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# The Allograft Reaction as an Index of Genetic Diversity in Inbred Chickens[1]

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**Summary.** The main purpose of the study was to use the skin allograft reaction as a possible biological tool to estimate genetic diversity (inbreeding) in a population and to determine the relative influence on the B locus blood group locus on variation in graft rejection. In chickens, the B locus is the major histocompatibility system. One outbred and five inbred lines were used in 12 skin grafting experiments. Graft exchange also were made between certain line crosses. The study was based on 2172 allografts performed on 712 chickens.

Incompatibility of the B blood group locus accounted for 3/4 of the total variance in homograft rejection within lines. For the compatible allografts only, lines accounted for more than 90% of the total variation.

Graft acceptance in terms of mean survival time (MST) was highest in the most highly inbred lines and lowest in the noninbred control. The regression of genetic diversity, as a function Wright's inbreeding coefficient, as a percentage of allograft rejection may be a useful biological index of genetic diversity.

## Introduction

A biological method to estimate the level of genetic diversity in a population would be a valuable check on Wright's classical mathematical method of computing inbreeding coefficients. One possibility is to measure the degree of compatibility of skin graft exchanges between members of a population. In chickens, exchanges between closely related individuals are usually successful. In a highly inbred line, Cock and Clough (1956) reported that only 6 of 86 graft exchanges were rejected.

The idea of using skin graft exchanges to measure genetic diversity in chickens was tested by Craig *et al.* (1960) who reported a strong correlation between the degree of relationship of donor and host and the rate of rejection of skin grafts. The immune response was slowest between full-sib exchanges and fastest between unrelated individuals. Earlier, Craig and Hirsch (1957) reported that the level of circulating lymphocytes, as an immune response to a skin allograft, was similarly correlated with relationship. On the other hand, Berry and Craig (1959), using year-old birds, were unable to distinguish between wide levels of genetic diversity. Thus, it seemed that young chicks less than 40 days of age were required for success. Even then, rates of graft rejection varied greatly among experiments. These workers suggested that age effects and differences among populations may have accounted for the limited success of their experiments.

In the studies reported by Craig and coworkers, no consideration was given to the *B* blood group locus. It is now well recognized that this is the major histocompatibility locus in chickens (Schierman and Nordskog, 1961, 1964; Gilmour, 1962; Craig and McDermid, 1963; and Gleason and Fanguy, 1964). Also the C blood group locus is associated with a transplantation antigen, but is weaker than the B locus (Schierman and Nordskog, 1965).

The objectives of the present study were to determine the relative importance of major and minor histocompatibility loci (*H* genes) on skin graft rejections in pure-line and cross-line populations of chickens and to relate this to the genetic relationship or diversity between individuals compatible at the *B* blood group locus.

## Assumptions and Theory

In all mammalian species surveyed, there is one major histocompatibility locus and several minor loci (Bodmer, 1973). In man, the major system is *HL-A* and, in mice, the *H-2* locus.

When skin from a $B^1B^1$ chicken is grafted on a $B^1B^2$ individual, the graft accepts. In contrast, when a $B^1B^2$ donor is grafted on a $B^1B^1$ host, the graft rejects because the host produces an antibody against the $B^2$ antigen in the donor skin.

In general, graft exchanges between sibs from the matings, $AA \times AA$, $aa \times aa$, and $AA \times aa$, would be compatible. On the other hand, 3/4 the graft exchanges between sibs from matings $AA \times Aa$ or $Aa \times aa$ would be successful, and 5/8 from the sib group progeny of the mating, $Aa \times Aa$. Thus, the genotypic variance of skin graft acceptance or rejection is expected to be relatively less but essentially proportional to the theoretical genotypic values among histocompatibility loci in the population.

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We assume that, aside from the major histocompatibility locus ($H$-locus), there are $n_0$ segregating minor ($h$) loci in some base population, each with equal and additive effects, that determine the success or failure of a skin allograft. An allograft is defined as a tissue transplant from one individual to another in an interbreeding population. We assume that, for a allograft to be permanent, there are no incompatibilities between the donor and host at any of the minor loci or at the $H$-locus. For a donor-host incompatibility at the $H$-locus, graft rejection would be rapid; for a difference at any minor locus, graft rejection would be slow. For multiple differences at the $n_0$ minor loci, graft rejection time would decrease proportionately. The distribution of the $h$ genes among the members of an unselected interbreeding population follows the Hardy-Weinberg law. Thus, there would be a range of allograft reactions between pairs of individuals chosen for grafting. The expected value of a graft reaction is the mean of the population and would be proportional to the average number of segregating loci. When the inbreeding coefficient in a line derived from the base population reaches $F$, $n_0F$ loci become fixed and $n_0(1-F)$ loci continue to segregate. Finally, we assume that, for some short-term observation period of $d$ days, the mean level of allograft reaction would be an unbiased indicator of the level of reaction over an extended period.

For pedigreed chickens of one generation per year and with mating pens of 1 male and m females per male, the population derived is family- or sibstructured: any 2 progeny with parents of the same pen are either full-sibs (2 parents in common) or half-sibs (a common sire). Any two individuals from different pens are called nonsibs because they have no parent in common. Inbreeding in the line is $F$ and, for this discussion, $(1-F)$ is the index of genetic diversity. For any sib group, the average level of allograft reaction is proportional to $1-(F+\Delta F)$, where $\Delta F$ is the increase in inbreeding that would result in the progeny if members of the sib group were mated together.

For full-sibs, $\Delta F = 1/4(1+2F+F') - F$, where $F$ is the average inbreeding in the sib-group and $F'$ the average inbreeding of the sib's parents. For half-sibs, $\Delta F = 1/8(1+6F+F') - F$ and for nonsibs, $\Delta F$ would be small and assumed to be zero.

As lines become more highly inbred, genetic diversity $(1-F)$ decreases and the difference between sib groups in $(1-F)$ approaches zero. This leads to the testable hypotheses that as $F \to 1$, the level of allograft reaction decreases in the line and that the spread between sib groups in allograft reaction decreases.

There seems no adequate theory in the literature to describe, in general, the genetic diversity in a population derived from a cross of 2 lines not completely inbred. In the extreme case in which the parental inbreds are 100% inbred, the genotypic variance in the $F_1$, as well as in the parental lines themselves, is zero, and in the $F_2$, the additive genetic variance in a randombred base population with all gene frequencies of a 1/2 is restored.

It can be shown for a single locus situation, that, if the genotypic variance within 2 inbred lines is proportional to $1-F_1$ and $1-F_2$, the arithmetic mean, $1-(F_1+F_2)/2$ is a measure of the genetic diversity.

Consider a locus ($A$, $a$) and an arbitrary segregation score, $s$, which we assign to the progeny of all possible mating types:

| Mating Type | Segregation | Score |
|---|---|---|
| $AA \times AA$<br>$aa \times aa$<br>$AA \times aa$ | none | $s = 0$ |
| $AA \times Aa$<br>$aa \times Aa$ | intermediate | $s/2$ |
| $Aa \times Aa$ | maximal | |

If $F$ is the inbreeding coefficient in a population, then the probability that locus $A$ is heterozygous is $1-F$, and the mean segregation score from all possible matings is $4(F/2)(1-F)(s/2) + (1-F)^2 s = (1-F)s$.

Letting $F_1$ and $F_2$ be the inbreeding coefficients in lines 1 and 2, the segregation score in the line cross progeny is $[1-(F_1+F_2)/2]s$. Thus, the expected amount of segregation at a single locus in the progeny of a cross between 2 lines is proportional to the average segregation in the 2 parental lines. This hypothesis will be tested with the data at hand.

## Materials and Methods

The experimental birds came from 5 inbred and 1 outbred lines. The lines and the coefficients of inbreeding ($Fx$) in 1970 were,

| Breed and Line | No. | Year of origin | $Fx$ |
|---|---|---|---|
| Leghorn | 8 | 1940 | .93 |
| Leghorn | 9 | 1940 | .91 |
| Leghorn | GH | 1953 | .58 |
| Leghorn | HN | 1953 | .86 |
| Spanish | SP | 1953 | .65 |
| Leghorn | R | 1969 | 0 |

Leghorn Line 8, segregating for the sex-linked barred plumage gene, was mass mated for 8 generations starting in 1954 and pedigree mated after 1962. Leghorn Line 9, also pedigreed since 1962, segregated at the dominant white locus, $I_i$. Plumage color segregations in Lines 8 and 9 were designed intentionally for other purposes (Smith and Nordskog, 1963). The White Leghorn Line GH, used in earlier transplantation studies (Schierman, 1962), was pedigreed throughout. The White Leghorn line, HN had been pedigreed through 17 generations up to the time of this study. The Spanish line (SP), with black and barred plumage obtained from the University of

Minnesota in 1953, was nonpedigree-mated for the first 9 and the last 2 of 16 generations.

The control for this study was Line R, an $F_1$ cross of two noninbred Leghorn Lines, $S_1$ and $S_2$ but with known *B* locus blood group genes (Nordskog *et al.*, 1973); inbreeding was assumed to be zero. The procedures for the production of blood-typing reagents used in this study followed those discussed by Marangu (1970).

### *Matings*

In the 1969 preliminary experiment, no birds were blood typed, but in 1970, all inbreds and controls were either blood typed or otherwise identified as to *B* locus compatibility between graft exchanges.

Heterozygous males and females were mated to produce 3 different genotypes. For example, in Line HN, the mating of blood types $B^6B^7 \times B^6B^7$ gave progeny: $B^6B^6$, $B^6B^7$, and $B^7B^7$ expected in the ratio of 1:2:1. The progeny from such matings required blood-typing to identify blood group genotypes. In some lines, homozygous males were mated to a pen of females each homozygous for a known allele. Progeny genotypes were then deduced from pedigree alone without blood typing. Lines 8 and SP were not blood typed because segregation at the B locus could not be detected in preliminary tests. However, this is not positive proof of homozygosity at the *B* locus. Chicks from all lines and crosses were pedigreed and sexed at hatching.

In 1970, cross line progeny from the 5 different lines were also skin grafted. The crosses were GH × L9, L9 × L8, L8 × SP, SP × HN, and HN × GH. The plan was to make graft exchanges only between individuals compatible at the *B* locus, but because L8 and SP were not typed it is possible that some exchanges within those cross line groups where these lines were involved were incompatible. Thus, only for crosses GH × L9 and HN × GH were we certain that no incompatible graft exchanges were made.

### *Grafting*

Skin graft exchanges were subjectively scored according to Polley *et al.* (1960). A full-thickness skin graft was cut with a dissecting scissors and transferred to a petri dish containing gauze and physiological saline. To obtain a uniform graft size, a standard 10 mm × 10 mm jig was used. Finally, a plastic bandage was securely placed on the graft. On the 7th postoperative day the bandages were removed, and the graft reaction was scored daily for 1 week and then on alternate days from the 9th through the 28th postoperative day.

Exchanges were made only between 17-day-old chicks of the same sex. Each chick exchanged grafts with 4 others. The 4 grafts placed on a chick included 1 each from a full-sib, a half-sib, a nonsib (no sire and dam in common), and one from a different line. A replicate set of 8 chicks were all from the same line and consisted of 2 full-sib pairs (2 × 2) of 2 sires. Each replicate set was handled as a separate experimental unit.

In 1969, grafts were exchanged also between chicks of different lines in an attempt to estimate the compatibility between lines. All were consistently rejected so that exchanges between lines were discontinued in 1970. Transplantation immunity was measured as the percentage of grafts surviving and also at the mean survival time to 26 days post-grafting (Brownlee and Hamre, 1950).

## Results

### *1969 Experiments*

The *B* blood group locus was ignored. Rejections ceased earliest in Line HN, ($F = .85$), and the level of accepted grafts at the end of the 26th day was highest (60–75). Graft rejections were highest in L9, ($F = .91$), with only about 25% of the grafts intact at 26 days. The mean survival time by lines and sib-groups is given in Table 1. Mean survival time averaged 16.9 days for the nonsibs but 2.1 days longer for the half-sibs and 3.5 days longer for the full-sibs. Differences between lines, sib-groups, and lines × sib-group interaction were all statistically significant (Table 2). In general, the results of the different experiments were repeatable, with each line showing distinct characteristics of graft rejection.

Table 1. *Mean survival time by lines and sib groups (1969 data)*

| | No. Reps | Full-sibs | Half-sibs | Nonsibs | Av. |
|---|---|---|---|---|---|
| L8 | 6 | 21.2 (90)[1] | 19.2 (86) | 12.5 (83) | 17.6 |
| L9 | 4 | 15.6 (74) | 15.6 (74) | 13.7 (74) | 15.0 |
| GH | 5 | 20.8 (78) | 16.3 (78) | 14.1 (78) | 17.0 |
| HN | 4 | 20.3 (32) | 19.8 (32) | 21.5 (31) | 20.5 |
| SP | 2 | 24.0 (27) | 24.0 (23) | 22.8 (26) | 23.6 |
| | Av. | 20.4 (301) | 19.0 (293) | 16.9 (292) | 18.7 |

[1] Fig. in parentheses are the number of graft exchanges.

Table 2. *Analysis of variance of mean survival time (1969 data)*

| Source | df | Mean Squares |
|---|---|---|
| Lines | 4 | 97.8** |
| Sib Groups | 2 | 97.5** |
| Lines × Sib Groups | 8 | 22.5** |
| Error | 48 | 3.8 |

** $P < .01$.

### *1970 Experiments*

A marked influence of the *B* locus "*H* antigen" was demonstrated in all lines, as shown by the difference in the mean survival time between the compatible and incompatible grafts in Table 3.

Table 3. *Influence of the B blood group locus on mean skin graft survival in four lines (1970) data*

| Line | No. Reps | *B*-locus[1] | Mean survival time (days) Full-sibs | Half-sibs | Nonsibs | Av. |
|---|---|---|---|---|---|---|
| GH | 3 | C | 23.5 (44) | 22.9 (40) | 21.3 (40) | 22.5 |
| | | I | 7.9 (17) | 8.3 (23) | 8.5 (22) | 8.2 |
| L9 | 2 | C | 24.0 (27) | 21.7 (24) | 20.1 (27) | 22.0 |
| | | I | 8.0 (21) | 7.6 (21) | 9.1 (18) | 8.2 |
| HN | 3 | C | 25.1 (30) | 25.4 (27) | 24.7 (23) | 25.1 |
| | | I | 8.6 (19) | 8.7 (20) | 9.7 (23) | 9.0 |
| R | 3 | C | 14.8 (35) | 12.9 (34) | 11.9 (19) | 13.6 |
| | | I | 7.9 (18) | 8.3 (20) | 7.4 (29) | 7.9 |
| | Av. | C | 21.6 | 20.6 | 19.6 | 20.8 |
| | | I | 8.1 | 8.2 | 8.7 | 8.3 |

[1] C – Compatible at the *B* blood-group locus
I – Incompatible at the *B* blood-group locus.

Table 4. *Analysis of variance of mean survival time (1970 data)*

| Source | df | Mean squares | |
|---|---|---|---|
| | | *B* locus Compatible | *B* locus Incompatible |
| Lines | 3 | 80.32** | 0.68 |
| Sib Groups | 2 | 5.52* | 0.36 |
| Lines × Sib Groups | 6 | 0.64 | 0.30 |
| Error | 21 | 0.43 | 0.21 |

* $P < .05$
** $P < .01$

For Line GH, the mean survival time of the *B* locus compatible grafts was 22.5 days and 8.2 days for the incompatible grafts. Averaging the 4 lines, the compatible grafts survived 20.8 days and, the incompatibles, 8.3 days. The mean survival times averaged over all compatible grafts were 21.6, 20.6 and 19.6 days for the full-sibs, half-sibs, and nonsibs, respectively. For the incompatible grafts, the rather small differences between sib groups were in reverse order from expectation.

An analysis of variance for these data is given in Table 4. The assumption is that sib-groups and lines are random effects. On the whole, the B locus accounted for about 3/4 of the total variance in graft survival time. For the compatible grafts only, lines accounted for about 93% of the total variation and sib-groups for less than 5%.

*Reaction patterns of individual lines*

Line R — This is the control line with an assumed $F = 0$. Fig. 1a shows the percentages of accepted

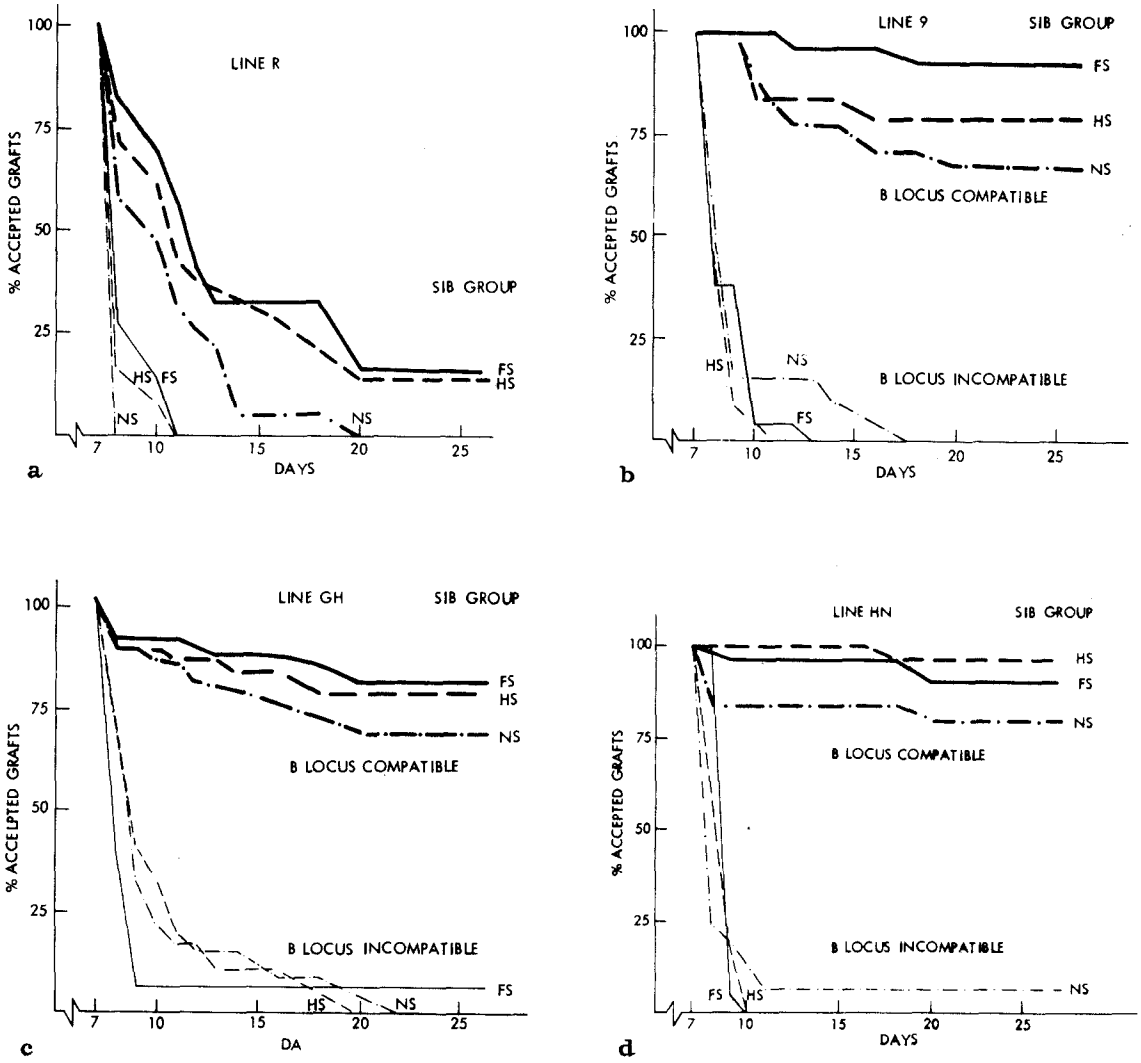


Fig. 1. Percentages of accepted grafts by days in 4 lines. Heavy lines represent B locus histocompatibles; light lines represent histoincompatibles. Sib groups: full-sib (FS), halfsib (HS) and nonsib (NS)

grafts over the 25-day observation period. For the *B* locus incompatibles, total rejection occurred by the 10th postoperative day. Among the *B* locus compatibles, more than 75% of the grafts were rejected by the 14th day. Differences between full and half-sib groups were small with about 85% rejected on the 25th day. For the nonsib graft exchanges, all were rejected by the 20th day.

Line 9 — The rejection response among the *B* locus incompatibles was rapid (Fig. 1b). Eighty-five percent were rejected by the 10th postoperative day. Half-sibs and nonsibs were totally rejected by the 11th and 16th day, respectively. The remaining nonsib incompatibles were totally rejected by the 18th day. The number of surviving *B* locus compatible grafts was high compared with the incompatibles. Rejections started about the 10th postoperative day, and each sib group proceeded at a different rate. Rejections were few among the full-sibs, leveled off at 78% for the half-sibs commencing at the 9th and 11th postgrafting days, but for the nonsibs, continued with slow rejections reaching 68% by the 26 th postoperative day.

Line GH — Nearly 90% of the *B* locus incompatible grafts were rejected by the 13th postoperative day in this line with the calculated inbreeding of 0.58 (Fig. 1c). By the 22nd day, all half-sib and nonsib incompatible grafts were rejected. One incompatible full-sib survived through the 26th day, which could have been a blood-typing error. Among the *B* locus compatibles, rejections were slow but persistent. The full-sibs had the highest number of surviving grafts on the 26th postoperative day with 82% followed by the half-sibs with 79% and the nonsibs with 69%.

Line HN — The number of surviving grafts from the *B* locus compatible individuals was the highest in this line ($F = 0.86$). Ninety percent of the graft exchanges between full-sibs and halfsibs survived compared with 80% for the nonsibs. Line HN was unique in its rejection pattern (Fig. 1d). Grafts exchanged between B locus incompatibles were rejected between the 7th and 10th day, after which no further rejections occurred. One exception, recorded as an incompatible, survived through the entire test observation period; this is believed a blood-typing error.

### *Reactions of line crosses*

Table 5 gives the mean survival time for the various line crosses. The 8 × SP cross had the shortest mean survival time (13.6 days), and the GH × 9 the longest (24.4 days). Within line crosses the grafts between fullsibs survived about 3 days longer than those between half-sibs and about 4 days longer than the nonsibs. The L8 × SP was exceptional in that the survival time between sib groups was not in order of expectation. This may be a sampling effect.

Fig. 2 shows the response patterns of graft acceptance for the 5 line cross groups. For the GH × L9 cross with the highest acceptance level, 1 parental line (L9) was highly inbred but all cross-line progenies were compatible at the *B* locus. On the other hand, the cross L8 × SP with the lowest acceptance level was not expected since 1 of the parent lines was highly inbred (L8) and one moderately inbred (SP). It is possible that some segregation occurred at the B blood group locus even though preliminary tests failed to show this.

Table 5. *Mean survival time of sib-groups from five line crosses*

| Cross[1] | Full-sibs | Half-sibs | Nonsibs | Av. |
|---|---|---|---|---|
| GH × L9 | 25.0 | 24.2 | 24.1 | 24.4 |
| L9 × L8 | 24.2 | 16.0 | 15.2 | 18.5 |
| L8 × SP | 11.5 | 15.1 | 14.1 | 13.6 |
| SP × HN | 23.9 | 15.6 | 16.1 | 18.6 |
| HN × GH | 21.5 | 18.0 | 14.5 | 18.0 |
| Av. | 21.2 | 17.8 | 16.8 | 18.6 |

[1] Each cross is represented by about 15 individuals in each sib-group. For each sib group about 40 grafts were exchanged.

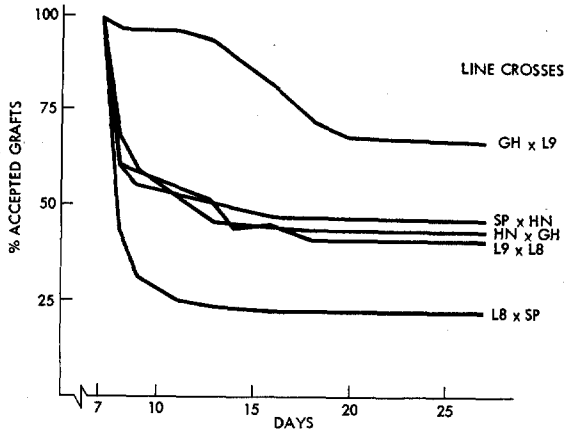


Fig. 2. Overall line cross mean percentages of accepted grafts by different days of observation

### *Relationship of graft survival to inbreeding*

Fig. 3 summarizes the data on graft rejection collected in 1970. Genetic diversity within sib groups, defined as $1 - F$ is plotted against observed graft rejection. The 3 inbred lines were averaged for each of the 3 sib groups. The mean of the sib groups of the inbreds and outbreds were then connected with a straight line.

The averages of the 5 line crosses are also plotted by sib groups where genetic diversity is estimated as $1 - (Fi + Fj)/2$ for the cross of line $i$ × line $j$. In all cases, genetic diversity of the crosses seem underestimated. In particular, if $(1 - F)$ measures the genetic diversity in an inbred line, $1 - (F_1 + F_2)/2$ seems not to be a useful measure of genetic diversity in a cross. Thus, it seems that the level of graft rejection of the crosses, when projected on the regression line might give a better estimate of genetic diversity.

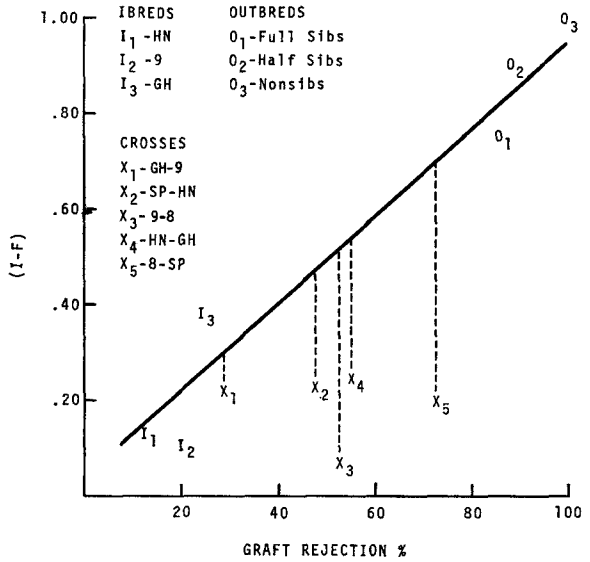


Fig. 3. Genetic diversity ($1 - F$) regressed on % graft rejections using the mean values of the inbreds and outbreds. The expected values of the crosses are shown when the genetic diversity is estimated as $1 - (F_1 + F_2)/2$ where $F_1$ and $F_2$ are the inbreeding coefficients of the parents of the cross. In general, this formula seems not to fit the regression equation giving too low values

## Discussion

The main objective of this study was to re-examine the question whether skin transplantation might be a useful biological tool to measure genetic diversity in inbred lines of chickens.

Of all body tissues, according to Billingham and Silvers (1971), the skin is the most exacting for successful transplantation. Prolonging the life of a skin allograft is a much more formidable problem than that of any other organ transplant. This high degree of sensitivity should enhance the value of skin allografting as a biological indicator of genetic diversity.

Wright's Coefficient of Inbreeding ($F$), derived from pedigree relationships in a population, is not valid for comparing populations of different origin. For each population, $F$ is relative to the genetic diversity, always unknown, in an arbitrarily chosen base population. How well $F$ measures true genetic diversity in a real population is hypothetical. Factors such as heterozygote superiority and variable mutation rates would cause $F$ to be biased downward. Studies with populations of chickens show that inbred lines may continue to segregate at blood group loci more often than expected. For example, Briles, Allen, and Millen (1957) reported continuing segregations at the $B$ locus in 71 of 73 closed populations. Gilmour (1959) found segregations at the $B$ locus even in lines with computed inbreeding coefficients of over 95%. Also the possibility of pedigree errors, such as cross-line individuals being inadvertently included as members of a pure line, cannot be ignored. Clearly, a biological measure of genetic diversity would be a useful check on Wright's coefficient of inbreeding.

For allograft reactions to be a valid indicator of genetic diversity in a population, it is necessary to assume that the number of $H$ loci are large, that they behave additively, and that they have the same mutation rate as non $H$ genes. As already indicated, the hypothesis of one major and several minor $H$ loci seem to fit all species investigated. In chickens very little is known of the number of $H$ genes. In mice, the number of minor $H$ loci have been estimated to be 30—100 (Hildemann and Cohen, 1967). Evidently, the number estimated from strain differences are gross underestimates (Bailey and Kohn, 1965). $H$-genes seem to act as semidominants (Snell, 1953), and the "strength" of an incompatibility, according to Hildemann and Cohen (1967) is a function of the interallelic combination rather than the $H$ locus involved. As a consequence, additive or augmentative effects are exhibited.

Differences between sublines of highly inbred lines are the result of segregation or mutation. In mice, the mutation rate for H genes has been estimated to be $5.4 \times 10^{-3}$ as compared with $.75 \times 10^{-6}$ to $10 \times 10^{-7}$ for coat color genes (Bailey and Kohn, 1965). If the mutation rates for the former are the same as for the latter, then the estimated number of $H$ loci would be 322—1500. On the other hand, if the number is much lower, then the mutation rate of H genes is of a high order.

Silvers and Gasser (1973) were unable to detect H incompatibilities in sublines of mice separated 29 to 42 generations and only slight incompatibilities between sublines separated by more than 120 generations. Greeneberg (1970) concluded that the mutation rate was high for skeletal differences in mice.

It is not clearly established, therefore, that the mutation rate of $H$ genes is greater than that of non-H loci in mice, and moreover, nothing is yet known of the mutation rate of H genes in chickens. These facts support the idea that allografting is a valid biological approach to estimate genetic diversity in partly inbred lines of animal and avian species.

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