Theor. Appl. Genet. 49 (1977) 111-115

Effects of Linkage and Interaction in a Comparison of Theoretical Populations Derived by Diploidized Haploid and Single Seed Descent Methods

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Summary. Comparisons were made between the genetic means and variances of a quantitative trait determined by 8 loci in simulated populations of lines derived by diploidizing haploids (DH) on the one hand and by single seed descent (SSD) on the other.

In the absence of linkage no differences between the populations were observed, but when linkage was present, recombination was more frequent in the SSD populations as indicated by the relative differences in variance between these and the DH populations. In addition, differences in means between the populations derived by the two methods were observed when non-allelic interaction was present. The direction and magnitude of the differences in both means and variances depended upon the linkage phase, the recombination frequency and the presence or absence of interaction.

The conclusion was drawn that the SSD method was to be preferred from theoretical considerations although in practice the choice of method will also depend upon practical and technical factors.

Introduction

The development of methods for haploid production in higher plants has implications not only for genetical research but also for the rapid development of true-breeding lines amongst which selection can then be practised in a practical breeding programme. In barley, *Hordeum vulgare* L., high frequencies of haploids have been observed following inter-specific hybridizations between *H. vulgare* (2n = 2x = 14) and *H. bulbosum* (2n = 2x = 14) (see Kasha 1974 for review), and techniques for the production of barley haploids and their subsequent chromosome doubling have since improved sufficiently for the utilisation of doubled haploids in breeding programmes (Kasha and Reinbergs 1972; Park et al. 1976). Recently Barclay (1975) showed that haploids could also be produced in wheat by chromosome elimination after the inter-generic cross between *Triticum aestivum* (2n = 6x = 42) and *H. bulbosum* (2n = 2x = 14 and 2n = 4x = 28).

Alternatively, rapid production of near-homozygous lines can be achieved by the single seed descent (SSD) procedure (Goulden 1939; Brim 1966; Kaufmann 1961, 1971; Riggs and Hayter 1976). In this method segregating generations are rapidly advanced in the glasshouse with no selection, each line being continued by a single seed in each generation.

Comparisons in plant breeding practice of SSD with early generation selection and testing have been made by several workers in a range of crops (Knott and Kumar 1975; Boerma and Cooper 1975; Casali and Tigchelaar 1975). In most cases SSD was considered to have considerable merit because of the generally short time required to develop homozygous lines, the large genetic variance observed amongst these lines, and the genetic gains obtained after selection. Snape and Riggs (1975) described the genetical consequences of SSD and concluded that whilst genetic advance may not be as great in some genetical situations as might be expected after efficient pedigree selection, the method nevertheless had considerable advantages. Similar conclusions were drawn by Bliss and Gates (1968) from a computer simulation study comparing SSD with mass selection. Jinks and Pooni (1975) showed that the performance of inbred lines derived by single seed descent could be predicted from the genetic parameters estimated from the parents, F_1, F_2 and backcross generations.

Using computer simulation, Walsh (1974) compared lines generated by the DH and pedigree selection methods and concluded that the DH method would be superior only when selecting for a quantitative trait with low heritability.

Park et al. (1976) reported comparative measurements made in populations of homozygous barley lines produced by SSD and by DH methods. They observed significant differences between SSD and DH population means for grain yield, plant height and heading date. Since previous work had indicated the random-

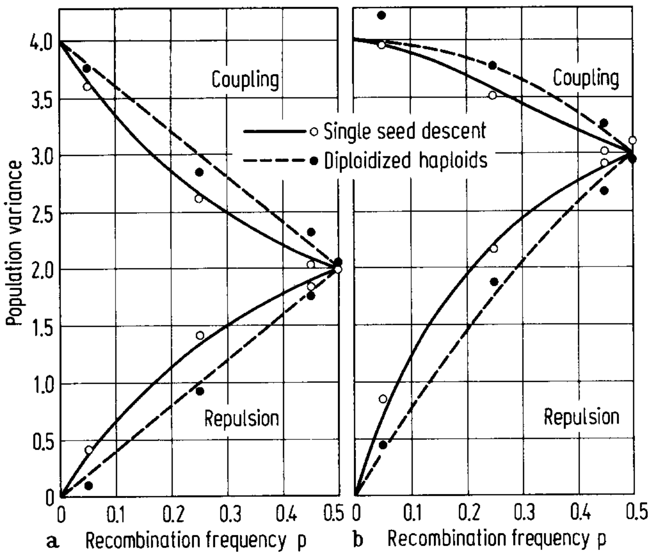

Fig. 1. The 2-locus model: expected variances with change in recombination frequency (p), and observed values for simulated populations.
a) No interaction,
b) Complementary interaction
——— o single seed descent
------ • diploidized haploids

ness of DH populations, they inferred that some selection was inherent in the SSD method as practiced.

In testing the assumption that haploid production results in a random sample of gametes produced from a heterozygous genotype, workers have looked for similar ranges and variances in DH populations and populations derived by selfing. However, when linkage is present, the compositions of the two populations would differ even assuming no differential survival of gametes. Snape (1976) has shown theoretically that when linkage is present the genetical consequences of the DH and SSD methods will be diffferent: for a 2-locus model the frequency of recombinants was greater in populations produced by SSD.

Technical and practical difficulties may be experienced in the use of either method and the breeder's choice may be determined by the availability of facilities and expertise and the relative time-scales of the two methods. He may also have no prior knowledge as to the degree of linkage between the genes under selection. Nevertheless an understanding of the differing genetical consequences of the two methods when linkage is present may be important in choice of method or interpretation of results.

In this paper we extend the findings of Snape (1976) to a multi-locus model and discuss the results of computer simulation studies aimed at comparing the variances in populations derived respectively by DH or SSD when linkage is present. The effects of complementary non-allelic interactions on the population means and variances are also described.

Methods

The simulation procedure was carried out for 8 loci assorting independently in the absence of linkage or, when linkage was present, distributed as 2 loci in each of four linkage groups, as 4 loci in each of two linkage groups or as 8 loci in a single linkage group. All loci were of equal effect.

Recombination frequencies (p) between adjacent loci in the same linkage group were set at 0.45, 0.25 or 0.05 and interference was assumed absent.

The F_1 between the parents was heterozygous at all loci, which were arranged in coupling ($\frac{++++++++}{--------}$), repulsion ($\frac{+-+-+-+-}{-+-+-+-+}$) or in mixed coupling and repulsion phases ($\frac{++++-+-+}{----+-+-}$).

Two situations of gene action were assumed:
(i) additive effects with complete dominance for increasing alleles;
(ii) additive effects with complete dominance for increasing alleles and complementary gene interactions.

Genotype values were calculated using $m = 10.0$, $d = \pm 0.5$, $h = + 0.5$ and for complementary gene interaction between homozygous loci, $i = + 0.5$.

Gametogenesis in the F_1 was simulated using the method of binary masks as described by Fraser and Burnell (1970).

In generating the diploidized haploids each gamete was simply duplicated to form a homozygous diploid individual. A population of 500 such individuals was generated and the mean and variance computed.

For the simulation of single seed descent an F_2 of 500 indivuduals was first generated. Single progeny descent was then simulated for three generations. In the F_6, families of 10 progeny were generated from each F_5 individual, and the family means and variances computed. The overall population mean and the between-family variance was then determined.

The computer programme for these operations was written in FORTRAN IV and run on the IBM 370/165 computer at the Cambridge University Computer Laboratory.

Results and Discussion

Mean data are presented from two runs of the programme for each combination of genetical situation and linkage arrangement. Residual heterozygosity in the SSD lines was generally very low and consequently within-family variances were absent or negligible, and would not materially affect the results.

The four linkage group situations simulated here can be considered to constitute four replications of the 2-locus model of Snape (1976). Thus the observed change in variance with change in p can be compared with the theoretical expectations. Figure 1 shows the

Table 1. Variances for populations derived by SSD or DH when linkage, but not interaction, is present

	Initial linkage phase						
		Coupling		Repulsion		Mixed coupling/repulsion	
	p	SSD	DH	SSD	DH	SSD	DH
No linkage	0.50	1.999	2.141	-	-	-	-
2 linkage groups of 4 loci	0.45	2.017	2.171	1.951	1.688 *	1.972	2.081
	0.25	3.482	4.078 *	1.132	0.742 ***	2.288	2.471
	0.05	6.184	7.608 **	0.341	0.149 ***	3.432	3.685
1 linkage group of 8 loci	0.45	2.114	2.502 *	1.862	1.707	2.074	2.001
	0.25	3.819	5.157 ***	1.166	0.816 ***	2.020	2.184
	0.05	10.413	12.575 *	0.298	0.123 ***	2.920	2.501 *

*, **, ***: F - ratio significant at $P < 0.05$, 0.01 and 0.001% respectively, $N_1, N_2 = 499$

Table 2. Variances for populations derived by SSD or DH when linkage and complementary interaction are present

	Initial linkage phase						
		Coupling		Repulsion		Mixed coupling/repulsion	
	p	SSD	DH	SSD	DH	SSD	DH
No linkage	0.50	3.107	2.945	-	-	-	-
2 linkage groups of 4 loci	0.45	3.138	3.700 *	2.801	2.753	3.119	2.782
	0.25	4.237	5.063 *	2.204	1.487 ***	3.228	3.275
	0.05	6.643	7.561	0.646	0.266 ***	3.712	3.544
1 linkage group of 8 loci	0.45	3.109	3.402	2.779	2.736	3.053	3.001
	0.25	4.640	5.494 *	2.064	1.608 **	3.048	2.779
	0.05	10.955	12.590	0.683	0.212 ***	3.406	3.493

observed variances, together with curves showing the theoretical trends, when initial linkage was in either coupling or repulsion and interaction was either absent (Fig. 1a) or present (Fig. 1b). The agreement shown in both figures was satisfactory.

Considering the other linkage arrangements, Table 1 shows the observed variances when no interaction was present. As expected, low recombination frequencies were associated with high population variances when the initial linkage phase was in coupling and low variances when linkage was in repulsion. A higher proportion of recombinants in the SSD population was indicated by consistently lower variance relative to the DH population, when initial linkage was in coupling, and higher variance when linkage was in repulsion.

In the cases of one or two linkage groups of 8 or 4 loci respectively, differences from the 2-locus model are clearly apparent in that restricted recombination has a proportionately greater effect on the variance when initial linkage was in coupling than when in repulsion. Clearly the 2-locus model provides a symmetrical situation where recombination results in a shift from one linkage phase to the other. In a multi-locus model with loci of equal or similar effect, infrequent cross-over events are seen to have relatively less effect on population variance when linkage is initially in repulsion than when it is in coupling. This is a scaling effect due to the high variance between homozygotes in coupling, and can be seen also in the populations having mixed coupling and repulsion phase linkage groups, where there was a positive trend in the variance as p decreased.

In the presence of complementary interaction (Table 2), variances were generally higher as expected (Snape and Riggs 1975). For the 2-locus model the change in variance with p is expected to be less when linkage is initially in coupling than when in repulsion (Fig. 1b). However, for the multilocus situations the

Table 3. Means for populations derived by SSD or DH when linkage and complementary interaction is present

	Initial linkage phase						
		Coupling		Repulsion		Mixed coupling/repulsion	
	p	SSD	DH	SSD	DH	SSD	DH
No linkage	0.50	10.07	9.98	-	-	-	-
2 linkage groups of 4 loci	0.45	10.10	10.26	9.91	9.75	10.03	10.01
	0.25	10.82	11.01	9.38	8.95 ***	10.04	10.02
	0.05	11.67	11.70	8.38	8.13 ***	10.05	10.00
1 linkage group of 8 loci	0.45	9.12	9.22	9.88	9.81	9.95	10.03
	0.25	9.54	9.87 *	9.28	9.05 **	9.05	9.90 ***
	0.05	11.78	11.80	8.41	8.11 ***	9.95	9.76

*, **, ***: significance at $P < 0.05$, 0.01, 0.001 % respectively for differences between SSD and DH population means s.e. difference = $\sqrt{\frac{VSSD + VDH}{500}}$

response to change in p for the coupling arrangements was again very marked. This further illustrates the proportionately greater effect of recombination when more than 2 loci are involved in coupling linkage.

From Tables 1 and 2 it can be seen that the difference in variance between SSD and DH populations, though not always statistically significant, indicated a consistently higher frequency of recombination in the SSD populations when linkage was either in coupling or repulsion. This was particularly the case, however, for repulsion linkages in these simulations, and was maintained at the lowest recombination frequency of 0.05 %. Interaction makes it difficult to discern differences with coupling linkages but inflates the differences with repulsion linkages. Where equal numbers of loci were involved in coupling and repulsion phase linkage, no consistent difference could be observed between the variances of the SSD and DH populations, nor would this be expected.

In the absence of interaction, no difference was expected between the means of the SSD and DH populations, which, apart from small fluctuations due to sampling, took the mid-parental value for all runs. When homozygote × homozygote (i) type interactions were present, however, differences in the population means were apparent as shown in Table 3.

The direction and magnitude of the difference was shown by Snape (1976) for 2 loci to depend upon the initial linkage phase and the recombination frequency respectively. With coupling linkage the means of both populations increased as p decreased. The means of the DH populations were always higher or equal to those of the SSD populations with the greatest difference at the intermediate value of p. This agrees with the theory for the 2-locus model (Snape 1976).

This was also the case for repulsion linkage: the means were generally lower than those when linkage was in coupling, and in this case the means of the SSD populations exceeded those of the corresponding DH populations. All the means decreased with decrease in p. Again the difference between the two populations was large at the intermediate recombination frequency ($p = 0.25$).

When equal numbers of loci were involved in coupling and repulsion phase linkages, population means were intermediate and, with one exception, did not differ markedly between populations derived by the different methods.

Production of haploids from an F_1 hybrid limits the opportunity for recombination between loci to a single meiosis. In the absence of linkage an unbiased sample from all possible genotypes should be obtained but when linkage is present, the frequency of recombinant genotypes in a population of diploidized haploids will be relatively lower and the degree of linkage disequilibrium greater than in an equal sized population of lines derived by single seed descent (Tables 1 and 2).

Differences detected in practice between DH and SSD populations (e.g. Park et al. 1976) could be due to linkage and/or unconscious selection in the development of lines by either method. Linkage with no interaction would lead to differences in the variances only, whilst selection, or linkage with interaction, would result in differences in variances and means.

The higher proportion of recombinant genotypes in populations derived by SSD when linkage is present, could be important whether the several loci involved control the expression of only one character, as assumed here, or of two or more characters. Indeed, the enhanced opportunity provided by SSD for the breaking of associations between characters might be the more important advantage.

From the breeder's point of view the main interest will generally be in obtaining the maximum variation from a cross between parents usually complementing each other for strengths and weaknesses and therefore likely to produce an F_1 with loci mainly in repulsion. If he wishes to accelerate the production of homozygous lines from such a cross, theoretical considerations suggest that the breeder should use SSD rather than the DH method, since linkage may be present. In addition, the DH method may require considerable expertise, and it may be difficult in some circumstances to produce and diploidize haploids in sufficient numbers to adequately exploit the hybrid. This consideration apart, the DH method might be more suitable for species having a relatively large number of chromosomes where new variation would be released mainly as a result of reassortment of chromosomes, and the importance of linkage would be relatively less than in species with fewer chromosomes.

Although the rate of failure in diploidized haploid production in barley may be fairly high, there is apparently no evidence that the losses are genotype specific (Kasha and Reinbergs 1972; Kasha 1974). Losses during SSD may also be high and there is some evidence for barley that these losses may not be randomly distributed (Riggs and Hayter 1976). The seriousness of this problem will depend upon the crop concerned and the agronomic methods used.

Acknowledgement

We are grateful to Dr. R.J. Baker for an introduction to computer simulation techniques.

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Received July 27, 1976
Communicated by R. Riley

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