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Genetic analysis of antibody responsiveness to sheep erythrocytes in crosses between lines of mice selected for high or low antibody synthesis

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Summary. Two selective breedings of mice for minimal or maximal agglutinin response to heterologous erythrocytes were carried out (selection I and II). Preliminary reported data indicated that for both selections the heritability was between 0·18 and 0·36 and the number of relevant loci from 7–13. The results reported in this article are definitive since the data of ten to twenty generations at selection limit are available and large populations of interline hybrids F_1, F_2 and both backcrosses were analysed. The character 'high response' was partially dominant in F_1 hybrids of both selections, the degree of this incomplete dominance was 0·27 in selection I and 0·54 in selection II. In selection I, 38% of the F_2 variance was due to genetic factors (*VG*) and 62% to environmental effects (*VE*). The partition of phenotypic variance of F_2 and backcrosses into additive variance (*VA*) and dominance variance (*VD*) was made according to three methods and the mean results were: $VA = 0{\cdot}72$ and $VD = 0{\cdot}05$. The resulting mean heritability was 0·35 and the number of relevant loci about ten. In selection II, 69% of the F_2 variance was due to *VG* and 31% to *VE*. The three methods of variance calculation give somewhat discordant results. According to the more probable estimation (see discussion) $VA = 0{\cdot}95$, the mean heritability was 0·23 and the number of loci, about 6. The results obtained in crosses between homologous lines of the two selections indicate that the two 'high' lines have probably identical homogeneous genetic constitution while the two 'low' lines contain some different 'high' effect loci.

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INTRODUCTION

High (H) and low (L) responder lines of mice were produced by selective breeding for maximal or minimal agglutinin response to heterologous erythrocytes. Two selections have been made, selection I, selection II, starting from distinct foundation populations of outbred albino mice. In such experiments, the reproductibility of the response to selection is an important finding, since the frequency distribution of the relevant genes in the foundation stock is unknown (Falconer, 1973).

In both selections I and II the selective breeding produced a progressive divergence between H and L lines during thirteen to sixteen consecutive generations, at which point, the selection limit was attained. This finding demonstrated that the character 'quantitative antibody responsiveness' is under polygenic regulation. The assortative mating pro-

duced a progressive increase of the frequency of high effect alleles in H line and of low effect alleles in L line until all the relevant genes had accumulated in each line at the selection limit. At this stage the two lines were considered as homozygous for the character investigated. The constancy of the interline separation in all the generations at selection limit justifies this assumption.

In preceding publications the selective breeding was described up to the twenty-second generation and the seventeenth generation in selection I and II respectively. The realized heritability (h^2) was calculated and a preliminary estimation of the number of relevant loci (n) was proposed. The h^2 ranged between 0·18 and 0·36 and n between 7 and 13 (Feingold, Feingold, Mouton, Bouthillier, Stiffel & Biozzi, 1976).

The selective breeding was continued, and at present data are available for thirty-six generations of selection I and twenty-two generations of selection II. In these generations the interline separation remained unchanged, confirming that the selection limit was correctly interpreted. These additional results are of importance since the analysis of a large number of homozygous generations permits a precise evaluation of the environmental variance (VE).

MATERIALS AND METHODS

Selective breeding: general procedure

In both selections I and II the selective breeding was carried out individually for the character 'agglutinin titre' by mating at every generation the mice giving the highest titre (H line) or the lowest titre (L line). For each line, at least six pairs were formed at every generation. Brother–sister mating was excluded.

Selection I. This was founded on a population of sixty-two random bred albino mice of both sexes (F_0) obtained from several breeders. The mean of F_0 ($\bar{X}F_0$) is 9·69 and its variance (VF_0) is 2·56. The mice were weaned at 30 days of age and immunized 20–30 days after weaning. The first six generations were immunized with sheep erythrocytes (SE). Then it was recognized that the interline separation was also effective for responsiveness to unrelated pigeon erythrocytes (PE) the selective breeding was consequently continued until the 36th generation alternating SE and PE at each generation in order to avoid the interference of maternal antibodies passively transmitted to the progeny (Biozzi, Stiffel, Mouton, Bouthillier & Decreusefond, 1970; Biozzi, Stiffel, Mouton, Bouthillier & Decreusefond, 1971). The mean number of mice per generation was 50 ± 15 in H line and 46 ± 15 in L line.

Selection II. This was founded on a population of fifty random-bred albino mice of both sexes from Charles River (Elbeuf, France), ($\bar{X}F_0 = 10{\cdot}12$; $VF_0 = 2{\cdot}43$). Sheep erythrocytes only were used for immunizing successive generations (Stiffel, Mouton, Bouthillier, Heumann, Decreusefond, Mevel & Biozzi, 1974; Feingold *et al.*, 1976). A long time interval (60–70 days) was set between weaning and immunization in order to minimize the effect of maternal antibodies. The mean number of mice per generation was 56 ± 13 in H line and 58 ± 14 in L line.

Interline hybrids:

$$[(H \times L) = F_1;\ (F_1 \times F_1) = F_2;\ (F_1 \times H) = BcH;\ (F_1 \times L) = BcL)]$$

F_1, F_2 and backcrosses of both selections were produced in our animal breeding unit. Mice used for crosses belonged to the homogeneous generations of H and L lines at the selection limit. A similar number of reciprocal crosses (ten to fifty pairs) were mated. Mice were weaned when 30 days old and immunized 3–6 weeks later. Since there was no demonstrable difference in data obtained from reciprocal crosses, the results from these animals have been pooled.

Immunization and agglutinin assay

Mice were immunized intravenously with optimal immunizing doses of erythrocytes: 5×10^8 SE and 10^8 PE. Individual blood samples were obtained from the retro-orbital venous plexus at different times after immunization. The serum agglutinin titre was measured using a micro-agglutination technique on standard plates. To 0·05 ml of serial doubling dilutions of serum in 0·15 M phosphate buffer (pH 7·3) was added 0·05 ml of erythrocyte suspension containing 2×10^8 SE/ml or 2×10^7 PE/ml. The agglutinin titre was scored 24 h later and expressed either as the highest serum dilution giving positive agglutination or as log 2 of this dilution starting from undiluted serum = 0.

In our preceding publications (reviewed in Biozzi, Stiffel, Mouton & Bouthillier, 1975), the

agglutinin titre was calculated starting from 1 = 1/10 serum dilution, 2·333 should thus be added to the log 2 values of agglutinin titres reported in our previous publications in order to convert them into the scale used here.

The character 'agglutinin titre' shows a normal frequency distribution when expressed as log 2 of the highest serum dilution giving a positive agglutination, therefore the mean ($\bar{X}$) and the variance (V) of the various populations studied were calculated on a log 2 scale. Although a small sex effect has been observed (females higher than males by 0·5 log 2), male and female data have been pooled for calculations since every population consisted of about equal numbers of males and females.

Methods of genetic analysis

The following are the definitions of the terms used in the present article. For more detailed explanations and worked examples see Falconer, 1960; Cavalli-Sforza & Bodmer, 1971; Bodmer & Cavalli-Sforza, 1976.

The *selection differential* (S) is the difference between the mean of the selected parents and the mean of their generation. It is a measure of the genetic pressure exerted by the assortative mating. In order to equalize the contribution of each pair to the descendent population, the value of S for each pair has been weighted for the number of offspring.

The *response to selection* (R) is the difference between the mean of two consecutive generations of selective breeding.

The *realized heritability* (h^2) is measured by the least square linear regression of R on S during the interline separation from F_0 to the selection limit

$$h^2 = \frac{Rg}{Sg}$$

where Rg is the mean response per generation and Sg is the mean selection differential per generation.

H and L lines at the selection limit were considered as genetically homogeneous at all the loci controlling antibody responsiveness (Homozygous lines). The *interline separation at the selection limit* (RT) is the total response to selection. $RT = \bar{X}H - \bar{X}L$ (homozygous lines).

The *additive effect* (a) is the phenotypic difference between the homozygous lines and the heterozygous F_1 hybrids in the absence of dominance. It results from the effect of all 'high' or 'low' homozygous loci.

$$a = (\bar{X}H - \bar{X}L)/2 \text{ then } RT = 2a$$

The *global dominance deviation* (d) resulting from the dominance effect of all the heterozygous loci in F_1 hybrids is

$$d = F_1 - (\bar{X}H + \bar{X}L)/2$$

The proportion of the global dominance to the additive effect is expressed by the ratio d/a.

The expected contribution of all the homozygous loci to the variance of F_2 hybrids in the absence of dominance is called *additive variance* (VA); $VA = (\Sigma a^2)/2$.

The contribution of all the heterozygous loci with their dominance effect to the variance of F_2 hybrids is called the *dominance variance* (VD); $VD = (\Sigma d^2)/4$.

The variance resulting from any environmental effect is called the *environmental variance* (VE). Since H, L, at selection limit, and their F_1 hybrids are considered as genetically homogeneous.

$$VE = (VH + VL + VF_1)/3 \qquad (1)$$

The total phenotypic variance of F_2 hybrids (VF_2) is

$$VF_2 = VA + VD + VE \qquad (2)$$

In each backcross the variance is due to the difference between homozygotes and heterozygotes. If VBc is the addition of VBcH + VBcL

$$V\text{Bc} = VA + 2VD + 2VE \qquad (3)$$

From Equations 2 and 3 the values of VA and VD may be calculated as follows:

$$VA = 2VF_2 - V\text{Bc} \qquad (4)$$

$$VD = V\text{Bc} - VF_2 - VE \qquad (5)$$

The ratio VD/VA may be calculated in another way using differences between the means rather than variances. If we assume that the variability in the effects of individual genes is negligible or follows a fixed pattern, the following formula may be used:

$$\frac{VD}{VA} = \frac{1}{2}\left(\frac{d}{a}\right)^2 \qquad (6)$$

Expressing VD as $VA[(d/a)^2/2]$ in Equations 2 or 3, VA and VD may be calculated from VF_2 or VBc respectively.

The heritability (h^2) of the character is measured by the ratio between VA and the total phenotypic

variance of F_2 or backcrosses according to the formula

$$h^2 = VA/(VA + VD + VE) \qquad (7)$$

The number of loci controlling the character investigated n may be calculated as follows

$$n = a^2/2VA \qquad (8)$$

The estimate of n is very approximate due to large sampling and experimental errors in variance analysis, and because the effect on the variance produced by gene interactions (VI) is disregarded in calculations made with Equation 6 but taken into account in calculations following Equations 2 and 3. Moreover, the calculation of n is based on a simplified theoretical model which postulates that each locus is completely independent and that it may be occupied only by two alleles, each of which is endowed with equivalent 'good' or 'bad' effects on the character studied. (For detailed discussion on all the limitations that surround the meaning of n see Falconer, 1960.)

RESULTS

In both selections the fecundity and fertility remained roughly constant in all the generations. This minimizes the intervention of natural selection on the response to the selective breeding.

Figure 1 represents the kinetics of agglutinin responses in groups of fifteen mice of H and L lines of selection I and selection II immunized with an optimal dose of SE (5×10^8 i.v.). The mice used in this experiment belonged to F_{20} and F_{22} generations of selections I and II respectively. This experiment was designed to determine the most appropriate time after immunization for the genetic analysis.

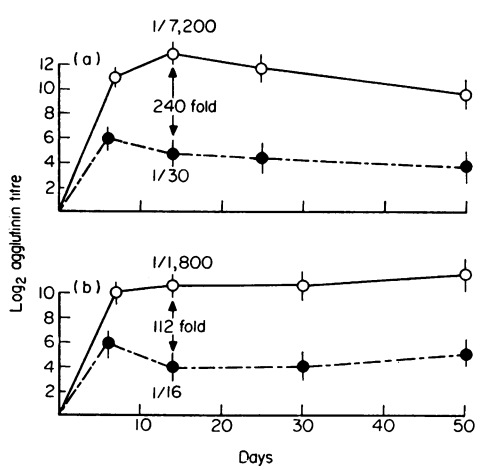

Figure 1. Kinetics of agglutinin response in H (——) and L (– – –) lines of selection I (a) and II (b) after i.v. immunization with 5×10^8 SE.

The kinetics are very similar in both selections I and II. In H line the agglutinin titre increased up to the fourteenth day post-immunization, during which time it had fallen in the L line. The larger phenotypic difference between H and L lines was therefore observed 14 days post-immunization: the difference was 240-fold for selection I and 112-fold for selection II. Consequently, we have chosen to carry out the genetic analysis using the agglutinin titres established 14 days post-immunization.

Results of selective breeding

The results of selection I are presented in Fig. 2. The selective breeding was continued for thirty-six consecutive generations. In order to eliminate the effects of environmental factors affecting both lines, the analysis of the selective breeding has been made according to the interline divergence. The value of S reported in the ordinates is the sum of the S values established separately in H and L lines at each generation, cumulated in successive generations. During the sixteen generations of selective breeding required to reach the selection limit, the mean value of S per generation (Sg) was 2·5. The cumulated R indicated on abscissae increased until F_{16} when it reached its maximal value $RT = 7{\cdot}8$. The mean value of R per generation (Rg) was 0·48. From F_{16} onwards, the response to selection ceased although the continuation of the selective breeding produced a steady increase of cumulated S. This means that all the alleles concerned with the regulation of agglutinin responsiveness had segregated in each line by the sixteenth generation which was therefore genetically homozygous for the character. The phenotypic variance of the successive generations (F_{16}–F_{36}) was only due to environmental factors (VE) and thus had no effect on R.

At the selection limit the mean agglutinin titre of all the homozygous generations either immunized with SE or PE was (12·71 ± 1·38) (1/6,698) in 852 H line mice and (4·91 ± 0·9) (1/30) in 885 L line mice. This corresponds to an interline difference of 233-fold in agglutinin titres. The agglutinin response to

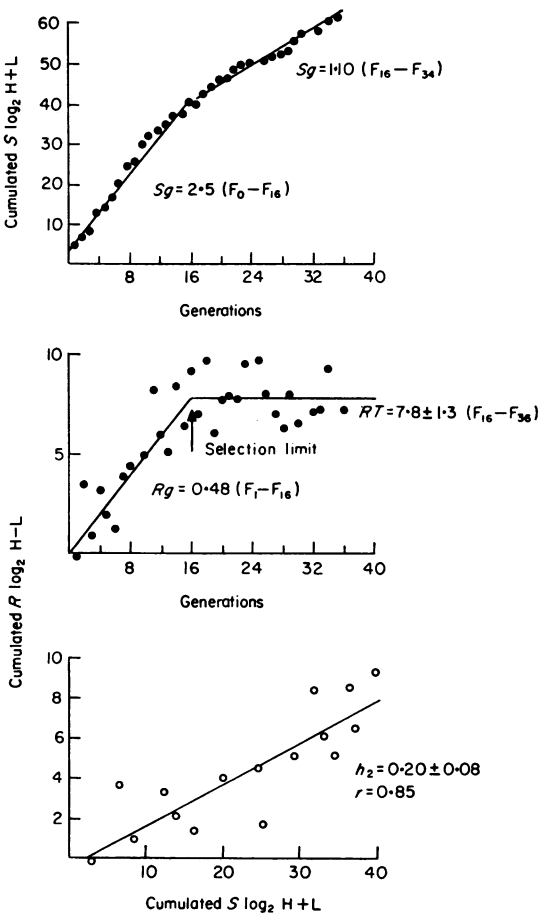

Figure 2. Results of selection I expressed as interline divergence: cumulated *S*, cumulated *R* and plot of *R* on *S*.

SE and to PE was very similar: the mean agglutinin titre of the generations between F_{16} and F_{36} immunized with SE was (12·50 ± 1·29) (1/5,790) in 472 H line mice, and (4·67 ± 0·75) (1/25) in 497 L line mice. The equivalent responses to the two antigens justify the cumulation of the data of every generation irrespective of the antigen used.

The realized heritability (h^2) of the character investigated was 0·20 ± 0·08 which means that about 20% of the parental deviation was actually inherited by the offspring at each generation. The h^2 value measures the proportion of the total phenotypic variance due to additive genetic effects.

The response to selection, *R*, may be calculated separately for H and L lines taking into account the level of responsiveness of the foundation population $F_0 = 9·7$ (1/830). From this value it may be calculated that antibody responsiveness was increased eight-fold in the H line and decreased twenty-eight-fold in the L line. The selective breeding thus produced an asymmetrical effect since the responsiveness of L line has been modified to a larger extent than that of H line, perhaps a result of the incomplete dominance of high over low responsiveness (Table 1), since dominance deviation reduces the response to selection. The value of h^2 was also calculated separately in each line, with reference to the level of F_0, the *RT* was 3·01 in the H line and 4·79 in the L line. The cumulated *S* in F_{15} was 20·22 in the H line and 19·72 in the L line. Thus h^2 was 0·15 in the H line and 0·24 in the L line. These values also reflect the asymmetrical effect of the selection.

The results for selection II are presented in Fig. 3 and are very similar to those of selection I. At the selection limit, the mean agglutinin titre in the generations considered as homozygous F_{14}–F_{22} was 11·6 (1/3,100) in the H line and 4·9 (1/30) in the L line. The interline difference in agglutinin titre was 103-fold, that is, about half the value obtained in selection I, but this is a minor difference (14% in relation to the total range of interline separation of selection I).

The asymmetrical effect observed in Selection I was also observed in selection II. Compared with the mean agglutinin titre of F_0 10·12 (1/1, 115), the antibody responsiveness of the H line at the selection limit was increased 2·8-fold while it was decreased thirty-seven-fold in the L line. Consequently, the h^2 value calculated separately for each line was higher in the L than in the H line. In the H line $RT = 1·48$; $SF_{13} = 12·01$; $h^2 = 0·12$. In L line $RT = 5·22$; $SF_{13} = 19·64$; $h^2 = 0·26$.

The asymmetrical response to selection of H and L lines and their difference in h^2 value are due to the incomplete dominance of higher responsiveness since the response of F_0 is identical to that of F_1 hybrids (see Table 2).

Results of interline crosses

The analysis of the data obtained in interline crosses are based on the probable assumption that in both Selections, H and L lines at selection limit are homozygous at the level of all the loci involved in the quantitative regulation of antibody responsiveness.

The mean agglutinin titres and the variance of H

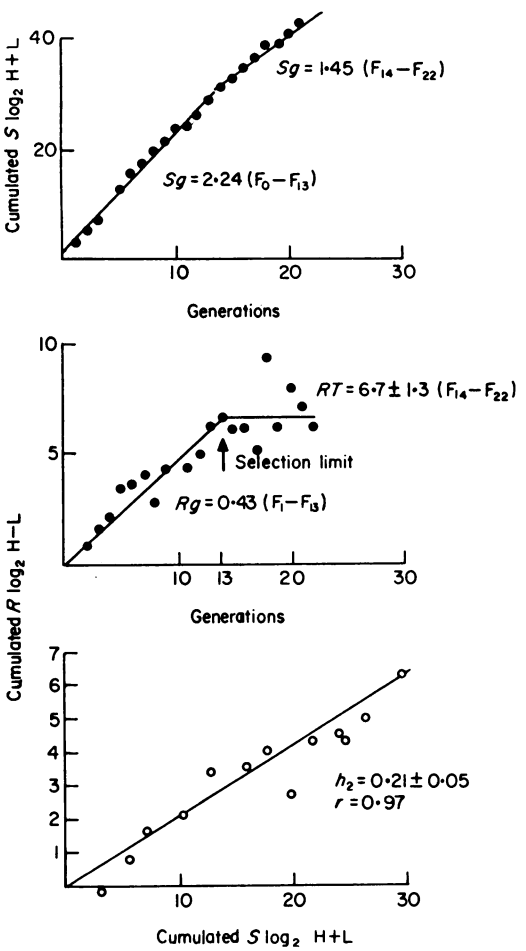

Figure 3. Results of selection II expressed as interline divergence: cumulated *S*, cumulated *R* and plot of *R* on *S*.

and L lines are calculated as the mean values obtained in F_{16}–F_{36} generations of selection I and in F_{14}–F_{22} generations of selection II. These data and the results obtained in interline crosses are reported in Table 1.

Since the F_0, the majority of generations in selection I and all the generations in selection II were immunized with SE, the interline hybrids were immunized with this antigen.

(a) *Study of the global dominance.* The study of the dominance of polygenic characters is only a measure of the dominance resulting from the unknown dominance deviation at the level of every separate heterozygous locus in F_1 hybrids, it is therefore called global dominance. The data presented in Table 1 show that in both selections, the mean value of F_1 was closer to that of H than L line, hence there was an incomplete global dominance of High over Low responsiveness. The degree of this dominance effect was measured by the ratio d/a. The theoretical value of d/a was between 0 (no dominance) and +1 (complete dominance of High response in the absence of overdominance).

As shown in Table 1, the degree of global dominance of High responsiveness was two-fold higher in selection II than in selection I ($d/a = 0{\cdot}54$ and $0{\cdot}27$ respectively). In both selections, the mean response of F_0 was very close to that of F_1 hybrids (selection I: $\bar{X}F_0 = 9{\cdot}7$, selection II: $\bar{X}F_0 = 10{\cdot}12$). Since the dominance effect limits the efficiency of the selection, the partial dominance of High responsiveness explains the asymmetrical effect of the selective breeding. The higher degree of dominance found in selection II was responsible for the larger asymmetrical effect observed in this selection compared with selection I.

The mean response of the other interline hybrids: F_2, BcH and BcL was influenced by the dominance effect measured in the F_1. The mean value of d/a measured in F_1, F_2, BcH and BcL was $0{\cdot}30 \pm 0{\cdot}18$ in selection I and $0{\cdot}51 \pm 0{\cdot}23$ in selection II. Because of the dominance of High responsiveness, the variance of BcL in both selections was larger than that of BcH.

(b) *Partition of the phenotypic variance in segregant hybrids, calculation of the heritability* (h^2) *and evaluation of the number of loci* (n). All the mice used in the present studies were bred in the same animal department with identical feeding and breeding conditions. The populations analysed always consisted of equal numbers of males and females immunized at approximately the same age. In spite of these controlled experimental conditions, the importance of non-genetic variability due to environmental factors (*VE*) is remarkable (selection I: $VE = 1{\cdot}22$; selection II $VE = 1{\cdot}31$; see Table 2).

The phenotypic variance of F_2 and Bc results from genetic factors (*VG*) and environmental factors (*VE*). The relative importance of environmental and genetic factors was calculated. In selection I, $VF_2 = 38\%$ $VG + 62\%$ VE. The importance of environmental factors was smaller in F_2 hybrids of Selection II where $VF_2 = 69\%$ $VG + 31\%$ VE. The

Table 1. Mean agglutinin titres and variances in H, L and interline hybrids of selection I and selection II, 14 days post i.v. immunization with 5×10^8 SE

Selection I*					Selection II†				
			Agglutinin titre log 2					Agglutinin titre log 2	
Line	Number of mice	Generations	Mean ($\bar{X}$)	Variance (V)	Line	Number of mice	Generations	Mean ($\bar{X}$)	Variance (V)
H line	472	F_{16}–F_{36}	12·50	0·74	H line	466	F_{14}–F_{22}	11·60	1·01
L line	497	F_{16}–F_{36}	4·67	1·30	L line	455	F_{14}–F_{22}	4·90	1·47
F_1 (H × L)	211	from F_{18}–F_{29}	9·63	1·62	F_1 (H × L)	88	from F_{21}	10·06	1·46
F_2 ($F_1 \times F_1$)	363		8·62	1·97	F_2 ($F_1 \times F_1$)	171		9·54	4·22
BcH (F_1 × H)	166		11·36	0·99	BcH (F_1 × H)	88		10·27	1·76
BcL (F_1 × L)	168		7·59	2·30	BcL (F_1 × L)	146		7·47	5·89

*a, 3·915; d, 1·045; d/a, 0·27.
†a, 3·35; d, 1·81; d/a, 0·54.

evaluation of genetic variance (VA), dominance variance (VD), and the results of calculation of h^2 and n are reported in Table 2.

Selection I. The calculation of VA and VD by the three methods used (A, B and C) gave very similar results; the differences found are probably insignificant. Due to the large number of mice in each group and their small variance, the total sampling variance was relatively small: 0·13 in calculation A.

The data in Table 2 show that the genetic variance of F_2 and Bc was almost entirely due to additive effect, the contribution of dominance (VD) was very small (15% of VA according to calculation A, and 4% according to calculations B and C). The h^2 calculated by Equation 7 was somewhat higher but of the same order of magnitude as that realized during the selective breeding (See Fig. 2). The number of loci controlling the character calculated according to Equation 8 is very approximate. The results obtained fit with a theoretical model where about ten independent loci, endowed with equivalent effect, regulate the quantitative antibody response to SE.

Selection II. In this selection the calculation of VA and VD was much less satisfactory than in selection I because the values obtained differ considerably according to the method of calculation used. Due

Table 2. Comparison of the different estimates of the variance components, the heritability and the number of loci, in selection I and selection II

	Environmental variance (VE) from Equation 1	Method of calculation	Additive variance (VA)	Dominance variance (VD)	Heritability (h_2) from Equation 7	Number of loci (n) from Equation 8
Selection I	1·22	(A) From Equation 4	0·65	0·10	0·32	11·7
		(B) From Equations 6 and 2	0·72	0·026	0·37	10·6
		(C) From Equations 6 and 3	0·79	0·028	0·39	9·7
Selection II	1·31	(A) From Equation 4	0·95	1·94	0·23	5·9
		(B) From Equations 6 and 2	2·50	0·38	0·60	2·3
		(C) From Equations 6 and 3	3·70	0·56	0·66	1·5

to the large variance of F_2 and Bc in relation to the size of populations analysed, the total sampling variance was large, it was 0·75 in calculation A. Consequently the results of Table 1 are difficult to interpret, nevertheless they deserve some comments. Calculations B and C gave a *VA* value considerably larger than that resulting from calculation A. These large *VA* values give a high h^2 (0·50–0·60) and a low number of loci (1·5–2·3). Such a high h^2 value diverges sharply from the realized h^2 measured during the selective breeding ($h^2 = 0{\cdot}21 \pm 0{\cdot}05$, see Fig. 3). In fact if the character were controlled by such a small number of loci with so high heritability a rapid interline separation should have been produced by the selective breeding and only four to five generations would be required to reach the selection limit. It is therefore very likely that calculations B and C tend to overestimate both *VA* and h^2 and as a consequence to undervalue *n*. On the other hand, the h^2 value obtained by calculation A and Equation 7 ($h^2 = 0{\cdot}23$) was in agreement with that obtained during selective breeding ($h^2 = 0{\cdot}21 \pm 0{\cdot}05$). We are thus inclined to consider that the number of 6 loci obtained by calculation A is the best estimation. The reasons for the discrepancy between the results of calculation A and calculations B and C will be discussed later.

Crosses between homologous lines of selection I and selection II

The genetic constitution of the lines of mice resulting from the method of selective breeding used in this study depends essentially on the distribution of the relevant alleles in the F_0 population and on the degree of inbreeding resulting from the close colony breeding. In spite of the systematic selection of parents from different families at each generation, the consanguineity of each line increased during the selection. In selection I the mean inbreeding coefficient at selection limit was 0·52 in HF_{16} and 0·66 in LF_{16} (Feingold *et al.*, 1976).

The inbreeding and random drift may lead to the loss of alleles represented at low frequency. For these reasons the homologous lines: the two H lines resulting from selections I and II, and the two L lines resulting from selections I and II, may not have identical genetic constitutions even at the level of selected genes. In order to verify this hypothesis the following experiment was carried out. High responders of selection I (HF_{29}) were mated with High responders of selection II (HF_{13}). Low responders of both selections belonging to the same generations were also mated. Their progeny were not tested, seven pairs were formed at random for each line. They produced fifty-seven mice (HI × HII) and sixty-four mice (L I × L II). These two groups of mice were submitted to selective breeding for responsiveness to SE according to the method used in selection II; they constitute the two foundation populations (F_0) of the selection. This was carried out for High responsiveness in H line and for Low responsiveness in L line.

The results in Table 3 show that the agglutinin response of the mice produced by crossing the two H lines (F_0H) was similar to that of the two parental lines. In H responder line the selective breeding was without effect since the cumulated *R* value did not increase during the five generations of selection. The cumulated *S* value which was 3·12 in F_4 does not correspond to a selective pressure but it is only produced by the environmental variance. In fact the standard deviation of HF_0–F_5 was similar to that of the lines of origin which were considered as homozygous. These results indicate that the two H lines resulting from selection I and selection II have identical genetic constitution as far as the genes regulating antibody responsiveness are concerned.

A different result was obtained in mice produced by crossing the two L lines resulting from selection I and selection II. The agglutinin titre of F_0 was 2·56 higher than the mean of the two parental L lines. The standard deviation of F_0 was also larger than that of the two homozygous lines of origin. Therefore the variance of F_0L was produced by both genetic and environmental factors. The phenotypic variance of F_0L was 3·61, since the mean *VE* of the two parental lines was 1·38, the *VG* of F_0L was 2·23. Consequently 62% of VF_0L was due to genetic factors. Due to this genetic component, the selective breeding for Low responsiveness operated. A cumulated selective pressure of 8·16 in F_4 produced a cumulated response of 2·61 in F_5. After five generations of selective breeding the responsiveness has been lowered to the same level as that of parental lines of origin. The difference between the mean response of the two parental L lines and that of the selected generations F_0 to F_3 is highly significant ($P < 0{\cdot}001$) and becomes insignificant in F_4 and F_5. The results of the selective breeding of crosses of homologous lines expressed in terms of *R* and *S* are illustrated in Fig. 4.

Table 3. Results of selective breeding of homologous crosses between selection I and selection II

	High responder line (H sel. I × H sel. II)					Low responder line (L sel. I × L sel. II)				
Generation	Number of mice	Generation mean log 2 ± SD	Mean* of selected parents log 2	Cumulated R log 2	Cumulated S log 2	Number of mice	Generation mean log 2 ± SD	Mean* of selected parents log 2	Cumulated *R* log 2	Cumulated *S* log 2
F°	57	12·06±0·7	13·20			64	7·34±1·9	3·89		
F_1	39	12·7 ±1·3	13·69	0·64	1·14	33	7·94±2	5·53	−0·6	3·45
F_2	30	11·06±0·7	11·13	−1·0	2·13	34	5·25±1·4	3·83	2·09	5·86
F_3	17	11·62±1·1	12·23	−0·44	2·20	16	6·48±1·7	6·08	0·86	7·28
F_4	28	10·41±0·75	10·72	−1·66	2·81	28	4·58±0·9	4·10	2·76	7·68
F_5	23	11·23±0·73	—	−0·83	3·12	36	4·73±1·1	—	2·61	8·16

H line selection I $F_{16}-F_{36}=12·50\pm0·86$ — L line Selection I $F_{16}-F_{36}=4·67\pm1·14$
H line selection II $F_{14}-F_{22}=11·60\pm1·0$ — L line Selection II $F_{14}-F_{22}=4·90\pm1·21$
$\bar{X}$ of the two H lines $=12·05\pm0·93$ — $\bar{X}$ of the two L lines $=4·78\pm1·17$
* Weighted for the number of offspring of each pair; SD, Standard deviation.

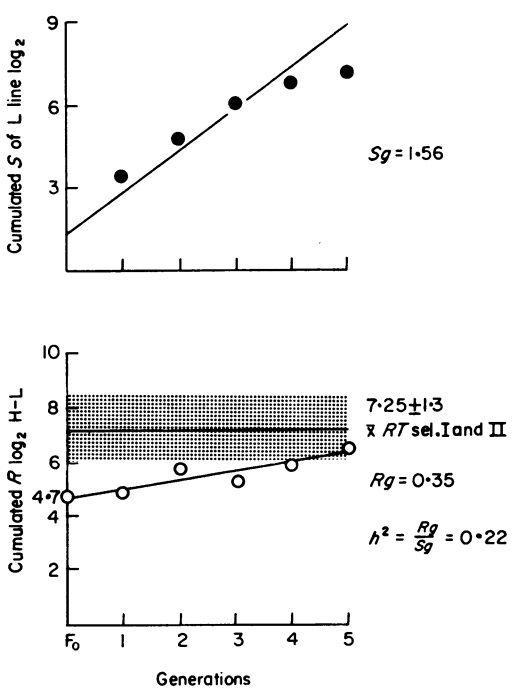

Figure 4. Results of selection performed from homologous crosses between H or L lines of selection I and II: cumulated *S* in L line and cumulated *R* expressed as interline divergence (see text). Horizontal line and stippled area indicate the mean value of *RT* in selection I and II and its standard deviation.

The mean and standard deviation of the interline difference of all the homozygous generations of both selections are indicated in ordinates of cumulated *R* (7·25 ± 1·3). The interline difference of homologous crosses F_0 is only 4·72 due to the increased agglutinin response of F_0L (Table 3). Since only L line responded to the selection, the cumulated *S* was only calculated in this line. The cumulated *R* value, however, was calculated from the interline difference, even if the H line did not respond to selection, in order to eliminate the environmental factors affecting both lines alike. *Rg* and *Sg* represent the mean value of *R* and *S* per generation calculated by a least square linear regression. Therefore the value of $h^2=0·22$ only concerns the L line resulting from homologous crosses. This value is close to the h^2 calculated separately in L lines of both selections which is 0·26. (See paragraph: Results of selective breeding). The results in Fig. 4 show that four to five generations of selective breeding were required to lower the agglutinin responsiveness of homologous L crosses to the mean level of the two L parental lines.

Both L lines resulting from selection I and selection II at selection limit are considered as homozygous at the level of all the relevant loci. They have very similar means and variances (Table 3). Therefore it is probable that each L line contains some different loci homozygous for 'high effect' alleles. The effect of these alleles is 2·56 in F_0. This

figure compared with the mean *RT* of both selections and the number of postulated loci would be equivalent to the effect of two to three homozygous 'high' loci. Other possibilities will be discussed later.

DISCUSSION

It is to be remembered first that, for uniformity, and in order to facilitate the comparison between the present results and those obtained in other selection experiments recently published (Siqueira, Bandieri, Reis, Sant'Anna & Biozzi, 1976; Siqueira, Esteves, Ibanez, Ferreira, Sant'Anna, Reis & Biozzi, 1977; Biozzi, Siqueira, Mouton, Sant'Anna, Stiffel, Esteves, Bouthillier, 1977; Passos, Siqueira, Reis, Ferreira, Ibanez, Sant'Anna & Biozzi, 1977) the scale of agglutinin titre used in our preceding publications (Biozzi *et al.*, 1975) has been modified as indicated in Materials and Methods.

Except for some details that will be discussed later, selections I and II give similar results, despite the different origin of their foundation populations and the antigens used. Selection II was carried out for agglutinin response to SE only whereas in selection I, from F_6 onward, SE and PE were alternated at each generation.

The frequency distribution of the alleles controlling the character in the two F_0 populations is unknown but it is probably equivalent as suggested by the similarities of means and variances as well as identical responses to selection.

The results in Fig. 1 indicate that the group of relevant loci regulates both the level and the duration of the immune response modifying the rate of multiplication and differentiation of small lymphocytes produced by antigen stimulation. (Biozzi, Stiffel, Mouton, Bouthillier & Decreusefond, 1972).

The total range of interline separation and the h^2 calculated from interline divergence are very similar in both selections (Figs 2 and 3). Even the asymmetrical effect of selective breeding producing a greater downward than upward response has been observed in both Selections. This asymmetry of response is not due to a scale effect modifying the variance as a result of the change of mean since the values of selection differential is similar in H and L lines of both selections. The asymmetry of response is in fact due to the genetic properties of the F_0 populations. These may concern either the dominance effect or the gene frequencies. In the last hypothesis, the more frequent alleles at each locus would affect the character in one direction, then the response to selection will be more rapid in the direction of the less frequent alleles. In both selections the mean value of F_0 is very close to that of F_1 hybrids (Table 1) which are supposed to be at gene frequency of 0·5. Therefore it is probable that the asymmetrical effect observed is due to directional dominance of High over Low responsiveness rather than to gene frequency differences. In fact the asymmetrical effect is stronger in selection II where the dominance deviation is greater than in selection I (Table 1).

Inbreeding depression may also produce an asymmetrical effect. The character investigated, however, does not seem to be affected by inbreeding depression. In fact the mean responses to SE in various inbred lines of mice is within the range of those observed in the outbred F_0 populations. These populations, on the contrary, have larger variances than inbred lines due to their genetic heterogeneity.

The analysis of the results obtained in all the interline hybrids is based on the assumption that H and L lines at selection limits are homozygous at the level of all the relevant loci. This assumption however has not already been confirmed and counter-selection experiments are under way to prove or disprove it. A result is already available indicating that the genetic homogeneity has been attained at selection limit. Starting from F_{18} of selection I brother–sister inbreeding has been initiated in both H and L lines and was continued for eighteen generations. The variance of the inbred sub-lines was similar to those observed in H and L lines reported in Table 1 and to those observed in various inbred lines (C57Bl, AKR, DBA/2) assayed in the same experimental conditions (A. M. Heumann, personal communication). The calculation of VE from Equation 1 is thereby justified. Moreover, the large number of mice included in the homozygous generations contributes to the validity of *VE* estimate. The estimation of the number of loci made in the present study deserves some comments. It must be stressed that such an estimation is very approximative since subject to large experimental errors, moreover it is based on a simplified theoretical model, the limitations of which are discussed in detail by Falconer (1960). Nevertheless some experimental data justify the application of this theoretical model to the study of the character investigated. In selection I the number of independent loci is approximately 10:

according to the model, they should have an equivalent effect, each of them should account for 10% of the *RT*. This has been confirmed experimentally for two of these loci, one linked with the H-2 locus (Stiffel *et al.*, 1974) and another linked with the allotype marker of Ig (Biozzi, Asofsky, Lieberman, Stiffel, Mouton & Benacerraf, 1970; Lieberman, Stiffel, Asofsky, Mouton, Biozzi & Benacerraf, 1972). The Ig allotype linked locus accounts for 10% of the *RT* value (Biozzi, Stiffel, Mouton, Bouthillier & Decreusefond, 1974) and the H-2 linked locus accounts for about 10–20% of *RT* (Stiffel *et al.*, 1974).

In selection I, the number of loci (n) calculated by the three methods A, B and C (Table 2) is very similar (9·7–11·7). This evaluation based on the variance analysis of interline crosses is in agreement with our previous calculation made from the result of selection, where *VA* was estimated as the heritable portion of VF_0. This study concluded that in both selections I and II the number of relevant loci was about 10 (range 7–13) (Feingold *et al.*, 1976).

In selection II, the results obtained by calculation A ($n = 5·9$, Table 2) are also acceptable with regard to the above-mentioned estimate. The discrepancy between the results of calculation A and those of calculations B and C in selection II has been partially discussed earlier but it deserves some further comments. The calculation of n depends primarily on the estimation of *VA* since a is a well established experimental result. The meaning of *VA* obtained from calculation A is somewhat different from that obtained from calculations B and C, because there is another source of genetic variance arising from gene interaction (*VI*). Assuming that *VI* is similar in F_2 and in Bc the calculation of *VA* from Equation 4 (used in calculation A) eliminates *VI*. On the other hand the calculation of *VA* from Equation 6 (used in calculations B and C) includes *VI* at least partially, thus it tends to overestimate *VA*, and underestimate n. In selection I the effect of *VI* might be negligible while it might play a role in selection II where a smaller number of loci intervene since it is admitted that the importance of *VI* increases as the number of loci decreases (Falconer, 1960). Another reason for a greater discrepancy in selection II might be the greater sampling error observed. Since the estimation of heritability resulting from calculation A is identical to that realized during the selective breeding, we consider that six loci is the most valid estimate. In order to confirm this evaluation, the study of the quantitative contribution of H-2 and Ig allotype linkages is under way in selection II.

The results in Table 3 and Fig. 4 indicate that H lines from the two selections have probably identical homogeneous genetic constitution. This is not true for L lines since responsiveness has been increased in crosses between L lines from selection I and II. The interpretation of this finding is not yet clear. Three hypotheses could explain the observed results. (a) Both L lines contain different homozygous 'high effect' alleles which have a cumulative effect in homologous cross. (b) Some loci in each L line are heterozygous with an overdominance effect of low responsiveness giving rise to high effect after homologous cross. (c) Responsiveness is increased by an interaction effect favoured in the heterogeneous genetic background of homologous cross. Counter-selection experiments under way will help in discriminating between these hypothesies.

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