THE SKIN-GRAFT REACTION AS A MEASURE OF GENETIC DIVERSITY IN CHICKS

by

CARL R. POLLEY

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INTRODUCTION

All mating systems may be described in terms of relationship or degree of genetic diversity between individual parents or mating groups. The results of a mating system are commonly measured in terms of progeny performance.

A technique which would measure genetic diversity of parents or mating groups might be of valuable assistance to the population geneticist in indicating matings to be made in order to reduce heterozygosity or to maximize heterozygosity. Other applications might include the use of such a method to check the reliability of calculated relationship and inbreeding coefficients within inbred lines.

The major objective of this study was to determine if a technique could be developed which would measure genetic diversity over a wide range with relatively good reliability, yet require less time and labor than current techniques. Other objectives included: a comparison of the homograft reaction severity between two different breeds of chickens, estimates of sex of host and age effects, estimates of the heritability of the homograft reaction, exploration of the possibility of sex-linkage and/or maternal effects, and an estimate of the importance of the bursa of Fabricius on homograft reactions.

REVIEW OF LITERATURE

Loeb and Wright (1927) using guinea pigs, and Loeb and King (1927) with rats, were able to demonstrate reductions in genetic heterozygosity due to inbreeding by means of tissue transplant studies. They found an inverse association between degree of genetic relationship of donors and hosts and

severity of immune reactions based on subjective scores of histological sections. Loeb and McPhee (1931) exchanged tissue in guinea pigs over a wide range of genetic diversity and found the severity of reaction, as measured by histological sections, directly associated with genetic diversity. Koselka (1932) working with integumental grafts in one- to three-day-old chicks showed a clear-cut association between percentage permanent survivals, i.e., grafts which persisted over a one year period, and relationship. Loeb and Siebert (1935) working with chickens, found that as genetic diversity increased, the severity of lymphocytic attack on the transplanted tissue increased and the time required for lymphatic infiltration after grafting decreased. Blumenthal (1939) counted total leucocytes and percentages of lymphocytes in the circulating blood of several species prior to and following tissue transplantation, and found higher postoperative counts and percentages as genetic relationship of donor and host decreased.

Craig and Hirsch (1957) and Berry et al. (1958a) using chickens, modified Blumenthal's (1939) technique and were able to demonstrate significant inverse associations between the relative increase in circulating lymphocytes following skin grafting and relationship coefficients of donors and hosts. Craig and Hirsch (1957) using five-week-old chicks, also found greater responses to grafts between breeds than to those made within a breed. However, Berry and Craig (1959) using year-old chickens in an extensive experiment, were unable to demonstrate any greater reaction to skin grafts exchanged between breeds than when exchanges were between essentially unrelated birds of the same closed strains, suggesting that a plateau of response had been reached. Blumenthal (1939) postulated a plateau of response when he indicated there was a limit to the power of the host to discriminate

between different degrees of relationship of the donor. His theory was that after a certain threshold of genetic diversity had been reached, the reaction was maximal and could not be increased by transplants of tissue from donors of greater genetic diversity. This theory applied to heterografts (grafts made between species).

Agdahl and Varco (1956) and Berry et al. (1958b) utilized a technique of fluorescein removal from skin grafts as a measure of the homograft reaction. The latter authors found this unsatisfactory for distinguishing between wide levels of genetic diversity.

Cock and Clough (1956) found the homograft reaction to be absent when skin exchanges were made between full sibs and non-full sibs of highly inbred lines of chickens, although a small percentage of the grafts did disappear at an early stage.

Taylor and Lehrfeld (1955) describe a simple in vivo technique utilizing a dissecting microscope to determine vascular blood stasis in the graft which they define as the end-point of graft viability. This technique has been used successfully in both mice and rabbits.

Snell (1953) suggests that susceptibility and resistance to tumor transplants is governed by multiple dominant Mendelian genes called "histocompatibility genes" which have been designated as "strong" or "weak" depending on the ability of a tumor carrying the gene to survive on a host lacking it.

Edgerton et al. (1957) postulate that determination of length of homograft survival by gross slough of all grafted tissue or cessation of all graft circulation will lead to widely varying results. They used instead, the sudden reduction in caliber of the large graft vessels and the slowing

of blood flow in these vessels as the end-point of graft viability.

Anderson et al. (1951) report the relative tolerance of skin grafts made between monozygotic and dizygotic twins in cattle. They found chronic low grade reactions to skin grafts between some but not all dizygotic twin pairs. Billingham et al. (1952) found a large proportion of dizygotic twin calves were completely tolerant to skin exchanges between them and concluded that this technique was unsuitable for differentiation between monozygotic and dizygotic twin calves. Anderson et al. (1951) and Billingham et al. (1952) suggest the failure of the expected homograft reaction to appear is due to the vascular anastomosis of placental blood vessels as found in the cases of twin calves reported by Owen (1945) and earlier by Lillie (1917). Owen (1945) also estimated the number of monozygotic twins to be small compared to dizygotic twins. He found placental anastomosis between bovine twins which resulted in the calves having the same blood antigens although one or both might be lacking genes necessary for these antigens. Owen et al. (1946) reported a case involving quintuplet calves (four bulls and one heifer). Although the blood of all five, when tested for inherited cellular antigens, was found to be identical, the authors contend the calves developed from five different fertilized eggs, and shared a common circulation due to placental anastomosis.

Cannon et al. (1954) working with chicks, postulated two independent factors responsible for the homograft reaction:

1. antigenicity of the transplanted tissue which is developing at the time of hatching and is complete by the fourteenth day post-hatching, and

2. the immunity response potential of the host which is also developing at hatching but is complete by the seventh day post-hatching.

Terasaki et al. (1957) and others have reported a "dosage effect" on the survival of homografts. These investigators found larger grafts survive longer in chicks and cite Billingham et al. (1956) theory that a large graft on a relatively weak immunological system may survive longer by having a greater capacity for absorbing out the antibodies produced against it. Edgerton et al. (1957) found no "dosage effect" in mice for first-set homografts.

Many authors (Woodruff, 1954; Billingham et al., 1955; Billingham and Brent, 1957; and others) report the induction of acquired tolerance of homografts by injection of a suspension of the proposed donor's cells into very young intended hosts. Billingham et al. (1955) suggest tolerance is acquired because the antibody-producing ability of very young animals is immature or not completely developed. Cannon et al. (1954) working with chicks, presented evidence that both the antigenicity of skin and the antibody-producing ability is immature but developing at hatching. The age of donor and host may, therefore, have an important effect on the homograft reaction. Billingham and Brent (1957) propose that the involution of lymph nodes observed in mice following the inoculation of adult cells may be the outcome of immunological reactions produced by those cells against tissue antigens present in the host. Billingham (1959) labels the physiological effects of the immunological response of the injected tissues against the host as runt disease. Simonsen (1957) has shown that grafts may also react against the host.

The reaction of the host to donor tissue is specific and the antibodies produced against foreign tissue remain, at least for a short period of time, readily available for attack on a second graft. Medawar (1946) and Egdahl and Varco (1956) with rabbits, and Berry et al. (1958b) with chickens have shown, using mature hosts, that a second graft from the same donor is sloughed much more rapidly than was the first (called the "second-set phenomena").

Several authors including Eichwald and Silmser (1955), Eichwald et al. (1957), Hirsch (1957), Short and Sobey (1957), Marino and Benaim (1958), Krohn (1958), and others have reported a sex effect on host reaction to grafted tissue which they have attributed to a Y-linked or multiple Y-linked histocompatibility genes. These authors have all found a lower survival of male donor tissue on female hosts in mammals than on the other three combinations of donor to host tissue exchanges. Kozelka (1932) reported less permanent survival (found to be significant in a chi-square test made by the author) of female donor tissue on male hosts in chickens, which could be expected as the heterogametic sex is the female in chickens, rather than the male as it is in mammals. Craig et al. (1960) working with day-old White Leghorn chicks found no sex-of-host effect on reaction to homografts when only the sex of the hosts was known. Danforth (1935) transplanted skin between sexes in all four combinations using pheasants and found each donor-host combination gave rise to a feather-type which was separate and distinct from the others, which might indicate a hormonal influence. Lustgraaf and Eichwald (1959) found no maternal influence on reaction to skin grafts in mice.

Billingham and Silvers (1959) propose that homografts are permanently acceptable to a host only if every antigen present in the donor is also

present in the host. They question the reliability of inbreeding coefficients and stress the value of skin grafting as the only obtainable evidence of the degree of genetic diversity.

Because the bursa of Fabricius has been shown to have an effect on antibody production by Chang et al. (1955), Glick et al. (1956), Chang et al. (1957), Chang (1957), it was postulated that the bursa might also be involved in the homograft reaction.

To the author's knowledge, there is no literature on the heritability of the severity of homograft reaction.

MATERIAL AND METHODS

All chicks used in this study were from the Regional NC-47 randombred control population of Rhode Island Reds (RIR)[1], the Cornell randombred control population of White Leghorns (WL), see King et al. (1959) and crossbreds between these populations. Depending on space availability and fertility, natural mating or artificial insemination was used to obtain fertile eggs, which were held in an egg cooler until a sufficient quantity was accumulated for setting. The eggs were not held longer than two weeks. All chicks were pedigree hatched and wing banded for identification. They were intranasally vaccinated for bronchitis and Newcastle disease on the day of hatching.

Chicks to be grafted at one-day-old were held overnight in the same room where they hatched, otherwise they were moved to battery brooders in another building and brought back on the day of grafting.

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[1] This population was formed by intercrossing four commercial strains of egg-producing Rhode Island Reds and one commercial strain of New Hampshires. Chicks of this population will be referred to as Rhode Island Reds (RIR).

The grafting technique is an adaptation of those of Cannon and Longmire (1952) and Short and Sobey (1957). A description of the technique is as follows:

The down is removed from the backs of both donor and host chicks shortly after hatching. Flexible collodion is applied several minutes prior to grafting to "stiffen" the skin of both donor and host. Physiological saline is injected subcutaneously to separate the skin from the underlying tissues (Plate I, figure 1) and a rectangular full-thickness skin graft (approximately eight by ten millimeters) is removed with a mouse-tooth forceps and placed flat on a scalpel handle which has a drop of physiological saline on it (Plate I, figure 2). The graft is then fitted into a previously prepared graft bed on the host (Plate I, figure 3) and a dressing[1], consisting of a plasticized vinyl chloride film coated with a pressure sensitive adhesive and with a bleached, unnapped flannel pad in the center, is then placed over the graft (Plate I, figure 4). Since these dressings are round, they may require trimming to avoid over-lapping other graft-bed sites on the host. No suturing is necessary. Chicks are held by an assistant during these operations and no anaesthetic is used. The grafts are reversed, i.e., turned one hundred and eighty degrees to provide positive identification of viable grafts since the feather-pattern of the grafts will be in reverse to the normal pattern.

Several grafts may be placed on each host. In this study, four grafts, each from a different donor, were randomly assigned to a position on the host's back designated as sites one, two, three, and four. The necessary

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[1]This dressing is available commercially from Johnson and Johnson Company, New Brunswick, New Jersey, under the name Plastic Band-Aid Spots, 7/8 inch diameter.

EXPLANATION OF PLATE I

Figure 1. The injection of physiological saline subcutaneously. Down has been removed and flexible collodion applied to the back of the chick.

Figure 2. The removal of a full-thickness skin graft from the mid-dorsal back area of a donor.

Figure 3. Skin graft which has been turned 180° being placed in position on the back of a host from a scalpel handle.

Figure 4. One host has a dressing in place over a graft in the number one position and the other has all four dressings in place.

PLATE I

Figure 1

Figure 2

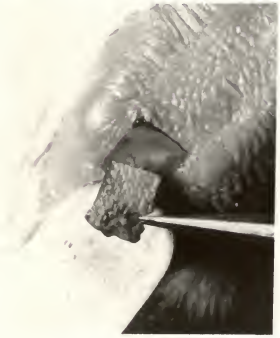


Figure 3

Figure 4

equipment for this operation is relatively simple and inexpensive (Plate II, figure 1).

A macroscopic, subjective, numerical scoring system was devised to estimate the severity of the homograft reaction and allow the rapid evaluation of graft status daily (Table 1). All grafts were scored at about the same time daily by the same observer, without knowledge of the genetic relationships involved, for a period of at least twenty days postgrafting.

One improvement in the technique made during this study was the use of tissue taken from the dorsal midline of the donor in the last two experiments instead of from approximately the same area as the tissue was to occupy on the host (Plates III and IV). This was thought to be an improvement since feather follicles are more densely concentrated along the dorsal midline than in the area immediately to either side.

Three experiments comprise this study.

Experiment I

This experiment was undertaken to determine if the plateau of response to grafted tissue more genetically diverse than unrelated donors of the same strain, reported in year-old chickens by Berry and Craig (1959) was present in young chicks. The adequacy of surgery and scoring system were tested. The accidental loss of grafts, percentage initial "take" (healthy grafts), and reversed feather growth were considered as possible measurements of methodology. The efficiency of the scoring system was tested by the repeatability of scoring between observers and by comparison of the scores with results obtained utilizing the microscopic *in vivo* technique described by Taylor and Lehrfeld (1955) with a slight modification[1].

[1]Corn oil was substituted for mineral oil.

EXPLANATION OF PLATE II

Figure 1. Photograph showing all necessary equipment used in skin grafting with chicks. Small bottle contains physiological saline, a two ml tuberculin-type syringe with 26 gauge needle, large bottle contains flexible collodion, Plastic Band-Aid Spots (trimmed), mouse-tooth forceps, cotton swab (for applying collodion and blotting blood), dissecting scissors, and scalpel handle.

Figure 2. WL host showing four viable grafts with feathers growing in reverse to normal feather pattern. Feathers from surrounding area have been plucked for easier identification.

Figure 3. WL host with a viable graft from an RIR donor showing red feathers growing in reverse to the normal feather pattern.

PLATE II

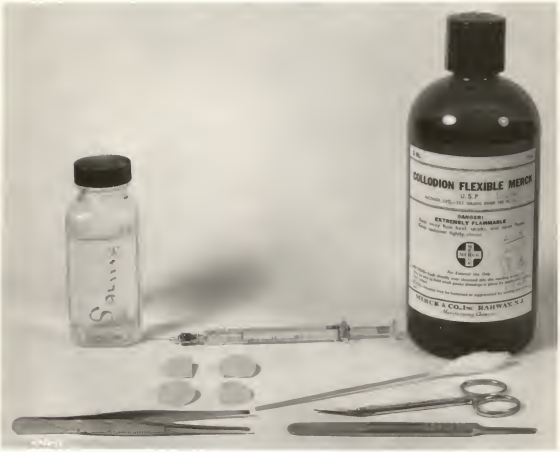


Figure 1

Figure 2

Figure 3

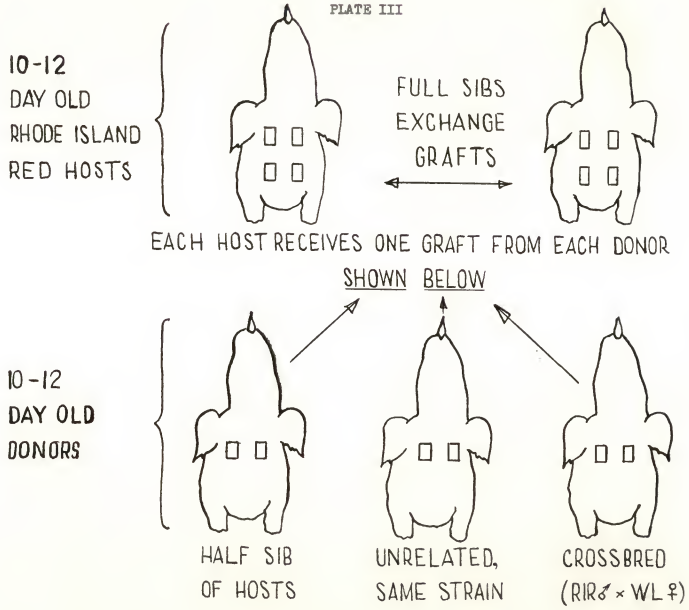
Table 1. Macroscopic numerical scoring system devised to estimate the severity of the homograft reaction.

Score	Description
5	Smooth, bright, and healthy appearing.
4	Some discoloration and/or inflammation apparent, but smooth.
3	Shows 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, hard, crusty, and becoming detached at the edges.
0	Graft sloughed off.
X	Graft missing but not sloughed (faulty operative technique or accidental loss).

EXPLANATION OF PLATE III

Schematic diagram showing the donor to host graft exchanges made in Experiment I.

PLATE III


10-12
DAY OLD
RHODE ISLAND
RED HOSTS
FULL SIBS
EXCHANGE
GRAFTS
EACH HOST RECEIVES ONE GRAFT FROM EACH DONOR
SHOWN BELOW
10-12
DAY OLD
DONORS
HALF SIB
OF HOSTS
UNRELATED,
SAME STRAIN
CROSSBRED
(RIR♂ × WL♀)

Ten- and twelve-day-old RIR chicks hatched March 6, 1959, were used as hosts. Forty chicks, representing twenty sires' families with one full-sib group per sire received grafts as shown schematically in Plate III. Within each pair of full sibs, each host chick received a graft from a full-sib, half-sib, unrelated (same strain), and crossbred donor. The crossbred donor chicks were sired by unrelated RIR males and were out of WL dams. Two surgical teams of two persons each and a fifth person acting as a coordinator completed eighty grafts on twenty chicks from ten sires on day ten and on the other twenty chicks from the remaining ten sires' progenies, on day twelve post-hatching.

Bandages were removed on the fourth postoperative day and grafts were scored between one and five p.m. each afternoon from the fourth through the twentieth days.

Experiment II

This experiment was designed to estimate: 1. response differences over a wide range in genetic diversity. 2. importance of three ages at grafting on the severity of the reaction in the populations used, and 3. relative severity of the homograft reaction in WL and RIR populations.

Eight sires' families of WL and nine sires' families of RIR were represented at each of three different ages at grafting as hosts. Hosts and donors were divided between two different hatches to provide more nearly consecutive days for grafting. Birds to be grafted at one and two days of age were hatched August 23, 1959, and birds to be grafted at seven and eight, and fourteen and fifteen days of age were hatched August 12, 1959. Because only four grafts were placed on the back of each host and because it was

desired to use more than four donor relationships, the kinds of grafts were arbitrarily divided into two classifications: 1. "close", comprising autograft, full sib, half sib, and unrelated (same strain), and 2. "distant", including unrelated (same strain), reciprocal crossbreds, i.e., from matings of RIR ♂ x WL ♀ and WL ♂ x RIR ♀, and different breed (which was RIR donors on WL hosts and WL donors on RIR hosts). One half of each host group from each breed received grafts from the "close" donor classification and the other half received grafts from the "distant" donor classifications. Plate IV shows a schematic drawing of the donor-host exchanges for the WL hosts.

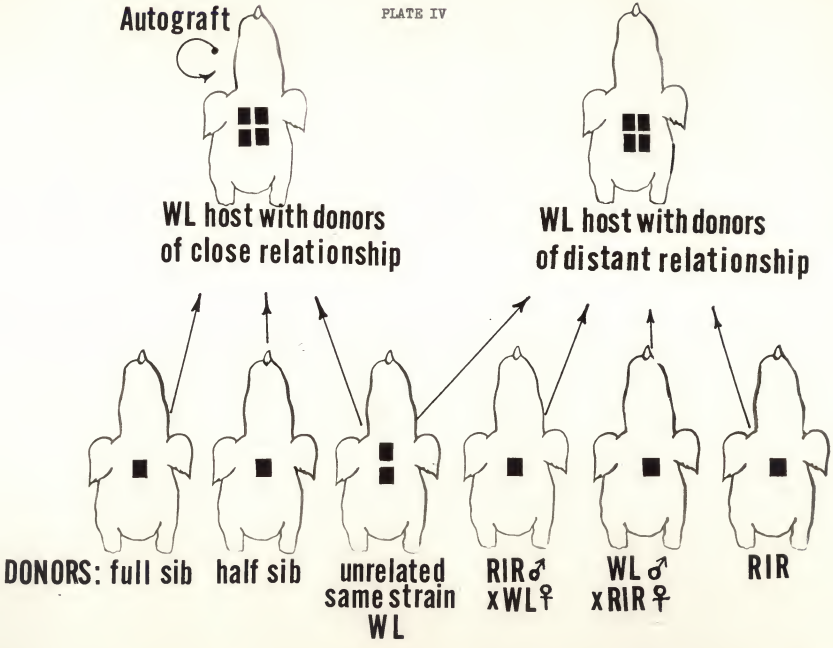
There were seventeen WL hosts and six RIR hosts in each classification of the birds grafted at one and two days of age, fourteen WL hosts and six RIR hosts in each classification of the birds grafted at seven and eight days of age, and thirteen WL hosts and six RIR hosts in each classification of the birds grafted at fourteen and fifteen days of age.

Experiment III

The third experiment was designed in such a manner that a comparison of the severity of homograft reaction between the two breeds at two ages could be made. Another consideration was the effect of sex of host on the homograft reaction. The bursa of Fabricius was recovered from all host birds and weighed at the end of the observation period on an electronic balance. Body weight was taken and sex was determined by autopsy at this same time. These same measurements were also obtained from twenty non-grafted birds (approximately ten of each sex) from each age-breed subgroup from the same hatch, to be used as controls. Hosts three and four days of age at grafting were killed and the measurements made on December 2, 1959.

EXPLANATION OF PLATE IV

Schematic diagram showing the donor to host graft exchanges made within WL hosts. Similar exchanges were made within RIR hosts.


Autograft
PLATE IV
WL host with donors of close relationship
WL host with donors of distant relationship
DONORS: full sib
half sib
unrelated same strain WL
RIR♂ xWL♀
WL♂ xRIR♀
RIR

Measurements were made on the controls December 3, 1959. The same data were collected from the hosts grafted at ten and eleven days of age on December 9, 1959, and the controls for this group on December 10, 1959. This experiment was also designed to explore the possibility of sex-linked and/or maternal effects.

Five WL sires' families and seven RIR sires' families, divided into two age groups, were used as hosts. The balanced design of this experiment may be seen from Table 2. These chicks were hatched on November 6, 1959. The grafting was again completed by two surgical teams, of two persons each, and a coordinator. Ten host pairs (five WL and five RIR host pairs) were grafted each day by each team. Each host carried transplanted tissue with relationships of: unrelated (same strain), crossbred female donor from the mating RIR♂ x WL♀, crossbred female donor from the mating WL ♂ x RIR♀, and different breed, i.e., WL donor on RIR hosts and RIR donor on WL hosts. Unrelated (same strain) and different breed donors were not sexed. Only female crossbred donors were used because the female is the heterogametic sex in chickens and would, therefore, carry an X-chromosome from the sire.

STATISTICAL ANALYSES

With the large number of birds per host group (n = thirty-seven to forty) in Experiments I and III, it was believed that the rate of decline in graft viability was measured with a high degree of accuracy for all groups. It should also be pointed out that since all treatments, i.e., different degrees of genetic diversity for the various kinds of donors, were applied to all hosts within each of these two experiments, each chick represented a replicate of the same experiment. Due to the differences in

Table 2. Disposition of sires' and dams' families in Experiment III.

PARENTS WL	PROGENY[1] Age at grafting, days 3-4	10-11	PARENTS RIR	PROGENY[1] Age at grafting, days 3-4	10-11
	No.	No.		No.	No.
Sire 1			Sire 6		
Dam 1	2	2	Dam 21	2	2
Dam 2	2	2	Dam 22	2	2
Dam 3	2	2	Dam 23	2	2
Dam 4	2	2			
Sire 2			Sire 7		
			Dam 24	2	2
Dam 5	2	2	Dam 25	2	2
Dam 6	2	2	Dam 26	2	2
Dam 7	2	2			
Dam 8	2	4	Sire 8		
Sire 3			Dam 27	2	2
			Dam 28	2	2
Dam 9	2	0	Dam 29	2	2
Dam 10	2	4			
Dam 11	2	2	Sire 9		
Dam 12	2	2			
Dam 13	2	0	Dam 30	2	2
			Dam 31	2	2
Sire 4			Dam 32	2	2
Dam 14	2	2	Sire 10		
Dam 15	2	2			
Dam 16	2	2	Dam 33	2	2
			Dam 34	2	2
Sire 5			Dam 35	2	2
Dam 17	2	2	Sire 11		
Dam 18	2	2			
Dam 19	2	2	Dam 36	2	2
Dam 20	2	2	Dam 37	2	2
			Dam 38	2	2
			Sire 12		
			Dam 39	2	2
			Dam 40	2	2
TOTALS	40	40		40	40

[1]Progeny used as hosts.

individual host's reactivity to grafts in general, statistical efficiency is greater than if a comparable number of grafts had been made, but with each chick receiving only one treatment. Mean daily graft scores for each kind of donor tissue were therefore calculated since it was assumed that the individual bird effects would sum to a value near zero.

Preliminary examination of graphs (Plate V, figure 1) showing changes in mean graft scores by days postgrafting indicated essentially exponential curves. It was observed that in Experiment I the mean graft score for one kind of donor tissue reached 0.0 on day fifteen. It was decided, therefore, to analyze data from Experiment I through the fifteenth day only. Hosts grafted at three and four days of age in Experiment III were analyzed over the entire twenty day observation period although the mean graft scores for donor tissue from WL had reached 0.0 on the RIR hosts on the fifteen postoperative day. Hosts grafted at ten and eleven days of age were analyzed through the fifteenth postoperative day because two kinds of donor tissue had reached 0.0 and four kinds of donor tissue closely approached 0.0 on the fifteenth postoperative day (see Table 7). Assuming these curves to closely approximate exponential curves, the percentage of perfect score (Y) remaining on day k for a given group would be: $Y = \alpha \beta^{k}$ where α and β are constants peculiar to each donor group. In order to obtain least squares estimates of α and β for each group, the experimental values of Y were converted to logarithms, transforming the equation to: $\log Y = \log \alpha + (k) \log \beta$. Thus, linear regression techniques were applicable to the transformed data (Plate V, figure 2).

The effect of sex of host was tested for significance in Experiment III. Since smaller numbers were present within the two sexes and since

EXPLANATION OF PLATE V

Figure 1. Mean graft scores expressed as percent of perfect score by days postgrafting for each of four donor groups in Experiment I.

Figure 2. The logarithm transformation of percent of perfect score (shown in figure 1 of this plate) by days postgrafting for each of four donor groups in Experiment I.

PLATE V

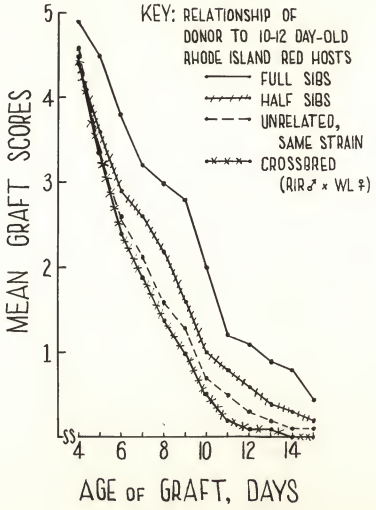


Figure 1

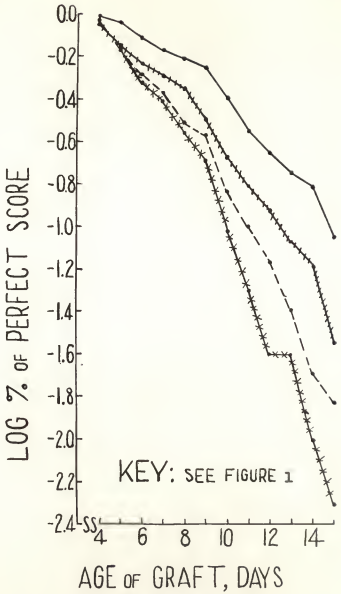


Figure 2

individual host differences would become more important where different host groups were involved, it was felt that the curves obtained within sexes would be of lesser reliability. Therefore, an analysis of variance was applied. It was felt that the prerequisite assumptions of normal distribution and homogeneous variance are best met when the mean of all individual graft scores are in the middle of the range. Therefore, the average day postgrafting when such scores were obtained within each experiment was computed. This was accomplished by finding the day on which the average score for all grafts was 2.5. The total score for each graft on each host was then calculated, using a three-day period, centering on the day of fifty percent of perfect score, i.e. day on which the mean score of all grafts was 2.5, in Experiment I and the hosts grafted at three and four days of age in Experiment III. Because the day of fifty percent of perfect score fell between the sixth and seventh postoperative days on the hosts ten and eleven days of age at grafting in Experiment III, and because these were the first two days of observation, a two-day period was used to calculate a total score for these hosts.

The variance component analysis of King and Henderson (1954) was used to estimate the heritability of graft reaction in Experiment III. Because the analysis requires normal distribution and homogeneous variance, the two and three day total scores computed at the point of fifty percent of perfect score were used. The correlations involving graft scores, body weights and bursa of Fabricius weights in Experiment III are also based on these same two and three day total scores.

Differences between the bursa weights of grafted hosts and non-grafted controls were tested for significance using the R x 2 analysis of variance

with disproportionate subclass numbers (see Snedecor, 1957) in Experiment III. This same analysis was applied to test for differences between age groups within the controls and the grafted birds.

RESULTS AND DISCUSSION

Surgical Success of the Technique

Initial "take" of all grafts and the percentage survival of autografts over the observation period would appear useful as measures of the success of the surgical technique. Table 3 presents the percentage of healthy grafts for all experiments on the sixth postoperative day. In Experiment I, scoring was started on the fourth postoperative day and these percentages are also included. It is apparent that as age of host at grafting increases, the percentage of healthy grafts on the sixth postoperative day decreases. These lower percentages found with older hosts are attributed to an earlier onset of homograft reaction rather than to failure of grafts to take initially due to operative trauma or faulty technique. The lack of any such phenomena in the autografts (see Table 3) supports the conclusion that all grafts became established initially, regardless of age or breed of host. Only one autograft out of sixty-two was lost. This graft was accidently pulled off when the bandage was removed. The ecchymotic conditions reported due to operative trauma by Cannon and Longmire (1952) were not found in these experiments. The inflammation and discoloration observed by them might be interpreted as caused by the onset of the homograft reaction prior to their initial observations at the ninth or tenth postoperative day.

Of the one thousand, two hundred and eighty grafts made in these three experiments, five grafts, including the one autograft previously mentioned,

Table 3. Percentage of healthy grafts on the sixth postoperative day.

Experiment	Age of host, days[1]	Breed of host	No. of hosts	Donors			
				Full sib	Half sib	Unrelated (same strain)	Crossbred (RIR♂xWL♀)
I	10 and 12	RIR	40	100[2] 60	85[2] 35	95[2] 25	93[2] 13
				Autograft	Full sib	Half sib	Unrelated (same strain)
II	1 and 2	WL	17	100	100	100	100
	1 and 2	RIR	6	100	100	100	100
	7 and 8	WL	14	100	86	86	64
	7 and 8	RIR	6	100	100	50	50
	14 and 15	WL	13	100	21	14	21
	14 and 15	RIR	6	100	33	33	17
				Unrelated (same strain)	Crossbred (RIR♂xWL♀)	Crossbred (WL♂xRIR♀)	Different Breed (WL or RIR)
II	1 and 2	WL	17	100	100	100	100
	1 and 2	RIR	6	83	100	100	100
	7 and 8	WL	14	64	86	57	64
	7 and 8	RIR	6	83	50	67	50
	14 and 15	WL	13	14	00	00	00
	14 and 15	RIR	6	33	17	00	00

Table 3. (con't.)

Experiment	Age of host, days[1]	Breed of host	No. of hosts	Donors			
				Unrelated (same strain)	Crossbred (RIR♂ x WL♀)	Crossbred (WL♂ x RIR♀)	Different Breed (WL or RIR)
III	3 and 4	WL	39	92	92	90	85
	3 and 4	RIR	37	89	84	78	62
	10 and 11	WL	38	50	39	32	45
	10 and 11	RIR	38	63	18	21	21

[1]Age at grafting.

[2]Percentage of healthy grafts, 4th postoperative day.

were lost accidently. All were lost because of adhesion to the bandage when it was removed.

Reversed Feather Growth

Experiment I contained no surviving homografts at the end of the twenty-day observation period and, therefore, no reversed feathers. Fifty of the sixty-one surviving autografts made in Experiment II displayed feathers growing in reverse on the twentieth postoperative day. Of the eleven autografts which did not show feather growth on the twentieth postoperative day, eight developed feathers growing in reverse by the twenty-eighth postoperative day, and three did not. There were four hundred and thirty-four homografts made in the second experiment in addition to the autografts. Fifty-seven of the one hundred and thirty-five homografts surviving on the twentieth postoperative day showed reversed feather growth. Fifteen of the seventy-eight homografts displaying no feather growth on the twentieth postoperative day had reverse-growing feathers by the twenty-fourth postoperative day.

Turning the graft one hundred and eighty degrees to allow positive identification of surviving grafts by reversed feather growth, as suggested by Cannon and Longmire (1952), did not provide the desired identification as a number of clearly viable grafts did not show feather growth by the twentieth postoperative day. Whether all surviving grafts would eventually have developed feathers is not known from these observations.

Evaluation of Subjective Scores

To measure the repeatability of the scoring system (see Table 1) between observers, two individuals independently scored eighty grafts in

Experiment I each day for an eight-day period. A highly significant correlation ($r = 0.975$) was obtained between total scores of the two observers for each graft over this period. These results indicated that the scoring system utilized in this study could be used successfully by other workers.

Another eighty grafts in the same experiment were scored by one observer while the microscopic *in vivo* technique of Taylor and Lehrfeld (1955) was employed independently by a second person to ascertain the period of viability of the grafts, in an attempt to test the reliability of the scoring system. Number of days of graft survival as judged by the microscopic technique and the number of days until a score of less than five was consistently obtained were correlated to a highly significant degree ($r = 0.907$). A similar correlation ($r = 0.875$, $P < 0.01$) was obtained between the microscopic measurement and the number of days until a score of less than four was consistently observed. The scoring system was, therefore, considered sufficiently reliable for further use.

Sex of Host Effect on Graft Reaction

The host birds of Experiment III were the only sexed hosts used in this study. Table 4 presents the analyses of variance results based on total two and three day scores centering on the day of fifty percent survival of all grafts (see analysis section of Material and Methods). No sex of host effects on graft reaction are indicated. These results are in agreement with the findings of Craig *et al.* (1960) who worked with one- and two-day old WL hosts.

Importance of Sex-Linked and/or Maternal Effects

It may be seen in Experiment III (Table 5) that in all cases the crossbred female donor tissue carrying the sex chromosome of the breed other than that of the host was rejected more rapidly than the tissue from the reciprocal crossbred female donor. This difference in severity of reaction between the reciprocal crossbred female donor tissues was significant in two of the four age and breed host groups. The difference in severity of reaction against reciprocal crossbred female donor tissues was in the direction to be expected from sex-linked gene effects.

Sex-linkage might be expected to be relatively more important in chickens than in most other warm-blooded animals, assuming that the genes involved in transplantation antigenicity are randomly distributed throughout the chromosome complement of the organism. This hypothesis is based on the results of Newcomer (1957) whose observations lead him to believe that there are only six major pairs of chromosomes in the domestic chicken. Although various workers have reported Y-linkage and multiple Y-linked histocompatibility genes, the only report in the literature dealing with possible sex-linked effects, to the author's knowledge, is that of Eichwald et al. (1958) who were unable to demonstrate X-linked histocompatibility genes using tumor transplants in mice.

Maternal effects on skin graft reactions might be postulated on the basis of cytoplasmic inheritance for either male or female donors and also on the basis of Y-linked inheritance for female donors. Maternal effects would be expected to cause female crossbred donor tissue from dams of the same breed as the host to have a longer period of survival than tissue from reciprocally crossbred female donors. Since sex-linked effects would be

Table 4. Analysis of variance for differences due to sex, by age and breed of host groups.

Source	d.f.	Mean Squares	F
		3- and 4-day-old WL	
Sexes	1	26.8	0.08
Error	37	316.5	
		3- and 4-day-old RIR	
Sexes	1	33.8	0.23
Error	35	146.5	
		10- and 11-day-old WL	
Sexes	1	6.4	0.16
Error	36	41.3	
		10- and 11-day-old RIR	
Sexes	1	31.5	1.38
Error	36	22.8	

Table 5. Multiple range tests for regressions of mean graft scores [1] on number of days postgrafting in Experiment III.

Breed of host	Grafting age, days		Donors			
RIR	3 and 4	Regression Coefficients	Unrelated (same strain)	Crossbred ♀ (RIR♂ xWL♀)	Crossbred ♀# (WL♂ xRIR♀)	Different Breed (WL)
			-0.06040	-0.07094	-0.10570	-0.18558
RIR	10 and 11	Regression Coefficients	Unrelated (same strain)	Crossbred ♀ (RIR♂ xWL♀)	Crossbred ♀# (WL♂ xRIR♀)	Different Breed (WL)
			-0.17752	-0.18442	-0.23627	-0.24157
WL	3 and 4	Regression Coefficients	Unrelated (same strain)	Crossbred ♀ (WL♂ xRIR♀)	Crossbred ♀# (RIR♂ xWL♀)	Different Breed (RIR)
			-0.03181	-0.04596	-0.05466	-0.05682
WL	10 and 11	Regression Coefficients	Unrelated (same strain)	Different Breed (RIR)	Crossbred ♀ (WL♂ xRIR♀)	Crossbred ♀# (RIR♂ xWL♀)
			-0.09560	-0.13565	-0.16784	-0.18839

NOTE: Any two regression coefficients not underscored by the same single line are significantly different at the 0.05 percent level of probability (see Duncan, 1955). Since regression coefficients are being tested for significance, Duncan's significant ranges are multiplied by the standard error of the regression coefficient (s_b) rather than that of the mean ($s_{\bar{x}}$).

[1] Mean graft scores are expressed as log percentages of perfect score.

Crossbred ♀ carrying the sex chromosome of the breed other than that of the host.

expected to cause, and apparently did cause, the opposite reaction, sex-linked effects are concluded to be more important than maternal effects in influencing skin graft reactions in chickens. Although it was not possible in this experiment to conclusively separate effects due to sex-linked or to maternal influences, it is evident that if maternal effects were present, they were of relatively minor importance since they did not prevent expression of differences in severity of homograft reactions hypothesized on the basis of sex-linkage.

Effect of Age of Host at Grafting

The important effect of age of host on time of onset and severity of homograft reactions to skin are clearly evident in Experiment II, Table 6. The day-by-day percentages of perfect score obtained from the various age groups in Experiments I and III are shown in Table 7. Regression of percentage perfect score, transformed to log percentage, on days postgrafting were computed from the data of Experiment III and are shown in Table 8, along with their ninety-five percent confidence limits. It may be seen from Table 8 that the regression coefficient for any one kind of donor tissue does not fall within the ninety-five percent confidence limits for that same donor tissue in another age group in the sixteen possible comparisons, within breeds. These results indicate a positive association between age at grafting and severity of homograft reaction. They also agree with the findings of Cannon et al. (1954) that the severity of the homograft reaction increased as age of donor and host at grafting increased. Birds two weeks of age at grafting were the oldest hosts used in this study. It was postulated from these data that hosts grafted at two weeks posthatching were approaching an

Table 6. Percentage of perfect graft score at four day intervals beginning on the sixth postoperative day, arranged by breed of host, age of donor and host, and kind of donor, Experiment II.

A. WL Hosts

Age of donor and host at grafting, days	No. hosts	Donors: Close: Auto-graft	Close: Full sib	Close: Half sib	Close: Unrelated[1]	Distant: No. hosts	Distant: Unrelated[1]	Distant: Crossbred RIR♂ x WL♀	Distant: Crossbred WL♂ x RIR♀	Distant: Different Breed (RIR)
6 Days postgrafting										
1 and 2	17	99	100	100	99	17	100	96	96	93
7 and 8	14	100	89	91	74	14	83	87	71	76
14 and 15	13	100	57	55	45	13	48	45	45	40
10 Days postgrafting										
1 and 2	17	100	98	93	87	17	94	79	87	75
7 and 8	14	100	74	74	44	14	54	57	40	37
14 and 15	13	100	29	34	29	13	20	17	14	09
14 Days postgrafting										
1 and 2	17	100	100	91	80	17	91	66	78	61
7 and 8	14	100	51	64	33	14	34	50	21	27
14 and 15	13	100	11	08	03	13	00	00	00	00
18 Days postgrafting										
1 and 2	17	100	100	82	68	17	91	56	64	51
7 and 8	14	100	54	53	29	14	27	43	21	20
14 and 15	13	100	08	08	00	13	00	00	00	00

Table 6. (con't.)

B. RIR Hosts

Age of donor and host at grafting, days	No. hosts	Donors: Autograft	Close: Full sib	Close: Half sib	Close: Unrelated[1]	No. hosts	Distant: Unrelated[1]	Distant: Crossbreds RIR♂ x WL♀	Distant: Crossbreds WL♂ x RIR♀	Different Breed (WL)
6 Days postgrafting										
1 and 2	6	100[2]	97	100	97	6	90	100	93	93
7 and 8	6	100	90	73	63	6	80	67	70	80
14 and 15	6	97	53	57	47	6	47	50	47	47
10 Days postgrafting										
1 and 2	6	100	73	67	60	6	53	70	63	60
7 and 8	6	100	60	37	23	6	37	20	30	37
14 and 15	6	100	30	30	23	6	13	27	20	20
14 Days postgrafting										
1 and 2	6	100	60	47	53	6	30	57	50	47
7 and 8	6	100	37	17	03	6	23	00	07	20
14 and 15	6	100	10	00	03	6	07	03	00	03
18 Days postgrafting										
1 and 2	6	100	57	37	43	6	17	37	30	33
7 and 8	6	100	03	00	00	6	00	00	00	00
14 and 15	6	100	00	00	00	6	00	00	00	00

[1] Unrelated (same strain).

[2] Five hosts, one autograft pulled off accidently.

age when reactions to grafted skin would be of such a severe nature that differentiation of genetic diversity by macroscopic subjective scoring would become increasingly difficult.

Effect of Breed of Host

Breed of host appeared to be of considerable significance in its effect on the severity of skin-graft reactions in Experiment II, as the RIR hosts produced a more severe homograft reaction than did the WL hosts, Table 6. Steeper regression lines of graft reaction on days postgrafting were found in Experiment III for the RIR hosts as compared to the WL hosts, Table 8. Regression coefficients for neither of the two breeds were included within the ninety-five percent confidence limits of the comparable regression coefficients of the other breed, in fourteen of the sixteen possible comparisons. The exception involved donor tissue from female crossbred donors, from the mating RIR♂ x WL♀ within the hosts ten and eleven days of age at grafting. Thus, it is clearly and significantly demonstrated that the RIR breed sample reacted more severely to skin grafts than the WL breed sample.

Differentiation Among Levels of Genetic Diversity

The ability to differentiate among different levels of genetic diversity, by skin graft reactions of host chicks, of two breeds and two ages, is indicated by the results presented in Tables 9 and 5 for Experiments I and III, respectively. The results are in the form of regression coefficients of mean graft scores, expressed as log percentage of perfect score, on number of days postgrafting. Duncan's (1955) New Multiple Range Test was used to test for significant differences among regression coefficients in these two

Table 7. Percentage of perfect score by days postgrafting, within each kind of donor, arranged by age at grafting and breed of host.

Donors	Days postgrafting															
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Experiment III 3- and 4-day-old RIR hosts (n=37)																	
Unrelated (same strain)			90	76	68	61	41	37	31	26	25	21	17	16	15	15	14
Crossbred ♀ (RIR♂ x WL♀)			84	68	53	41	35	30	25	21	17	16	15	12	09	09	09
Crossbred ♀ (WL♂ x RIR♀)			80	60	52	44	33	26	23	18	14	11	08	06	04	03	03
Different Breed (WL)			72	53	39	26	19	13	08	03	01	00	00	00	00	00	00
Experiment III 3- and 4-day-old WL hosts (n=39)																	
Unrelated (same strain)			95	90	84	82	77	73	68	66	58	53	50	43	42	38	34
Crossbred ♀ (RIR♂ x WL♀)			90	81	71	64	60	55	49	45	35	32	28	26	25	23	23
Crossbred ♀ (WL♂ x RIR♀)			90	81	67	56	50	46	40	31	30	26	22	22	20	18	15
Different Breed (RIR)			90	76	67	58	53	45	41	30	29	27	23	19	17	16	16
Experiment III 10- and 11-day-old RIR hosts (n=38)																	
Unrelated (same strain)			74	48	38	33	22	16	11	08	04	01					
Crossbred ♀ (RIR♂ x WL♀)			60	41	31	23	19	10	06	04	03	01					
Crossbred ♀ (WL♂ x RIR♀)			52	28	24	18	09	04	02	02	00	00					
Different Breed (WL)			53	28	23	17	11	05	03	01	00	00					

Table 7. (con't.)

Donors	Days postgrafting																	
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Experiment III 10- and 11-day-old WL hosts (n=38)																		
Unrelated (same strain)			65	48	42	33	24	18	16	13	13	08						
Crossbred ♀ (WL ♂ x RIR ♀)			57	34	28	22	17	12	07	05	04	01						
Crossbred ♀ (RIR ♂ x WL ♀)			63	43	35	26	18	12	06	04	03	01						
Different Breed (RIR)			61	41	35	25	18	12	09	06	05	04						
Experiment I 10- and 12-day-old RIR hosts (n=40)																		
Full sib	98	90	77	67	61	56	40	28	22	18	16	09	07	04	02	03	02	
Half sib	88	72	58	52	44	32	21	16	12	08	06	03	02	00	00	00	00	
Unrelated (same strain)	92	68	52	42	31	25	14	10	06	04	02	02	00	00	00	00	00	
Crossbred (RIR ♂ x WL ♀)	90	69	48	38	20	10	05	02	02	01	00	00	00	00	00	00	00	

Table 8. Regression coefficients of mean graft scores[1] on number of days postgrafting and their confidence limits for donors in Experiment III.

Age and Breed of Host	Donors	Regression Coefficients	95 Percent Confidence Limits Lower	Upper
3-4-day old RIR	Unrelated (same strain)	-0.06040	-0.06714	-0.05366
	Crossbred ♀ (RIR♂ x WL♀)	-0.07094	-0.07688	-0.06500
	Crossbred ♀ (WL♂ x RIR♀)	-0.10670	-0.11255	-0.10850
	Different Breed (WL)	-0.18558	-0.21878	-0.15238
3-4-day old WL	Unrelated (same strain)	-0.03181	-0.03440	-0.02922
	Crossbred ♀ (RIR♂ x WL♀)	-0.05466	-0.05827	-0.05105
	Crossbred ♀ (WL♂ x RIR♀)	-0.04596	-0.04950	-0.04242
	Different Breed (RIR)	-0.05682	-0.06540	-0.05310
10-11-day old RIR	Unrelated (same strain)	-0.17752	-0.22156	-0.13348
	Crossbred ♀ (RIR♂ x WL♀)	-0.18442	-0.21161	-0.15723
	Crossbred ♀ (WL♂ x RIR♀)	-0.23627	-0.26936	-0.20318
	Different Breed (WL)	-0.24157	-0.27676	-0.20638
10-11-day old WL	Unrelated (same strain)	-0.09560	-0.10632	-0.08488
	Crossbred ♀ (RIR♂ x WL♀)	-0.18839	-0.23735	-0.13943
	Crossbred ♀ (WL♂ x RIR♀)	-0.16784	-0.20273	-0.13295
	Different Breed (RIR)	-0.13565	-0.14391	-0.12739

[1] Mean graft scores are expressed as log percentages of perfect score.

experiments. Experiment II, used for exploratory purposes to suggest hypotheses for further testing, contained relatively small numbers of observations per subgroup and has, therefore, been excluded from detailed statistical analysis. The results obtained in Experiment II (see Table 6) suggested, however, that with very young hosts and with WL hosts in particular, due to lack of reaction to grafted tissue, there is some difficulty in differentiating among relatively close genetic relationships, e.g., all full-sib skin grafts survived as well as autografts. Variation from experiment to experiment in relative severity of skin graft reactions is indicated, however, from a comparable experiment with day-old WL hosts, of the same strain as used here, in which highly significant differences were obtained between reactions to donors of close genetic relationships (see Craig et al. 1960). Difficulty in differentiation among all levels of genetic diversity was experienced, in Experiment II, using hosts as old as fourteen and fifteen days of age at grafting. However, in these older birds, the difficulty was apparently due to very severe homograft reactions. It seems evident that a plateau of response to grafted tissue arose in older hosts due to a very rapid and severe homograft reaction in which differentiation was not measurable because of inadequate techniques. Because of the clear separation of four different kinds of donor tissue by RIR hosts ten and twelve days of age at grafting (evident from Table 9 and Plate V), and because of limitations indicated above for one- and two- and fourteen- and fifteen-day-old hosts, the decision was made to use hosts of intermediate ages, viz. three- and four- and ten- and eleven-day-old WL and RIR chicks as hosts in Experiment III.

Table 9. Multiple range test for regressions of mean scores[1] on number of days postgrafting in Experiment I.

Breed of host	Grafting age, days		Donors			
			Full sib	Half sib	Unrelated (same strain)	Crossbred (RIR♂ x WL♀)
RIR	10 and 12	Regression Coefficients	-0.09170	-0.12612	-0.16541	-0.20554

NOTE: Any two regression coefficients not underscored by the same double line are significantly different at the 0.01 percent level of probability (see Duncan, 1955). Since regression coefficients are being tested for significance, Duncan's significant ranges are multiplied by the standard error of the regression coefficient (s_b) rather than that of the mean ($s_{\bar{x}}$).

[1] Mean graft scores are expressed as log percentages of perfect score.

Separations among levels of donor-host genetic diversity were not as clear-cut in Experiment III (see Table 5) as in Experiment I. It did not appear, however, from Experiment III that any serious plateauing effect of response to grafted tissue, genetically more diverse than that of unrelated (same strain), was encountered. Such a plateau of response was reported by Barry and Craig (1959) using year-old WL and RIR strains and appears to be characteristic of older hosts such as used in that experiment and of the fourteen- and fifteen-day-old hosts in Experiment II. However, Craig and Hirsch (1957) were able to differentiate between breeds using three- and five-week-old hosts. Also easily discernible from Tables 9 and 5 is the rejection, in four of the five age and breed groups, of donor tissue in order from the less diverse to the genetically more diverse levels. The only exception to the orderliness of skin graft reactions involved tissue from the different breed (RIR) which was rejected less rapidly than tissue from either crossbred donor in WL hosts grafted at ten and eleven days of age. Chance alone is the only explanation offered for this result, since it happened in only one of the twenty donor-to-host classifications in Experiments I and III. It is, therefore, concluded, particularly from the results of Experiments I and III, that the skin graft reaction scores may be used with host chicks between three and twelve days of age, to successfully demonstrate differences in homograft reactions due to levels of genetic diversity of donor and host, ranging from full sibs to different breeds.

Influence of the Bursa of Fabricius

The bursa of Fabricius has been shown by Chang et al. (1955), Glick et al. (1956), Chang et al. (1957), Chang (1957), and others to be

associated with antibody production against Salmonella antigens. It was, therefore, postulated that the bursa may also produce antibodies against the antigens of grafted skin. All hosts and an additional eighty non-grafted birds from the same hatch, utilized as controls, were autopsied following the observation period in Experiment III and the bursae weighed because it was thought that the postulated antibody production of the bursae against grafted skin might be reflected by their weights.

Table 10 contains the correlations calculated among body weights, bursa weights, and scores of the homograft reactions. As may be seen from the table, all hosts grafted at three and four days of age were autopsied at twenty-six days of age and non-grafted controls were autopsied one day later. The hosts of both breeds, ten and eleven days of age at grafting, were autopsied at thirty-three and non-grafted controls at thirty-four days of age. Correlations within age groups were tested for heterogeneity and pooled correlations calculated, since significant differences were lacking.

A pooled correlation of 0.66 ($P < 0.01$) was obtained between body weight and bursa weight in chicks autopsied at twenty-six and twenty-seven days of age. Correlations derived from these same measurements on hosts and controls at thirty-three and thirty-four days of age yielded a highly significant pooled correlation of 0.29. Significant correlations might well be expected between the weights of two parts of the body. Because the bursa of Fabricius is known to regress in size beginning at ages ranging from four to nine and one-half weeks of age (see Glick, 1956), the smaller pooled correlation found in the birds autopsied at thirty-three and thirty-four days of age may reflect the fact that regression had started previously. The difference in mean bursa weights (Table 11) between the two ages of control birds

Table 10. Correlations among the weight of the bursa of Fabricius, body weight, and homograft scores in Experiment III.

Breed of host	Sex	Grafted or control	Age at autopsy, days	d.f.	Body wt. and bursa wt.	Body wt. and score	Bursa wt. and score
			Hosts 3 and 4 days of age at grafting				
WL	M	G	26	17	.97**	-.10	-.28
WL	F	G	26	18	.74**	-.70**	-.72**
WL	M	C	27	9	.50		
WL	F	C	27	7	.49		
RIR	M	G	26	11	.68**	-.23	-.39
RIR	F	G	26	22	.40*	-.09	-.07
RIR	M	C	27	8	.91**		
RIR	F	C	27	8	.49		
POOLED							
Correlations					.66**	-.31**	-.38**
d.f.					107	71	71
			Hosts 10 and 11 days of age at grafting				
WL	M	G	33	16	.48*	-.43	-.56*
WL	F	G	33	18	.44*	.26	-.02
WL	M	C	34	9	-.19		
WL	F	C	34	7	.51		
RIR	M	G	33	19	.54**	-.21	.15
RIR	F	G	33	15	.08	.27	.14
RIR	M	C	34	8	-.32		
RIR	F	C	34	8	.02		
POOLED							
Correlations					.29**	.04	-.08
d.f.					107	71	71

* Indicates significance at the 5 percent level of probability.
** Indicates significance at the 1 percent level of probability.

was significant. The weighted difference was 0.29 gm. heavier for the twenty-seven-day-old than for the thirty-four-day-old controls (see Table 12B). These results indicate that the bursae of the older birds were regressing. The difference in mean bursa weights (Table 11) between the two ages of grafted hosts was also significant. The weighted difference was 0.15 gm. heavier for the twenty-six-day-old than for the thirty-three-day-old hosts (Table 12C).

Table 10 shows a highly significant negative pooled correlation ($r = -0.38$) between bursa weight and score in hosts autopsied at twenty-six days of age (grafted at three and four days of age), which would indicate that birds found to have larger bursae at the end of the experiment showed greater reactions to skin grafts. The significant difference obtained between bursa weights of grafted hosts and non-grafted controls (shown in Table 12A) indicates that bursa weight is increased as a result of skin grafting. The correlation between bursa weight and score ($r = -0.08$) in hosts autopsied at thirty-three days of age (grafted at ten and eleven days of age) is negative but not significant. This could be explained by the hypothesis that a stress placed on the antibody producing centers of the chick before these centers are mature enough to function properly is handled by the antibody producing bursa of Fabricius. As the antibody producing centers near maturation, there is less need for the "reserve production site," the bursa, and it regresses. It could then be postulated that skin grafts placed on a host when the antibody producing centers are more mature would place less stress on the reserve production site and consequently a smaller correlation between bursa weight and score in the older hosts would be expected. Glick (1956) has suggested that stress factors may stimulate the bursa to extend its growth and continue its antibody production.

Table 11. Bursa of Fabricius weights arranged by breed, sex, and age of host at grafting.

Breed of host	Age at autopsy, days	Hosts 3 and 4 days of age at grafting: No.	Mean wt., gms.	Age at autopsy, days	Controls: No.	Mean wt., gms.
WL	26	19	1.17	27	11	1.11
WL	26	20	0.97	27	9	1.11
RIR	26	13	1.47	27	10	1.27
RIR	26	24	1.20	27	10	1.06
Total number and unweighted mean		76	1.20		40	1.14
		Hosts 10 and 11 days of age at grafting: No.	Mean wt., gms.		Controls: No.	Mean wt., gms.
WL	33	18	1.16	34	11	1.08
WL	33	20	0.95	34	9	0.78
RIR	33	21	1.15	34	10	0.97
RIR	33	17	0.95	34	10	0.53
Total number and unweighted mean		76	1.05		40	0.84
Grand totals and unweighted mean		152	1.13		80	0.99

Table 12. A. Analysis of variance for bursa of Fabricius weights of grafted and non-grafted birds.

Source	d.f.	Mean Square	F
Grafted vs. control	1	0.954	5.64*
Host groups[1]	7	0.857	5.07***
Interaction	7	0.155	0.92
Individuals	216	0.169	

Weighted difference: Grafted - Control = 0.14 gm.

B. Analysis of variance for effect of age on bursa of Fabricius weights in control birds.

Between ages[2]	1	1.732	14.93***
Host groups[1]	3	0.450	3.88*
Interaction	3	0.217	1.87
Individuals	72	0.116	

Weighted difference: 27-day-old weight - 34-day-old weight = 0.29 gm.

C. Analysis of variance for effect of age on bursa of Fabricius weights in grafted birds.

Between ages[3]	1	0.798	4.09*
Host groups[1]	3	0.691	3.54*
Interaction	3	0.225	1.15
Individuals	144	0.195	

Weighted difference: 26-day-old weight - 33-day-old weight = 0.15 gm.

[1] See Table 11 for identification of host groups and mean bursa weights by groups.

[2] Bursa weights at 27 vs. 34 days of age.

[3] Bursa weights at 26 vs. 33 days of age.

* Indicates significance at 0.05 percent level of probability.

*** Indicates significance at 0.005 percent level of probability.

The highly significant negative correlation ($r = -0.31$) found between body weight and score on birds autopsied at twenty-six days of age (grafted at two and three days of age) is thought to be associated in some way with the high correlation between body weight and bursa weight and the negative correlation between bursa weight and score in the birds autopsied at twenty-six days of age. The essentially zero correlation of bursa weight and score in the birds autopsied at thirty-three days of age may be due to the apparent regression of the size and function of the bursa in the older birds.

Analysis of variance (Table 12) also shows significant host group effects on bursa weights of both control and grafted birds. Breed and sex are confounded in the host group effect, but their individual contributions to bursa weights were not analyzed. Glick (1956) has reported significant differences in bursae weight due to breed.

Heritability of Graft Reaction Severity

Table 13 shows analyses of variance of graft scores within breed and age of host in Experiment III. To meet the requirements of analysis of variance, the two and three day total scores centering on the day of fifty percent of perfect score (see analysis section of Material and Methods) were used. The "hierarchal" classification of the analysis of variance with disproportionate subclass numbers, with variance components broken out for computation of heritability estimates, described by King and Henderson (1954), was used. Although significance was not found, the mean square for sire families and for dam families within sires were larger than for full sibs in six of eight comparisons. The two host breeds were pooled within age groups in an effort to increase the numbers involved and arrive at more

Table 13. A. Analysis of variance of graft reaction severity by means of graft scores[1], Experiment III.

Breed of host	Age at grafting, days	Source	d.f.	Mean Square	F
RIR	3 and 4	Sires' progenies	6	108.25	0.70
		Dams within sires	13	153.69	1.40
		Full sibs	17	147.85	
WL	3 and 4	Sires' progenies	4	544.02	1.39
		Dams within sires	15	390.25	2.00
		Full sibs	19	195.18	
RIR	10 and 11	Sires' progenies	6	45.53	1.81
		Dams within sires	13	25.22	1.80
		Full sibs	18	14.03	
WL	10 and 11	Sires' progenies	4	49.70	1.36
		Dams within sires	13	36.46	0.89
		Full sibs	20	40.95	

B. Estimates of heritability graft reaction severity based on sire and dam components of variance after breeds have been pooled within ages at grafting.

Age at grafting	Heritability	
	Sire component	Dam component
3 and 4 days	0.00	0.99
10 and 11 days	0.32	0.17

[1]Three-day total scores were calculated for the hosts 3 and 4 days of age at grafting, centering on the day of 50 percent of perfect score (2.5) which was the 12th postoperative day for WL hosts and the 8th post-operative day for RIR hosts.

Two-day total scores were calculated for the hosts 10 and 11 days of age at grafting since the day of 50 percent of perfect score fell between the 6th and 7th postoperative days for both breeds.

accurate estimates of heritability. The sire component of variance yielded estimates of heritability of 0.00 in the hosts three and four days of age at grafting, and 0.32 in the hosts ten and eleven days of age at grafting. Estimates of heritability obtained from the dam components were 0.99 and 0.17 for hosts three and four days of age at grafting and for hosts ten and eleven days of age at grafting, respectively, The variability among estimates is considered to result from the limited numbers involved.

SUMMARY AND CONCLUSIONS

Three hundred and twenty chicks from two populations, with extremely broad genetic bases, were used in a series of skin grafting experiments. Each chick received four skin grafts representing four different levels of genetic diversity. The objectives of this study were: to determine whether a technique could be developed which would differentiate among levels of genetic diversity over a wide range with relatively good reliability; to explore the possibilities of sex, breed, and age of host effects on severity of the homograft reaction; to explore possible sex-linked and/or maternal effects; to estimate the importance of the bursa of Fabricius in skin-graft reactions; and to estimate the heritability of the severity of the homograft reaction.

A skin transplantation technique has been developed which has the advantage of practically no accidental loss of grafts and a reduction in host-to-host variation, since several grafts may be placed on each host so that each host represents a replicate of the same experiment. Turning the graft one hundred and eighty degrees before it was placed on the host did not provide positive identification, by reversed feather growth, of viable grafts

since a number of viable grafts did not show feather growth by the end of the observation period.

The macroscopic numerical scoring system developed was found to be highly repeatable between observers and was considered to be reliable since it was found to be highly correlated with in vivo microscopic measurements of graft viability.

Sex of host was found to have no significant effect on homograft reactions.

Differences due to breed of host were found. RIR hosts demonstrated significantly more severe skin-graft reactions than WL hosts in fourteen of sixteen possible comparisons.

Large and significant positive associations between age of donor and host at grafting at various ages up to 14 days of age, and the earliness of onset and severity of the homograft reaction have been demonstrated.

Reciprocally crossbred females (the heterogametic sex in chickens) were used to estimate possible sex-linked and/or maternal effects on skin-graft reactions. All four breed and age groups of hosts used, demonstrated a greater reaction to crossbred female donor tissue carrying the X-chromosome of the breed other than that of the host breed, than to the reciprocally crossbred female donor tissue. These differences were significant in two of the four cases. Because maternal effects would have acted in the opposite direction, it was concluded that if they were present, they were of minor importance.

Some difficulty in differentiating among relatively close levels of genetic diversity was found in very young hosts (one and two days of age) due to weakness of reaction to grafted tissue. The difficulty in differ-

entiation among widely diverse genetic levels found in older hosts (fourteen and fifteen days of age) was due to very severe homograft reactions. Hosts of intermediate age (three and four and ten and eleven days of age) rejected grafted tissue in order of genetic diversity with the exception of one kind of donor tissue in twenty donor-host combinations.

Hosts three and four days of age at grafting and their controls, and hosts ten and eleven days of age at grafting and their controls were autopsied at twenty-six, twenty-seven, thirty-three, and thirty-four days of age, respectively. Correlations among skin-graft scores, body weights and bursa of Fabricius weights were tested for heterogeneity within age groups and then pooled. Highly significant pooled correlations between body weight and bursa weight were obtained in both age groups. However, the difference in magnitude of the two correlations suggested that atrophy of the bursa had begun previous to the age of autopsy of the older group. Birds autopsied at twenty-six and twenty-seven days of age were found to have significantly heavier bursae than birds autopsied at thirty-three and thirty-four days of age.

Weight of the bursa of Fabricius and skin-graft scores showed a highly significant negative pooled correlation in hosts autopsied at twenty-six days of age, indicating that birds found to have larger bursae at the end of the experimental period showed greater reaction to skin grafts. The pooled correlation of bursa weight and score in hosts autopsied at thirty-three days of age was also negative but was not significant. Significant differences found between bursa weights of grafted hosts and non-grafted controls indicated that bursa weights were increased as a result of skin grafting.

Body weight and skin-graft score were significantly and negatively correlated in hosts autopsied at twenty-six days of age and an essentially zero correlation was obtained from the hosts autopsied at thirty-three days of age.

Heritability estimates of skin-graft reaction were obtained from sire and dam components of the analysis of variance within age groups. Although significance was not found, the mean squares for sire families and for dam families within sires were larger than for full sibs in six of eight comparisons. The variation among estimates of heritability from these components (0.00, 0.17, 0.32, and 0.99) is considered to be a result of the limited numbers involved. These estimates indicate, however, that the severity of the homograft reaction is probably heritable.

A skin transplantation technique was developed in conjunction with a subjective macroscopic scoring system which can be used on young host chicks to determine differences in genetic diversity over a wide range. Both age and breed of host were found to have an effect on homograft reaction while sex of host did not. Although it was not possible to determine the presence of maternal effects, sex-linkage was found to be of importance in the homograft reaction in chickens. The bursa of Fabricius is indicated as a site of antibody production against skin-graft antigens using chicks as hosts. Heritability estimates, while not considered reliable due to the limited numbers involved, suggest that the severity of the homograft reaction is heritable.

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THE SKIN-GRAFT REACTION AS A MEASURE OF GENETIC DIVERSITY IN CHICKS

by

CARL R. POLLEY

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One thousand two hundred and eighty skin grafts were placed on host chicks from two populations, with extremely broad genetic bases, in a series of experiments in which each host chick received four skin grafts representing four levels of genetic diversity.

A skin transplantation technique and a macroscopic scoring system were developed and used successfully. The scoring system was found to be repeatable between observers and highly correlated to in vivo microscopic measurements of graft viability.

Sex of host was found to have no significant effect on homograft reactions.

RIR hosts were shown to have significantly more severe reaction to grafted tissue than WL hosts in fourteen of sixteen possible comparisons. Large and significant positive associations between age of donor and host at grafting up to fourteen days of age and the earliness of onset and severity of the homograft reaction were demonstrated.

All four breed and age groups of hosts showed a greater reaction to crossbred female donor tissue carrying the X-chromosome of the breed other than that of the host breed, than to reciprocally crossbred female donor tissue. These differences were significant in two of the four cases. Because maternal effects would have acted in the opposite direction, it was concluded that sex-linkage was relatively more important in skin-graft reactions in chickens.

Some difficulty in differentiating among relatively close levels of genetic diversity was found using very young hosts, due to weakness of reaction to grafted tissue. Contrarily, difficulty in differentiation among widely diverse genetic levels found in older hosts was apparently

due to very severe homograft reactions. Hosts of intermediate age rejected grafted tissue in order of genetic diversity with the exception of one kind of donor tissue in twenty donor-host combinations.

All hosts and an additional eighty non-grafted controls were autopsied following the observation period and sex, body weight, and bursa of Fabricius weight were recorded. Hosts three and four days of age at grafting and their controls, and hosts ten and eleven days of age at grafting and their controls were autopsied at twenty-six, twenty-seven, thirty-three, and thirty-four days of age, respectively.

Correlations among these measurements were tested for heterogeneity within age groups and then pooled. The difference in magnitude between the two highly significant pooled correlations for body weight and bursa weight in the two age groups indicated regression of the bursa had begun previous to autopsy at thirty-three and thirty-four days of age. Significantly heavier bursae were also found in the younger birds at autopsy.

Weight of the bursa of Fabricius and skin-graft score showed a highly significant negative pooled correlation in the younger hosts indicating birds found to have larger bursae at the end of the experimental period also showed greater reactions to grafted tissue. The pooled correlation of bursa weight and score in older hosts was negative but not significant. Significant differences found between bursa weights of grafted hosts and non-grafted controls in both age groups indicated that bursa weights were increased as a result of skin-grafting.

Body weight and skin-graft scores were highly and negatively correlated in younger hosts and there was essentially no correlation in older hosts.

Variation among heritability estimates of skin-graft reactions obtained from sire and dam components of the analysis of variance were considered to be a result of the limited numbers involved. These estimates indicated, however, that the severity of the homograft reaction is probably heritable.