Theor. Appl. Genet. 54, 169-175 (1979)

Effect of Reciprocal Crossing of Selected Lines of Mice*

M. Kownacki
Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Mroków (Poland)

Summary. Mass selection of mice was conducted in populations of various size for 16 generations. Each selected population (E) corresponded to an analogous unselected population (C). The experiment was conducted in three replicates. After the 16th generation the replicates of the selected and control lines were crossed.

Reciprocal crossing within the control lines gave better results than reciprocal crossing of the selected lines, despite the fact that the selected mice were characterised by a higher inbreeding coefficient. Larger effects were also obtained when crossing smaller rather than larger populations. This result is understandable since the animals from the smaller populations were characterised by higher inbreeding coefficients.

The effect of heterosis was higher upon crossing the control lines rather than the selected ones and this caused a decreased the response to selection in almost all the traits investigated.

Key words: Mice – Reciprocal crossing of lines – Accumulated response to selection free of inbreeding

Introduction

The evaluation of heterosis based on the crosses of various lines of animals is often a subject of investigation. This problem is, undoubtedly, of considerable importance for animal production, as skilful utilisation of heterosis may stet favourable economic effects. Although the practical results of crossbreeding animals have been known for a long time, the results of various investigations have not been in agreement. The effect of crossbreeding undoubtedly depends on the inbreeding of the parents, the degree of their genetical differentiation and on the interaction of genes received from both parents.

It is impossible to present the results of all the extremely numerous investigations devoted to this problem, so I shall review only a part of the publications in this field. Rutledge et al. (1974) crossed mice from selected and control lines and obtained an insignificant effect of crossing, i.e., of heterosis. However, the lines used for crossing were characterised by low inbreeding coefficients. For selected mice the inbreeding coefficient reached 0.13; for the control ones, 0.07. White et al. (1975) demonstrated a significant influence of heterosis on the body weights of 42 and 56 day old mice and on their weight gains between the 21st and 42nd days. Those results were obtained when crossing 4 highly inbred lines of mice (F = 92%). Naso et al. (1975) obtained similar results of heterosis when crossing 4 highly inbred lines of rats. In this case, heterosis demonstrated a most positive influence on the weight gains of animals between the 20th and 50th day of life. Bakker et al. (1976) analysing the effects of heterosis when crossing selected and unselected lines (control), did not observe a greater influence of heterosis in the selected lines when they were compared with the control ones, although the selected lines were characterised by a higher inbreeding coefficient. McNew and Bell (1974) demonstrated an increase in heterosis in *Tribolium* when crossing two, pure, selected lines for 24 generations and a decrease in heterosis when crossing those lines in further generations.

In the investigations reported in this paper, an attempt was made at evaluating the influence of crossing lines which had been selected for 16 generations and of crossing unselected lines. The crossing was conducted between 3 analogous selected and 3 analogous unselected lines. The cumulative response to selection was also examined after reciprocal crossing of lines (replicates) of animals selected and free from inbreeding.

* This work was partly supported by U.S. Department of Agriculture, Grant No. FG-Po-348/JB-13/, Project No. PL-ARS-68

0040-5752/79/0054/0169/$ 1.40

Material and Methods

Before starting the experiment, 10 inbred strains of mice were mated at random through three generations. From the third generation, 1500♂ and 1500♀ were chosen at random to constitute the initial population. From this population the experimental (E) populations were formed using the following animals (parents): 10/5♂ + 5♀/, 20/10♂ + 10♀/, 50/25♂ + 25♀/, 100/50♂ + 50♀/, 150/75♂ + 75♀/, 200/100♂ + 100♀/. For each of the (E) populations there was a corresponding, unselected, control (C) population. These E and C lines were repeated in three concurrent experiments.

Selection of breeders within a given population, at the age of 6 weeks, was based on individual weight gains between the 21st and 42nd days of life. Mice were weighted at the age of three weeks. Males were then separated from females and both were subsequently weighted at the age of 6 weeks. The animals with the best weight gains in each particular population were used as parents for the next generation. Full-sib mating was avoided to minimize inbreeding. The number of mice in each litter was reduced to 8 shortly after birth.

After generation 16, all reciprocal crosses were made between the three replicates of each population of selected and control lines in order to measure the accumulated responses free of inbreeding depression. The reciprocal crosses were carried out in two generations according to the following method: 1st crosses among replicates A, B and C

A♂ X B♀
C♂ X A♀
B♂ X C♀
2nd crosses
AB♀ X C♂
CA♀ X B♂
BC♀ X A♂

In the 16th generation and in the reciprocal crosses the number of mice in litters was not reduced at birth. In all populations the following traits were examined: weight at 21 and 42 days of age, weight gain, litter size and mortality from birth to 6 weeks old.

The mice room was lighted for 12 hours per day, temperature was maintained at about 20-22°C and humidity at about 60%. The standard pelleted feed contained about 20% of crude protein.

All the traits mentioned and also the responses to selection for individual traits were subjected to statistical analysis.

The following mathematical model was used to calculate the analysis of variance of the variables examined:

$$Y_{ijklt} = m + a_i + b_j + ab_{ij} + c_k + ac_{ik} + bc_{jk} + abc_{ijk} + d_l + ad_{il} + bd_{jl} + cd_{kl} + abd_{ijl} + acd_{ikl} + bcd_{jkl} + abcd_{ijkl} + e_{ijklt}$$

where:

m = total mean
a_i effect of generation $i = 1, 2, 3$
b_j effect of sex $j = 1, 2$
c_k effect of selection $k = 1, 2$
d_l effect of population $l = 1, 2 \ldots 6$
ab_{ij}, ac_{ik}, ... effects of interaction
e_{ijklt} random effect $t = 1, 2, 3$

For litter size and mortality the effect of sex was not included in the model. In turn, in the calculations of the response to selection the effect of the type of selection was not taken into consideration.

In the analysis of variance empirical numbers of litters were applied in replicates as weight corrections, proportional to the variance of means. Calculations were made applying a non-orthogonal system.

Results

The mice used for crossing were characterised by varying inbreeding coefficients (Table 1). The selected mice had higher inbreeding coefficients than the unselected (control) ones which is understandable, since selection prefers specific genotypes and diminishes genetic variability. According to Robertson (1961), if the trait under selection is heritable, then inbreeding will be enhanced in selected lines relative to control lines. Also, in small populations higher inbreeding occured than in the large ones. The dif-

Table 1. Coefficients of inbreeding in 16-th generation

Population's size (number of parents)	Average coefficients of inbreeding	
	Selected	Unselected
10	32.6	29.1
20	26.2	20.1
50	16.5	10.8
100	9.9	6.1
150	8.6	5.2
200	7.8	4.2

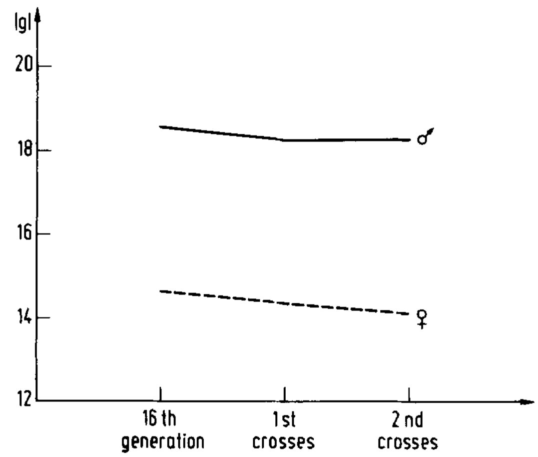

Fig. 1. Postweaning weight gain of selected mice

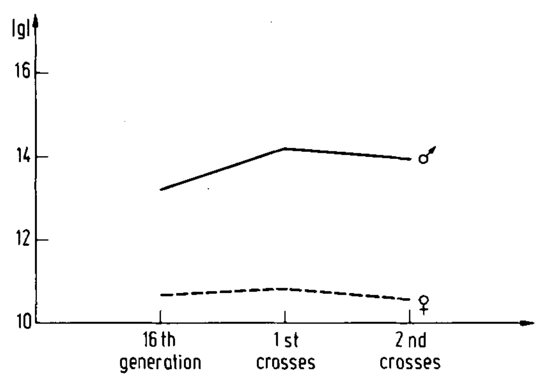

Fig. 2. Postweaning weight gain of unselected mice

Table 2. Unbiased mean results of reciprocal crossing (grams)

Gene-rations	Population's size (Number of parents)	Males 21st day wt. E	Males 21st day wt. C	Males 42nd day wt. E	Males 42nd day wt. C	Males Weight gain E	Males Weight gain C	Females 21st day wt. E	Females 21st day wt. C	Females 42nd day wt. E	Females 42nd day wt. C	Females Weight gain E	Females Weight gain C	Litter size E	Litter size C	Mortality up to 42 days in % ♂ + ♀ E	Mortality up to 42 days in % ♂ + ♀ C
	10	7.86	7.36	21.54	18.66	13.68	11.25	7.65	7.47	19.22	18.32	11.63	10.57	6.58	7.54	20.96	27.04
	20	11.72	9.37	26.98	21.06	15.27	11.73	11.21	9.12	23.76	19.64	12.55	10.50	8.71	7.07	4.56	9.75
	50	11.41	9.29	29.67	22.26	18.51	12.95	10.97	8.92	25.22	18.98	14.21	10.21	9.34	7.77	8.08	10.80
16th	100	10.58	9.04	29.01	22.12	18.34	12.94	10.27	8.90	24.88	19.45	14.57	10.51	8.62	7.05	11.64	14.78
	150	10.15	8.50	29.13	21.77	18.97	13.17	10.00	8.41	25.00	19.01	15.02	10.57	9.37	7.94	12.69	10.15
	200	10.58	8.85	29.97	22.27	19.28	13.35	10.33	7.86	25.80	19.36	15.39	10.62	8.75	7.62	14.41	22.72
Mean		10.57	8.79	29.14	22.10	18.54	13.22	10.30	8.63	24.99	19.34	14.65	10.66	8.69	7.53	12.40	16.54
	10	11.15	9.16	28.47	25.40	17.27	16.41	10.85	9.16	23.92	21.83	13.09	12.75	6.68	8.61	16.30	33.69
	20	8.98	7.62	26.32	21.86	17.16	13.65	8.95	7.49	22.64	18.53	13.57	10.67	7.60	6.50	24.96	33.54
	50	11.07	8.93	29.28	22.83	18.21	13.91	10.77	8.58	24.73	19.07	13.96	10.46	9.74	7.82	13.83	12.45
1st	100	11.68	10.19	30.52	25.10	18.79	14.89	11.19	9.93	25.80	20.77	14.66	10.84	9.38	8.03	18.53	9.97
crosses	150	10.90	9.84	28.88	23.27	18.27	13.61	10.47	9.44	24.97	20.43	14.50	10.99	9.63	7.84	25.23	20.12
	200	11.19	10.55	29.79	24.67	18.58	14.14	10.56	10.15	25.27	20.67	14.71	10.54	9.85	7.57	13.55	8.56
Mean		10.87	9.49	29.12	23.74	18.26	14.21	10.48	9.21	24.81	20.09	14.33	10.83	9.19	7.74	18.93	17.77
	10	10.74	10.06	28.59	25.11	17.86	15.05	10.67	9.72	24.27	21.32	13.80	11.60	8.22	9.45	18.36	24.83
	20	12.51	10.77	30.77	27.02	18.28	16.24	12.03	10.41	25.59	22.28	13.58	11.84	8.71	7.35	10.06	7.72
	50	12.25	10.59	31.70	24.33	19.44	13.74	11.74	10.24	26.12	20.50	14.34	10.27	10.08	8.40	14.96	14.73
2nd	100	11.44	10.69	29.53	24.30	18.11	13.65	11.22	10.30	24.96	20.65	13.72	10.44	10.11	8.05	13.76	16.86
crosses	150	12.27	9.91	29.27	23.59	16.97	13.67	11.91	9.65	25.89	20.34	13.89	10.68	10.07	8.59	14.27	16.34
	200	10.33	9.95	28.93	22.77	18.60	12.83	10.07	9.67	24.42	19.27	14.49	9.59	10.34	8.49	13.89	11.72
Mean		11.61	10.35	29.93	24.28	18.31	13.94	11.27	10.02	25.36	20.54	14.10	10.54	9.88	8.36	14.00	15.53

E = experimental populations, C = control populations ferences between the selected and control populations, as also between the smallest and the largest ones, were quite considerable. In the smallest selected populations the coefficient of inbreeding reached an average of 32.6, while in the control it was only 29.1. In the largest population those values reached 7.8 and 4.2, respectively. The degree of inbreeding in individual populations undoubtedly influenced the results of crossing (Table 2), as will be presented later.

An analysis of the effect of reciprocal crosses on the traits was used to compare body weights of animals at the ages of 21 and 42 days and the weight gains from generation 16 with those obtained after the first and second crossing. The tendency to increase (Table 2) can be clearly seen only at an age of 21 days in both the selected and the control animals when comparing the mean results from all the 6 populations for both males and females. Therefore, after the first and second crossings, the tendency of a higher 42-day weight and weight gain can't be distinctly observed (Table 2, Fig. 1-2). When comparing the mean effect of crossing in all the 6 populations in the 16th generation selected animals showed an decrease of weight gain in males obtained from the 1st crossing (by 1.62%) and from the second (by 1.24%). In the case of females, these values were 2.98% and 3.75%, respectively. In the unselected animals, the 1st crossing produced an increase and the 2nd decrease in body weight gains (Table 2, Fig. 2).

As a result of reciprocal crossings of the replicates, the differences between the smallest and the largest populations decreased. In the case of weight gains in the selected males, the mean differences between the smallest and the largest population reached 4.86 g in the 16th generation but in the 1st and 2nd crossing were only 0.99 g and 0.45 g. A similar decrease in those differences occurred between the females of the smallest and largest populations – in generation 16 those differences reached 3.02 g, while after crossing they were only 1.24 g and 0.3 g. In the unselected populations, after 1st and 2nd crossing, the body weight at 42 days and weight gains were greater in the smallest population than in the largest. From those it can be clearly seen that the crossing of small populations, where the inbreeding coefficients are higher than in larger populations, resulted in a higher heterosis.

As seen from the statistical analysis (Table 3) all three traits discussed were highly significantly ($P \leq 0.01$) influenced by selection and sex. Crossing highly significantly influenced the weight on the 21st and 42nd day and population size influenced highly significantly only the weight on the 21st day. Also, significant and highly significant interactions were recorded between crossing and selection, likewise between crossing and population size, for all three traits discussed. Highly significant interactions were also ascertained for weight gains between sex and crossings, sex and selection, selection and population size and between crossing, selection and population size, for weight on the 42nd day; between sex and selection, population size and selection, for the 21st day between selection and population size, crossing, selection and population size.

Litter size increased after reciprocal crossing of the replicates, both in the selected and control lines (Table 2). In turn, mortality increased after the first crossing and, subsequently, fell after the second.

It is shown by the statistical analysis (Table 4) that litter size and mortality were hightly significantly influenced by crossing and by population size, while selection influenced, highly significantly and positively, litter size. The influence of selection on mortality was not ob-

Table 3. Analysis of variance for body weight at 21 and 42 days of age and gain in body weight

Source	d.f.	Weight at 21st day M.S.	Weight at 42nd day M.S.	Weight gain M.S.
Crosses (C)	2	1150.44[a]	939.46[a]	20.12
Selection (S)	1	4020.79[a]	60181.28[a]	33448.33[a]
C × S	2	30.91[b]	279.30[a]	118.08[a]
Sex (X)	1	163.74[a]	28758.90[a]	24363.98[a]
C × X	2	3.41	90.53	63.68[a]
S × X	1	2.24	474.07[a]	422.94[a]
C × S × X	2	0.46	19.89	21.21
Population size (P)	5	80.30[a]	57.38	10.74
C × P	10	1992.47[a]	537.33[a]	168.95[a]
S × P	5	49.01[a]	330.97[a]	321.06[a]
X × P	5	1.17	31.43	20.48
C × S × P	10	31.53[a]	23.21	41.99[a]
C × X × P	10	1.19	29.78	26.15
S × X × P	5	0.17	5.54	6.66
C × S × X × P	10	0.67	4.90	4.75
Residual	138	12.76	34.86	16.37

[a] differences highly significant ($P \leq .01$), [b] differences significant ($P \leq 0.5$)
d.f. = degrees of freedom, M.S. = mean square

Table 4. Analysis of variance for litter size and mortality

Source	d.f.	Litter size M.S.	Mortality M.S.
Crosses (C)	2	367.17[a]	4910.39[a]
Selection (S)	1	1814.46[a]	2029.97
C × S	2	11.59	1946.50[b]
Population size (P)	5	135.02[a]	4129.57[a]
C × P	10	13.92	5769.02[a]
S × P	5	109.89[a]	1700.99[b]
C × S × P	10	6.08	1460.45[a]
Residual	72	16.52	551.41

[a] differences highly significant ($P \leq .01$), [b] differences significant ($P \leq .05$)
d.f. = degrees of freedom, M.S. = mean square

Table 5. Unbiased mean direct and correlated responses to selection (grams)

Generations	Population's size (number of parents	Males			Females			Litter size	Mortality up to 42 days in %
		21st day wt.	42nd day wt.	Weight gain	21st day wt.	42nd day wt.	Weight gain		
	10	0.37	2.89	2.58	0.06	0.84	1.25	–0.87	–6.44
	20	2.45	6.16	3.27	2.19	4.19	1.99	1.77	–6.06
	50	2.15	7.40	5.52	2.06	6.23	3.98	1.60	–2.65
16th	100	1.53	6.84	5.35	1.35	5.40	4.04	1.57	–3.08
	150	1.72	7.45	5.82	1.67	5.97	4.44	1.39	+2.49
	200	1.74	7.69	5.92	1.65	6.44	4.76	1.12	–8.30
Mean		1.81	7.14	5.38	1.68	5.72	4.04	1.20	–3.99
	10	2.01	3.08	0.88	1.74	2.10	0.37	–1.95	–16.94
	20	1.58	5.02	3.25	1.68	4.49	3.00	1.06	–9.73
	50	1.76	6.55	4.43	2.19	5.75	3.61	1.86	1.47
1st	100	1.50	5.41	3.89	1.26	5.02	3.81	1.36	8.57
crosses	150	1.10	5.63	4.70	1.03	4.55	3.51	1.36	5.02
	200	0.32	5.08	4.40	0.40	4.54	4.15	2.28	5.05
Mean		1.26	5.52	4.10	1.29	4.84	3.59	1.39	1.40
	10	0.74	3.57	2.85	0.99	2.99	1.98	–1.12	5.07
	20	1.69	3.60	1.99	1.58	3.29	1.74	1.38	1.86
	50	1.66	7.35	5.67	1.51	5.60	4.05	1.71	0.32
2nd	100	0.72	5.19	4.45	0.89	4.26	3.24	2.09	–3.20
crosses	150	2.02	5.65	3.29	2.25	5.52	3.20	1.50	–2.12
	200	0.38	6.17	5.80	0.37	5.17	4.92	1.86	+2.08
Mean		1.17	5.71	4.47	1.21	4.86	3.62	1.57	0.17

served. However, ascertained was an influence of the interaction between crossing and selection and crossing and population size on mortality and between selection and population size on litter and mortality. A three way, highly significant interaction was found only in the case of mortality.

Mean response to selection is compared in Table 5 for 6 populations of generation 16 with the response to selection after the first and second crossing. A clear decrease is noticed after the first, and next after the second crossing, for body weights at 21 days of age, both in the case of males and females. The response to selection for body weights of 42 day old mice and for weight gains (Fig. 3), decreases after the first crossing then increases after the second, but still remains below the mean response to selection obtained in generation 16.

This fact undoubtedly indicates that crossing had a more positive effect on body weights of 21 and 42 day old mice and their weight gains in the control populations than in the selected ones.

As seen from statistical analysis (Table 6), the response to selection for the weights at the 21st and 42nd days and weight gain were significantly influenced by crossing and population size. Sex highly significantly ($P \leqslant 0.01$) influenced weight at the 42nd day and weight gain. Significant interactions ($P \leqslant 0.05$) were recorded only between crossing and population size for weight gain.

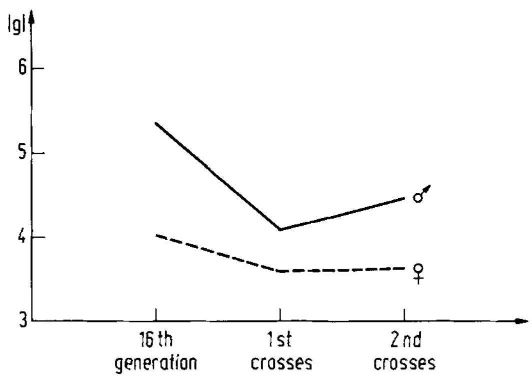

Fig. 3. Response to selection for postweaning weight gain

Table 6. Analysis of variance of cumulative response to selection for body weights at 21 and 42 days of age and gain in body weight

Source	d.f.	Weight at 21st day M.S.	Weight at 42nd day M.S.	Weight gain M.S.
Crosses (C)	2	105.28[b]	520.24[a]	208.63[a]
Sex (X)	1	0.29	918.16[a]	795.66[a]
C × X	2	2.08	38.59	40.17
Population size (P)	5	92.73[b]	514.89[a]	519.84[a]
C × P	10	49.33	49.89	69.23[b]
X × P	5	0.38	9.02	12.69
C × X × P	10	1.84	9.75	8.44
Residual	72	29.51	79.68	32.88

[a] differences highly significant ($P \leqslant .01$), [b] differences significant ($P \leqslant .05$)
d.f. = degrees of freedom, M.S. = mean square

Table 7. Analysis of variance of cumulative responses to selection for litter size and mortality

Source	d.f.	Litter size M.S.	Mortality M.S.
Crosses (C)	2	22.90	4110.52[b]
Population size (P)	5	173.96[a]	1284.22
C × P	10	11.34	3328.70[a]
Residual	36	26.56	899.38

[a] differences highly significant ($P \leqslant .01$), [b] differences significant ($P \leqslant .05$)
d.f. = degrees of freedom, M.S. = mean square

The response to selection for litter size increased slightly after crossing (Table 5) but those differences were not significant (Table 7). Crossing was found to have a positive influence ($P \leqslant 0.05$) on the response to selection for the animals mortality. The population size had a highly significant positive influence ($P \leqslant 0.01$) on the response to selection for litter size and no influence on mortality.

Discussion

The results of the reciprocal crossing of the control animals were slightly better than those from reciprocal crossing of selected lines. An interpretation of this phenomenon is rather difficult as the selected lines were more inbred (an average of 32.6 to 7.8%) than the control ones (an average of 29.1 to 4.2%) and one could expect that after crossing the selected lines, the heterosis would be greater than when crossing the control animals. Similar results were obtained by Bakker et al. (1976) when crossing selected and control lines. He did not observe a greater influence of heterosis in selected lines when compared with unselected. It might be explained on the basis of Wright's adaptive peaks. It could be that selected lines tend towards a co-adapted genome which tends to be broken down in crossing.

The crossing of animals in populations of various sizes, with differing inbreeding coefficients, generally resulted in a decrease in differences between the mean values for the traits investigated. Animals from small populations, with higher inbreeding coefficients, generally demonstrated, after crossing, an increase in the value of the traits investigated, while the animals from large populations showed a decrease in the traits. The decrease in the differences after crossing seems, in this case, plausible, as animals with higher inbreeding coefficients in small populations demonstrated greater heterosis. Animals in large populations were characterised by greater selection and small populations by a greater influence of heterosis. As a result of these facts the differences between the populations were rather small.

The more positive effect of crossing the control than the selected lines caused a decrease in the response to selection in almost all the traits investigated. Only the response to selection for litter size and mortality increased slightly after crossing.

Acknowledgement

The author is grateful for helpful suggestions from Dr. Gordon E. Dickerson, Co-operating Scientist Agricultural Research Service, U.S. Department of Agriculture, at University of Nebraska.

Literature

Bakker, H.; Nagai, J.; Eisen, E.J.: Average genetic and heterotic effects on growth in mice selected for large 6-week body weight or rapid postweaning gain. J. Anim. Sci. **43** (6), 1145-1155 (1976)

McNew, R.W.; Bell, A.E.: Crossbred response from purebred selection, an experimental check on selection theory with *Tribolium*. Theor. Appl. Genet. **44**, 100 (1974)

Naso, R.B.; Baker, F.T.; Butcher, D.; Kaczmarczyk, W.: Weight gain and heterosis at different stages of development of the rat. Growth **39** (3), 345-361 (1975)

Robertson, A.: Inbreeding in artificial selection programs. Genet. Res. **2**, 189-194 (1961)

Rutledge, J.J.; Eisen, E.J.; Legates, J.E.: Test for heterosis in topcross progeny of replicate selected lines of mice. J. Hered. **65** (2), 121-122 (1974)

White, J.M.; Jamison, M.G.; Vinson, W.E.: Heterosis, sex and maternal interaction in crosses among inbred lines of mice. Canad. J. of Genet. Cytol. **17** (4), 563-571 (1975)

Received May 20, 1978
Accepted September 19, 1978
Communicated by L.D. Van Vleck

Dr. Miroslaw Kownacki
ul. Długa 24 m 12
00-238 Warszawa
(Poland)