ISTITUTO DI GENETICA
UNIVERSITÀ DI PAVIA
VIA SANT'EPIFANIO, 14
PAVIA

Telefoni: 23.029; 29.038 - Indirizzo telegrafico: Istgenetica - Pavia

US Atomic Energy Commission Contract AT(30-1)-2686

INVESTIGATIONS ON MUTABILITY OF POLYGENES

Chief Investigator: R.E.SCOSSIROLI, Institute of Genetics, University of Pavia, Via Sant'Epifanio 14, Pavia, Italy.

F i n a l R e p o r t (NYO 10,620)

1) Introduction
2) Experimental work
3) Results and conclusions
4) Complete list of reports and papers

[redacted]

to: US Atomic Energy Commission, Washington 25, D.C., USA.

December 31, 1963

RESEARCH CONTRACT

no.AT(30-1)-2686

INVESTIGATIONS ON MUTABILITY OF POLIGENES

Final Report (NYO 10,620)

1) Introduction:

a) The aim of the work proposed was to obtain information about the effect of spontaneous and induced mutations in quantitative traits, using as mutagenic agent hard X-rays applied to seed or gametes.

Contract AT(30-1)-2686 was handled directly with the AEC Office during the first year (1961) and during the first renewal (1962). For further continuation it had been suggested by the Commission that the research work might be supported through the IAEA, Vienna, under Contract AT(30-1)-2466 which was already in operation with the recipient under IAEA number 61/US.

b) Information available before the research under the above contract was started was representes by the work performed by the proponent on drosophila and by few other research workers, as it was listed in the Research Proposal. A need of more data on mutability of factors responsible for quantitative traits was stressed: 1) by the importance of an induced increase of genetic variation for production traits in plants and animals of economic value, and 2) by the interest of knowing much better the mechanisms which may affect in Nature the speed of evolution of plant or animal populations.

c) A discussion of the work performed was given in the research proposal previously submitted.

d) Under this final report the results obtained with the researches carried on the following organisms will be summarized:

Solanum pimpinellifolium (wild tomato);
Medicago sativa (alfalfa);
Habrobracon juglandis (a parasitic Braconid wasp);
Zea mays (mais).

2) Experimental work:

Details on the experimental plan and methods were given in the Research Proposal. The realization of the experimental work followed the program proposed, and was given in part also in the Report submitted to the Commission on October 1,1961.

3) Results and conclusions:

Solanum pimpinellifolium. The results obtained with this plant have been given in the report submitted on October 1, 1961 and in a paper by D.Palenzona (1962).

The results obtained indicated that under inbreeding the population taken into consideration, represented by seeds from a monoploid plant, showed to contain a large amount of genetic variation, while it was expected to be completely homozygous. Because the seeds used, obtained through the courtesy of Professor Burdick, Purdue University, had been stored for a few years, it was suggested that such unexpected variation might come from spontaneous mutations accumulated in seed during aging. However the amount of genetic variation found was too large for being referred to this phenomenon only. For this reason it was proposed that the work should be continued on Solanum lycopersicon with the goal to identify in a first stage haploid plants, screening a very large number of seedling, and to produce autodiploid lines to be used for continuation of the work. Subsequently it was planned to follow the rise of genetic variation under inbreeding in this material.

Variety S.Marzano was used for this work, haploid seedlings were identified on the basis of morphology and further checked by cytological examination and by test of sterility of the initial flowering branches. Autodiploid seed were next obtained from latest flowering branches derived from the callus after topping the plants. The work was discontinued at this stage as a consequence of a reduction of the financial support operated during the transfer of the research program from the AEC to the IAEA.

Medicago sativa (alfalfa)

A first series of results obtained in this crop were given in the report submitted October 1, 1961. Further results were obtained from the genetic analysis of the size of leaflets and were given in the report submitted to IAEA on July 1, 1963. This work is continued under IAEA Contract and considers observations on plants derived from seeds obtained under inbreeding and under outcrossing in plants from treated and untreated seeds. The analysis of these data is in progress. The results will be reported to IAEA.

Habrobracon juglandis (parassitic Braconid wasp)

Extensive experiments have been performed on this organism. The experimental program and methods used were described in the report submitted October 1, 1961, as well as the results obtained in a first series of experiments. X-ray treatments were applied to males of a line which was previously inbred for six generations up to an expected homozygosity level of 97%. Selection performed for longer wings resulted successful. No difference in response to selection was found however when the results of selections on the treated groups were compared to those obtained in the untreated ones. An analysis of phenotypic variation in the treated and untreated groups showed that the 40% of phenotypic variance in females and the 20% in males was of genetic origin in spite of the fact that the population used after inbreeding was expected to be genetically uniform. Because of the success of selection, it was hypothesized that selection for longer wings in the inbred strains was made possible by either:

1) maintenance of genetic variation by persistance of heterozygosis, or
2) high spontaneous mutation rate for quantitative traits during or after the process of inbreeding.

It was than decided to continue the experiments in order to collect more data on the phenomenon of persistance of genetic variability under prolonged inbreeding. The work was started during the first renewal of the AEC Contract AT(30-1)-2686 and continued under Contract AT(30-1)-2466, operated through the IAEA Contract 61/US. For this reason the results which are being reported here have been given also in the report

submitted on July 1, 1963 to IAEA, Vienna.

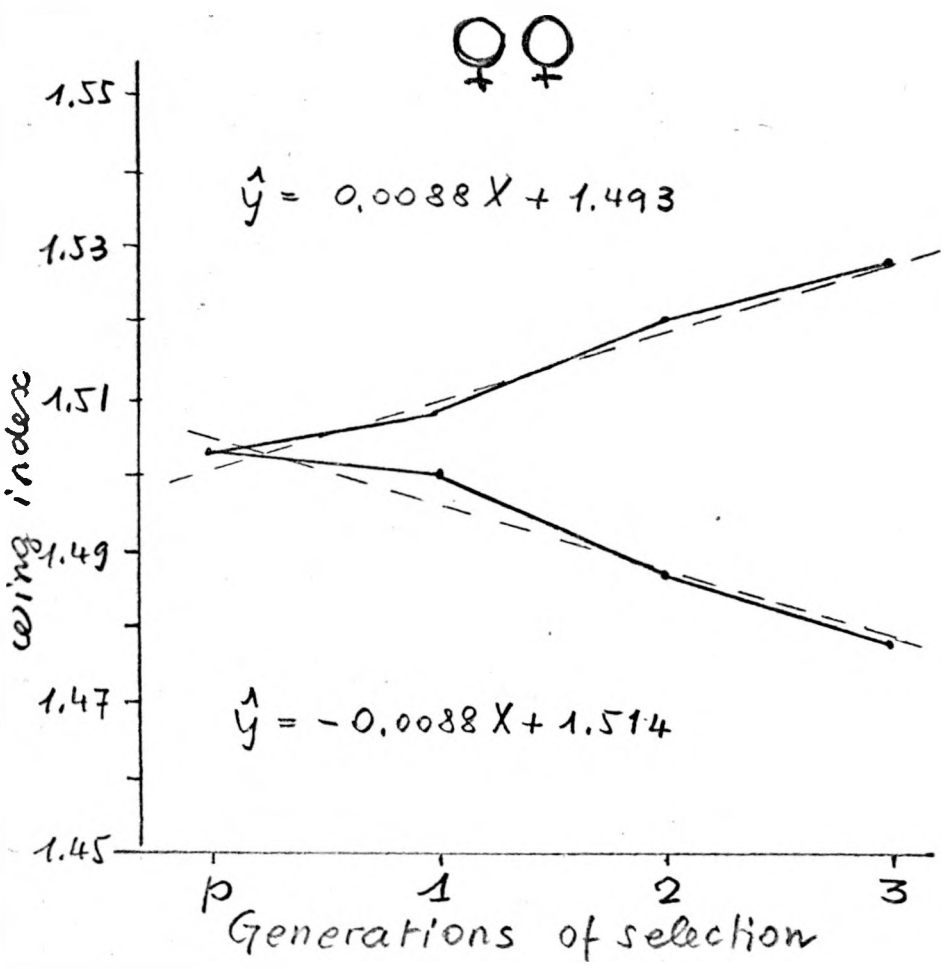
In order to collect more data on the above problem, long- and short winged lines were selected from a small wing, white eye (sw, wh) stock not subjected to any special inbreeding mating scheme. A family method of selection was used, with a selection pressure variable from generation to generation from 0.868 to 1.166 standard deviations from the mean, according to the number of families available. It had not been possible to impose a constant selection pressure through all the experiment since a high infertility fluctuated from generation to generation and the number of families fluctuated accordingly. The selection pressure was applied to every other generation to allow reassortment of favourable genes through genetic recombination. During selection, inbreeding was avoided by means of planned matings among the selected wasps.

The results of this selection are given in fig.1. It may be seen that selection for long and for short wings are effective and the responses symmetric.

Selection under identical conditions was repeated after three generations of inbreeding by back-crossing to the same haploid male of daughters from subsequent generations: the homozygosity level was estimated to be 0.875. It was again possible to select long- and short- winged lines. The results of this selection in two independent replicates are given in fig. 2. The selection for long wings is still effective, while the short wing selection shows an immediate strong response and later it seems to have reached a plateau.

After 15 generations of inbreeding, when an homozygosity coefficient higher than 0.99 was expected, selection for long- and short wings was again performed, using three independent replicates for each selection. The outcome of this experiment is given in fig. 3. In the long-wing selection, in spite of the repeated reproduction of families with the longest wings, a reduction of the mean wing length is found in females, while in the males the wing length responds in the direction expected according to the selection applied. In the analysis of the regression in males the linear regression component is found to be significantly above P 0.01, while the deviation from linearity component is found to be unimportant. In the selected short wing population the females repeat the behaviour shown

Fig. 1 - Progress of selection for long and short wings in Habrobracon before starting inbreeding



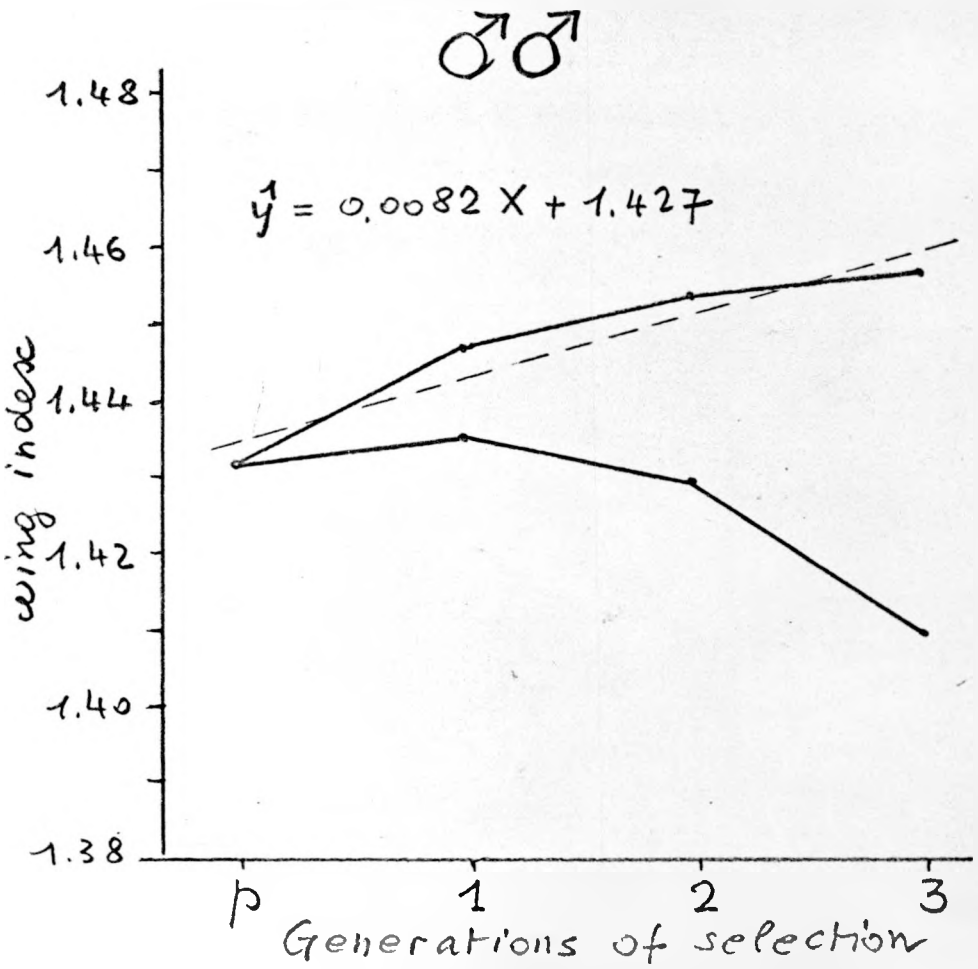
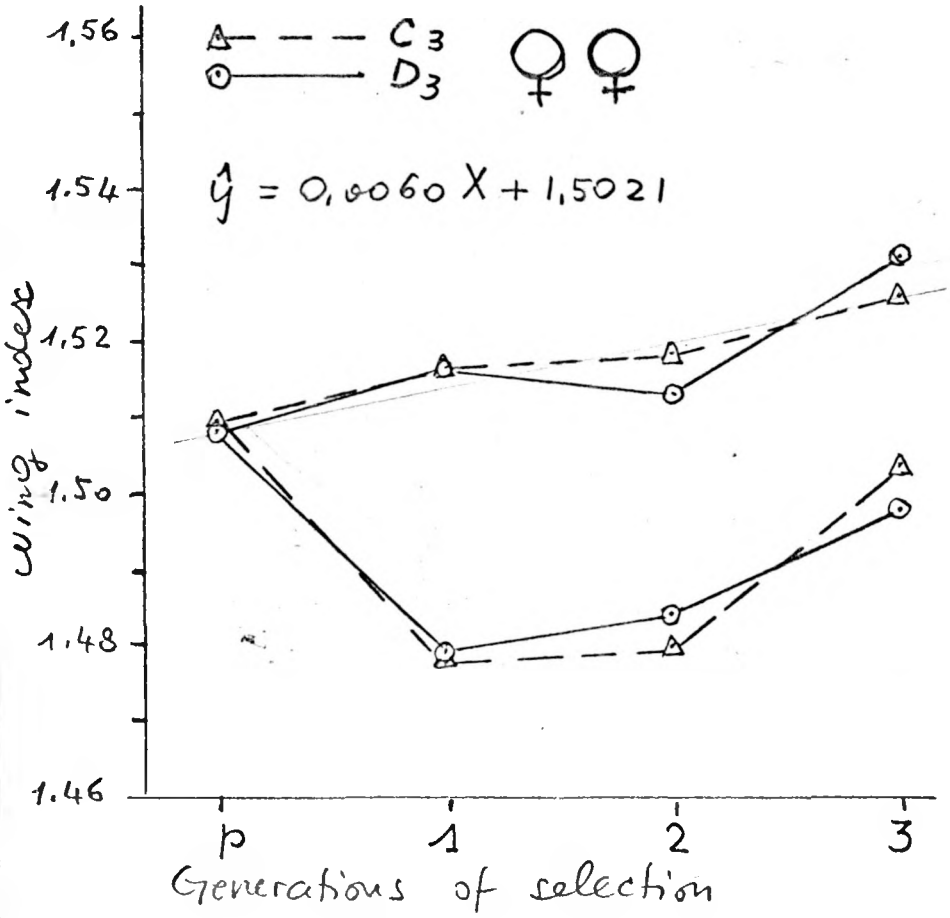


Fig. 2 - Progress of selection for long and short wings in Habrobracon after 3 generations of inbreeding



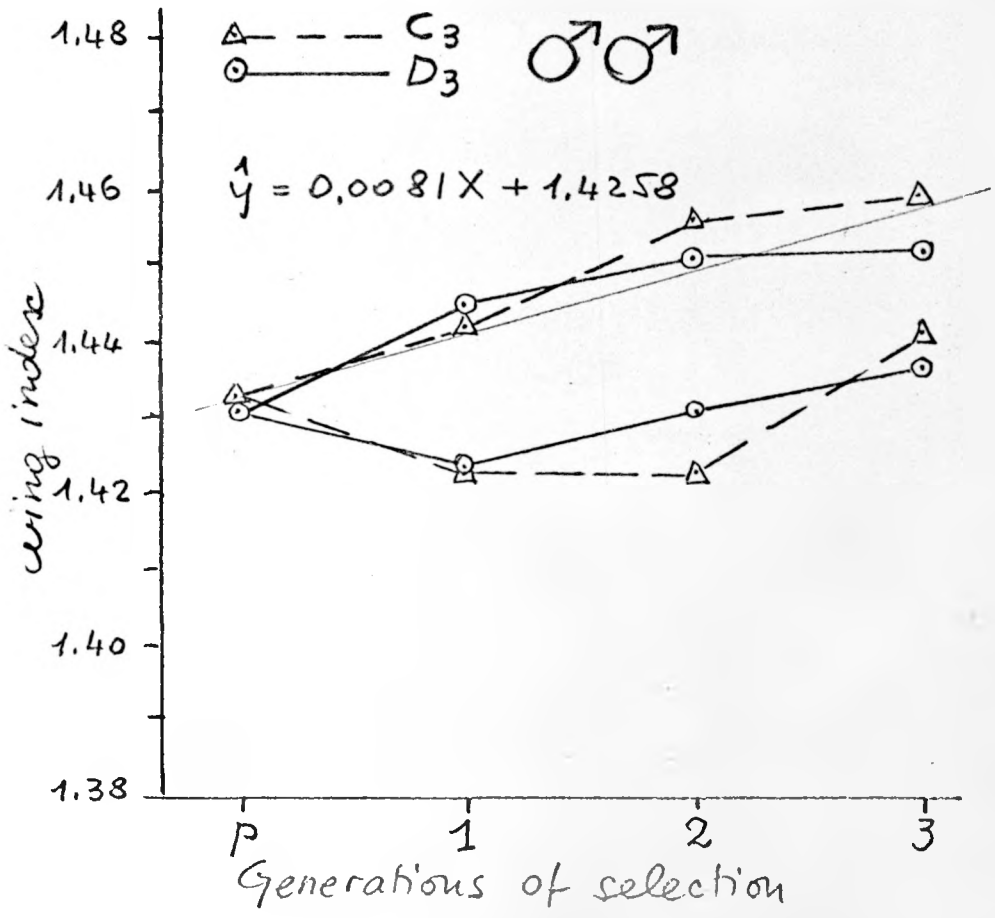
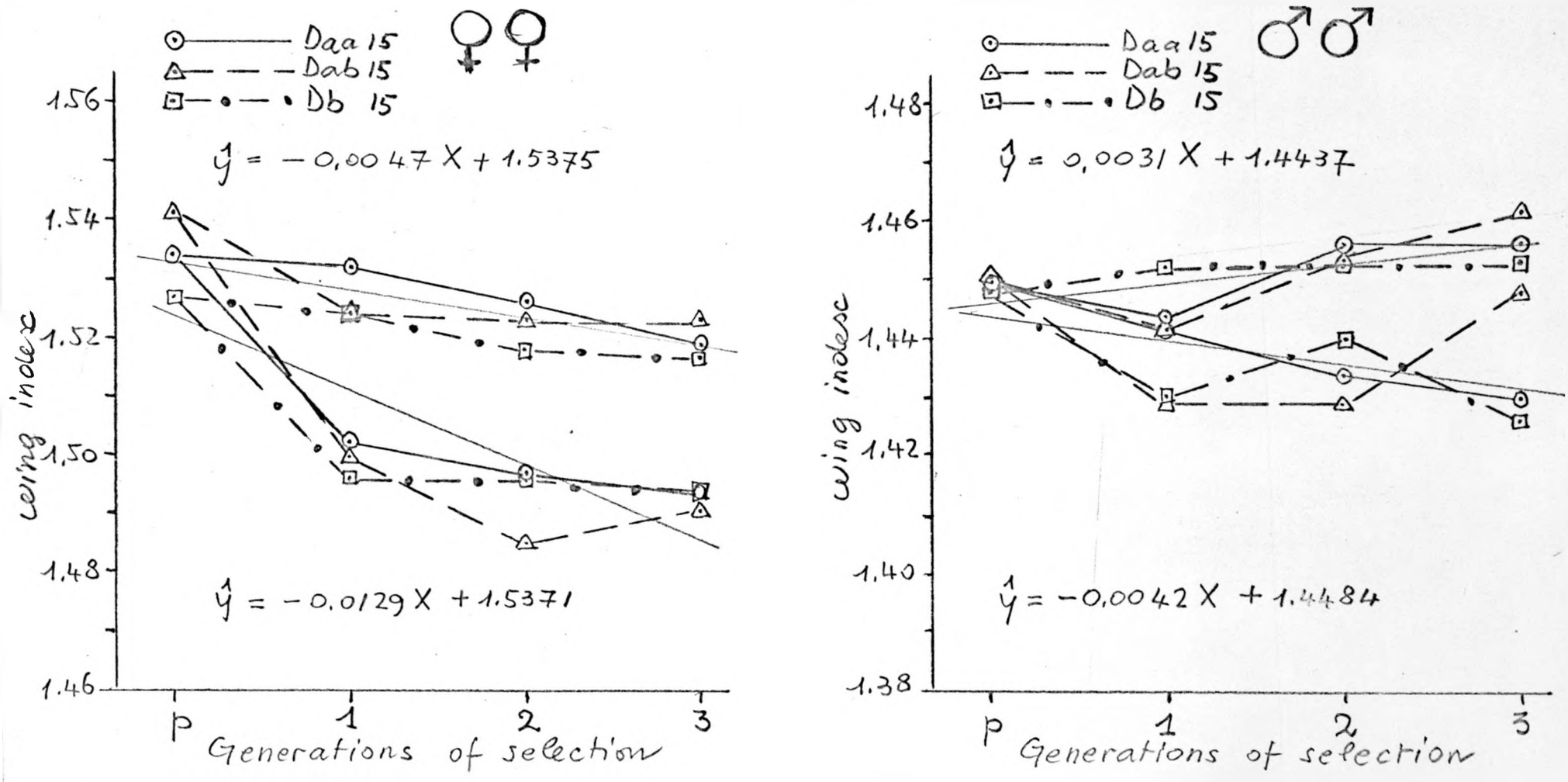


Fig. 3 - Progress of selection for long and short wings in Habrobracon after 15 generations of inbreeding



previously after three generations of inbreeding. In the males a persistent response in the expected direction is found.

In tables 1 and 2 the results of the genetic analysis performed on the basis of the following assumption are given.

Assuming that under family method selection the progress is measurable by:

$$\triangle G_{fa} = h^2_{fa} \, \bar{i} \, s_{fa}$$

where $\triangle G_{fa}$ = the progress of selection measured on family means, h^2_{fa} = the heritability for families, $\bar{i}$ = the intensity of selection and s_{fa} = the standard deviation for families, an estimate of heritability based on family means is given by:

$$h^2_{fa} = \frac{\triangle G_{fa}}{\bar{i} \, s_{fa}}$$

and a reliable estimate of additive genetic variability may be further obtained by:

$$s^2_{Gfa} = h^2_{fa} \cdot s^2_{Pfa}$$

considering that heritability represents the ratio between genetic (σ^2_G) and phenotypic ($\sigma^2_P = \sigma^2_G + \sigma^2_E$) variability of a population. In the above formula, s^2_{Gfa} represents the estimate of the genetic component of variation on the basis of family variance, and s^2_{Pfa} indicates the observed variance for families.

The picture which may be drown from figs. 1,2 and 3 and from tables 1 and 2, is that in spite of the continuation of inbreeding and of expectations in terms of the assumed level of homozygosity reached, the selection performed for quantitative traits shows that a large amount of genetic variability is present in the population even after 15 generations of close inbreeding. While the heterogeneity persisted for the selection for shorter wings, homozygosity was approximated for the characters affecting the lengthening of the wings. These data indicate

Table 1 - Selection gain ($\triangle_{Gfa}$) and differential ($\bar{i}_{fa}$), average phenotypic (s_{Pfa}) and genetic (s_{Gfa}) standard deviation for family means and heritability (h^2_{fa}) after different inbreeding generations. ♀♀

Selection	Generat. of inbreed.	Lines	$\triangle_{Gfa}$	$\bar{i}_{fa}$	s_{Pfa}	s_{Gfa}	h^2_{fa}
LONG WINGS	Parental population		0.0088	0.990	0.0164	0.0121	0.54
	3	C	0.0054	1.034	0.0164	0.0093	0.32
		D	0.0076	0.932	0.0133	0.0104	0.61
		Aver-age	0.0060	0.983	0.0149	0.0095	0.41
	15	Daa Dab Db	Response obtained in the direction contrary to the expected.				
SHORT WINGS	Parental population		0.0088	0.990	0.0199	0.0135	0.46
	3	C D	Non linear regression				
	15	Daa	0.0126	0.977	0.0167	0.0147	0.77
		Dab	0.0128	0.958	0.0177	0.0175	0.97
		Db	0.0051	0.934	0.0204	0.0147	0.52
		Aver-age	0.0129	0.955	0.0200	0.0164	0.68

Table 2 - Selection gain ($\triangle_{Gfa}$) and differential ($\bar{i}_{fa}$), average phenotypic (s_{Pfa}) and genetic (s_{Gfa}) standard deviation for family means and heritability (h^2_{fa}) after different inbreeding generations. ♂♂

Selection	Generat. of inbreed.	Lines	$\triangle_{Gfa}$	$\bar{i}_{fa}$	s_{Pfa}	s_{Gfa}	h^2_{fa}
LONG WINGS	Parental population		0.0082	0.990	0.0192	0.0126	0.43
	3	C	0.0092	1.034	0.0147	0.0114	0.61
		D	0.0070	0.932	0.0240	0.0134	0.31
		Average	0.0081	0.983	0.0200	0.0128	0.41
	15	Daa	0.0032	0.957	0.0158	0.0073	0.21
		Dab	0.0045	0.982	0.0130	0.0077	0.35
		Db	0.0016	0.970	0.0149	0.0050	0.11
		Average	0.0031	0.972	0.0155	0.0070	0.21
SHORT WINGS	Parental population		0.0072	0.979	0.0200	0.0123	0.38
	3	C	Non linear regression				
		D					
	15	Daa	0.0066	0.977	0.0138	0.0097	0.49
		Dab	0.0006	0.958	0.0135	0.0029	0.05
		Db	0.0056	0.934	0.0194	0.0108	0.31
		Average	0.0042	0.955	0.0167	0.0086	0.26

that the variability for longer wing length is not brought about by a high spontaneous mutation rate for quantitative characters. The genetic variation that is present in the inbred lines as indicated by the selection for shorter wings may be attributed to maintenance of genetic variance by persistence of heterozygosity. The sex determination mechanism of Habrobracon is such that heterozygosity in the region of the sex locus is mandatory. It is possible that a cluster of characters associated with a shortening of the wing is located near the sex locus, in spite of the extremely loose linkage which is found for the sex chromosome.

Zea mays (Maize)

The plan of research and the results obtained in a pilot experiment for the study of the influence of radiation on the combining ability of inbred lines in maize have already been given in the report submitted on October 1, 1961. In the 1962 season a larger series of crosses was made which have been tested in 1963 season under the IAEA Contract. The results will be given in a report to IAEA.

4) Complete list of reports and paper.

1) Research proposal: Investigations on mutability of polygenes.

2) Report of Scientific activity for the first year contract and plan of research for the second year contract. Submitted to AEC, Washington, on October 1, 1961. Contract AT(30-1)-2686.

3) Report of Scientific activity for the second year and plan of research for the third year contract. Submitted to IAEA, Vienna, on August 1, 1962. Contract 61/US.

Palenzona, D.L. - Effetti della selezione intrasomatica in relazione agli effetti mutageni delle radiazioni ionizzanti sui caratteri poligenici. Genetica Agraria, 15:390-396, 1962.

Scossiroli, R.E. - L'impiego delle radiazioni ionizzanti per aumentare la variabilità genetica nelle piante coltivate.

Atti Accademia Econ. Agraria dei Georgofili, Firenze 9:1-23, 1962; riprodotto in Agricoltura d'Italia, 8:13-37, 1962.

Scossiroli, R.E. - L'impiego pacifico dell'energia nucleare: le radiazioni ionizzanti ed il miglioramento delle piante. Pavia Economica n.5:30-34, 1962.

Scossiroli, R.E. - Inincrocio ed omozigosi in popolazioni. 32° Conv. Unione Zool. Ital. Bologna, Boll. Zool. 29:121-142, 1962.

Scossiroli, R.E. e Von Borstel, R.C. - Esperimento di selezione in Habrobracon dopo inincrocio. Atti Ass.Genet.It., Pavia, 7:191-197, 1962.

Scossiroli, R.E. - Problemi di selezione nell'erba medica. Sementi Elette 9:432-437, 1963.

Scossiroli, R.E. and Von Borstel, R.C. - Persistent genetic variation after inbreeding in Habrobracon. (in preparation).

Sarti, A. e Ferrari, A. - Ricerche sulla variabilità genetica nella medica dopo trattamenti con raggi X applicati al seme. (in preparation).

Scossiroli, R.E. and Von Borstel, R.C. - Selection experiments after inbreeding in Habrobracon. Genetics Today. Proc. XI Int.Congr.Genetics, I:152. 1963.