

71-7300

MARANGU, John Paul, 1935-
ALLOGRAFTING AS A BIOLOGICAL MEASURE OF
INBREEDING IN CHICKENS.

Iowa State University, Ph.D., 1970
Biology-Genetics

University Microfilms, Inc., Ann Arbor, Michigan

ALLOGRAFTING AS A BIOLOGICAL
MEASURE OF INBREEDING IN CHICKENS

by

John Paul Marangu

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Poultry Breeding

Approved:

Signature was redacted for privacy.

In Charge of Major Work U

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1970

TABLE OF CONTENTS

INTRODUCTION	1
REVIEW OF LITERATURE	4
Genetic Basis of Immune Response in Allografts	4
Studies in man and other animals	4
Variation among histocompatibility loci and their effects	9
Sex linked transplantation antigens	10
Number of histocompatibility loci in animals	12
Immunological Basis for Histocompatibility	13
Leukocyte interaction and histocompatibility in chickens	14
Development of immunological competence	16
Inbreeding	17
MATERIALS AND METHODS	22
Description of Inbred Lines	22
Reagent Synthesis and Blood Typing Techniques	24
Mating Plans	24
The Grafting Pattern	25
Skin Graft Technique (Chicken)	28
Line Crosses	29
Skin Grafting in Coturnix	30
Mixed Leukocyte Cultures	31

RESULTS	34
Skin Graft Acceptance Among Sib-Groups Not Blood Typed	34
Effect of the B Locus Blood Groups on Skin Graft Reaction of Different Inbred Lines	38
Results of individual lines	42
Line R	42
Line 9	45
Line GH	45
Line HN	49
Results of Line Crosses	49
SP X HN	49
8 X SP	49
HN X GH	53
9 X 8	53
GH X 9	53
Results of Coturnix Study	57
Mixed Leukocyte Cultures	60
DISCUSSION	67
A Biological Measure of Genetic Diversity	67
Crossing a tester line	68
Empirical Models for Measuring Genetic Diversity	70
Method A	70
Method B	71
Coturnix for Immunogenetics Studies	74
Mixed Leukocyte Cultures	75

CONCLUSIONS	77
SUMMARY	78
GLOSSARY	81
BIBLIOGRAPHY	82
ACKNOWLEDGMENTS	93

INTRODUCTION

The late 1960's marked the beginning of accelerated human experimentation with the transplantation of major organs such as heart, lung and liver. Although there has been great accomplishment in the development of techniques, the complete prevention of the rejection of a transplant is far from sight. Thus, there is need for continued studies in this area which holds promise to prolong the life span in man.

Transplantation immunity has clearly been shown to be controlled by histocompatibility (H) genes. H genes direct the synthesis of transplantation antigens which evoke an immune response when they are introduced into an incompatible recipient. The H loci in various species have been shown to be highly complex usually with multiple alleles. Transplantation antigens are found in many tissues; they may or may not be present on red cells (Amos, 1969).

On the basis of their ability to stimulate an immune response, the H antigens are designated strong or weak; this is measured in terms of median survival time. In mice the H-2 system is a strong histocompatibility locus (Gorer et. al. 1948), while other H systems are designated weak H loci (McKhann 1962, 1964a, b, Graff et al. 1966). In chickens, Schierman and Nordskog (1961) showed that the B locus is the major H system while the C locus is a minor system. In humans, blood group A and B of the ABO system are all also H antigens while the HL-A system contains both weak and strong alleles (Amos, 1969).

Transplantation techniques might also be useful in a selection program to hasten the development of histocompatible lines. Such lines,

especially of mice, have proved to be valuable for fundamental studies on transplantation, particularly as regards the area of immune suppressors.

Transplantation techniques may have application in biological fields in addition to medicine. For example, a biological method to estimate the level of heterozygosity in a population would be a useful supplement to Wright's classic mathematical method for the genetic study of populations. Such a method could be based on the degree of compatibility of skin graft exchanges. The hypothesis is that the success of graft exchanges among individuals in a population depends on the degree of relationship among the individuals.

One of the consequences of inbreeding in a small population is the increase of homozygotes at the expense of heterozygotes. This applies to all loci if we assume no selective advantage of the heterozygotes. Thus we should expect more fixed histocompatibility loci in inbred lines than in non-inbreds.

The objectives of this study were:

1. To measure the survival fraction of graft exchanges between individuals of the same and of different families, with known inbreeding coefficients, belonging to the same inbred line. In a sense this should give a biological measure of inbreeding.
2. To determine the importance of the major histocompatibility B locus in different inbred lines as a factor in intra-line skin graft rejection.
3. To test the application of the mixed leukocyte culture technique in chickens as an in vitro measure of histocompatibility.

4. To apply the skin grafting technique used in chickens on a small randomly bred Coturnix population.

REVIEW OF LITERATURE

Genetic Basis of Immune Response in Allografts

Studies in man and other animals

When tissues are surgically exchanged between two individuals of the same species, the transplants are usually destroyed by an immune response. However, when tissues are exchanged between monozygotic twins, the recipient accepts the homografts (Merrill et al. 1956). This "recognition of identity" phenomenon is a clear indication that the acceptance or rejection of a transplant is under genetic control. Thus, the fate of an allograft depends on the genetic relationship between the donor and the recipient. This relationship is determined by transplantation genes or histocompatibility (H) genes. The genetic identity of the donor and host usually assures success while genetic diversity does not necessarily mean that the graft will be destroyed by the host (Hasek et al. 1961).

The genetic variation among individuals can be expressed as antigenic differences (Owen, 1959). Theoretically, genetic variation is low in a highly inbred line. Consequently members of an inbred line usually tolerate homograft exchanges. If a major transplantation locus is segregating, this will be reflected by Mendelian segregation of the graft reactions. An individual reacts against an antigen lacking in the individual. Therefore, graft exchanges among F_1 hybrids of two highly inbred lines are usually successful as demonstrated by Little and Johnson (1922). The success of such allografts clearly indicates that possible genetic disparity of the graft is not sufficient in itself for evoking an immune response in the recipient (Hasek et al. 1961).

During the early stages of transplantation studies in mice the highly

complex nature of the major H-2 locus became evident. Tyzer (1909) showed that susceptibility to mammary adenocarcinoma of a strain of Japanese waltzing mice had a heritable basis. When a resistant strain was crossed with a susceptible strain, the F_1 's were all susceptible to tumor challenge as though indicating that susceptibility was dominant to resistance. However, in the succeeding two generations no susceptible mice could be found; inheritance on a classical Mendelian basis therefore, seemed questionable. Little (1914) showed that such results would be expected if resistance and susceptibility were determined by the interaction of different gene loci.

Interest in genetic studies of transplantation was greatly stimulated by Snell's studies of histocompatibility genes in mice (Snell, 1948). The essential features of his method are to mate a susceptible strain (A) to a resistant strain, and produce an F_2 generation. The F_2 offspring are inoculated with tumor cells to which strain A is susceptible, which eliminates all susceptible offspring. The resistant F_2 individuals are then backcrossed to A repeatedly for twelve to fourteen generations. In this way, isogenic resistant (IR) lines of mice may be obtained. The availability of IR lines as well as the congenic strains also produced by Snell (1958) made possible quantitative studies in transplantation. In general, studies with mice provided the model for transplantation studies in other species.

The discovery of linkage between genes for an erythrocytic antigen and a transplantation antigen in mice provided a useful genetic marker for transplantation studies. Gorer (1937) first reported this observation which now is currently referred to as Histocompatibility-2 or H-2 in mice. Shortly thereafter, Gorer, Lyman, and Snell (1948) demonstrated a close linkage between genes controlling the H-2 locus and an abnormal tail condition "Fused"

located in the IX linkage group. An interesting genetic aspect of this linkage is that when an F_1 of Fused and H-2 is mated to a mouse with a different H-2 allele, the progeny can be examined for the phenotype trait, i.e. fused tail, for agglutination, and for the ability to accept a tumor. Thus, selection for a desired H-2 allele can be made from an agglutination test or by the fused tail phenotype. This method of selection is efficient because the time interval necessary for the tumor to express itself, i.e. to take its toll, is eliminated.

As the genetic evidence for transplantation genes in mice has accumulated, greater complexity, especially in the major H-2 locus, has been found. The transplantation antigens of this locus are widely distributed among several tissues, but highest concentrations are found in the organs of the reticulo-endothelial system (Basch and Stetson, 1962). This highly polymorphic locus is responsible for the production of 27 antigenic specificities which may appear in 18 allelic combinations (Shreffler and Snell, 1969). Although H-2 locus products are inherited as a unit, evidence now indicates that this region is composed of at least 3 or 4 separate loci (Shreffler and Klein, 1970).

In man the relationship of erythrocytes to transplantation antigens was suggested more than a half a century ago (Shawan, 1919). He noted that skin graft survival could be prolonged by applying blood typing techniques. Blood groups in man have been known to be under genetic control for decades. Recent evidence now substantiates that the A and B blood groups are also transplantation antigens. Their importance was first clearly shown when a blood group O recipient rejected a blood group B kidney almost immediately (Hume et al. 1955). The documented clinical studies by Starlz et al. (1964, 1965)

on kidney transplantation are a part of the overwhelming evidence that A and B blood groups are H antigens or are closely linked to them. Furthermore, according to Gleason and Murray (1967) the Kidney Transplant Registry reaffirms the significance of blood groups in transplantation. Three years of data on 565 kidney transplants, representing studies of Registry participants throughout the world, indicate that ABO incompatibility tends to increase the incidence of non-functional transplants. Incompatibility of the P blood group system seems to have no effect on initial function of a transplant but the limited data now available indicates a decline in function with time (Gleason and Murray, 1967).

Other important H antigens in man include the HL-A system. This too is a highly complex system as other histocompatibility systems. Nearly 20 possible crossovers in the HL-A group have been recorded by Batchelor and Chapman (1967). In an excellent review on human transplantation systems, Amos (1969) expressed the need for further studies on crossovers in the HL-A system.

A considerable amount of research done with chickens has helped elucidate the genetic role in immune response. One of the earliest demonstrations of tissue acceptance between related individuals in chickens was by Kozelka (1929). In his study of sexual dimorphism using comb, spur, and feather follicles, he observed fewer rejections among related birds.

Using the increase in circulating lymphocytes as an indicator of severity of immune response, Craig and Hirsch (1957) demonstrated that the level of response was correlated with the degree of relationship between the donor and host. They suggested that this technique might be a useful check on calculated relationship coefficients from pedigree information in populations.

Valid comparisons however, would require a non-inbred control population originating from the same foundation stock as the inbred populations.

A strong relationship between levels of genetic diversity of a donor and host and the rate of rejection in the skin grafts was demonstrated by Craig et al. (1960). Rate of immune response in young chicks was slowest between full sib exchanges, and fastest between unrelated individuals of the same strain as determined by a macroscopic system of scoring skin grafts.

Homograft exchanges between highly inbred Reaseheath chickens showed a relatively high degree of acceptance (Cock and Clough, 1956). Only six of a total of 86 homografts failed to survive in inbred line I with an inbreeding coefficient of 98.75%. The significant difference between homografts and autografts in this study might be due to one or more very weak histocompatibility loci. According to Schierman (1962), this line is still segregating at A blood group locus, which so far has not been established as an H locus.

Since the discovery of blood groups on chicken red blood cells by Landsteiner and Miller (1924) eleven different systems have been categorized: A, B, C, D, E, H, I, J, K, and P (Briles, 1962). The genetic aspects of these have been reviewed by Briles (1962) and Gilmour (1962), while the study to be reported here deals only with the B system.

The key role of the B system associated with transplantation genes was recognized and demonstrated by Schierman and Nordskog (1961, 1964). Using a moderately inbred White Leghorn line, they reported that graft exchanges between B blood group compatibles were accepted whereas the incompatibles at this locus were rejected. Other blood group loci including A and D had no effect on skin transplants. These results were subsequently verified by

Gilmour (1962), Craig and McDermid (1963), and Gleason and Fanguy (1964). Evidence for a second H locus in chickens was presented by Schierman and Nordskog (1964): they found that the C blood group locus was also associated with a transplantation antigen but evoked a weaker reaction than the B locus.

The B locus is not only a major H locus in chickens, but also is associated with physiological fitness. Briles (1954) reported that individuals with heterozygous B blood group alleles were superior in hatchability, growth rate, and egg production. Similar results indicating superior reproductive fitness in B locus heterozygotes were reported by Gilmour (1958). More recently, Rishell (1968) observed consistently poor egg production and high mortality of homozygous B^1B^1 individuals.

The complexity of the B locus in chickens is comparable to the H-2 locus in mice. Out of 12 lines derived from 9 different stocks, Briles et al. (1957) identified 21 alleles in this system.

Variations among histocompatibility loci and their effects

Studies with coisogenic lines in mice, have shown that the intensity of the immune response evoked by various H loci differs: some are strong and some weak. The strong reaction leads to rapid graft rejection while the weak reaction leads to a prolonged period of rejection. Differences at the H-2 locus in mice lead to rapid rejection while differences at other loci such as H-1 or H-3 lead to slow rejection (Counce et al. 1956).

Further studies on strong and weak H factors have been studied by McKhann (1964a, 1964b). Using coisogenic lines of mice he immunized the recipients with spleen cells of donors and challenged them at different days with skin grafts to test the presence of immunity as indicated by early

rejection. He found that "immunization with spleen cells in the presence of only an H-2 incompatibility was rapid in onset but of short duration, while immunization across the weaker H-3 barrier was slow in onset and of longer duration." This was interpreted to mean that strongly antigenic lymphoid cells, transferred between mice differing at the H-2 locus, are destroyed within a few days by the recipient. On the other hand, the weak antigenic cells provoke a weak response allowing them to survive a little longer.

Cummulative effects of H loci are a well established fact. The additive effects of non H-2 loci were demonstrated by Graff et al. (1966). They found that the median survival time (MST) of grafts exchanged between individuals differing, for example, at three weak loci was significantly shorter than the MST of grafts exchanged between individuals differing at only 1 or 2 such weak loci. There is, however, a maximum response such that, once attained by the host against the allografts, no further H antigen accumulation can accelerate rejection.

Sex-linked transplantation antigens

Eichwald and Silmsler (1955) reported the consistent rejection of male to female skin grafts in C57BL mice. This indicates the existence of Y-linked histoantigens. Another line, A/Jax in the same experiment, showed inconsistent results from intra-line male to female skin grafts; this was attributed to a generation break in brother-sister matings. Billingham, Silvers and Wilson (1965) confirmed the existence of a Y-linked histocompatibility (H-Y) system. They deduced that H-Y is a minor histocompatibility gene based on the ease it can be suppressed. So far, no additional alleles

at this locus have been found (Billingham and Silvers, 1963).

Billingham and Silvers (1959) also reported a Y-linked H locus in the B.N. strain of rats. Sex-linked histoantigens have not yet been reported in man.

A possible sex-linked H factor in chickens was reported by Kozelka (1932). He noticed that more homografts were rejected from female to male transplants than from other sex combination transplants. Well over thirty years elapsed before Bacon and Craig (1966) reported similar results with two inbred White Leghorn lines. These workers found this histoantigen to be lacking in a third inbred line. Further evidence for a W-linked histoantigen was presented by Gilmour (1967). Using two highly inbred Reasenheath chickens matched at the locus B, the intra-line male to female graft exchanges showed a moderate degree of reaction. Bacon and Craig (1966) presented evidence supporting the hypothesis "that acceptance or rejection of female skin graft by males within lines is associated with a genetic difference in the capacity of the recipient to respond to weak histoantigens."

The presence of a sex-linked transplantation antigen was accidentally observed by Bailey (1963) in a cross of two strains of mice, BALB female X C57BL male; he found F_1 individuals which rejected allografts from the paternal strain while accepting the allografts from the maternal side. He concluded that a sex-linked factor or factors were present in the X chromosome of the male. The MST in these grafts exchanges were rather high indicating a rather weak nature.

Number of histocompatibility loci in animals

Under certain conditions the immune response to allografts is precise and very repeatable. This is because genetic entities acting individually or collectively are involved in the rejection mechanism. Snell (1948) realized this fact early which led to his ingenious "isolation method" (Bailey, 1963) for a single locus and by which he was able to isolate several loci. This method however, requires great patience and would take many years to study a few loci.

An operationally useful method to estimate the number of H loci in animals proposed by Billingham, Hodge and Silvers (1962) requires the following assumptions: (1) that histocompatibility alleles express themselves even when in heterozygous condition, i.e., they are co-dominant; (2) that the loci segregate independently when interstrain F_1 hybrids are mated to produce an F_2 generation, i.e., they are not linked; (3) that the antigen determined by each allele is singly sufficient to bring about the rejection of a graft when present in the donor but absent in its host. With these assumptions all possible genetic combinations would be expected to segregate on a Mendelian basis if the F_2 population is large. Since an individual normally responds to the antigen which it lacks, the proportion of successful grafts in the F_2 -parental strain exchange is $(3/4)^N$ where N represents the number of H loci present in the donor strain against which the other parental strain could react (Billingham et al. 1962). Therefore the estimated number, N, of H loci is: $(\ln S)/(\ln 3/4)$ where \ln is the natural log, and S is fraction of successful grafts. Other methods of estimating the number of H loci have been discussed by Bailey (1963), and Elandt-Johnson (1969).

Immunological Basis for Histocompatibility

That an allograft rejection is an immune response is based on at least three different kinds of evidence: (1) the "accelerated second set" phenomenon, (2) the ability of the immunologically competent cells to confer sensitivity when transferred to a new host, and (3) the induction of specific immune tolerance (Hasek et al. 1961).

The sequence of events following the first set of skin grafts in man is similar to that seen in experimental animals (Rapaport and Converse, 1958). Following transplantation, the skin graft in man becomes vascularized, grows and proliferates. This healthy state is short lived; generalized thrombosis ensues and the epidermis becomes necrotic, and finally turns into a scar (Converse and Rapaport, 1956). In general, the approximate time for the first set skin graft in most species is seven to ten days according to Billingham et al. (1954). After rejection of the first set, the individual is said to be sensitized.

When a second set of grafts is applied from the same donor to the sensitized recipient, the response is an accelerated rejection. This phenomenon was first observed by Medawar (1944, 1945) in rabbits, and has been observed in rats, guinea pigs and man (Billingham and Sparrow, 1954; Rapaport and Converse, 1958). Eichwald et al. (1966) characterized the second set of grafts as showing either: (1) no vascularization, usually referred to as white grafts; (2) engorgment, hemorrhage, epithelial necrosis and absence of lymphocytic infiltration; or (3) marked lymphocyte infiltration and a hyperplastic epithelium. These authors interpret categories (1) and (2) as the result of a marked sensitization usually encountered when there is a strong genetic difference between donor and recipient.

The category (3) response is also interpreted as a reflection of a weak genetic difference between the donor and the recipient.

Homograft reaction resembles a delayed type of hypersensitivity with respect to time of onset of reaction and histological features according to Hasek et al. (1961). Furthermore, the sensitivity of either phenomenon is transferable to other individuals by lymphoid cells of the sensitized donor (Michison, 1953; Billingham et al., 1954).

The type of immunity observed in the homograft reaction is cellular rather than the classical antibody-mediated response. Brent et al. (1958) reported that cellular immunity may be expressed in guinea pigs by a delayed type of hypersensitivity. Indeed, the role played by this fundamental immune mechanism in homograft rejection is unequivocal. Using deoxyribonuclease treated leukocytes, Lawrence et al. (1960) demonstrated that skin homograft destruction can be accomplished by the delayed type of hypersensitivity independent of the circulating antibodies.

Leukocyte interaction and histocompatibility in chickens

A need for a fast, efficient and reliable method for screening prospective tissue or organ donors to assure success of transplants has been recognized particularly by the medical workers. In response to this need, several tests for histocompatibility have been formulated in the past decade.

Brent and Medawar (1963) suggested the normal lymphocyte transfer could be used as an index of histocompatibility based on the immunological capacity of white blood cells. Lymphocytes from recipients were injected intradermally into a panel of prospective donors. Inflammatory reactions

of unequal intensities among the recipients closely paralleled the reaction intensities of the homografts of donors with subsequent recipients.

Wilson et al. (1963) took a different approach using the same principle. An individual C is sensitized with skin from A, and challenged with skin from B. If C responds to B in a second set type of rejection, then A and B are assumed to share common antigens.

One criticism of these H tests is the possible danger of disseminating an infectious disease by cellular transfer (Brent and Medawar, 1963). Other factors such as skin thickness and vascular responsiveness may cause errors of ranking the donors.

Phytohemagglutinin (PHA), a protein commonly extracted from leguminous plant seeds, has been used as an agent to separate leukocytes from whole blood. More specifically, it initiates mitotic activity in normal human leukocytes (Nowell, 1960). In its presence, mitosis occurs in 3 to 5 days, whereas in its absence, no mitosis occurs. This phenomenon was confirmed by Marshall and Roberts (1963). They found that cultures from single donors stimulated with PHA showed 95% or more cell transformation after 3 to 5 days.

Shrek and Donnelly (1961) reported that cultures of "mixed blood from two patients with hemochromatosis" showed mitotic activity. In five days a small number of large cells was observed in their culture.

Bain et al. (1964) found that the transformation of leukocytes such as that observed by Shrek and Donnelly (1961) also occurs in normal human mixed leukocyte cultures. Later, Elves et al. (1963) indicated that this transformation is associated with the production of antibodies.

Using the immunological property of lymphocyte interaction in mixed

cultures, Bach and Hirshhorn (1964) demonstrated that this method could be a useful tool as an in vitro histocompatibility test. The test is based on the hypothesis that grafts between individuals, whose cells do not stimulate the other in mixed leukocyte culture (MLC), should survive longer. Bach and Kischen (1967) observed this in an experiment with two pairs of siblings: one compatible and the other incompatible. Without prior knowledge as to which pair was compatible, they successfully matched them on the basis of the MLC reaction; the pair showing the least stimulation survived much longer than the other. Silvers et al. (1967) applying the MLC test to rats, demonstrated its success for this species as well.

In using the MLC test for histocompatibility, the donor cells are treated with mitomycin C (Bach and Voynow, 1966). This permits a one way stimulation: the recipient cells are stimulated by the donor cells but not the reverse.

Development of immunological competence

The development of immunological competence in various animals has been studied by several workers. Schinkel and Ferguson (1953) reported that the foetal lamb is capable of making an immune response against a foreign tissue. In cattle, Billingham and Lampkin (1957) showed that new born calves have a fully developed immunological capacity to reject homografts. Although milk colostrum contains maternal antibodies, these are not a necessary factor for transplantation response of calves. In man, immunological development seems to be complete at birth according to Woodruff (1957) but the information is difficult to come by because of the

obvious difficulty to study transplantation experimentation with children or infants.

The development of immunological maturity in chickens has been reported by Cannon and Longmire (1952) to be well developed about 3 days post-hatch. A permanent take of 5-10% surviving grafts was observed when the donor-recipients were no older than 3 days.

Gallinaceous birds (including chickens and quail) and pigeons differ from other birds and mammals in that they have no lymph nodes; instead they have an accumulation of lymphocytes in various diverse tissues including the skin (Yoffey and Courtice, 1956). Billingham and Silvers (1959) demonstrated that lymphoid cells from adult skin are capable of inducing splenomegaly in recipient embryos when transplanted to the chorioallantoic membrane. Using chicks of varying ages, Solomon (1963) showed that the earliest appearance of transplantation antigen of immunologically competent cells occurs in three day old chicks, confirming the previous work of Cannon and Longmire (1952).

Inbreeding

Inbreeding may be defined as the mating together of individuals more closely related than the average relationship among random members of the whole population (Kempthorne, 1969). In natural populations the degree of inbreeding is mainly a function of effective size of the population (N_e). In artificial populations, inbreeding is a function of both the mating scheme and N_e . As a rule the population N_e is the single primary force in determining the amount of inbreeding. However, if the mating scheme prescribes, say, sib-avoidance, the level of inbreeding may lag one generation

(Tolman, 1969).

The major consequence of inbreeding is the increase in the frequency of homozygous genes within a population and concomitantly, the decrease in heterozygosity. Continued inbreeding leads to fixation of genetic characters. If, for example, we have a group of animals heterozygous for two gene pairs (AaBb), inbreeding in this group could lead to the production of AAbb, aaBB, aabb, and AABB non-segregating genotypes. Each of these genotypes would breed true and would display possible distinct genetic features. Thus, inbreeding increases the uniformity in phenotypic expression for highly heritable traits, a feature of importance to commercial animal breeders as well as to fanciers. Inbreeding helps to identify and eliminate undesirable recessive genes in a population.

A knowledge of the amount of inbreeding in domesticated species of animals is a useful index of the genetic variation of a population. The coefficient of inbreeding of an individual is defined to be the probability that two genes possessed by an individual at a locus are identical by descent (Malecot in Kempthorne, 1969). Earlier, Wright developed his classical path coefficient method of calculating inbreeding. Wright defined inbreeding in terms of the degree of correlation of uniting gametes in a population but this definition is completely equivalent to Malecot's. Wright's formula, given in a slightly modified form by Lush (1945) is:

$$F_x = \frac{1}{2} \sum \left[\left(\frac{1}{2} \right)^n (1 + F_A) \right]$$

where F_x is the inbreeding coefficient of animal X; Σ is the summation over all paths leading to a common ancestor between two parents of X; n is number of generations in a line by which parents are related to a common ancestor A; and F_A is the inbreeding coefficient of the common ancestor.

This formula requires complete pedigree information and is operationally useful only when the number of individuals in a population or line are not too large. As the number of common ancestors increases the calculation of inbreeding coefficients become very tedious and possible errors are likely to occur in the process. For complex populations, the inbreeding coefficient can be calculated from covariance charts as shown by Emik and Terrill (1949) using Lush's (1945) formula:

$$R_{xy} = \frac{\frac{1}{2}^n (1 + F_A)}{\sqrt{1 + F_x} \sqrt{1 + F_y}}$$

where R_{xy} is the coefficient of relationship of animals X and Y; n is the total number of Mendelian segregations in the path of descent through which X and Y are related; F_A , F_x , and F_y are inbreeding coefficients of a common ancestor, animal X and Y, respectively. Lush (1945) defined the coefficient of relationship as the probability that the two animals under consideration possess duplicate genes because of their common line of descent over and above those that are found in the base population. Even with a large number of animals in a pedigree, this formula can be programmed in the computer to obtain both inbreeding as well as relationship coefficients.

The inbreeding coefficients in non-pedigreed populations can be estimated when the number of sires and dams for each generation is known. This is possible through the use of the concept of effective population size (N_e) originally developed by Wright (1931). Falconer (1967) defined N_e as the number of individuals that would give rise to the sampling

variance or rate of inbreeding appropriate to the conditions if they bred in manner of an idealized population:

$$N_e = \frac{1}{\frac{1}{4N_m} + \frac{1}{4N_f}}$$

where N_m is the number of male and N_f is the number of female parents.

Using effective population number, Cockerham (1965) derived a recurrence formula to estimate inbreeding coefficients:

$$F_t = F_{t-1} + \frac{1-F_{t-1}}{2N_e} + \frac{1-F_{t-2}}{2N_e}$$

where F_{t-i} is the inbreeding coefficient in generation $t-i$.

Most of the experimental studies on the effects of inbreeding were done prior to 1960. King (1935) was able to maintain a brother-sister mating scheme through 93 generations in two Wistar strains of rats. Waters and Lambert (1936) ascribed the success of inbreeding in White Leghorn chickens to good foundation stock and to rigid selection of the best birds for breeders in each generation. However, some years later these same inbred chickens showed a slow but gradual decline in hatchability of fertile eggs as inbreeding increased. This confirmed earlier observations that inbreeding usually leads to poor reproductive fitness. Wilson (1948a) categorized the components of reproductive capacity as (1) egg production, (2) fertility, (3) hatchability and (4) viability. He reported that for

each percent of inbreeding there was a loss of .19% in hatchability, .02% in chick viability at eight weeks of age, and 1.4% in egg production (Wilson, 1948b). Stephenson et al. (1953) reported a 0.43% decrease in egg production for each percent of inbreeding. Indeed, any adverse effect on any one of these reproductive capacity components would have profound effect on the future of a line.

MATERIALS AND METHODS

Description of Inbred Lines

Five inbred lines were the sources of experimental birds for this study (Fig. 1). These lines have been inbred in varying degrees since 1954. The white Leghorn line, HN has been pedigreed through 17 generations and has an average inbreeding coefficient of 85.9 percent in the last generation. The Black Spanish line (SP), originating from the University of Minnesota in 1953, was maintained by non-pedigree matings for the first nine and the last two generations. This line was pedigreed only from generation 10 through 15. The inbreeding coefficient of Line SP averaged 65.9 percent in the last generation. The third inbred line, Leghorn GH previously described by Schierman (1961, 1962), has been pedigreed throughout; the experimental birds used in this study had an average inbreeding coefficient of 58.1 percent. Leghorn Line 9, had an inbreeding coefficient of 54.2 percent. Leghorn Line 8, a dominant white Leghorn segregating for the sex-linked barring gene, was maintained by mass mating for the first 8 generations and then pedigreed. The average inbreeding coefficient for Line 8 is now 65 percent.

The control for this study was an F_1 of a cross of two Leghorn Lines, S_1 and S_2 with known B locus blood groups. The development of the S lines have been described by Rishell (1968). For practical purposes the inbreeding of the F_1 cross was considered to be zero.

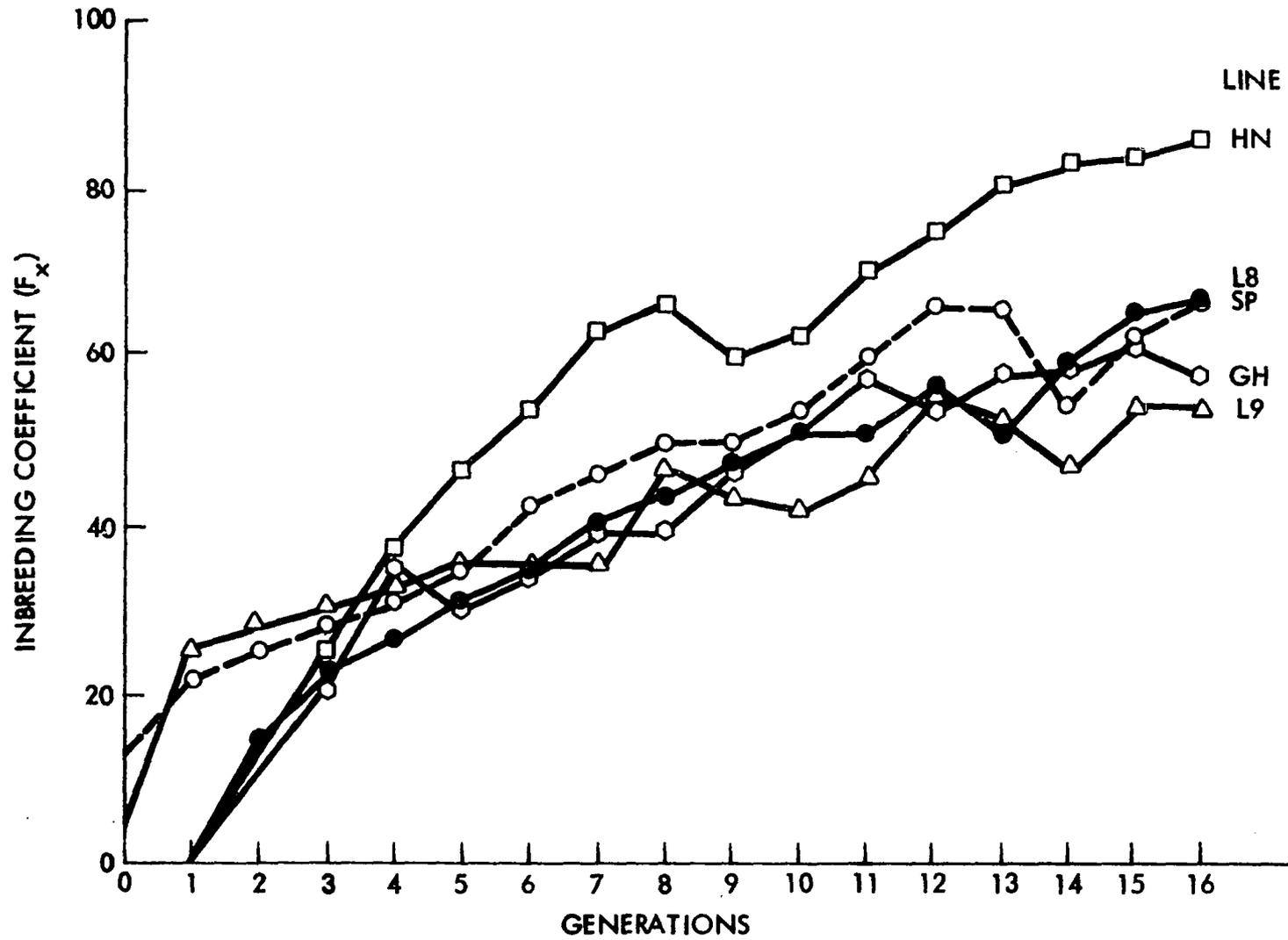


Fig. 1. Inbreeding coefficients by lines and generations. (The last generation shown is the experimental generation).

Reagent Synthesis and Blood Typing Techniques

The procedures for the production of blood typing reagents used in this study correspond to those discussed by Schierman (1961), and Rishell (1968).

Briefly, the antisera were produced by isoimmunization. Agglutination tests were performed on plastic plates.¹ Each plate contained 100 wells in 10 x 10 rows and columns. Single drops of each test reagent were placed in successive column wells. To each well a single drop of test cells in 2% suspension was added. Each plate was gently but thoroughly shaken to mix the cells and the test reagent. After setting the plates aside for 45 minutes, the agglutination test was read over a fluorescent light and recorded. If the cells lie as a mat covering the whole well, the test is positive. If the cells settle and appear as a red dot or "button" on the bottom of the well, the test is negative. Tilting the plate at a 45° angle, the contents of a negative well flows downwardly while the cells of a positive test tends to stick to the wall of the well. The technique proved to be fast and repeatable.

Mating Plans

Heterozygous males were mated to heterozygous females to produce three different genotypes. For example: in Line HN, the mating of blood types $B^{6,7} \times B^{6,7}$ gave the progeny: $B^{6,6}$, $B^{6,7}$ and $B^{7,7}$ in the ratio of 1:2:1, respectively. The above mating plan required blood typing the progeny to identify their genotypes. On the other hand, when a homozygous male

¹ Supplied by Lindbro Co., New Haven, Connecticut.

was mated to a pen of homozygous females containing both alleles, no blood typing was necessary because the blood type of the offspring could be classified on the basis of the parents. Lines 8 and SP were not blood typed at the B locus, but chicks from all lines were pedigreed and sexed.

The Grafting Pattern

Exchanges were made at 17 days between chicks of the same sex. For each chick, 4 grafts were exchanged with four others in the 1969 series of experiments as follows:

from a full sib

from a half sib

from a non-sib (no sire and dam in common)

from another line

A replication unit or block consisted of 10 chicks: eight of Line i, one of Line j, and one of Line k. The eight chicks of Line i consisted of:

2 full-sib pairs (2 x 2) of 2 sires = 2 x 2 x 2 = 8

In Figure 2 the graft exchanges within the replication unit are represented by edges of a cube while the chicks are represented by the corners. Note that each corner has a full-sib (F), a half-sib (H), and a non-sib (N) edge. In addition, tetrahedron corners were added to the top and bottom to accommodate graft exchanges with a chick of Line j and Line k. On the basis of results obtained in the 1969 experiments, the interline graft exchanges were excluded in the 1970 experiments. However, in 1970, B locus blood group differences were considered. The different comparisons when the birds are blood typed are shown in Figure 3.

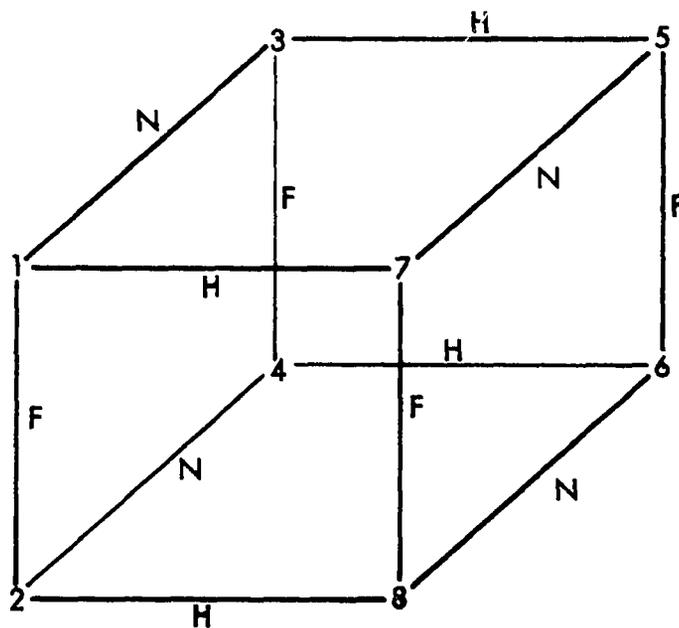


Fig. 2. The plan of graft exchanges: individuals are represented by the corners of the cube numbered 1 to 8. The edges of the cube represent the kind of exchange: F = full-sibs, H = half-sibs and N = non-sibs.

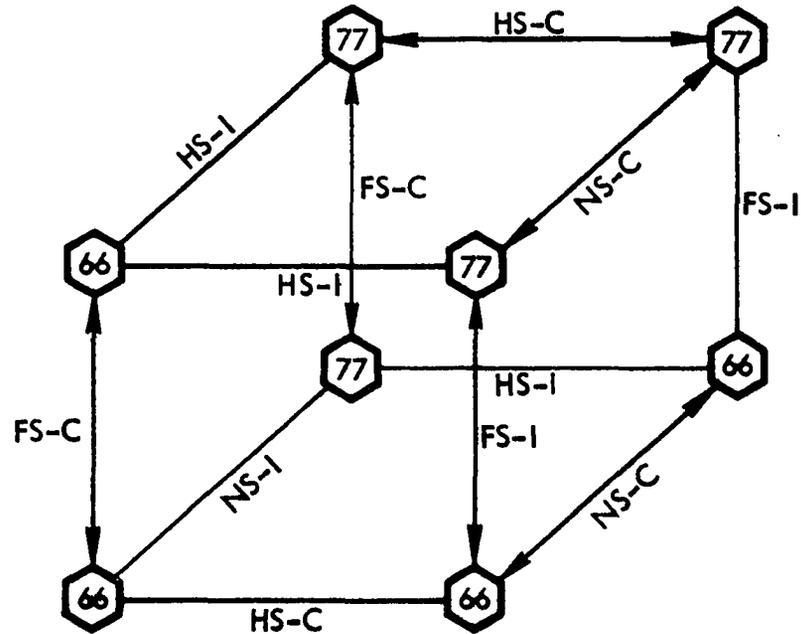


Fig. 3. The plan of graft exchanges when individuals are first blood typed at the B locus. The figure gives an example of two blood groups, B⁶/B⁶ and B⁷/B⁷. The arrows indicate the direction of compatibility. The kinds of exchanges represent:

1. Full-sib compatible (FS-C)
2. Full-sib incompatible (FS-I)
3. Half-sib compatible (HS-C)
4. Half-sib incompatible (HS-I)
5. Non-sib compatible (NS-C)
6. Non-sib incompatible (NS-I)

Skin Graft Technique (Chicken)

The skin graft exchanges were performed between appropriate individuals using the Polley et al. (1960) technique. Each experimental unit was handled together. Flexible collodion was applied to the back of each individual chick about 30 minutes prior to the operation. The down being left intact served to stiffen the graft. Each chick was placed on an operation rack and restrained with rubber bands. To obtain a uniform graft size, a standard 10mm x 10mm jig was used. A full thickness skin graft was cut with a sharp pointed dissecting scissors and transferred by clean forceps to a waiting petri dish containing gauze and physiological saline. The graft, to be exchanged with one already removed, was placed directly on the recipient after first turning it 180°; the first graft, earlier set in the petri dish, was then placed in similar fashion. Finally, a plastic bandaid was securely placed on the graft. Operationally, the following was the order between the 10 chicks in a replication unit:

Indiv. Chick #	Family ¹	Intraline			Between
		Full-sib	Half-sib	Non-sib	Lines
					BL
1	11	2	3	7	9
2	11	1	4	8	10
3	12	4	1	5	9
4	12	3	2	6	10
5	21	6	7	3	9
6	21	5	8	4	10
7	22	8	5	1	9
8	22	76	6	2	10
<hr/>					
9	X	1	3	5	7
10	Y	2	4	6	8

¹ A family ij is of sire i and dam j.

On the 7th postoperative day the plastic bandaids were carefully removed and the first reading of graft reaction was recorded. The scoring scale, similar to that of Polley et al. (1960) is shown on Table 1. Allografts were read and recorded daily for seven days, and on alternate days from the ninth through the 28th postoperative day.

Table 1 Macroscopic numerical scoring system to estimate the severity of a homograft reaction (Polley et al., 1960)

Score	
5	Smooth, bright and healthy appearing.
4	Some discoloration and/or inflammation apparent, but smooth.
3	Some deep brown or deep red color and may be slightly shrunken.
2	Brownish-black color and shrunken.
1	Brownish-black or black color, much shrunken, crusty, and becoming detached at the edges.
0	Graft sloughed off.
X	Graft missing but not sloughed off (faulty operative technique or accidental loss).

Line Crosses

Chicks from five different line cross matings included:

	<u>males</u>		<u>females</u>
1.	Line 8	X	Line SP
2.	Line 9	X	Line 8
3.	Line Gh	X	Line 9
4.	Line HN	X	Line Gh
5.	Line SP	X	HN

Since Line 8 and Line SP were not blood typed, the offspring of any mating involving them could not be tested with regard to a specific H locus effect. The number 3 mating was the only one in which homozygous Gh males and homozygous Line 9 hens were available. In #4 homozygous Gh hens were available but only one functional HN male was available. The other male

had to be heterozygous at the B locus. Thus, from the matings arranged above, only #3 produced F_1 progeny which were identical at the B locus. In others, the segregating B alleles had to be contended with.

The grafting pattern followed in this section was the same as previously followed. Graft exchanges were made between full-sibs, half-sibs, and non-sibs.

Skin Grafting in Coturnix

A small Coturnix population, panmictically bred for one and one-half years, was the source of experimental data. The grafting techniques used on these birds were similar to the chicken grafts but with some modification because of the smallness of the quail chicks. The steps followed were:

1. Flexible collodion was applied to the back of the chick.
2. The chick was held with its legs between two fingers of the left hand, with the thumb restraining one wing; the other wing usually does not interfere. Using a clean and sharp dissecting scissors, a graft of standard 10mm x 10mm size was cut and placed with clean forceps to a waiting petri dish containing gauze and physiological saline.
3. After the operation the bird was placed alone in a small waiting container.
4. Step #2 was repeated with the other bird of the pair. As with chickens, the grafts were exchanged after rotating each 180° on the graft bed. The bandaid spot was tightly applied to the graft and the chicks were returned to the brooder.

These procedures were repeated for all pairs. After seven days the bandaids were removed and scores were recorded as in chicken experiments.

Mixed Leukocyte Cultures

White Leghorn Lines Gh and L9 with known B locus blood types were used. The experiments were planned to show effects of one gene and of two gene differences among full-sibs on lymphocyte interaction in mixed cell cultures. Differences between full-sibs of the same B locus blood type and differences between lines served as test controls.

Lymphocyte mixtures were prepared from pairs of individuals of the following blood types or lines:

Experiment 1: a. B^1B^2 x B^2B^2

b. B^1B^1 x B^2B^2

c. B^1B^3 x B^2B^2

Experiment 2: a. B^8B^8 x B^8B^9

b. B^8B^8 x B^9B^9

c. Lines Gh x L9

d. Lines Gh x L9

Experiment 3: a. B^9B^9 x B^8B^8

b. B^9B^9 x B^9B^9

c. B^8B^9 x B^8B^9

Experiment 3: d. $B^1B^1 \times B^2B^2$

(cont.)

e. Lines Gh x L9

Experiment 4: a. $B^2B^3 \times B^2B^3$

b. Lines Gh x L9

The birds for each experiment were heart-bled using aseptic techniques. The feathers were plucked at the point of heart puncture, and the area was cleaned with 70% alcohol. Individual syringes, wetted in heparin to avoid blood coagulation, were used once only for collection of blood from individual chickens. The blood was immediately transferred into test tubes containing 0.5 ml of heparin and stoppered. Mixing of the blood with heparin was accomplished by inverting the tube several times. About 20 ml of blood were collected from each bird in labeled test tubes. Tubes were set aside at room temperature for 3 hours and then centrifuged at 400 rpm for 8 minutes to obtain approximately 6 ml of white blood cells in serum from each sample. Again using aseptic techniques, the buffy coat layer plus serum was removed from each tube and the selected pairs of cells were mixed in equal proportions in 50 ml labeled Erlenmeyer flasks. The serum mixtures in each flask were diluted with 4 volumes of warm Eagles media. From 3-fold dilutions of 1 ml aliquots of the sub-samples, cell counts were made using a Coulter counter. Each of the diluted aliquots of the sub-sample were counted 4 times. Cultures were incubated at 37° C during the counting process. Subsequent counts were made on 4th, 6th, and 8th day of incubation except in the case of Experiment 4 in which the cells were counted on 3rd, 5th, and 7th day. The averages of the counts were

converted into percentages based on the initial first day's count for each of the samples and plotted on a graph.

RESULTS

Skin Graft Acceptance Among Sib-Groups Not Blood Typed

The percentage of accepted grafts on specific observation days of each experiment was plotted on a graph in order to observe the trend of acceptance. In general, results were highly repeatable with each line tending to show distinct characteristics.

In Line HN the rejection rate declined slightly after about the 9th postoperative day; this was followed by "permanent" acceptance of the remaining grafts to the end of each experiment (Figure 4). This decline was characteristic among all groups: full-sibs, half-sibs, and non-sibs. Another unique characteristic of Line HN was the early feather growth first appearing on the grafts about the 16th postoperative day; by the 20th day practically all grafts had grown feathers. Early feather growth facilitated fast and accurate reading of graft survival in Line HN. The calculated mean survival time, using the Brownlee and Hamre (1950) method, was 20.5 days for all experiments (Table 2).

In Line 8 tested in six experiments in 1969, rejections generally took place over the entire test period. Unlike HN, considerable variation in graft acceptance was observed among the different sib-groups. The highest acceptance occurred between the full-sib exchanges with the half-sib exchanges second and the non-sib exchanges the lowest acceptance level. The overall mean survival time for Line 8 was 17.6 days. From the analysis of variance, a highly significant difference between sib-groups was found.

The overall mean survival time in the GH line was 20.6, 16.3, and 14.1 days for the full-sib, half-sib and non-sib groups, respectively. There

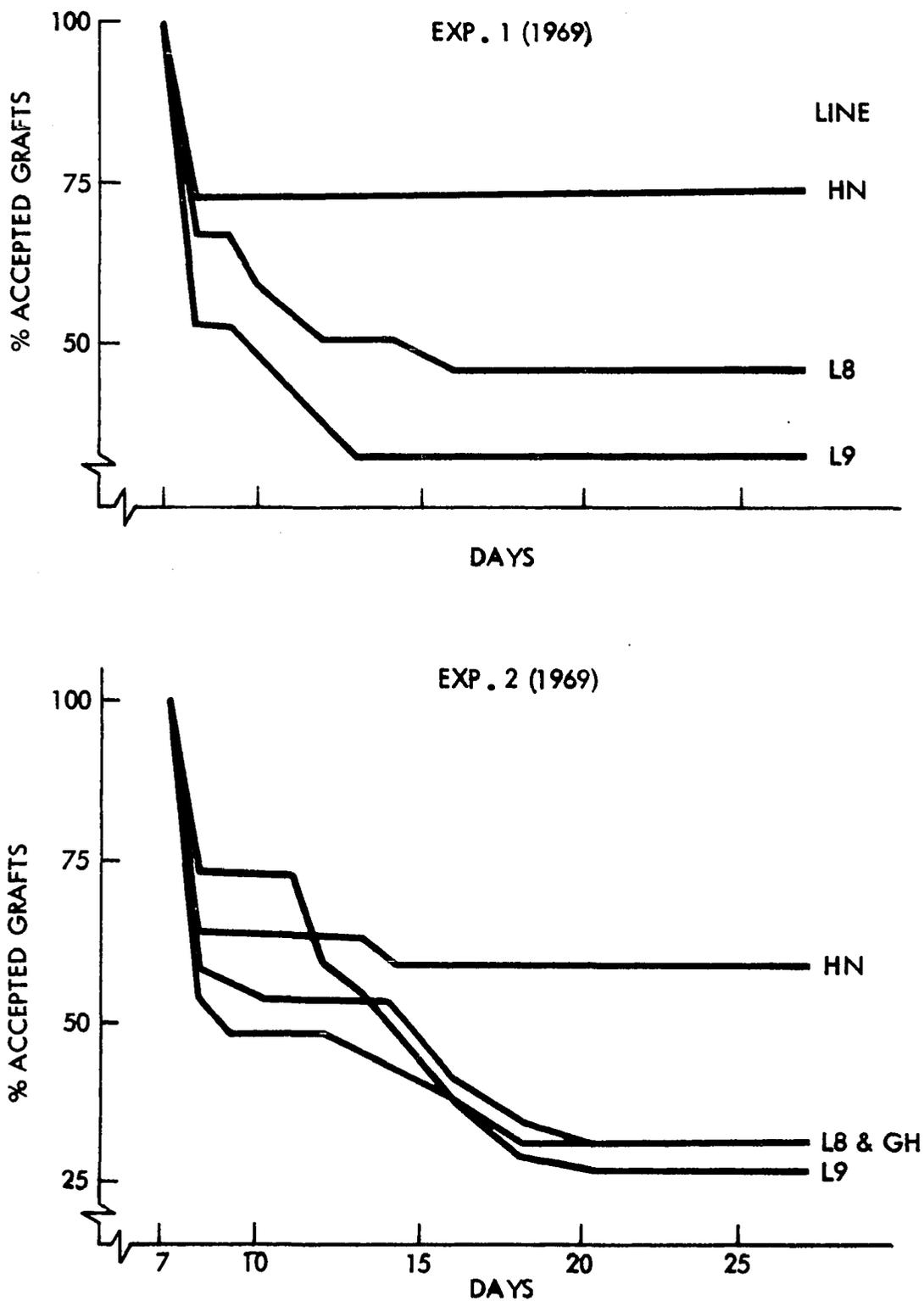


Fig. 4. Percentage of accepted grafts on the different days of observation for experiments 1 and 2.

was continuous rejection of skin transplants through the entire test period of each experiment in this line. The analysis of variance on the five experimental sets of data on this group was highly significant between sib-groups.

The results from Line 9 showed the lowest overall acceptance of all lines studied. This line also showed continuous rejection during the entire test period of the experiment (Figure 4). The mean survival time was 15.5, 15.1, and 13.9 days for the full-sib, half-sib and non-sib groups, respectively. Although this line has a relatively low acceptance rate, there was no statistical difference between the sib-groups.

Members of Line SP had, indeed, a unique response to the homografts. All grafts were accepted up to the 16th postoperative day, then rejections started. This type of response, not observed in other lines, was repeated in all five experiments conducted with Line SP (two in 1969 and three in 1970). The acceptance was occasionally followed by feather growth after the 18th postgrafting day. Some of the grafts which did not grow feathers maintained a chronic rejection state whereby the graft does not integrate with the surrounding skin and its original size tends to be maintained. The mean survival time for this line was the highest, being 23.9, 24.1, and 22.8 days for the full-sib, half-sib and non-sib groups, respectively.

The inter-line graft exchanges were practically all rejected by the 9th postoperative day. Graft reactions were so severe that in most cases no vascularization took place. However, in two cases grafts lingered on until the 14th day when they were sloughed off. In view of these results, inter-line graft exchanges were discontinued in the 1970 series of experiments.

Table 2 Mean survival time by sib-groups of 7 skin grafting experiments performed in 1969 on five lines, ignoring blood-groups

Mean Survival Time in Days					
Line	Exp. No.	Full-Sib	Half-Sib	Non-Sib	Pooled Av.
HN	1	18.9 (8) ^a	18.9 (8)	23.6 (8)	20.5
	2	19.2 (8)	17.0 (8)	19.8 (8)	
	4	21.4 (8)	21.5 (8)	20.9 (7)	
	5	21.8 (8)	21.8 (8)	21.8 (8)	
L8	1	21.3 (8)	19.3 (8)	8.9 (8)	17.6
	2	18.7 (16)	16.6 (16)	9.3 (16)	
	3	21.3 (16)	18.7 (16)	11.8 (16)	
	4	20.8 (16)	21.0 (16)	13.6 (16)	
	6	22.4 (20)	18.9 (19)	15.9 (14)	
	7	22.6 (14)	20.6 (11)	15.7 (13)	
GH	2	19.2 (16)	13.9 (16)	11.2 (16)	17.0
	3	19.1 (16)	17.8 (16)	16.4 (16)	
	4	21.6 (8)	18.8 (8)	13.9 (8)	
	5	24.9 (24)	17.5 (24)	14.5 (24)	
	6	19.4 (14)	13.6 (14)	14.6 (14)	
L9	1	13.8 (24)	13.2 (24)	13.5 (24)	15.0
	2	15.7 (16)	15.7 (16)	12.9 (16)	
	3	16.3 (16)	14.6 (16)	13.7 (16)	
	4	16.8 (16)	18.9 (16)	14.7 (16)	
SP	6	22.9 (11)	24.0 (7)	24.0 (10)	23.6
	7	25.0 (16)	24.1 (16)	21.6 (16)	
Mean		20.1	18.4	15.8	

^a Figures in parentheses are number of grafts.

Dates of experiments: #1 - 8/1, #2 - 8/15, #3 = 8/28, #4 - 9/13, #5 - 9/25, #6 - 10/24, and #7 - 11/8.

Table 2 summarizes the results of all experiments performed in 1969 in terms of mean survival time of grafts. The analysis of variance shows highly significant differences between lines between sib-groups and lines x sib-group interaction (Table 3). The regression of mean survival time

on inbreeding is shown in Figure 5. The relationship between inbreeding and mean survival time is clearly linear. The results indicate that for each 10 percent increase in inbreeding, mean survival time increased 1.6 days as an average for the populations used in this study.

Table 3 Analysis of variance of mean survival time on the 1969 data presented in Table 2

Source	df	SS	M S	F
Between lines	4	391.0743	97.7685	26.049**
Sib-groups	2	195.0867	97.5434	25.989**
Line & sib-groups	8	179.8203	22.4775	5.989**
Error	48	179.6732	3.7532	

** $P < .005$.

Effect of the B Locus Blood Group On Skin Graft Reaction of Different Inbred Lines

When the B locus was taken into account, a different type of histocompatibility response was obtained from graft exchanges. The effect of the H antigen was demonstrated in all lines as shown by the MST in Table 4. An analysis of variance for these data is given in Table 5 using the model

$$Y_{ijkl} = U + C_i + L_k + S_j + (CL)_{ij} + (SR)_{ik} + (LS)_{jk} + n(CLS)_{ijk} + e_{ijkl}$$

Y_{ijkl} = Observation on individual l of line k with a graft exchange in sib-group j and B locus compatibility i; i = 1, 2; j = 1, 2, 3; k = 1, 2, 3, 4; l = 1, 2 - - n.

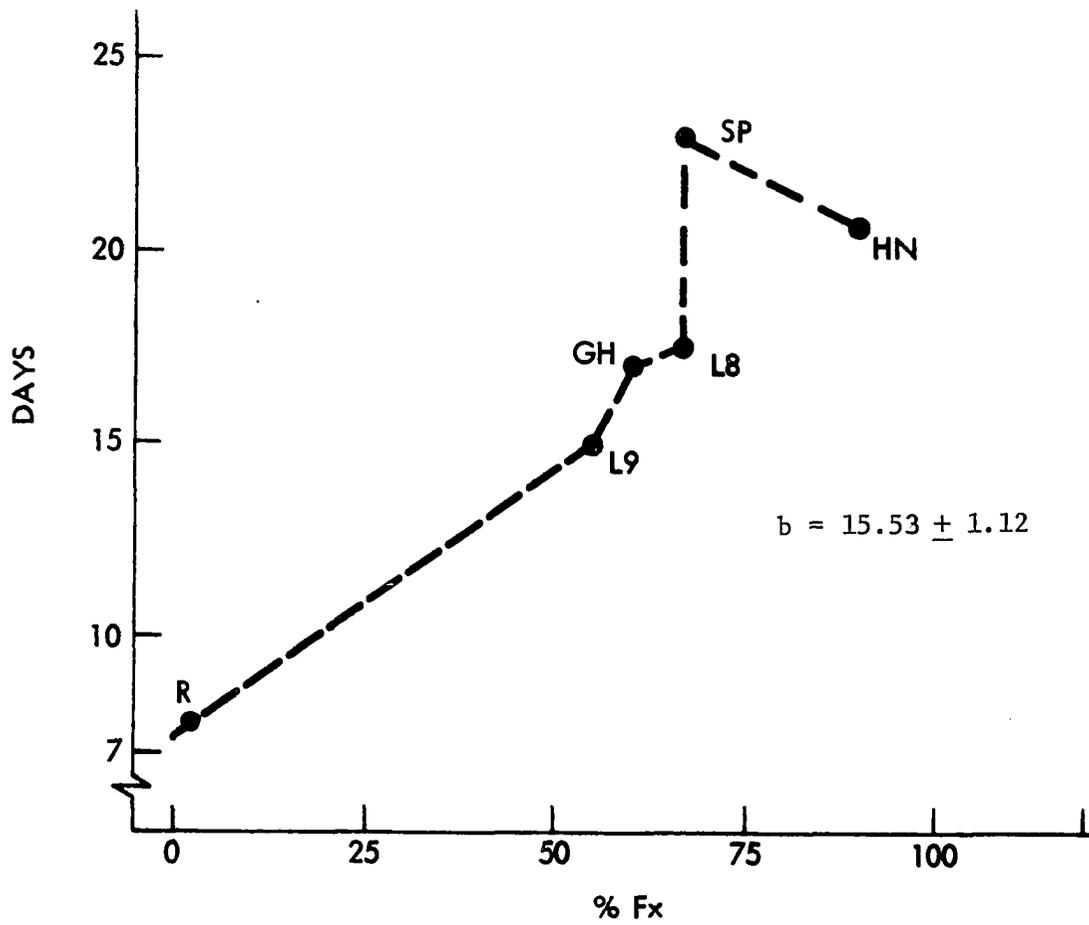


Fig. 5. Regression of mean survival time in days on percent inbreeding (F_x) by lines (1969 data).

Table 4 Summary of four experiments taking into account B locus bloodgroups

		Mean Survival Time in Days					
Line	Exp.	B-Locus Compatible			B-Locus Incompatible		
		Full-Sibs	Half-Sibs	Non-Sibs	Full-Sibs	Half-Sibs	Non-Sibs
GH	1	24.7 (12) ^a	23.9 (11)	22.9 (11)	8.0 (9)	9.2 (11)	9.4 (11)
	2	22.3 (18)	22.1 (18)	19.7 (17)	7.7 (6)	7.8 (6)	8.0 (7)
	3	23.4 (14)	22.7 (11)	21.3 (12)	8.0 (2)	8.0 (6)	8.0 (4)
L9	1	22.0 (11)	21.5 (11)	20.4 (16)	7.3 (13)	7.0 (13)	9.5 (8)
	2	26.0 (16)	22.0 (13)	19.8 (11)	8.6 (8)	8.2 (8)	8.7 (10)
HN	2	26.0 (9)	24.1 (10)	26.0 (8)	8.2 (8)	8.2 (6)	10.5 (8)
	3	23.2 (12)	26.0 (9)	22.2 (10)	8.0 (4)	8.3 (7)	8.5 (6)
	4	26.0 (9)	26.0 (8)	26.0 (5)	9.7 (7)	9.7 (7)	10.0 (9)
R	2	12.2 (6)	11.3 (7)	13.5 (2)	7.5 (2)	7.0 (7)	7.0 (6)
	3	13.9 (20)	13.4 (14)	9.9 (13)	8.5 (10)	8.4 (10)	8.0 (11)
	4	18.4 (9)	14.1 (13)	13.5 (4)	7.7 (6)	9.5 (3)	7.3 (12)
Mean		21.6	20.6	19.6	8.1	8.3	8.6
		<u>B-Locus Compatible</u>			<u>B-Locus Incompatible</u>		
GH		22.5			8.2		
L9		22.0			8.2		
HN		25.1			9.0		
R		13.6			7.9		

^a Figures in parentheses are number of grafts.

Table 5- Factorial analysis of variance of mean survival time by compatibility levels, lines and sib-groups

Source	df	Expected Mean Squares	
		Assuming C, S and L are all fixed	Assuming C and S fixed, L random
Compatibility (C)	1	2485.23**	$2\sigma_e^2 + 33k_c^2$
Between lines (L)	3	135.14**	$2\sigma_e^2 + 14k_L^2$
Sib-groups (S)	2	3.36	$2\sigma_e^2 + 22k_s^2$
C x L	3	99.03**	$2\sigma_e^2 + 8.25k_{eL}^2$
C x S	2	9.32*	$2\sigma_e^2 + 11k_{cs}^2$
L x S	6	1.26	$2\sigma_e^2 + 5.5k_{Ls}^2$
C x L x S	6	0.14	$2\sigma_e^2 + 2.75k_{csL}^2$
Error	42	1.92	$2\sigma_e^2$

**P < .001.

*P < .05.

U = Mean

C_i = B locus compatibility group of the i^{th} exchange

L_k = Line k effect

S_j = Sib-group j effect

(CL) $_{ik}$ = Compatibility i by line k interaction

(CS) $_{ij}$ = Compatibility i by sib-group j interaction

(LS) $_{jk}$ = Line k by sib-group j interaction

(CLS) $_{ijk}$ = Compatibility i by Line k by sib-group j interaction

Two models were considered. For the mixed model the assumptions are that compatibility levels and sib-groups are fixed effects and that lines and individuals are random effects with error effects, e_{ijkl} , normally and independently distributed. For the first model the assumptions are the same except that line effects are also fixed.

The partitioning of the source of variation in these data shows that the B locus compatibility level accounted for 75% of the total variance (Table 6). Of the remaining variation most is accounted for by lines and by the interaction of lines with the compatibility levels. Only a relatively small proportion of the total is unaccounted for, i.e. the error variance accounts for only two percent. Lines alone accounted for about ten percent of the total variation with sib-groups accounting for less than one percent.

Results of individual lines

Line R Figure 6 shows the percentages of accepted grafts over the 25-day observation period. For the B locus histoincompatibles, 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.

Table 6 Variance component estimates from Table 5 using the fixed model

			<u>Variance Component</u> ^a	<u>Percent Variance Component</u>
Compatibility	(C)	k_c^2	75.25	74.79
Between lines	(L)	k_l^2	9.52	9.46
Sib-groups	(S)	k_s^2	0.07	0.07
C X L		k_{cl}^2	11.78	11.71
C X S		k_{cs}^2	0.67	0.67
L X S		k_{ls}^2	1.26	1.25
C X L X S		k_{cls}^2	0.14	0.14
Error		σ_e^2	1.92	1.91
Total			100.61	100.00

^a The variance components in both the mixed and fixed models were essentially the same. The symbol k^2 is used for a fixed effect and σ^2 for a random effect.

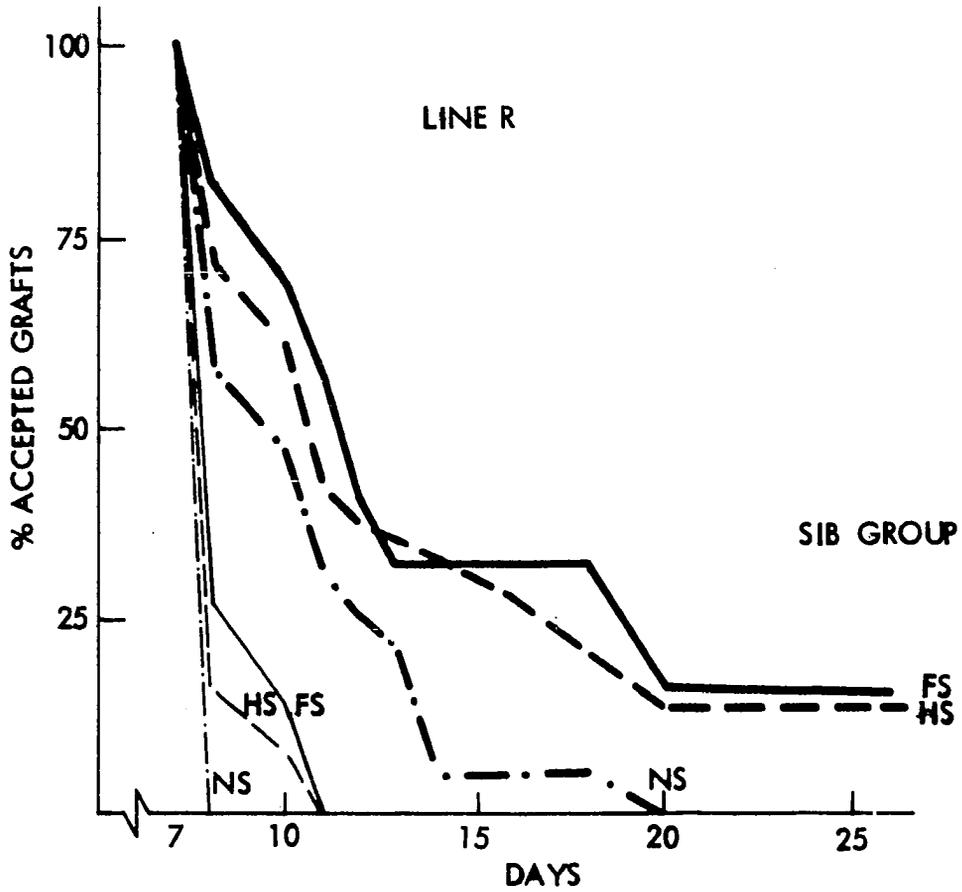


Fig. 6. Percentages of accepted grafts on the different days of observation in R line. Heavy lines represent graft exchanges between histocompatibles at the B locus. Light lines represent histoincompatibles. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS).

During this period, the sib-group effect seemed not to be important. However, by the 20th day all non-sib compatible grafts were rejected while 14% and 16% of half-sib and full-sib groups respectively, remained through the 26th day.

Line 9 In Line 9 the response among the B locus histoincompatibles was swift (Figure 7). Eighty-five percent were rejected by the 10th postoperative day. Half-sibs and non-sibs were totally rejected by the 11th and 16th day, respectively. The remaining non-sib incompatibles were totally rejected by the 18th day. Among the B locus histocompatibles, the number of surviving grafts was high in comparison to the incompatibles. Rejections started about the 10th postoperative day and proceeded at different rates for the various sib-groups. Rejections were few but gradual among the full-sibs and leveled off at 78%, after 20% rejection between 9th and 11th post-grafting days, whereas the non-sib groups continued with slow rejections finally reaching 68% by the 26th postoperative day. Grafts from down colored chicks to white chicks were accepted just as well as white to white feather grafts (Figure 8). As expected these grafts maintained the donor feather color. Figure 9 shows a graft being rejected.

Line GH Nearly 90% of the B locus histoincompatible grafts were rejected by the 13th postoperative day. By the 22nd day all half-sib and non-sib incompatible grafts were rejected. Oddly, one incompatible full-sib survived through the 26th day. Among the B locus histocompatibles, the rejection was slow but persistent for all sib-groups. However, the full-sibs had the highest number of surviving grafts with 82% followed by the half-sibs with 79% and the non-sibs with 69% being the lowest (Figure 10).

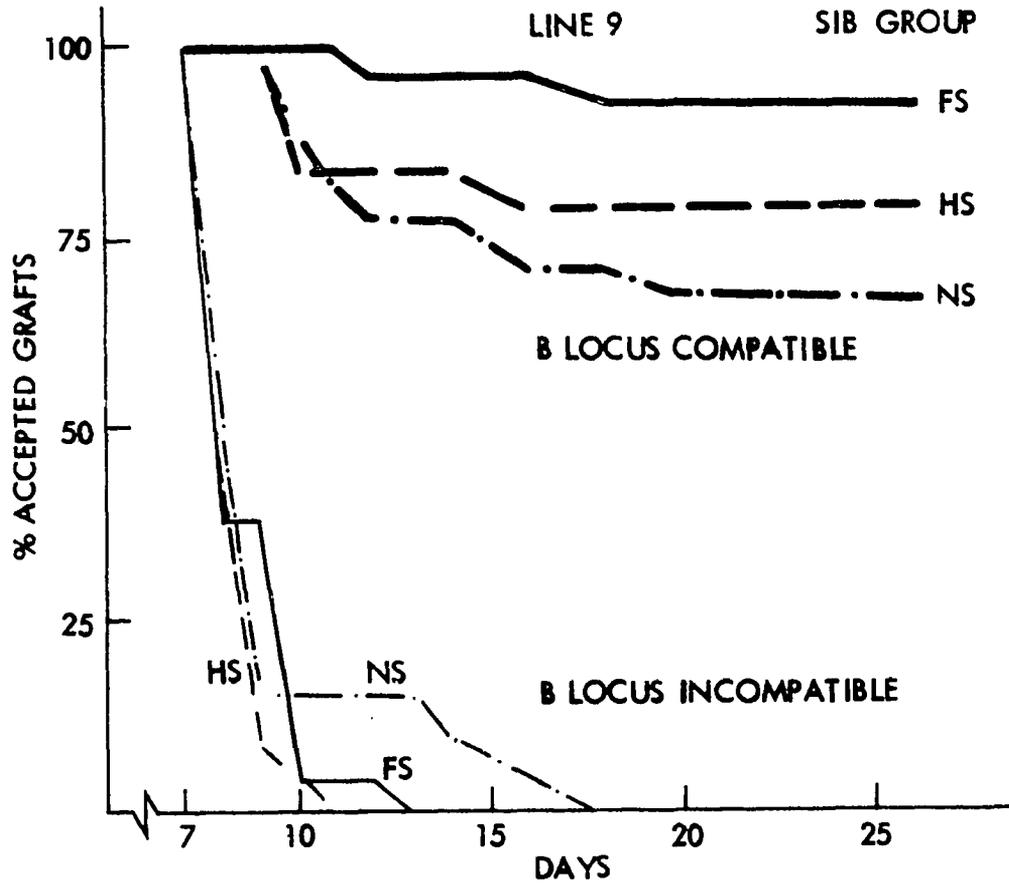


Fig. 7. Percentages of accepted grafts on the different days of observation. Heavy lines represent graft exchanges between histocompatibles at the B locus. Light lines represent histoincompatibles. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS).



Fig. 8. Line 9 host at 19 days post-grafting displaying a healthy dark feathered graft on a light feather host.

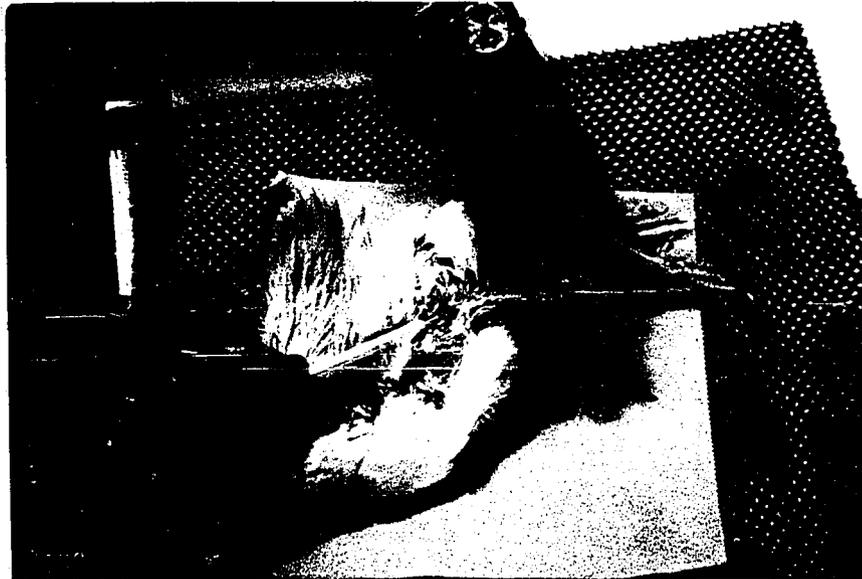


Fig. 9. Line 9 host displaying rejected allograft 13 days post-grafting.

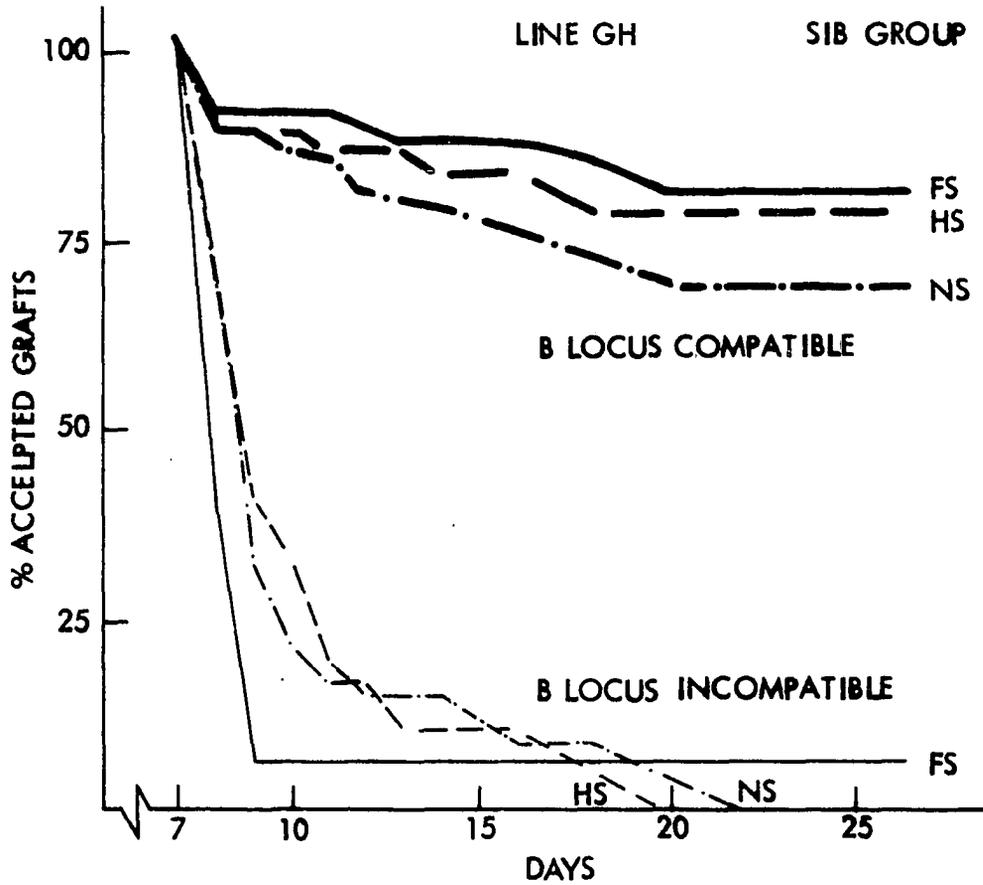


Fig. 10. Percentage of accepted grafts on different days of observation in the Gh Line. Heavy lines represent graft exchanges between histocompatibles at the B locus. Light lines represent histoincompatibles. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS).

Line HN The HN line responded uniquely: early rejections were generally followed by uninterrupted acceptance (Figure 11). Graft exchanges between B locus histoincompatibles were followed by rejections between 7 and 10th day after which no further rejections occurred. One exception to this was an incompatible graft which survived through the entire test observation period. This graft grew feathers just as all the compatibles and autografts.

The B locus histocompatible individuals responded similarly as described above. At the same time the number of surviving grafts was the highest in comparison with all other lines. The full-sibs and half-sibs had a high percentage of surviving grafts (90%) while the non-sibs were lower (80%)

Results of line crosses

Table 7 shows the mean survival time for various line crosses. Details for specific crosses are shown graphically.

SP x HN In this cross the acceptance of grafts among full-sibs was distinctly high--almost 80%. The 20% level of rejection was reached before the 9th postoperative day. The results of the half-sib and non-sib graft exchanges paralleled one another: the former was slightly higher in acceptance than the latter. No rejections occurred after the 16th postoperative day. The final acceptance levels, for both the half- and non-sibs were 38% and 35%, respectively (Figure 12).

B x SP This mating produced progeny with generally low acceptance patterns (Figure 12). The full-sibs were the lowest at 17% with the half- and non-sibs acceptance levels at 31% and 29%, respectively. A

Table 7 Mean survival time of sib-groups from five line crosses

<u>Mean Survival Time in Days</u>					
	<u>Cross</u>	<u>Full-Sib</u>	<u>Half-Sib</u>	<u>Non-Sib</u>	<u>Pooled Av.</u>
1	SP x HN	23.9	15.6	16.1	18.6
2	8 x SP	11.5	15.1	14.1	13.6
3	HN x GH	21.5	18.0	14.5	18.0
4	8 x 9	24.2	16.0	15.2	18.5
5	GH x 9	25.0	24.2	24.1	24.4
	Mean	21.2	17.8	16.8	

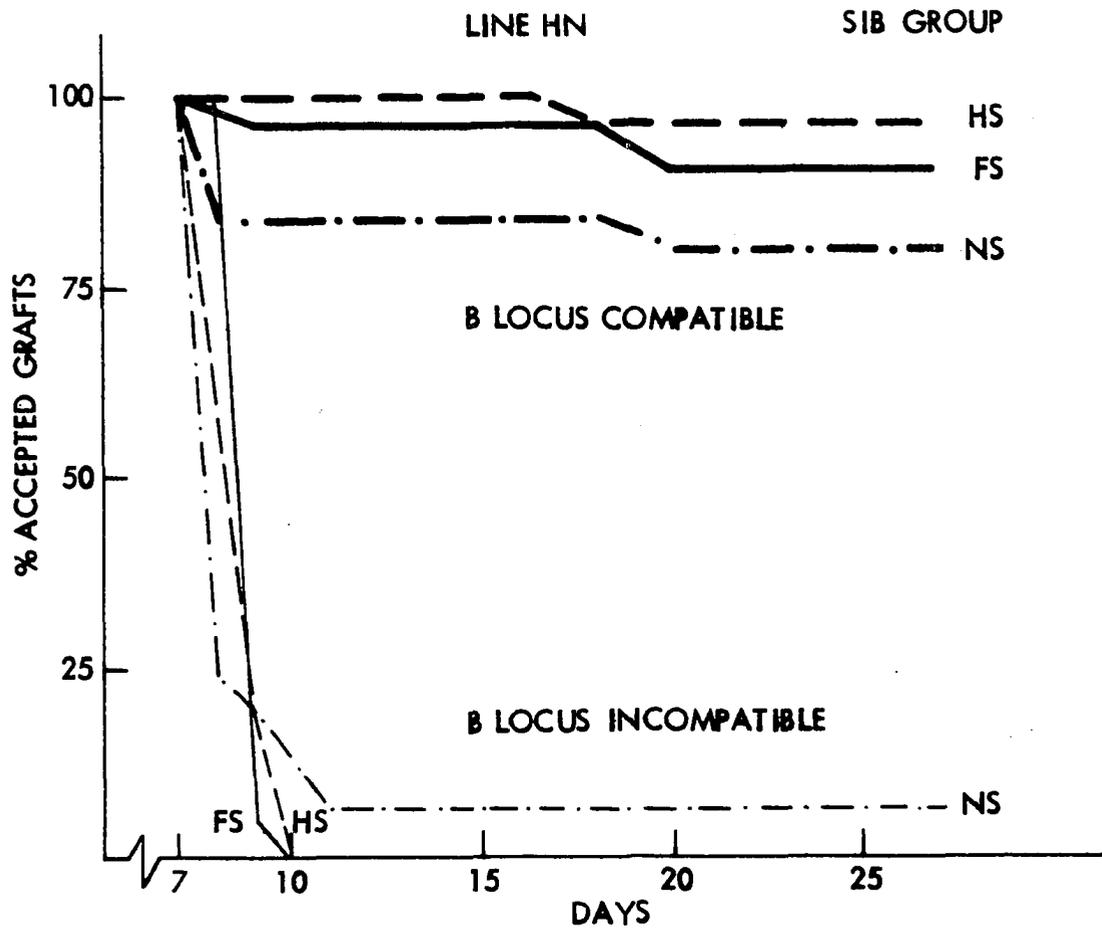


Fig. 11. Percentage of accepted grafts on various days of observations in line HN. Heavy lines represent graft exchanges between histo compatibles at the B locus. Light lines represent histoincompatibles. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS).

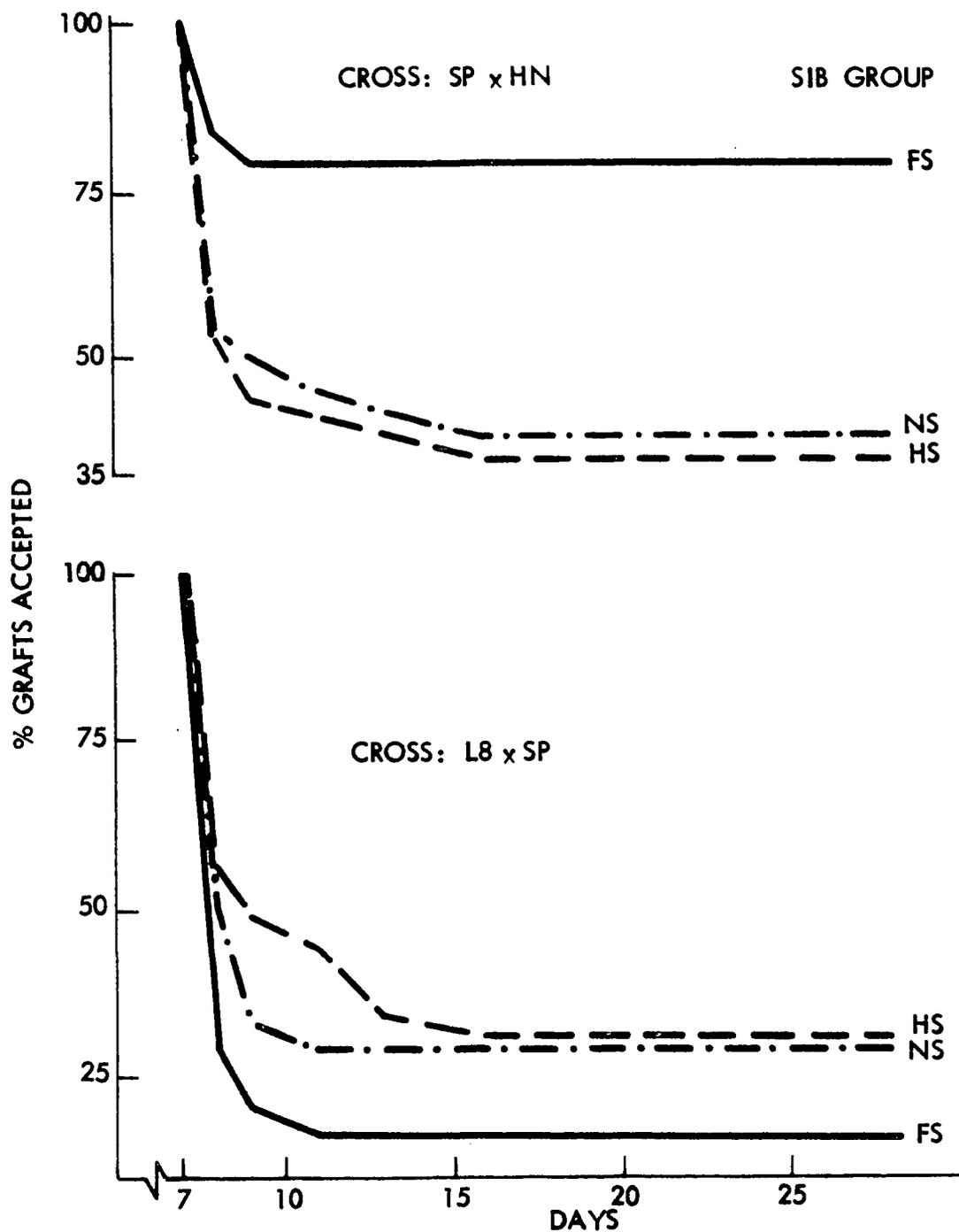


Fig. 12. Percentages of accepted grafts for the crosses, SP x HN and L8 x SP for different days of observations. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS). Note the low acceptance level in L8 x SP cross.

distinctive feature in this cross was a high percentage of rejections during the 7-10 postoperative days. After the 14th day no grafts were rejected to the end of the observational period.

HN x GH Among the progenies of this mating all graft rejections occurred before the 15th postoperative day. The full-sibs completed the experiment with a high acceptance level of 67%; the level for the half-sib was 43%, and that for the non-sibs was lowest at 29% (Figure 13).

9 x 8 The full-sib graft exchanges were accepted at a high level (79%). Most rejections occurred between the 7-14 postoperative days. Among the half-sibs, rejections occurred between the 7-10 post-grafting days with the acceptance level at 38% by the end of the experiment. The non-sib exchanges were slightly different in that rejections were continuous through the 18th day of the test (Figure 13). The level of acceptance in the non-sib group was 25% at the end of the experiment.

GH x 9 The reaction pattern of graft exchanges in this cross was completely different from all the other line crosses described. All three sib groups responded similarly between the 7-18 days (Figure 14). During this period the rejection level was slow but persistent with rather small differences between the sib-groups. No rejection occurred after the 20th post-grafting day. At the end of the experiment the acceptance level for full-sibs was 77% and for the half-sib and non-sib groups, 68%.

Figure 15 summarizes the response pattern of graft acceptance for the five line cross groups. The high acceptance level of the GH x L9 cross, in contrast to the low acceptance level of the 8 x SP cross, is mainly accounted for on the basis of a major H locus difference. In the first cross both the GH and L9 lines were homozygous at the B locus. Thus, the

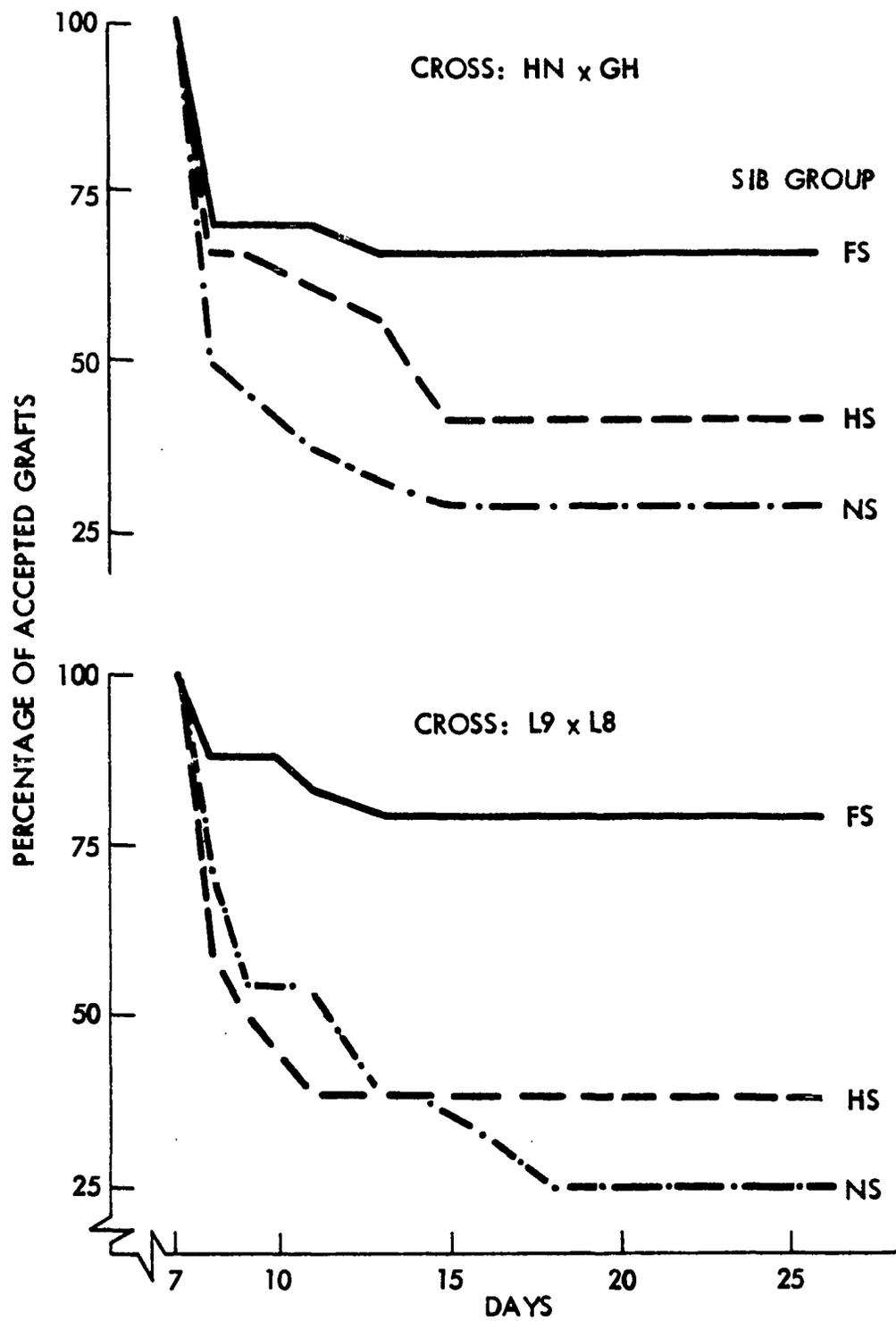


Fig. 13. Percentages of accepted grafts for the crosses HN x GH and L8 x L8 for different days of observations. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS).

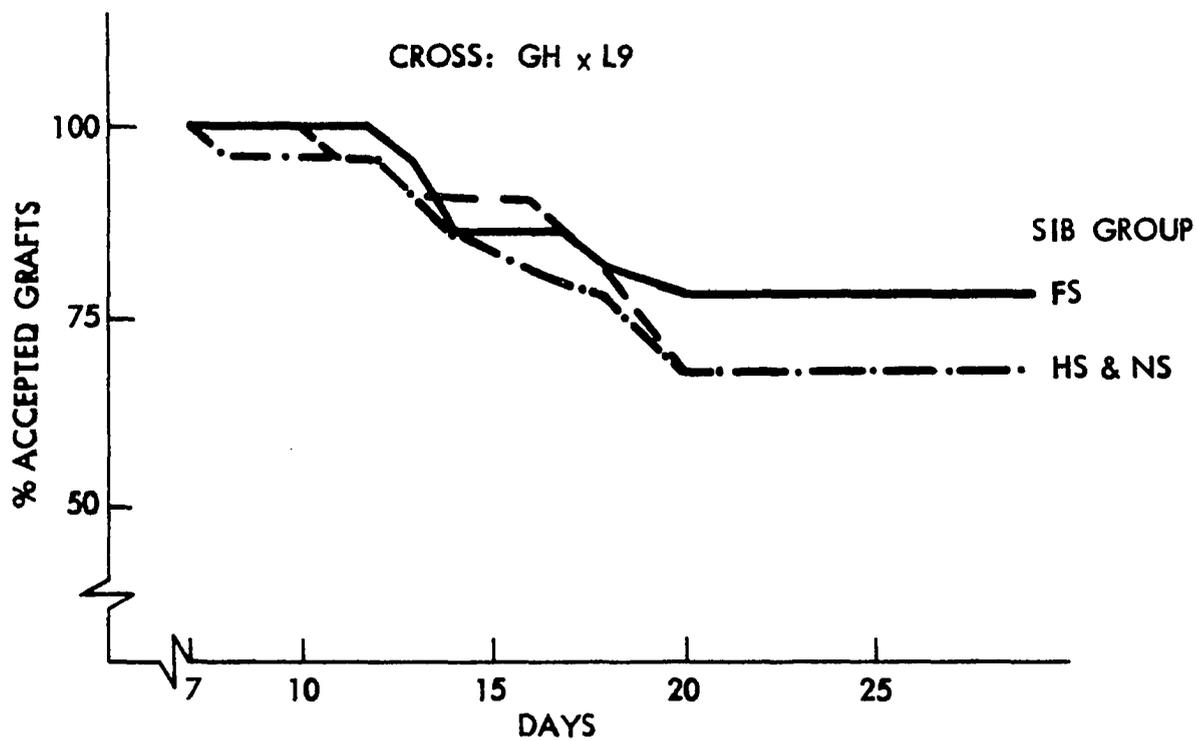


Fig. 14. Percentages of accepted grafts in GH x L9 cross for different days of observations. Sib groups: Full-sib (FS), half-sib (HS) and non-sib (NS).

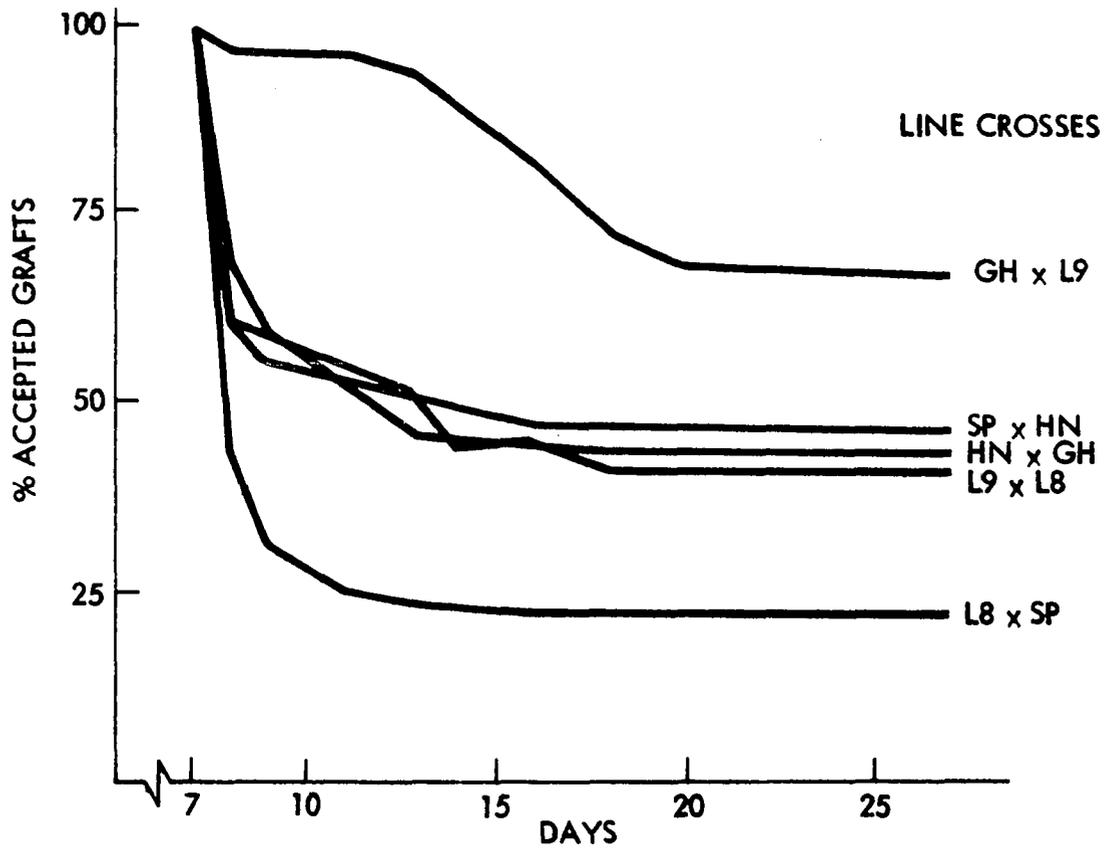


Fig. 15. Overall line cross mean percentages of accepted grafts by different days of observation. Note the contrast between GH x L9 and L8 x SP.

F_1 individuals should have identical antigens at the H locus, as Little and Johnson (1922) long ago demonstrated, and crosses between highly inbred lines should produce highly compatible progeny. In the 8 x SP cross the effects of segregating H alleles have reduced compatibility.

Results of Coturnix Study

Table 8 summarizes the results obtained in the coturnix study. Of eight birds operated on at one and two days of age, five drowned while the remaining three accepted the skin graft exchanges. By the 11th post-grafting day all grafts grew feathers. When birds of the same hatch were skin grafted on the 14th day of age all homografts were rejected and all autografts were accepted. A typical example of an accepted homograft is shown on Figure 16, and the rejected graft is shown on Figure 17.

An effort to determine age at which immunological competency develops was tested from the 4th day of age and older in the next experiment. Three out of four birds accepted graft exchanges which grew feathers when the operation was performed on the fourth day of age. Of four grafts made at six days of age two were rejected; one on the tenth post-grafting day and the other 26 days later.

Half of the graft exchanges on the seventh day of age were rejected 12 days post-grafting. The remaining grafts were permanently accepted to the end of the experiment with all showing feather growth. Only one of six grafts exchanged at eight days of age was rejected at six days of age, and three of six grafts were rejected on birds operated on the seventh day of age. It seemed advisable to check the acceptance level of five day old birds. In this case all grafts were accepted and grew feathers.

Table 8 Acceptance of graft exchanges in Coturnix

Date	Age in Days	Number of Birds	Postoperative Days							
			8	10	12	14	16	20	26	
3-14-70	2	3	3	3	3	3	3	3	3	3
3-26-70	14	Autografts	4	4	4	4	4	4	4	4
3-26-70	14	8	8	0	0	0	0	0	0	0
4- 4-70	4	4	4	4	3	3	3	3	3	3
4- 6-70	6	4	4	3	3	3	3	3	3	2
4- 7-70	7	6	6	4	3	3	3	3	3	3
4- 8-70	8	6	6	5	5	5	5	5	5	5
5-27-70	5	4	4	4	4	4	4	4	4	4

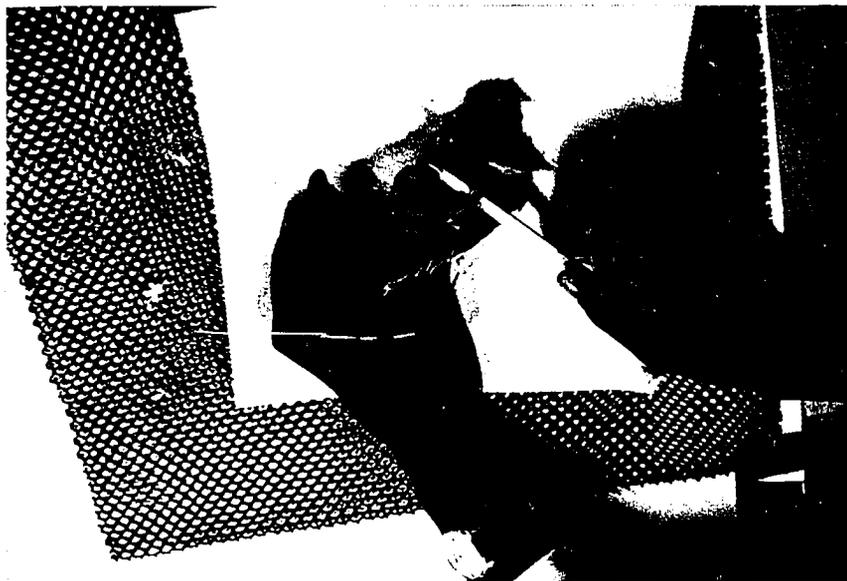


Fig. 16. Coturnix host displaying accepted skin graft 16 post-grafting days. Note feathers growing towards the head.



Fig. 17. Coturnix host displaying rejected graft 16 post-grafting days.

An interesting feature of the Coturnix skin graft is that at first sight they look dark as if they are undergoing rejection. Indeed, a young coturnix skin is dark but some variation may occur among individuals.

Coturnix develop feathers rapidly. Some show feather stubs as early as nine days after the operation. By the 20th post-grafting day all successful grafts developed feathers. Although it was not planned to follow up these skin grafts beyond 28 days, it is interesting to note that 20 grafts were still accepted 110 days later.

Mixed Leukocyte Cultures

The cell cultures in Experiment 1 were discontinued after five days. Figure 18 shows the cellular response in mixed leukocyte cultures (MLC) in Experiment 2. All cultures dropped in cell count from the initial count during the first four days but this was generally followed by an increase in cellular proliferation. Exceptions were the homologous MLC of genotype $B^8 B^9 / B^8 B^9$ in Experiment 2 and $B^2 B^3 / B^2 B^3$ in Experiment 4. These two types displayed a continuous decline in cellular proliferation. The inter-line MLC were most proliferative in all experiments (Figures 18 and 19). These were followed by cultures differing by two B locus alleles such as $B^8 B^8 / B^9 B^9$ or $B^1 B^1 / B^2 B^2$.

An attempt to re-feed the cultures by replacing the volume of supernatant with an equal volume of fresh Eagles Media 199 after centrifugation of cell suspension at 500 rpm for 5 minutes was unsuccessful. Thus, the cell counts after sixth day in Experiment 3 dropped sharply. The idea was to keep the media constant so that changes in the cell counts would reflect quantitatively the proliferation.

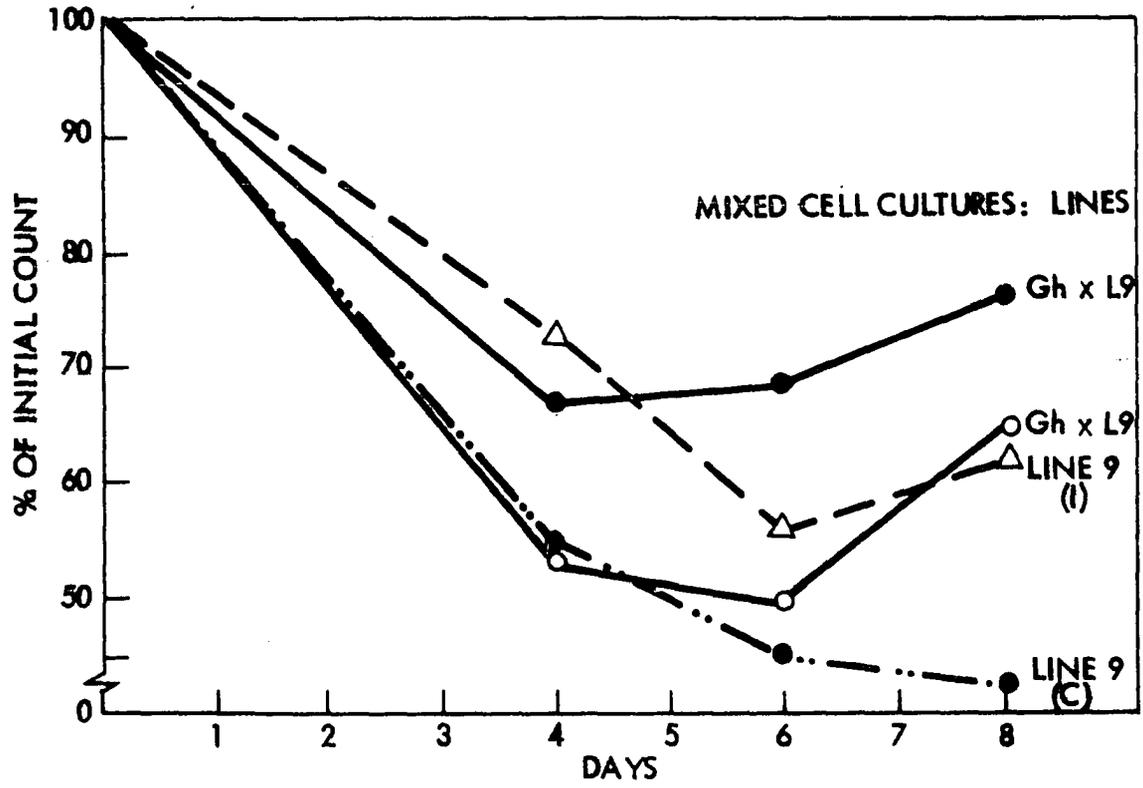


Fig. 18. Growth rates for mixed leukocyte cultures in Experiment 2.

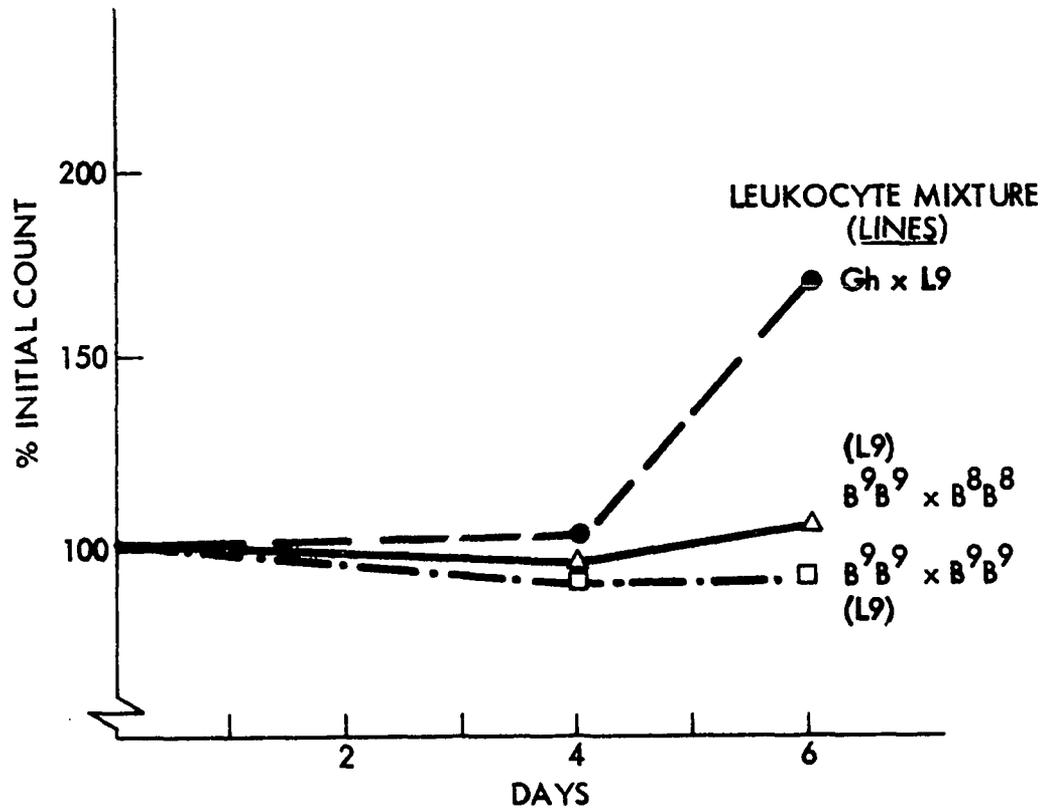


Fig. 19. Growth rates for mixed leukocyte cultures in Experiment 3. Note high cellular proliferation on Gh x L9 mixed cultures.

In view of the difficulties encountered, we tried to modify the re-feeding system. To the cell suspension of each culture an equal volume of warm Eagles Media was added. The counts were multiplied by a factor of 2 to account for the dilution. Again, the cell counts declined, as indicated in Figure 20, although less than experienced in the first trial.

In a number of cases the re-feeding of the cells was accompanied by contamination. Such cultures, or those suspected of contamination, were discarded and not used in plotting the graphs. Clearly, the chances of introducing a contaminant are increased by transfers or addition of the nutrient media.

Morphological studies of the cells in various cultures revealed some interesting results. Large cells (lymphoblastoids) were observed in mixed cell cultures Gh x L9 after three days of incubation. By the tenth day the 100% cellular transformation which had been reached about the 5th day was still very clear (see Figure 21). In $B^8 B^8 / B^9 B^9$ MLC both lymphoblastoid as well as small lymphocytes were observed from the fourth day on. No transformed cells were found in homologous $B^9 B^9 / B^9 B^9$ mixed leukocyte culture up to 8 days.

The data obtained in Experiment 2 were subjected to an analysis of variance (Table 9).

Differences between genetic groups were highly significant. This is reflective of the variation in antigenicity ranging from almost zero in the homologous $B^8 B^9 / B^8 B^9$ to the maximum in the L9/Gh line mixtures. Also differences between days and the interaction of days and groups were statistically significant.

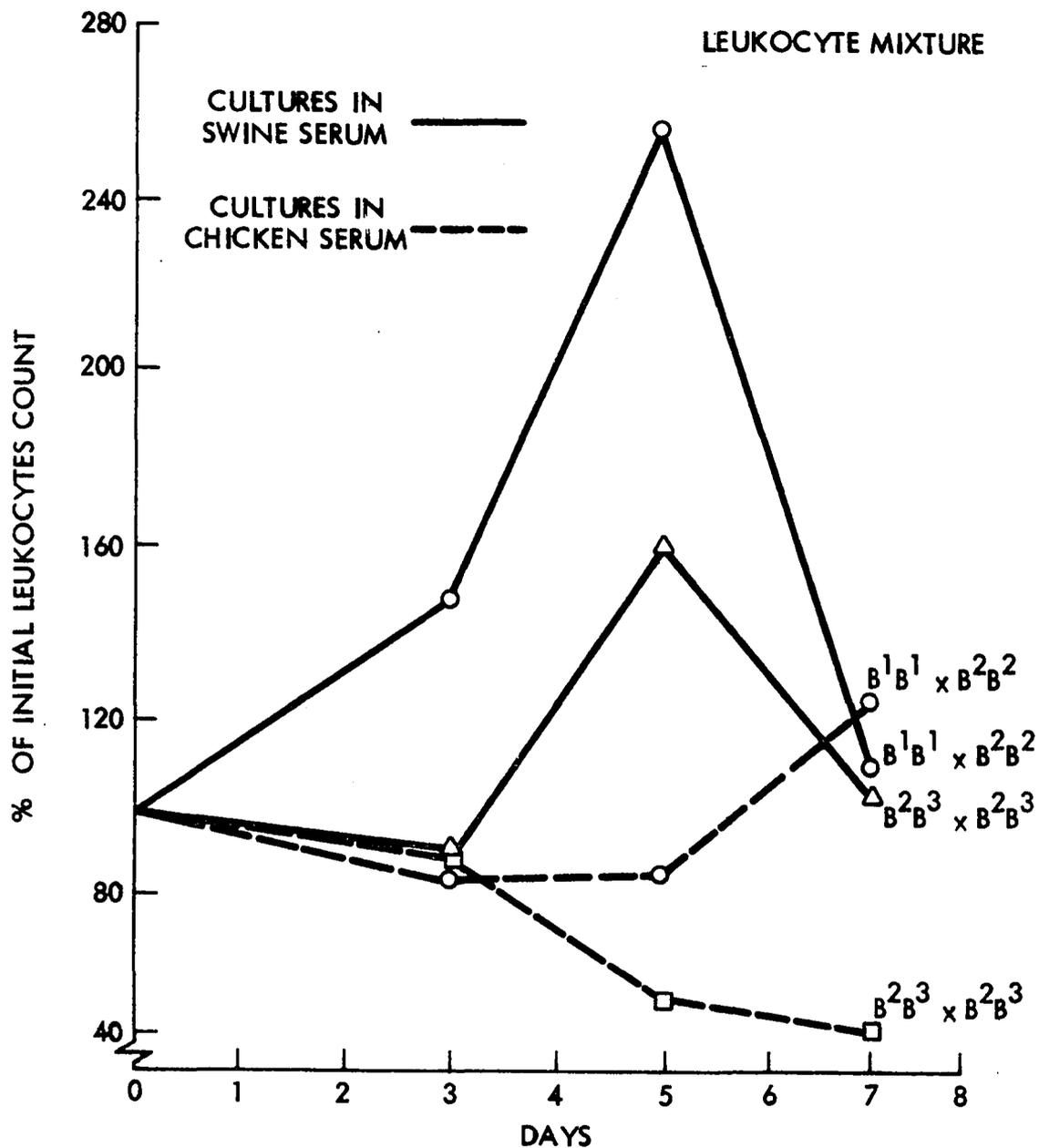


Fig. 20. Growth rates for various B locus combination cultures in Experiment IV. Cells cultured in swine serum are shown with solid line. Those cultured in chicken serum are shown with broken line. Note the drop in count after day 5, a possible dilution effect.



Fig. 21. Transformed cells in Gh x L9 mixed leukocyte cultures
5 days. Magnification: 400X

Table 9 Analysis of variance of cell proliferation mixed leukocyte cultures

Source of Variation	df	SS	MS	F
Between genetic groups	3	1649.550	549.83	19**
Between days	2	288.990	144.498	4.965*
Interaction	6	622.765	103.7	3.576*
Error	12	364.863	29.8	
Total	23	2908.168		

** P < .01.

* P < .05.

DISCUSSION

A Biological Measure of Genetic Diversity

The main purpose of this study was to determine whether skin transplantation might be used as a biological tool to measure genetic diversity among inbred lines of chickens. The standard measure of genetic diversity is a mathematical one (Wright's Coefficient of Inbreeding, F_x); it is based on the degree of relationship between mated individuals in a population. Wright's method is not valid for comparisons between populations of different origin, since for each population, F_x is relative to the amount of genetic diversity in some base generation. How well F_x measures true genetic diversity in a particular population, is really not known. Factors including heterozygote superiority and unknown mutation rates would cause F_x to underestimate genetic diversity. Studies in inbred populations of chickens show that inbred lines continue to segregate at blood groups more often than expected. For example, Briles, Allen and Millen (1957) found segregation at the B locus in 71 of 73 closed populations. Gilmour (1959) reported B locus segregation even in lines with inbreeding coefficients over 95 percent. Clearly, a biological measure of genetic diversity would be a useful supplement to Wright's classical coefficient of inbreeding.

A biological measure of genetic diversity was proposed by Berry and Craig (1959). Their method was based on lymphocyte counts following skin grafting between individuals of different relationships. They concluded that their method was not useful because it was insensitive to breed differences.

Measures of genetic diversity between lines could be developed from procedures suggested by Billingham et al. (1962) and Chai and Chiang (1963). These methods are designed to count H loci differences between two inbred lines. In a sense these are measures of relationship or dissimilarity between pairs of lines. They do not measure genetic diversity in a single line.

Crossing a tester line

From the same general principle as the Billingham et al. (1962) and Chai and Chiang (1963) methods, a tester inbred line might be used to obtain in biological measure of genetic diversity in a single population or inbred line. We require that the tester inbred is essentially isogenic. We assume that the H genes have equal, although not necessarily small effects, that the antigenic effects of the genes are discrete, and that we have multiple alleles at each locus. The number of segregating loci from the progeny of a cross of the tester line and an unknown line can be estimated. For example, consider a simple case of 3 H loci, A, B, and C.

$$\text{Genotype: } \begin{array}{c} \text{Tester Line} \\ \hline A_1 A_1 B_1 B_1 C_1 C_1 \end{array} \times \begin{array}{c} \text{Unknown Line} \\ \hline A_2 A_3 B_2 B_3 C_2 C_2 \end{array}$$

↓

Kinds of Progeny Genotypes

1. $A_1 A_2 B_1 B_2 C_1 C_2$
2. $A_1 A_2 B_1 B_3 C_1 C_2$
3. $A_1 A_3 B_1 B_2 C_1 C_2$
4. $A_1 A_3 B_1 B_3 C_1 C_2$

The probability of a successful graft exchange (S) is a function of the number of progeny genotypes, n

$$S = 1/2^n$$

If the unknown line is adequately sampled then the average number, \bar{n} , of segregating loci in the line from a random sample of several matings can be estimated from

$$\bar{S} = 1/2^{\bar{n}}$$

$$\bar{n} = \log \bar{S} / \log 1/2$$

The major difficulty in applying this method would be to develop an isogenic line. In mice near-isogenic lines are available. For chickens a reasonably highly inbred line might give useful approximate estimates.

The assumption that all loci have equal effects may not be realistic. We know that in both mice and chickens strong and weak histocompatibility antigens exist. Results found in this study, as well as results for mice reported in the literature, demonstrate that strong H genes mask effects of weak H genes. However, this problem could be handled step-wise: for an unknown population or line of chickens the B locus genes could first be identified by either or both blood typing and histocompatibility testing. The number of remaining segregating H loci all having weak effects could then be estimated. Finally, this method also requires the assumption that each weak allele can be uniquely identified by the effects of its corresponding weak antigen. Whether current laboratory procedures may be sufficiently sensitive to detect all single weak antigen effects remains yet to be investigated.

Empirical Models for Measuring Genetic Diversity

Genetic diversity may also be estimated empirically without counting H loci differences. In this study we obtained information on genetic diversity within lines in two ways: from graft exchanges between individuals in a line without regard to sib-groups (Method A), and from graft exchanges within sib-groups in a line (Method B).

Method A

We could use either one of two models. In Model I we assume that the phenotypic character is a threshold: the graft is either accepted or rejected but the underlying genetic scale is continuous, i.e. the effects of genes are cumulative.

In Model II we assume that the phenotypic character is a continuous quantitative trait such as the mean survival time for an arbitrary test period. Model II would seem to be operationally most practical and also would have the simplest underlying genetic mechanism.

In the case of either Model I or II the assumption is that the variance of compatibility is an unbiased sample measurement of the total genetic variance in the population.

The results shown in Figure 5 would correspond to Model II and its underlying genetic theory. The results suggest that the relationship between F_x and MST is linear. The regression line was fitted to the mean survival time as the dependent variable and the calculated inbreeding coefficient (F_x) as the independent variable. To apply these results to estimate the genetic diversity in a population then the independent and dependent variables would be reversed.

Method B

Sib-group differences in compatibility within a line may also be an indicator of genetic diversity. As with Method A the assumptions are that histocompatibility is due to many genes, that their effects are cumulative and that the effects of inbreeding on the variance of compatibility is equal to its effects on the overall additive genetic variance.

Let σ_G^2 represent the genetic variance for histocompatibility and let F_x be Wright's inbreeding coefficient. Then the additive variance within sib-groups would be:

$$\begin{aligned} \text{Full-sibs} &= 1/2 \sigma_G^2 (1 - F_x) \\ \text{Half-sibs} &= 3/4 \sigma_G^2 (1 - F_x) \\ \text{Non-sibs} &= \sigma_G^2 (1 - F_x) \end{aligned}$$

Thus, theoretically the genetic variance among individuals of full-sib groups is less than among half-sibs which in turn is less than among non-sibs. Since the major consequences of inbreeding is to reduce heterozygosity, genetic variance is reduced. Thus, for a non-inbred line the genetic variance for full-sibs, half-sibs, and non-sibs would be proportional to 1/2:3/4:1, respectively, and for a line with $F_x = .50$, proportional to 1/4:3/8:1/2. As inbreeding increases, the amount of genetic variance would decrease correspondingly for each of the three sib-groups. Hence, the relative differences between the compatibility levels of full-sibs (FS), half-sibs (HS), and non-sibs (NS) would become less as inbreeding increases. Based on this reasoning the magnitude of sib-group differences can be used to compare the relative amount of inbreeding between lines: for lines not highly inbred, sib-group differences

should be distinct with compatibility levels such that $FS > HS > NS$.

On the other hand, for lines highly inbred, the differences between sib-groups would be small and would likely not be consistent.

In using the empirical Methods A or B, the assumption that the H alleles have equal effects would not seem to be critically important. In fact, we could have assumed, alternatively, that the effects of the H genes were normally distributed. Also we would need to take into account the compatibility at the B locus. Since compatibility at the B locus accounted for 75% variance in graft acceptance in this study, the hypothesis of one major H locus with multiple alleles and no minor H loci seems to be the most realistic for the species G. domesticus. The highly inbred line HN (inbreeding (F_x) = 86%), with only two segregating alleles at the B locus, had a high graft acceptance level with small and inconsistent differences among the sib-groups. For L8 and GH with inbreeding coefficients of 65% and 58%, respectively, the order of sib-group compatibility was consistently $FS > HS > NS$. The results for these lines agree with genetic theory. However, for Line 9 with a moderate coefficient of inbreeding (F_x = 54%) and only 2 segregating B alleles, the acceptance level for all sib-groups was low. In this line the chicks for graft exchanges were picked at random from non-blood typed families. The results found would be expected only if there was no "interference" from strong H alleles. Cumulative effects of minor H genes have been reported by Graff et al. (1966) in mice. When we consider the control Line R, in which the B locus compatibility was controlled, the cause for rejection was assumed to be due to minor H genes. The rate of rejection of grafts, compatible at the B locus, was slow but persistent over the entire

observation period (Fig. 6). Counce et al., (1956) reported that rejection due to weak H genes in mice may be delayed for as long as 200 days after grafting.

If the major H gene in a population is fixed, the rejection response is expected to be slow. The results obtained in the SP line indicates that the B locus is fixed, and that the rejection pattern after 16 days is determined by minor H genes. The B locus fixation coupled with a high inbreeding level would seem to be prerequisite for the high mean survival time observed in all sib-groups in Line SP. Figure 10 shows that the estimated inbreeding coefficient (based on effective population size) was lower than expected from the empirical model. Because Wright's F_x method shows inbreeding relative to a specific base population, it may well be that Line SP is a highly homozygous line. Thus, SP may have been inbred before it was brought to Iowa State from University of Minnesota. It must be recognized F_x is not valid for comparisons of relative heterozygosity between lines unless they have common origin. In contrast, for a biological method such a comparison would be valid irrespective of the origin of the lines.

The results of graft exchanges between and among F_1 hybrids of two lines would be difficult to interpret unless one line is fixed for certain loci. If two lines are highly inbred, the F_1 crosses should show compatibility not much less than that of the inbred lines themselves. This was our underlying reason for making line crosses in our study. The GL x L9 cross showed that when the B locus is fixed in each line, the acceptance level in the cross may be high.

When the variance in B locus compatibility versus incompatibility

is taken into account the sib-group effect was a minor factor in determining acceptance or rejection of skin grafts. This is probably because the inbreeding level in all the lines used in this study except Line R was quite high.

Coturnix for Immunogenetics Studies

Among the avian species, the chicken has been the leading bird for immunological studies. The history of long association with man and its economic importance places it in a class by itself. Other species, in particular, pigeons and doves have been intensively used in some immunogenetic laboratories for immunogenetics studies. The Coturnix quail, although relatively new as a laboratory animal, is becoming of increasing importance for this purpose. So far it has been used little for immunological studies. Its short generation interval and high reproductive rate, in addition to its low cost maintenance requirements, make it an attractive species for consideration as a research animal for studies in immunogenetics.

The skin grafting experiment in this study with the Coturnix showed that immunological competency¹ develops well before the 14th day of age and possibly as early as 6th or 7th day. From the limited data collected, half the grafts exchanged between unrelated progeny accepted homografts. This suggests that the population used contained substantial inbreeding. On the other hand, immunological tolerance seems to be the likely

¹Here, my use of the term "immune competency" applies specifically to skin transplantation phenomena.

explanation for the nearly total acceptance of homografts at one to five days of age.

The fact that Coturnix matures in approximately 35 days after hatch leads to speculation that these birds may be immunologically mature at a very early age. There seems to be no relationship between early maturity and development of immunological competence. In chickens, for example, the development of immune responsiveness occurs at two days of age whereas reproductive maturity is reached after about 140 days. However, early maturity in Coturnix presents a special problem because the young males start to mount females at about four weeks of age, and this may interfere with feather growth and graft development in the females.

Mixed Leukocyte Cultures

An in vitro immune response due to antigenic stimulation in incompatible mixed leukocyte cultures was demonstrated in chickens. The level of genetic disparity between the two sources of cells in the mixed leukocyte culture is indicated by the rate of cellular proliferation. The mixed cell culture from two unrelated inbred lines displayed the highest growth rate while homologous cell cultures from the same line showed the lowest.

The report of Silvers et al. (1967) with rats showing the importance of a single gene difference at a major H locus on lymphocyte stimulation, was confirmed by our study with chickens. This increases the optimism that this technique may be useful as an in vitro histocompatibility test. In the case of chickens we have demonstrated that incompatibility at the B locus can be detected without breeding tests or

without blood typing. The technique we used for our study (Bach and Hirshhorn, (1964) lacks the sophistication of more recent methods. Bach and Voynow (1966) showed that mitomycin C can be used to inactivate one line of cells (stop proliferation) but still retain the capability to stimulate the other cell type. Thus, it is possible to test whether one type of cell is more antigenic than the other. However, the simplicity of the older technique which we used has merit: no complicated equipment or complex media are required.

The results obtained in Experiment 3 showed that the nutrient media influences cell proliferation in the leukocyte culture. Cell cultures in heterologous sera proliferate at a higher rate than in homologous serum cultures. In either case, a single B locus difference between the cell types stimulates cell proliferation. The rate of proliferation depends on the number of antigens of segregating H locus alleles including the B locus. The maximum stimulation is elicited by cell types widely differing while cell cultures of homologous types show little or no proliferation.

The mixed leukocyte technique might also be used as an index of genetic diversity within and between lines. It would seem that the method might be a faster and simpler method than the skin grafting technique. However, this is a problem that awaits experimental exploration.

CONCLUSIONS

1. Compatibility at the B locus was a prerequisite to successful skin graft exchanges in the lines used in this study. This is in agreement with previous studies (Schierman and Nordskog, 1961; Gilmour, 1962; and Craig and McDermid, 1964).
2. There was a linear relationship between mean survival time of skin grafts and Wright's inbreeding coefficient. This suggests that genetic diversity in a population can be estimated by skin graft exchanges among individuals in a population or inbred line.
3. Using mixed leukocyte cultures, the importance of a single major histocompatibility locus can be demonstrated in chickens. When cells of two different inbred lines were mixed, cell proliferation was maximal. These results are in agreement with an earlier study in rats involving a difference at a single histocompatibility locus (Silvers et al. 1967).
4. Coturnix may be a useful laboratory species for immunological studies. Early feathering of skin grafts, low maintenance cost, a high reproductive rate, early maturity, and rapid development including feather development on grafts makes Coturnix a desirable candidate for further experimental exploration.

SUMMARY

Four Leghorn inbred lines HN, L8, GH and L9, one Spanish inbred SP, and one non-inbred Leghorn control line R, were used in 12 skin grafting experiments conducted in 1969 and 1970. In addition, certain crosses between these lines were grafted in 1970. The pure line chicks were blood typed in 1970 experiments but not in the 1969 experiments. The total number of individuals grafted and total number of grafts were 299 and 897 in 1969 and 413 and 1275 in 1970, respectively. In addition, 18 GH and six L9 adult chickens were used in four mixed leukocyte culture experiments. The objectives of the study were (1) to investigate skin graft acceptance between individuals of a line as a method to estimate the degree of genetic diversity (inbreeding), (2) to determine the relative importance of the B locus blood group on the graft rejection pattern in different lines, (3) to compare the rejection pattern of the F_1 crosses of highly inbred lines with the pure lines, (4) to test the practicability of the chicken skin grafting technique in the Coturnix quail and (5) to test the application of mixed leukocyte culture technique in chickens as an in vitro measure of histocompatibility.

Graft acceptance was highest in the inbred lines as indicated by their mean survival time (MST) in comparison with the low inbred and with a control line. The relationship between Wright's inbreeding coefficient and MST of skin grafts was linear indicating that skin grafting might serve as a basis for a biological measure of inbreeding.

In the 1970 experiments incompatibility of the B blood group locus accounted for 75 percent of the total variance among all lines. The

results confirm the hypothesis that there is one major H locus and several minor H loci which corresponds to the histocompatibility systems in mice.

The results from the line crosses show that when two lines are highly inbred, compatibility of the F_1 cross is not much less than that of the inbred lines themselves. When the B locus is fixed in each line the acceptance level in the cross is usually high. This emphasizes the importance of the role of the B group locus as a major H system.

Possible methods to measure genetic diversity from skin graft reactions were considered theoretically. One method is to estimate the number of segregating H loci from skin graft acceptances among the F_1 of a known tester line and some unknown line under study. Ideally the tester line should be isogenic which is essentially attainable in mice but perhaps not in chickens. The assumption that each allele with weak effects can be recognized by a graft response in a short observation period may not be wholly realistic.

Other more practical but empirical methods to measure genetic diversity were considered. In Method A, genetic diversity is measured from graft exchanges between individuals in a line without regard to sib-groups. We assumed that the phenotypic character is a continuous quantitative trait such as the MST of a graft for an arbitrary period of time. This model seemed to fit the observed regression of survival time of grafts on calculated inbreeding.

In Method B genetic diversity in a line was based on sib-group differences in compatibility. The assumptions are that histocompatibility is due to many genes, that their effects are cumulative and that inbreeding reduces the variance of compatibility proportionately to its reduction on

overall additive genetic variance. With a random bred population rejection of graft exchanges within sib-groups would be expected in ratio of 1/2:3/4:1 for full-sibs, half-sibs, and non-sibs respectively. Since inbreeding reduces heterozygosity these ratios would change according to the level of inbreeding. Our observed results agree reasonably well with genetic theory; highly inbred lines accept graft exchanges between non-sibs just about as well as between full-sibs.

Chicken skin grafting techniques were successfully applied to the Coturnix quail. The short generation interval, high reproduction rate and low maintenance requirements makes the Coturnix a potentially useful laboratory animal for immunological research. The young Coturnix seems to maintain immune unresponsiveness up to at least five days of age. This is in contrast to chickens which are immune tolerant only to two days of age.

Using the mixed leukocyte culture technique the importance of a single major H locus (the B blood group locus) was demonstrated. This confirms a similar study showing the importance of a major H locus in mice (Silvers, et al. 1967). These studies strengthen the possibility of the mixed leukocyte culture technique as a useful in vitro measure of histocompatibility.

GLOSSARY

Allograft denotes a graft between genetically different individuals of the same species, for example grafts between two lines of chickens.

In this study it is used synonymously with homograft.

Autograft denotes a graft transplanted to a new site on the same individual.

Coisogenic strains are genetically identical except for a difference at a single locus. In practice coisogenicity is probably never attainable.

Congenetic strains are identical for certain groups of loci but are different at a single locus in this group.

Histocompatibility is relevant to the acceptance or failure of a tissue transplant to grow and survive.

Isoisogenic strains are strains with genetically identical members.

Mean survival time (MST) is the average survival time in days obtained by summing the days of acceptance for each graft and dividing by the number of grafts. The maximum given for each graft in this study was 26 days.

Median survival time refers to the time at which 50 percent of transplants are rejected. This method was not appropriate in this study because most graft exchanges among individuals of an inbred line survived so that the 50 percent rejection level was never attained.

BIBLIOGRAPHY

- Amos, D. B.
1969 Genetic and antigenic aspects of human histocompatibility systems in man. *Advances in Immunology* 10: 251-297.
- Bach, F. H.
1970 Transplantation: pairing of donor and recipient. *Science* 168: 1170-1179.
- Bach, F. H. and K. Hirshhorn.
1964 Lymphocyte interaction: a potential histocompatibility test in vitro. *Science* 143: 813-814.
- Bach, F. H. and W. A. Kiskan.
1967 Predictive value of results of mixed leukocyte cultures for skin allograft survival. *Transplantation* 5: 1046-1052.
- Bach, F. H. and N. K. Voynow.
1966 One-way stimulation in mixed leukocyte cultures. *Science* 153: 545.
- Bacon, L. D. and J. V. Craig.
1966 Evidence for a histocompatibility locus on the W-chromosome in chicken. *Poultry Science* 45: 1066. (Abstract)
- Bailey, D. W.
1963 Histoincompatibility associated with the X-chromosome in mice. *Transplantation* 1: 70-74.
- Bailey, D. W.
1970 Four approaches to estimating number of histocompatibility loci. *Transplantation Proceedings* 2: 32-38.
- Bain, B., M. R. Vas, and L. Lowenstein.
1964 The development of large immature mononuclear cells in mixed leukocyte cultures. *Blood* 23 :108-116.
- Basch, R. S. and C. A. Stetson.
1962 Quantitative studies on histocompatibility antigens of the mouse. *Transplantation* 1: 469-480.
- Batchelor, J. R. and B. A. Chapman.
1967 Genetic background and transplantation antigens. *J. of Clinical Pathology* 20: 415-422.
- Berry, J. E. and J. V. Craig.
1959 Estimation of genetic diversity among strains and breeds of chicken by lymphocytes increases following skin grafting. *Poultry Science* 38: 439-442.

- Berry, J. E., J. V. Craig and G. K. L. Underbjerg.
 1958 A comparison of the effects of skin implants on the cellular constituents of chicken blood. *Poultry Science* 37: 312-316.
- Billingham, R. E., E. L. Brent, and P. B. Medawar.
 1954 Quantitative studies on tissue transplantation immunity. II The origin, strength and duration of actively and adaptively acquired immunity. *Roy. Soc. Proc.* 143: 58.
- Billingham, R. E. and G. H. Lampkin.
 1957 Further studies in tissue homotransplantation in cattle. *J. Embryol. Exper. Morphol.* 5: 351-367.
- Billingham, R. E., B. A. Hodge, and W. K. Silvers.
 1962 An estimate of the number of histocompatibility loci in rat. *Nat. Acad. Sci. Proc.* 48: 138-147.
- Billingham, R. E. and W. K. Silvers.
 1959 Inbred animals and tissue transplantation immunity. *Transplantation Bulletin* 6: 399-403.
- Billingham, R. E. and W. K. Silvers.
 1963 Sensitivity to homografts of normal tissues and cells. *Annual Review of Microbiology* 17: 531-564.
- Billingham, R. E., W. K. Silvers, and D. B. Wilson.
 1965 A second study on the H-Y transplantation antigen in mice. *Proc. Royal Soc. Proc., Ser. B*, 163: 61.
- Billingham, R. E. and E. M. Sparrow.
 1954 Studies on the nature of immunity to homologous grafted skin with special reference to use of pure epidermis grafts. *British Journal of Experimental Biology* 31: 16-39.
- Brent, L., J. B. Brown, and P. B. Medawar.
 1958 Skin transplantation immunity in relation to hypersensitivity. *Lancet* ii: 561-564.
- Brent, L. and P. B. Medawar.
 1963 Tissue transplantation: a new approach to the typing problem. *Br. Med. J.* 129: 269-272.
- Briles, W. E.
 1954 Evidence of overdominance of the B blood group alleles in the chicken. *Genetics* 39: 961-962. (Abstract)
- Briles, W. E.
 1962 Additional blood group systems in the chicken. *New York Academy of Sciences Annals* 97: 173-183.

- Briles, W. E., C. P. Allen, and T. W. Millen.
1957 The B blood group systems of chickens. I Heterozygosity in closed populations. *Genetics* 42: 631-648.
- Brownlee, K. A. and D. Hamre.
1950 Studies on chemotherapy of Vaccinia virus. *J. Bacteriology* 61: 126-134.
- Cannon, J. A. and W. P. Longmire, Jr.
1952 Studies on successful homografts in the chicken. *Ann. Surg.* 135: 60-68.
- Chai, C. K. and M. S. Chiang.
1963 A method of estimating the number of histocompatibility loci in a sib-mating mouse population. *Genetics* 48: 1153-1161.
- Cock, A. G. and M. Clough.
1956 Successful skin homografts in inbred chickens. *Nature* 178: 136-137.
- Cockerham, C. C.
1965 Notes on quantitative genetics. Section 5. Unpublished mimeographed paper presented at 1965 Summer Institute of Quantitative Genetics, Brown University, Providence R.I., Raleigh, North Carolina, N. C. State University, Department of Statistics.
- Converse, J. M. and F. T. Rapaport.
1956 The vascularization of the skin autografts and homografts. An experimental study in man. *Ann. Surg.* 143: 306.
- Counce, S., P. Smith, R. Barth, and G. D. Snell.
1956 Strong and weak histocompatibility gene differences in mice and their role in rejection of homografts of tumors and skin. *Ann. Surg.* 144: 198-204.
- Craig, J. V. and L. J. Hirsch.
1957 Genetic relationship and reactions to skin grafts. *J. Heredity* 48: 235-237.
- Craig, J. V. and E. M. McDermid.
1964 Prolonged skin homograft survival and erythrocyte (B-locus) antigens in young chicks. *Transplantation* 1: 191-200.
- Craig, J. V., C. R. Polley, and S. Wearden.
1960 Estimation of genetic diversity by skin graft reactions in young chicks. *Poultry Science* 39: 1533-1540.
- Eichwald, E. J. and C. R. Silmsler.
1955 Communication. *Transplantation Bulletin* 2: 148-149.

- Eichwald E. J., W. Wetzel and E. C. Lustagraaf.
1966 Genetic aspects of second-set skin grafts in mice. *Transplantation* 4: 260-273.
- Elandt-Johnson, R. C.
1969 Survey of histocompatibility testing. *Biometrics* 25: 208-283.
- Elves, M. W., S. Roath, G. Taylor, and M. C. G. Israel.
1963 In vitro production of antibody by lymphocytes. *Lancet* i: 1292.
- Emik, L. O. and C. E. Terrill.
1949 Systematic procedures for calculating inbreeding coefficients. *J. Heredity* 40: 51-55.
- Falconer, D. S.
1967 Introduction to quantitative genetics. The Ronald Press Company, New York.
- Gilmour, D. G.
1963 Strong histocompatibility effects associated with the B blood group system of chickens. *Heredity* 8: 123 (Abstract).
- Gilmour, D. G.
1958 Maintenance of segregation of blood group genes during inbreeding in chickens. *Heredity* 12: 141-142. (Abstract)
- Gilmour, D. G.
1959 Blood groups in chickens: a review. Munich, Report of the 8th International Blood Group Congress. 50-79.
- Gilmour, D. G.
1962 Current status of blood groups in chickens. *New York Academy of Sciences Annals* 97: 166-172.
- Gilmour, D. G.
1967 Histocompatibility antigen in the heterogametic sex in the chicken. *Transplantation* 5: 699-706.
- Gleason, R. E. and R. C. Fanguy.
1964 The relationship of blood group to skin graft survival in chickens. *Transplantation* 2: 509-514.
- Gleason, R. E. and J. E. Murray.
1967 Report from kidney transplant Registry: Analysis of variables in the function of human kidney transplants. *Transplantation* 5: 343-359.
- Gorer, P. A.
1937 The genetic and antigenic basis of tumor transplantation. *J. Path. Bact.* 47: 231-252.

- Gorer, P. A., S. Lyman, and G. D. Snell
1948 Studies on the genetic linkage and antigenic basis of tumor transplantation. Linkage between a histocompatibility gene and 'fused' in mice. Roy. Soc. (London) Proc., Ser. B, 135: 499-505.
- Graff, R. J., W. K. Silvers, R. E. Billingham, W. H. Hildelman, and G. D. Snell.
1966 The cumulative effect of histocompatibility antigens. Transplantation 4: 605-617.
- Hasek, M., A. Lengerova, and T. Hraba.
1961 Transplantation immunity and tolerance. Advances in Immunology 1: 1-66.
- Hume, D. M., J. P. Merrill, B. F. Miller, and G. W. Thorn.
1955 Experiences with renal homotransplantation in the human: Report of nine cases. J. Clinical Investigation 34: 327-382.
- Kempthorne, O.
1969 An introduction to genetics statistics. Iowa State University Press. Ames, Iowa.
- King, H. D.
1935 (Quoted by Waters, N. F. and W. V. Lambert in Inbreeding in the White Leghorn fowl). Agricultural Experiment Station, Iowa State College of Agriculture and Mechanic Arts, Research Bulletin No. 202. June, 1936, Ames, Iowa.
- Kozelka, A. W.
1929 Integumental grafting in the domestic fowl. J. Heredity 20: 3-14.
- Kozelka, A. W.
1932 Integumental grafting as a means of analyzing the factors determining the secondary sexual characters of the Leghorn fowl. J. Exp. Zool. 61: 431-484.
- Landsteiner, K. and C. P. Miller.
1924 On individual differences of the blood of chickens and ducks. Proc. Soc. Exper. Biol. and Med. 22: 100-102.
- Lawrence, H. S., F. T. Rapaport, J. M. Converse, and W. S. Tillett.
1960 The transfer of homograft sensitivity (accelerated rejection) with DNase-treated leukocyte in man. New York Academy of Sciences Annals 87: 223-228.

- Little, C. C.
1914 A possible Mendelian explanation for a type of inheritance apparently non-Mendelian in nature. *Science* 40: 904-906.
- Little, C. C. and B. W. Johnson.
1922 The inheritance of susceptibility to implants of splenic tissue in mice. *Soc. Exper. Biol. and Med. Proc.* 19: 163-167.
- Lush, J.
1945 *Animal Breeding Plans*. 3rd. Edition. The Collegiate Press, Ames, Iowa.
- Marshall, W. H. and K. B. Roberts.
1963 Tuberculin induced mitosis in peripheral blood. *Lancet* i: 773.
- McKhann, C. F.
1962 Weak histocompatibility genes: the effect of dose and pre-treatment of immunizing cells. *J. Imm.* 88: 500-504.
- McKhann, C. F.
1964a Transplantation studies of strong and weak histocompatibility barriers in mice. I Immunization. *Transplantation* 2: 613-619.
- McKhann, C. F.
1964b Transplantation studies of strong and weak histocompatibility barriers in mice. II Tolerance. *Transplantation* 2: 620-626.
- Medawar, P. B.
1944 Behaviour and fate of skin autografts and skin homografts in rabbits. *J. Anat.* 78: 176.
- Medawar, P. B.
1945 A second study of the behaviour and fate of skin homografts in rabbits. *J. Anat.* 78: 176.
- Merrill, J. P., J. E. Murray, J. H. Harrison, and W. R. Guild.
1956 Successful homotransplantation of the human kidney between identical twins. *J. A. M. A.* 160: 277.
- Michison, N. A.
1953 Passive transfer of transplantation immunity. *Nature* 171: 267-268.
- Miller, R. A.
1938 Spermatogenesis in a sex-reversed female and normal males of the domestic fowl, Gallus domesticus. *Anatomical Record* 70: 155-183.

- Nowell, P. C.
1960 Phytohemagglutinin: An initiator of mitosis in cultures of normal human leukocytes. *Cancer Research* 20: 462-466.
- Owen, R. D.
1959 Genetic aspects of tissue transplantation and tolerance. *Journal of Medical Education* 34: 366-383.
- Polley, C. R., A. E. Grosse, and J. V. Craig.
1960 A skin grafting technique for use in genetic studies with chickens. *Transplantation Bulletin* 7: 425-428.
- Rapaport, F. T. and J. M. Converse.
1958 The immune response to multiple-set skin homografts. An experimental study in man. *Annals of Surgery* 147: 273-280.
- Rishell, W. A.
1968 Adaptive value of B locus blood group genes in White Leghorns. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Shawan, H. K.
1919 The principle of blood grouping applied to skin grafting. *Am. J. Med. Sci.* 157: 503-509.
- Schinkel, P. G. and K. A. Ferguson.
1953 Skin transplantation in foetal lamb. *Aust. J. Biol. Sci.* 6: 533-546.
- Schierman, L. W.
1961 Determination of blood group genotypes in an inbred line of Leghorn. Unpublished M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Schierman, L. W.
1962 Genetic analysis of transplantation immunity in the fowl. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Schierman, L. W. and A. W. Nordskog.
1961 Relationship of blood type to histocompatibility in chickens. *Science* 134: 1008-1009.
- Schierman, L. W. and A. W. Nordskog.
1964 Immunogenetic studies with fowl: Relationship of blood groups to transplantation immunity and tolerance. *New York Academy of Sciences Annals* 120: 348.

Schmid, W.

- 1964 DNA replication patterns of heterochromosomes in Gallus domesticus. Cytogenetics 1: 344.

Shreffler, D. C. and D. G. Snell.

- 1969 The distribution of thirteen H-2 Alloantigenic specificities among the products of eighteen H-2 alleles. Transplantation 8: 435-450.

Shreffler, D. C. and J. Klein.

- 1970 Genetic organization and gene action of mouse H-2 region. Transplantation Proceedings 2: 5-14.

Shrek, R. and W. Donnelly.

- 1961 Differences between lymphocytes of leukemic patients with respect to morphologic features, mobility, and sensitivity to guinea pig serum. Blood 18: 561.

Silvers, W. K., D. B. Wilson, and J. Palm.

- 1967 Mixed leukocyte reaction and histocompatibility in rats. Science 155: 703-704.

Snell, G. D.

- 1948 Methods for the study of histocompatibility genes. J. Genetics 49: 87-105.

Snell, G. D.

- 1958 Histocompatibility genes of the mouse. Production and analysis of isogenic resistant lines. J. Nat. Cancer Inst. 21: 843-877.

Solomon, J. B.

- 1963 Ontogenesis of immunological competence of lymphocytes in chicken. Transplantation 1: 327-333.

Starlz, T. E., T. L. Machioro, J. H. Holmes, G. Hermann, R. S. Brittain, O. H. Stanington, D. W. Talmage, and W. R. Waddell.

- 1964 Renal homografts in patients with major donor recipient blood group incompatibilities. Surgery 55: 195-200.

Starlz, T. E., T. L. Machioro, P. I. Terasaki, K. A. Porter, T. D. Faris, T. J. Herrmann, D. L. Vrederoe, M. P. Hutt, D. A. Ogden, and W. R. Waddell.

- 1965 Chronic survival after human renal homotransplantation. Ann. Surg. 162: 749-787.

Stephenson, A. B., A. J. Wyatt, and A. W. Nordskog.

- 1953 Influence of inbreeding on egg production in the domestic fowl. Poultry Science 32: 510-517.

- Tolman, H.
1969 Inbreeding in small populations of fowl undergoing selection. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Tyzer, E. E.
1909 A study of inheritance in mice with reference to their susceptibility to transplantable tumor. J. Med. Research 21: 519.
- Waters, N. F. and W. V. Lambert.
1936 Inbreeding in the White Leghorn fowl. Agricultural Experiment Station, Iowa State College of Agriculture and Mechanic Arts, Research Bulletin No. 202, Ames, Iowa.
- Wilson, W. O.
1948a Egg production rate and fertility in inbred chickens. Poultry Science 27: 719-726.
- Wilson, W. O.
1948b Viability of embryos and of chicks in inbred chickens. Poultry Science 27: 727-735.
- Wilson, R. E., L. Henry, and J. B. Merrill.
1963 A model system for determining histocompatibility in man. J. Clinical Investigation 42: 1497.
- Woodruff, M. F. A.
1957 Can tolerance skin be induced in the human infant at birth? Transplantation Bulletin 4: 26-28.
- Wright, S.
1922 Coefficients of inbreeding and relationship. Amer. Nat., 56: 330-338.
- Wright, S.
1931 Evolution in Mendelian populations. Genetics 16: 97-159.
- Yoffey, J. M. and F. C. Courtice.
1956 Lymphatics and lymphoid tissues. London, Edward Arnold.

ACKNOWLEDGMENTS

The author wishes to express sincere appreciation to Dr. A. W. Nordskog for his interest, understanding, and wise counsel during the course of this study and preparation of the manuscript. Also the author wishes to recognize Dr. M. L. Kaeberle for many valuable suggestions; and Dr. L. N. Hazel for the supply of swine serum used in cell culture experiments.

The valuable assistance given to the author by fellow graduate students and the staff members, Iowa State University Poultry Science Department is gratefully acknowledged.

Finally, sincere gratitude goes to the author's wife Leah Tirindi for her constant encouragement and understanding.