld, de planten van een ras brengen in mengteelt met andere rassen ongeveer 50 gram (korrels) op, echter in monokultuur misschien wel 75 gram, of misschien slechts 25 gram. Dit verschijnsel wordt veroorzaakt door verschillen in konkurrentievermogen van de verschillende rassen, en wordt intergenotypische konkurrentie genoemd. Intergenotypische konkurrentie kan van invloed zijn op de schattingen van de verschillende parameters, zodat de parameters eigenlijk alleen betrekking hebben op het genetisch heterogeen milieu, en helaas niet op het milieu van het toekomstige ras.

Dit proefschrift beschrijft onderzoek naar de voorspellende waarde van de schattingen van de kwantitatief-genetische parameters. Zoals gezegd, is deze voorspellende waarde van belang voor de opzet van een efficiënt veredelingsprogramma. Het onderzoek is gericht op de effekten van de individuele foutenbronnen. Het is uitgevoerd met veldexperimenten van zomertarwe, kasexperimenten met het modelgewas *Arabidopsis thaliana* (zandraket), en met computersimulatie.

In hoofdstuk 2 is de toevalsvariatie onder de loep genomen. Het hoofdstuk toont aan, dat een andere dan de gangbare schatter onder de meeste praktische omstandigheden een veel nauwkeuriger resultaat geeft. Tevens geeft het hoofdstuk een methode om een optimale populatie-indeling (het aantal lijnen, het aantal

planten per lijn) te bepalen, zodat de nauwkeurigheid van de schatter zo groot mogelijk is.

Hoofdstuk 3 onderzoekt de effekten van eventueel onjuiste veronderstellingen, hierboven genoemd onder punt 2. Hierin blijkt dat de voorspelling ernstig verstoord kan worden, wanneer, in tegenstelling tot de gangbare veronderstelling, slechts weinig genen betrokken zijn bij het onderhavige kenmerk. Heterogeniteit van de restvarianties blijkt slechts een geringe invloed te hebben op de kwaliteit van de betrouwbaarheidsuitspraak over de geschatte parameters. Verder blijkt dat de betrouwbaarheidsuitspraak over de geschatte parameters aan kwaliteit wint, wanneer deze geen betrekking heeft op de oorspronkelijke kruising, maar op het materiaal dat de veredelaar op dat moment in handen heeft. Deze winst is overigens alleen noemenswaardig bij een relatief hoge erfelijkheidsgraad.

De hoofdstukken 4 en 5, tenslotte, behandelen de invloed van intergenotypische konkurrentie op de schatters. Hierin komt duidelijk naar voren dat intergenotypische konkurrentie een belangrijke verstoring in de schattingen kan geven.

De konklusie van het onderzoek is, dat er geen twijfel is over de werkzaamheid van de voorspelprocedure. Er zijn echter twee uitzonderingen. 1) Als het aantal genen, dat bij de kwantitatieve eigenschap betrokken is, gering is, kan de voorspelling tot een onjuiste rangorde van de kruisingen in een veredelingsprogramma leiden. 2) Als de betreffende eigenschap onderhevig is aan een behoorlijk nivo van intergenotypische konkurrentie, zal de procedure tot foutieve resultaten leiden, zodat mogelijk de in werkelijkheid beste kruising niet als meest belovende uit de voorspelling naar voren komt.

Curriculum vitae

Johannes Willem van Ooijen is op 24 juli 1957 geboren in Middelburg. Direkt na het Atheneum is hij in 1975 begonnen aan zijn universitaire studie plantenveredeling aan de Landbouwhogeschool te Wageningen. Zijn praktijktijd heeft hij doorgebracht in Kenia. Op 2 februari 1982 is hij met lof afgestudeerd met het doctoraal vakkenpakket: plantenveredeling, fytopathologie, erfelijkheidsleer, en wiskundige statistiek. Na de militaire dienstplicht (juli 1983) was de arbeidsmarkt in een diep dal beland. Dit had tot gevolg dat Johan werkloos werd. Na precies een jaar (juli 1984) heeft hij een tijdelijke baan aan kunnen nemen als systeemprogrammeur c.q. -beheerder bij de Dienst Waterbeheer van de Provincie Gelderland te Arnhem. Na weer een jaar (juli 1985) kon hij overstappen naar het promotie-onderzoek bij de vakgroepen Erfelijkheidsleer en Plantenveredeling, dat werd gesubsidieerd door de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO), en waarvan dit proefschrift het resultaat is.

Sinds februari 1989 is hij als toegevoegd onderzoeker werkzaam voor de Landbouwuniversiteit Wageningen bij de vakgroep Erfelijkheidsleer. Hij verricht onderzoek naar de toepassingsmogelijkheden van moleculair genetische merkers als indirekt selektie-hulpmiddel bij de veredeling van zelfbevruchtende gewassen. Dit onderzoek gebeurt in opdracht van een aantal Nederlandse tuinbouw-plantenveredelingsbedrijven.