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Selection for Fresh Weight in *Arabidopsis thaliana* under Two Mating Systems[1]

D. G. PEDERSON and **D. F. MATZINGER**

Department of Genetics, North Carolina State University, Raleigh, North Carolina (USA)

Summary. Four races of *Arabidopsis thaliana* were used to produce two F_2 populations and these constituted the base populations of an artifical selection study. All plants were grown under aseptic culture conditions in a controlled environment and the character studied was fresh weight after 15 days' growth. For one section of the study, two selection intensities were applied to each F_2 population and the selected parents were randomly mated. In addition to the high selections a group of random selections were maintained as a control. In the second section of the study the procedure differed only in that mating was by self-fertilization.

For one population the selection response was as expected for a character of low heritability. A regular response was also observed for the other population when mating was at random, but the results for selection with self-fertilization indicated the presence of dominance effects. The overall conclusion was that selection with random mating and selection with self-fertilization produced approximately equal genetic gains.

Introduction

The theory of artificial selection has historically been associated with animal breeding and the majority of laboratory selection studies have correspondingly employed an outcrossing organism such as *Drosophila* or the mouse. This report is of an experiment in which the normally self-fertilizing plant *Arabidopsis thaliana* was selected in the laboratory under both complete random mating and complete self-fertilization as a demonstration of its usefulness for selection studies.

The expected response to selection with random mating has been described by many authors (e. g. Falconer 1960) and good agreement has generally been observed between prediction and observation, at least in the short term. For selection with self-fertilization the expected response has been briefly referred to in several papers (e. g. Hanson, Probst and Caldwell 1967) and a more detailed account has been given by Pederson (1969). In line with the latter paper, the present study aims at demonstrating that a continuous response is possible with self-fertilization and affords an illustration of the use of prediction formulae. At the same time, a comparison of the responses under the two mating systems gives some insight into the type of gene action operating.

A measure of the relative genetic gains under the two mating systems was obtained by intermating the previously self-fertilized plants after three generations of selection. The means of the resulting populations were compared with the means of the corresponding randomly mated populations. The relative merit of the two systems with regard to selection gain is of importance when determining optimum selection procedures for plants which are both self- and inter-fertile.

Materials and Methods

Seeds of the four races of *Arabidopsis thaliana* (L.) Heynh. designated TU, LU, RLD, and CT by Langridge and Griffing (1959) were obtained from the Genetics Section, C.S.I.R.O, Canberra. *Arabidopsis* is predominantly self-fertilizing in nature and each race was considered homozygous.

Two F_2 populations were produced by self-fertilization of crosses $RLD \times CT$ and $TU \times LU$. Four selection lines were subsequently developed from each of these populations, namely

(i) a random mated line with 10% selection
(ii) a random mated line with 20% selection
(iii) a self-fertilized line with 10% selection
(iv) a self-fertilized line with 20% selection.

The two populations were treated identically and the common procedure will now be detailed.

The selection procedure: For the initial cycle of selection, 200 plants were grown by the aseptic culture method of Langridge (1957), and this set of plants will be referred to as generation 0 of the selection process. Seed were first sterilized and then placed into 16 mm test tubes with "vacuum tweezers" (Feenstra 1965). After being kept at approximately 2 °C for 72 hours the tubes were placed into wooden blocks in a controlled-environment chamber. Initially, incandescent illumination was used but after the cotyledons had lifted from the agar medium, fluorescent illumination was supplied at an intensity of 2000 footcandles. An agar temperature of 25 °C was maintained. During growth of the plants each wooden block

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was moved to a new random position each day, this technique being used in preference to a stationary randomized design.

Growth proceeded for 15 days and the plants were then pulled from the agar, their roots blotted, and the fresh weights measured. All of the plants were stored after weighing, prior to selection of the best from within the population. The storage trays consisted of commercially made holders for "Dispo" assay trays, each covered with a sheet of plexiglass into which a number of 8 mm holes had been bored so that one tray corresponded to one of the wooden blocks used to hold test tubes. In use, the trays were filled with water and plants were placed one to a hole after weighing, in which position they would remain viable for at least 24 hours.

Four sets of plants were selected from the initial population, namely,

(i) the 20 plants with highest fresh weight
(ii) the 40 plants with highest fresh weight
(iii) 20 plants at random
(iv) 40 plants at random.

Some plants were common to two or more sets. The plants were potted in a sand-soil-peat moss mixture and grown to maturity. Selfed seeds were collected and random cross-fertilization was carried out within each set such that each plant was used once as a male parent and once as a female parent.

In subsequent generations no intermixing or intermating was permitted between the four sets. Two further cycles of selection were carried out within each set such that the mating-type and selection intensity were the same as for the first cycle of selection. The randomly-chosen control plants were maintained separately but were grown concurrently with the corresponding high selections, and population sizes were kept at 200 by growing ten plants of each self-family or full-sib family in the case of 10% selection and five plants of each family in the case of 20% selection.

A measurement was required of the relative selection gains under the two mating systems. Since self-fertilization leads to decreased heterozygosity, the third cycle of high selections from the previously self-fertilized populations were intermated in addition to being allowed to self. In this way the heterozygosity was raised to a level comparable to that of the continuously random-mated populations. The third cycle of random selections was treated similarly, and this was done for each of the two selection intensities.

In summary, from each of two F_2 populations, selection was carried out at two intensities with two mating types. After three cycles of selection a series of trials were carried out, each with two replications in time. The only character measured was fresh weight after 15 days' growth.

Evaluation of the selected material: The first trial consisted of parents TU and LU (12 plants of each per replication), their F_2 (24 plants), and the three generations of high and random selections derived by self-fertilization (120 plants per class per generation). Both levels of selection intensity were included, making a total of 768 plants per replication. The plants within each replication were completely randomized and the twenty wooden blocks holding the test tubes were moved to new random positions and orientations each day.

The second trial consisted of parents *RLD* and *CT* (24 plants of each), their F_2 (36 plants), and the three generations of high and random selections derived by random-mating at 20% selection intensity. Included was the material which had been selected under self-fertilization and then random-mated. In each replication 120 plants were grown of the first and second generation material and 160 plants of the third generation material. The third trial was similar to the second but consisted of the 10% selections from the cross of *RLD* and *CT*. The fourth trial was of the selfed material from the cross of *RLD* and *CT*, and the remaining two trials tested the random-mated material from the cross of TU and LU.

Results and Discussion

In each of the final trials the test tubes were inspected after eight days and any for which the seed had failed to germinate or the plant showed reduced vigor were eliminated from the trial. As a general rule, the survival percentages were lower for older seed.

All fresh weights were measured in milligrams and logarithmically transformed since the plants had been in the exponential growth phase. The parental and F_2 means, pooled over the complete set of trials, are shown in Table 1. The standard errors and indications of significance were obtained from analyses of variance of the genotypic means.

Table 1. *Parental and F_2 means pooled over the complete set of final trials*

Genotype	Mean log fresh weight
TU	1.368 ± .006
LU	1.279 ± .006*
F_2 (*TU* × *LU*)	1.283 ± .005*
RLD	1.318 ± .006*
CT	1.311 ± .006*
F_2 (*RLD* × *CT*)	1.349 ± .005

* Within each cross, means do not differ significantly at the .01 level of probability.

The absolute responses to selection: The complete set of response curves are given in Fig. 1. The control means with random mating are approximately constant over generations for the cross *RLD* × *CT*, but the F_2 generation for cross *TU* × *LU* has an inexplicably low value. However, it is apparent that selection under random mating has been effective in that the high selections always have a greater mean than the corresponding random selections. At the same time, the real effects are masked by the large fluctuations in mean from generation to generation, apparently due to the age of seed rather than chance variation since the comparable response curves within each cross closely parallel one another. It may be significant that there was an age effect on the viabilities of seeds and seedlings, with survival percentages of 44%, 80%, and 82% for the first, second, and third generation material of cross *TU* × *LU*. For cross *RLD* × *CT* the corresponding figures were 60%, 84% and 89% respectively.

For selection under self-fertilization the control means for cross *TU* × *LU* decrease only slightly over generations and there is a similar but larger effect for the other cross. However, the general conclusions are the same as for the random mated material, namely that selection has been effective but that the

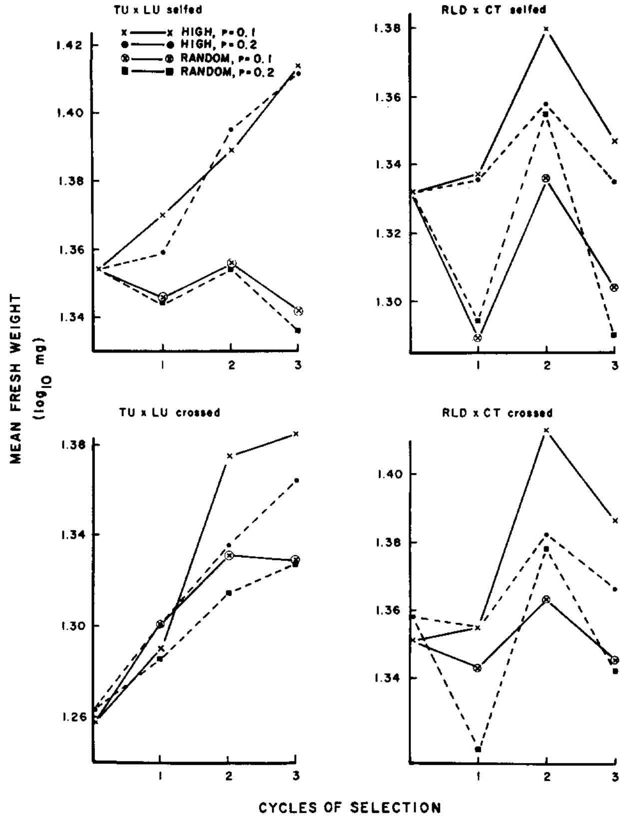

Fig. 1. Absolute responses ($\log_{10}$ mg) to selection with self-fertilization (upper pair of graphs) and random-mating (lower pair of graphs) for each of two base populations

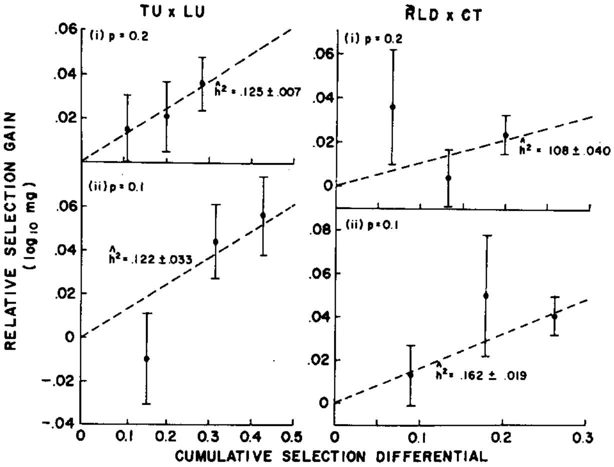

Fig. 2. Relative gains due to selection with random mating. The observed gains are shown ± standard error, and the estimated weighted regression coefficients are given adjacent to the dashed line

magnitude of the gain has been obscured by other factors.

A better measure of selection gain is, therefore, the deviation of the high selections from their corresponding random selections since this is expected to minimize seed effects. This measure will be considered for each cross in turn.

The relative response to selection — TU × LU: The relative selection gains are given in Table 2. The standard errors and indications of significance were obtained from analyses of variance using the appropriate mixed model.

In each case, selection with random mating gave a significant response after three cycles of selection. As is expected, the magnitude of the gain is directly dependent on selection intensity.

For 10% selection the cumulative selection differentials were 0.1554, 0.3179, and 0.4286 for the three cycles of selection. A weighted linear regression of observed gain on cumulative selection differential, passing through the origin, gives the estimate of slope

$$\hat{h}^2 = 0.122 \pm 0.033$$

This relationship is shown in Fig. 2. The symbol "$\hat{h}^2$" is used since this is an estimate of the heritability of individuals in the base population. If a model of non-interacting loci is assumed then

$$h^2 = \frac{A}{A + D + E},$$

where A, D, and E are the base population variances attributable to additive genetic, dominance, and environmental effects respectively.

The cumulative selection differentials for 20% selection with random mating were 0.1093, 0.1981, and 0.2821, and the fitting of a weighted linear

Table 2. *Deviations between the high and random selections for cross TU × LU*

Mating system	Selection intensity	Generation 1	2	3
Random	10%	−.010 ± .021 *NS*	.044 ± .017 *	.056 ± .018 *
	20%	.015 ± .016 *NS*	.021 ± .016 *NS*	.036 ± .012 **
Self	10%	.024 ± .029 *NS*	.033 ± .014 *	.072 ± .014 **
	20%	.015 ± .019 *NS*	.042 ± .012 **	.077 ± .023 *
Self & random	10%			.054 ± .017 **
	20%			.022 ± .015 *NS*

NS Not Significant

*, ** Significant at .05 and .01 levels, respectively.

regression (Fig. 2) gives

$$\hat{h}^2 = 0.125 \pm 0.007\,.$$

This estimate is consistent with that for 10% selection and we can, therefore, obtain a pooled value

$$\hat{h}^2 = 0.125 \pm 0.007\,. \tag{1}$$

Turning to selection with self-fertilization, for the higher selection intensity the selection differential for cycle 1 was 0.1450. This gives a realized heritability

$$\hat{h}_1^2 = 0.162 \pm 0.202$$

for this first cycle. The corresponding differential for 20% selection was 0.1086, giving

$$\hat{h}_1^2 = 0.138 \pm 0.172\,.$$

Again the two estimates are not significantly different and we obtain a pooled value

$$\hat{h}_1^2 = 0.148 \pm 0.131\,. \tag{2}$$

In terms of the base population variances, Pederson (1969) has shown that

$$h_1^2 = \frac{A + (1/2)\,D}{A + D + E}\,.$$

By combining the estimates (1) and (2), we can, therefore, derive

$$\begin{aligned} \hat{A} &= 0.125 \\ \hat{D} &= 0.046 \\ \hat{E} &= \underline{0.829} \\ \hat{A} + \hat{D} + \hat{E} &= \underline{1.000} \end{aligned}$$

These estimates will now be used to compare the observed and predicted responses for the second and third cycles of selection with self-fertilization. On the assumption of a low heritability and infinite population size, the predicted gain following two cycles is (Pederson 1969)

$$G_2 = i_1 \cdot \frac{A + (1/4)\,D}{A + D + E} + i_2 \cdot \frac{(3/2)\,A + (3/8)\,D}{(3/2)\,A + (3/4)\,D + E}\,,$$

where i_1 and i_2 are the selection differentials, and the predicted gain following three cycles is

$$G_3 = i_1 \cdot \frac{A + (1/8)\,D}{A + D + E} + i_2 \cdot \frac{(3/2)\,A + (3/16)\,D}{(3/2)\,A + (3/4)\,D + E} + i_3 \cdot \frac{(7/4)\,A + 7(/32)\,D}{(7/4)\,A + (7/16)\,D + E}\,.$$

Substitution of the appropriate selection differentials for 10% selection gives

$$\begin{aligned} G_2\,(\text{predicted}) &= 0.044; \\ G_2\,(\text{observed}) &= 0.033 \pm 0.014 \end{aligned}$$

and

$$\begin{aligned} G_3\,(\text{predicted}) &= 0.069; \\ G_3\,(\text{observed}) &= 0.072 \pm 0.014\,. \end{aligned}$$

The predicted values, therefore, agree well with the observed means. For 20% selection

$$\begin{aligned} G_2\,(\text{predicted}) &= 0.037; \\ G_2\,(\text{observed}) &= 0.042 \pm 0.012 \end{aligned}$$

and

$$\begin{aligned} G_3\,(\text{predicted}) &= 0.061; \\ G_3\,(\text{observed}) &= 0.077 \pm 0.023\,, \end{aligned}$$

and again the predicted values are in good agreement with the observed means.

When selection is with self-fertilization, but the final assessment is in a randomly mated population, it can be shown that the expected gain for a character of low heritability is

$$G_3' = i_1 \cdot \frac{A}{A + D + E} + i_2 \cdot \frac{(3/2)\,A}{(3/2)\,A + (3/4)\,D + E} + i_3 \cdot \frac{(7/4)\,A}{(7/4)\,A + (7/16)\,D + E}$$

The selection differentials for 10% selection were 0.1632, 0.1159, and 0.1205, and these give the predicted value

$$\begin{aligned} G_3'\,(\text{predicted}) &= 0.066; \\ G_3'\,(\text{observed}) &= 0.054 \pm 0.017\,. \end{aligned}$$

For 20% selection the differentials were 0.1135, 0.1119, and 0.1134, giving

$$\begin{aligned} G_3'\,(\text{predicted}) &= 0.057; \\ G_3'\,(\text{observed}) &= 0.022 \pm 0.015\,. \end{aligned}$$

The predicted value is appreciably greater than the observed value in this case.

However, the overall conclusion for this cross is that fresh weight behaves as a character of low heritability with a low level of dominance, and the observed results are satisfactorily explained by simple quantitative genetic theory. From Table 1 it is seen that the parent and F_2 generations in no way present a conflicting pattern since the parental means are significantly different and the F_2 is intermediate in mean value.

The relative response to selection — RLD × CT: The relative selection gains are given in Table 3, and it is observed that three cycles of selection with random mating produced a significant response at each intensity. The more intense selection produced a greater response.

For 10% selection with random mating the cumulative selection differentials were 0.0895, 0.1787, and 0.2621, and a weighted linear regression of observed gain on cumulative selection differential (Fig. 2) gives

$$\hat{h}^2 = 0.162 \pm 0.019\,.$$

The cumulative selection differentials were 0.0653, 0.1323, and 0.1982 for 20% selection, giving the estimate

$$\hat{h}^2 = 0.108 \pm 0.040\,.$$

The two estimates of heritability are different, but not significantly so, and they give the pooled estimate

$$\hat{h}^2 = 0.152 \pm 0.017\,. \tag{3}$$

A feature of selection with self-fertilization in this cross is that the response for each selection intensity was significantly different from zero after only one cycle. In no other case was this so. The selection differential was 0.0917 for the first cycle of 10% selection, and the realized heritability is therefore

$$\hat{h}_1^2 = 0.523 \pm 0.161\,.$$

The corresponding selection differential for 20% selection was 0.0623, giving

$$\hat{h}_1^2 = 0.660 \pm 0.159\,.$$

The two estimates of h_1^2 agree well and they give rise to the pooled estimate

$$\hat{h}_1^2 = 0.592 \pm 0.113\,. \qquad (4)$$

Table 3. *Deviations between high and random selections for cross RLD × CT*

Mating system	Selection intensity	Generation		
		1	2	3
Random	10%	.013 ± .014 *NS*	.050 ± .028 *NS*	.041 ± .009 **
	20%	.036 ± .026 *NS*	.004 ± .013 *NS*	.024 ± .009 **
Self	10%	.048 ± .015 **	.044 ± .009 **	.043 ± .011 **
	20%	.041 ± .010 **	.003 ± .015 *NS*	.045 ± .011 **
Self & random	10%			.039 ± .009 **
	20%			.031 ± .012 *

NS Not Significant

*, ** Significant at .05 and .01 levels, respectively.

The genetic interpretation of h^2 and h_1^2 are as described previously, but when the estimates (3) and (4) are equated to expectation a negative estimate of error is obtained. It is concluded that the error variance is relatively insignificant, and since $\hat{h}^2$ has a far smaller standard error than $\hat{h}_1^2$ we will assume that additive genetic effects contribute 15.2% of the total variation and that the remainder is due to dominance effects, yielding

$$\begin{aligned} \hat{A} &= 0.152 \\ \hat{D} &= 0.848 \\ \hat{E} &= 0.000 \\ \hat{A} + \hat{D} + \hat{E} &= 1.000 \end{aligned}$$

Thus the dominance variance predominates. These values will now be used to derive the expected gains from cycles 2 and 3 of selection with self-fertilization.

At the higher selection intensity we obtain

$$\begin{aligned} G_2\ (\text{predicted}) &= 0.106; \\ G_2\ (\text{observed}) &= 0.044 \pm 0.009 \end{aligned}$$

and

$$\begin{aligned} G_3\ (\text{predicted}) &= 0.139; \\ G_3\ (\text{observed}) &= 0.045 \pm 0.011\,. \end{aligned}$$

Each of the predicted values is considerably greater than the corresponding observed gain. For 20% selection the result is similar, with values of

$$\begin{aligned} G_2\ (\text{predicted}) &= 0.060; \\ G_2\ (\text{observed}) &= 0.003 \pm 0.015 \end{aligned}$$

and

$$\begin{aligned} G_3\ (\text{predicted}) &= 0.117; \\ G_3\ (\text{observed}) &= 0.045 \pm 0.011\,. \end{aligned}$$

For the material subjected to 10% selection under self-fertilization and then random mated the selection differentials were 0.0890, 0.1164, and 0.0920, giving

$$\begin{aligned} G_3'\ (\text{predicted}) &= 0.083; \\ G_3'\ (\text{observed}) &= 0.039 \pm 0.009\,. \end{aligned}$$

For 20% selection the differentials were 0.0576 0.0608, and 0.1007, and the predicted gain is

$$\begin{aligned} G_3'\ (\text{predicted}) &= 0.067; \\ G_3'\ (\text{observed}) &= 0.031 \pm 0.012\,. \end{aligned}$$

In each case the observed response is considerably, below the predicted value.

This population has, therefore, responded in a straightforward manner to selection with random mating, and the responses are repeatable in the sense that duplicate estimates of realized heritability agree well. The magnitude of these estimates is evidence against the presence of genes of large effect. Further, there is a significant response from a single cycle of selection with self-fertilization and again the realized heritability is a repeatable quantity. However, selection with continued self-fertilization gives a response which deviates markedly from expectation, and in fact, the selection gain for each intensity remained approximately constant after the initial significant response. If various combinations of variances are tested, it is found that a result of this type is possible if the additive genetic variance is very low and the dominance variance is very high, but even then the pattern of response is not identical with that obtained in the present study.

Reference to the parental and F_2 means (Table 1) reveals that races *RLD* and *CT* differed very little, but that the F_2 mean was significantly greater than that of either parent. One explanation is that the parents are homozygous for different dominant alleles at two or more loci, and if these loci are linked then the expected selection response could well be as observed since the base population is in a situation of extreme linkage disequilibrium. Further genetic analyses are required to test this hypothesis.

Conclusions

The results for cross $TU \times LU$ demonstrate that a continuous selection response is possible with complete self-fertilization. This raises the question of whether the same technique can profitably be applied to increasing yield in such crop plants as wheat or barley, with rigorous selection commencing in the F_2 generation. It would probably be necessary to increase heritability by selecting on the basis of self-family means rather than individual values.

Simple prediction formulae were found to give an adequate description of the results for the first cross, but the greater genetic gain which is expected with self-fertilization was not realized. In fact, for both crosses the genetic gains under complete random mating and complete self-fertilization were approximately equal.

It is probable that there are large dominance effects in the base population derived from cross $RLD \times CT$. However, this does not necessarily raise the question of the evolution of dominance since the population is not naturally occurring, but is a synthetic constructed for the purpose of this experiment. Griffing and Langridge (1963) studied the relationship between level of heterozygosity and response to high temperature in *Arabidopsis* and found that heterozygotes are generally more stable. It was suggested that this is a consequence of the combination in hybrids of the more thermostable alleles of different genes. In classical terminology, the more thermostable alleles are dominant and the combination of two or more dominant alleles in a hybrid produces a heterotic effect. Race *RLD* was among the five races tested by Griffing and Langridge (their race *R*), and it may be significant that this race, being the most thermostable, was assumed to possess the greatest number of thermostable alleles. Race *CT* was also among the five tested (their race *C*), and this was found to be the least thermostable.

Of particular note for the cross $RLD \times CT$ is the realized heritability of 0.152 with random mating and the realized heritability of 0.592 for cycle 1 with self-fertilization. This points up the fact that a heritability is not only a property of the unit of selection, but is also dependent on the mating design for selected parents. In fact, the two heritabilities specified are quite different functions of genetic and environmental variances.

Zusammenfassung

Vier Rassen von *Arabidopsis thaliana* wurden verwendet, um zwei F_2-Populationen herzustellen, die als Ausgangspopulationen für Untersuchungen über künstliche Selektion dienten. Alle Pflanzen wurden unter sterilen Kulturbedingungen in kontrollierter Umwelt gezogen, das untersuchte Merkmal war das Frischgewicht nach 15tägigem Wachstum. In einem Teil des Versuchs wurden zwei Selektionsintensitäten auf jede der F_2-Populationen angewandt und die selektierten Eltern zufällig gepaart. Zusätzlich zur strengen Selektion wurde eine Gruppe zufallsgemäß ausgewählter Individuen als Kontrolle gezogen. Im zweiten Teil des Versuchs unterschied sich das Vorgehen vom vorigen nur dadurch, daß die zufällige Paarung durch Selbstbefruchtung ersetzt wurde.

Für eine Population war der Selektionserfolg wie für ein Merkmal mit niedriger Heritabilität zu erwarten. Eine reguläre Antwort wurde auch für die andere Population beobachtet, wenn die Paarung zufällig erfolgte, jedoch zeigten die Ergebnisse der Selektion mit Selbstbefruchtung das Vorkommen von Dominanzeffekten an. Insgesamt wird gefolgert, daß Selektion verbunden mit zufälliger Paarung und Selektion verbunden mit Inzucht näherungsweise gleiche genetische Gewinne zur Folge hatten.

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D. G. Pederson
Department of Agricultural Botany
University of Sydney
Sydney, New South Wales (Australia)

D. F. Matzinger
Department of Genetics
North Carolina State University
Raleigh, North Carolina 27607 (USA)