THE INHERITANCE OF GRAYING IN HOLSTEIN-FRIESIAN CATTLE

by

JOSEPH WILLIAM MUDGE

B. S., Kansas State College of Agriculture
and Applied Science, 1942

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

TABLE OF CONTENTS

INTRODUCTION

Coat color is one of the distinguishing characteristics of a breed of dairy cattle. It could be called a breed trademark. It was the purpose of the early breeders of Holstein-Friesian cattle in Holland to develop, not only a good breed of dairy cattle, but one that had distinctive color markings (5). These markings would help to distinguish the breed from animals of mixed breeding.

Prawochenski (7) in 1928 concluded from studies of Holstein-Friesian cattle that there was no correlation between the amount of white markings and the amount of milk produced. Nevertheless, the production of animals that closely conform to the desired color pattern of the breed adds economic value because such animals often sell for higher prices than animals with less desirable color markings. This is emphasized often in the sale of young grade animals. Also, there is the added personal satisfaction of breeding animals which are pleasing to the eye.

The Holstein-Friesian Association of America (5), in describing desirable color markings, stated, "Black spots should be solid with no gray hairs intermixed". Gilmore (4) in a publication of the Holstein-Friesian Association of America, stated,

> "... Intermixture of Black and White. An intermixture of hairs may be the beginning of a graying process that results in the

> gradual disappearance of the black pigmentation. A sharp demarcation between the black and white areas is desired. An inherited trait underlies this decoloration. In some families the graying starts earlier than in others. While slight graying in old cattle is not objectionable it may start in yearlings which is definitely objectionable."

The Holstein-Friesian Association has taken positive steps to eliminate or control gray. Thus, Article 4, Section 12 of the By-Laws of the Holstein-Friesian Association of America reads, "The following colors shall bar the registration of males and females in this Association on and after January 1, 1949, to wit: (1) ...., ... (7) black and white intermixed to give grayish appearance (8) ...." However, this bar to registration eliminates only those animals which are gray at the time the registration application is submitted. Most animals are registered before they are six months of age. Many animals which were black and white at the time of registration, become gray later at various ages.

Since gray coat color is objectionable, a knowledge of its mode of inheritance, would help breeders reduce the incidence of graying, particularly in young animals. The purpose of this study was to determine characteristics of the graying trait, such as body areas on which it first appears, body areas on which it most frequently occurs, and the mode of its inheritance.

REVIEW OF LITERATURE

No reports of studies of gray color in Holstein-Friesian cattle have been found; therefore, this review will be limited to studies of hair structure in cattle and the graying of hair in other species of animals.

According to Fox (3), hair pigments are melanins, which are the products of the action of the enzyme, tyrosinase, on the amino acid, tyrosine. Bogart and Ibsen (1) found that hairs differed in the concentration of black pigments in the cortex, the outer part of the hair. The medulla, or center area of the hair, was usually all black. The cortices of black hairs were filled with closely packed granules of black pigment. White hairs had varying amounts of black pigment in the medullae, and about five per cent of them had small amounts of black in the cortices.

Hrdlicka (11) reported that graying of hair in humans was due to a decrease in the production of melanins in old age.

Lambert (6), in 1935, described a color variation in guinea pigs which he called "silver". This variation was described as the appearance of totally white hairs in a pigmented coat. The white hairs did not appear in the first hair coat; they developed only in later coats. Some individual animals continued to produce greater numbers of white hairs until two years of age. Lambert suggested that there were various thresholds for pigment production and that the

threshold changed with age. He postulated, from the results of his breeding tests, that the inheritance of silvering was controlled by one major recessive gene, but that the degree of silvering was influenced by plus and minus modifiers. Silvering was inherited independently from the white spotting gene and the factors of the extension series.

Wright (12) in 1947 described four different types of "silvering", only two of which were similar to graying found in cattle. The type which he called "stationary silvering" was similar in appearance to various shades of gray found in cattle, but it differed in that the silvering was present at birth and did not change with age. The type described by Lambert was called "grizzling" by Wright to differentiate it from other types. Wright's breeding tests to stock which he had obtained from Lambert confirmed Lambert's interpretation of the inheritance of "grizzling" as a major recessive gene with the degree of "grizzling" undoubtedly subject to minor factors. Wright stated, "With the usual modifiers, grizzling is completely or almost completely recessive and even in homozygotes may not appear until the animal is nearly a year old. A residual heredity can be built up by selection that enables grizzling to appear at two months or even less and to become very strong in time and which enables a trace of the character to appear in heterozygotes."

A non-congenital graying, which increased with age, in

rats was described by Castle (2) as a recessive.

Salisbury (10) in 1941 concluded that gray coat color in horses was due to a dominant gene epistatic to the basic colors. Horses known from breeding results to be homozygous turned white more rapidly than those which were heterozygous. Castle (2) also attributed gray in horses to the dominant gene, G.

Gray coat color apparently results from an intermixing of black and white hairs on a specific area of the body. It may be present at birth as in the one type of "silvering" described by Wright (12) or absent at birth, appear at later ages, and increase with age as in graying of humans, horses, rats, and "grizzling" type of graying in guinea-pigs. Castle (2) and Lambert (6) both called gray a mixture of white hairs with colored hairs, most of which are black. Wright (12) referred to white or light-colored hairs in the gray area. Possibly these light colored hairs had smaller amounts of dark pigment, and thus might have represented a form of hair structure intermediate to that of black and white. The degree of graying was dependent on the relative proportions of black and white hairs. According to descriptions of the horse by Castle (2) and the guinea-pig by Lambert (6), the numbers of white hairs on gray coat areas increased each time new hair grew after old hair was shed. This made the graying appear more intense.

Genetic explanations for graying vary with different species. Graying was described as a major or incomplete recessive, modified by various factors, in the guinea-pig, a recessive in rats, and a dominant, epistatic to the basic colors, in horses. The age of graying was changed to less than two months in guinea-pigs by selection of individuals used as breeding stock.

EXPERIMENTAL PROCEDURE

Collection of Data

The data for this study were collected by George R. Barrett, who was assisted with part of the data collection by Keith Huston. They visited between March 28, 1950 and August 8, 1950, 17 herds of registered Holsteins and one consignment sale. All animals of all ages were examined for the presence or absence of gray. The herds studied included nine in Wisconsin, four in New Jersey, two in Vermont, two in Maine, and one in Delaware. Barrett described selection of the herds studied as follows:

> "... Except for the ____herd, we had no prior knowledge as to the occurrence of gray in any of the herds studied. On the eastern tour, we attempted to include the fountainhead herds of as many different blood lines as possible. We felt that the result of the study was to include representatives of most of the important blood lines in the breed east of the Mississippi river. I think you can feel reasonably sure that these data were not biased by the deliberate selection of herds

> in which the occurrence of gray was known to be a breeding problem"...

The largest herd consisted of 430 animals and the smallest, 53, with a total of 2,704 animals studied. The number of animals of each age group in these 18 herds is presented in Table 1.

Six areas, nose, cheeks, jaws, neck, body, and escutcheon, of each animal were checked for gray. Barrett described the rating system as follows:

> "In the preliminary work which I did, I made no attempt to evaluate the degree of graying but simply marked them plus or minus depending on whether any gray was observable. This procedure left something to be desired, and I subsequently set up the arbitrary system of assigning a rating to indicate to some degree how much transformation had taken place in those areas. A grade of '1' was assigned to those areas in which a few scattered white hairs were observable upon close inspection but which might well be overlooked. A grade of '2' was assigned to those areas in which the graying was pronounced enough to be noted by any reasonably observant person. A grade of '3' was assigned when the graying had advanced to the point where it could hardly be overlooked by anyone. The grade of '4' was assigned only occasionally to those animals and those areas in which the white hairs probably outnumbered the black and in which the grade of '3' hardly seemed to describe the animal or the area."

Methods of Analysis

As the animals of each herd were examined, the ear tag number or name of each animal, its sire, dam, and dam's sire,

Table 1. The distribution in four age groups of the animals in the 18 herds of this study.

Herd	Animals 0-23 mos. old	Animals 24-47 mos. old	Animals 48-95 mos. old	Animals more than 95 mos. old	Total animals in herd
1	187	72	132	39	430
2	58	46	32	16	156
3	59	26	26	17	128
4	27	8	12	6	53
5	84	63	52	22	221
6	53	15	24	15	107
7	26	20	21	0	67
8	55	31	39	15	140
9	61	31	42	12	146
10	59	89	25	2	177
11	74	47	33	14	168
12	72	45	55	30	202
13	101	86	55	12	254
14	58	36	27	16	137
15	42	22	16	18	98
16	19	15	9	16	59
17	24	16	14	8	62
18	41	21	30	13	105
Totals	1100	689	644	271	2704

were listed on the data sheets. Opposite the name or ear tag number of each animal studied were spaces in which the presence or absence of graying on the six hair coat areas was indicated. Absence of graying was indicated by ---, and the presence of graying was indicated by numbers, previously described, to indicate the intensity of graying. Animals were grouped on the data sheets by sires. Later maternal sib groups

and full sib groups were determined from the data.

Since both the number of animals with gray and the number of gray coat areas on many animals increased with age, the animals were divided into four age groups. Group 1 consisted of animals less than 24 months of age. Group 2 included animals from 24 to 47 months old. Group 3 consisted of animals from 48 to 95 months old and group 4, of animals more than 95 months old. There were 1,100 animals in group 1; 689 in group 2; 644 in group 3; and 271 in group 4.

To determine characteristics of graying, the frequencies with which graying occurred on each of the hair coat areas of animals in each age group were tabulated. To determine the association of gray on one coat area with gray on another coat area, all possible combinations of two coat areas were tabulated as follows: gray on both areas; gray on one area only, gray on the other area only, and absence of gray on both areas.

To study the mode of inheritance of graying, tabulations were made of the numbers of affected (gray) and normal (black) progeny from different types of matings. Types of matings studied were normal with normal, normal with affected, affected with affected, normal with unobserved and affected with unobserved. Unobserved parents were those which were dead or sold from the herd before the herd was examined for this study.

RESULTS

Microscopic Studies of Holstein Hairs

In order to determine differences between hairs from gray areas of the coat and hairs from the black areas and the white areas of the coat, whole mounts of hairs from registered Holsteins of the Kansas State College herd were studied microscopically. The black hairs from black coat areas of animals with no gray appeared to have closely packed black pigment throughout the medullae and cortices. Hairs from white coat areas had black medullae with very little or no black pigment in their cortices. To the naked eye, single hairs from gray coat areas appeared to be either black or white. Microscopically, as whole mounts, many of the hairs from gray areas are indistinguishable from white hairs from white-spotted coat areas, or black hairs from black coat areas. A few hairs from gray coat areas appeared to be of intermediate form, having less black pigment in the cortices than those of black coat areas. A few hairs were found that had a brown appearance to the naked eye and appeared to be similar to those hairs often seen on young calves which haven't shed. Under the microscope these hairs showed some red pigment in the cortex.

From these observations, varying intensities of gray coat color appeared to be due to a variation in the ratios of black and white hairs within an area.

Frequency and Site of Occurrence of Gray on Hair Coat

Within Age Group and Herd. Animals possessing gray areas anywhere in their coat were first classified as gray. Within each age group in each of the 18 herds, the percentage of gray animals was determined. These percentages are presented by age group and herd in Fig. 1. The percentage of animals possessing gray in their coat was greater in the older age groups than in the younger.

There was much variation in percentage of gray animals within age groups among herds.

In one herd, 49 per cent of the animals younger than 24 months possessed gray. This was the highest percentage found in this age group in any herd. In all herds, the percentage of gray animals in the group comprised of animals older than 95 months exceeded 50 per cent. In one herd, all animals older than 47 months possessed gray coat areas.

Within Age Group and According to Location of Gray Area on Coat. Each animal had been inspected for the presence of gray in six general areas of the coat: the nose, cheeks, jaws, neck, body, and escutcheon. Within each age group, the percentage of animals possessing gray at each of the six coat locations was determined. These percentages are presented in Table 2. At each of the six coat areas, gray was found with a frequency which increased with the age grouping.

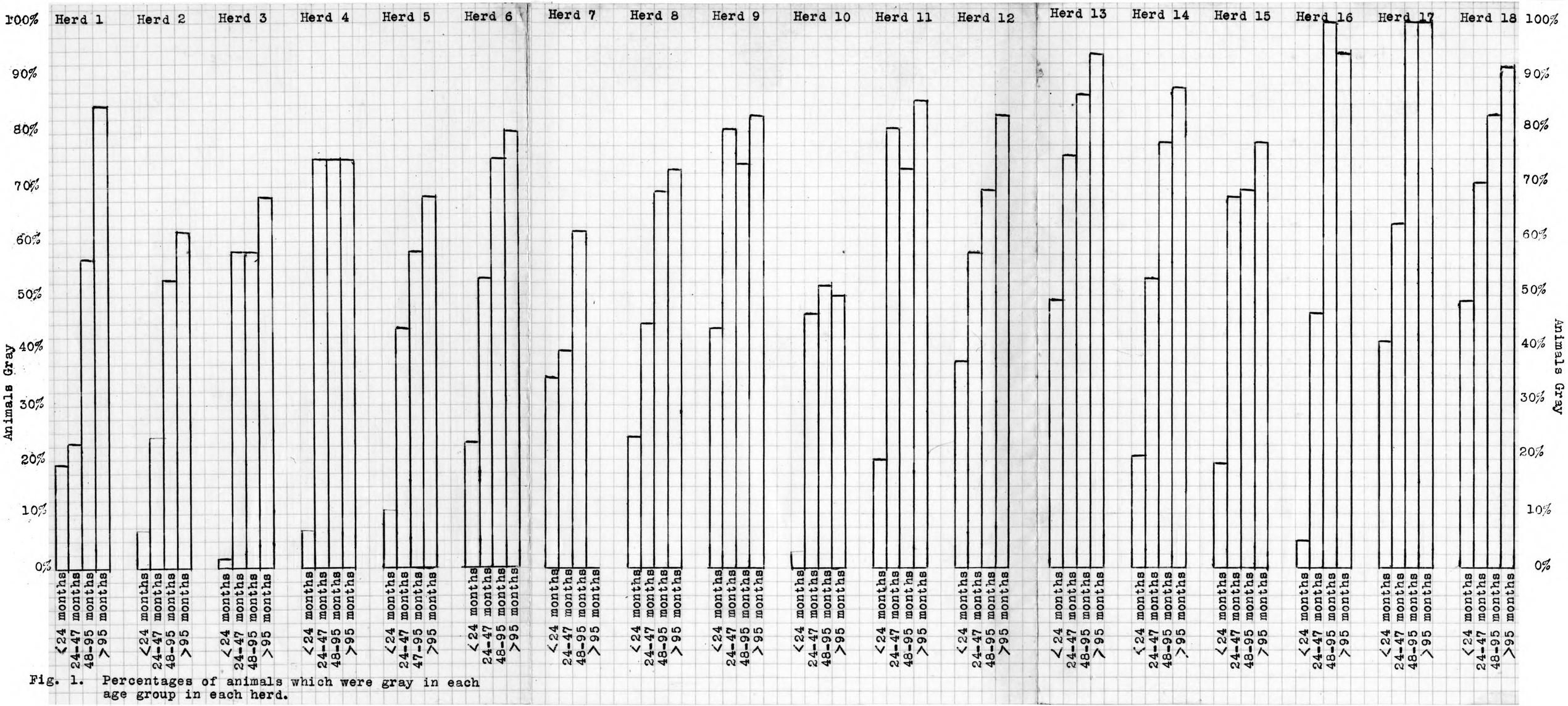

Fig. 1. Percentages of animals which were gray in each age group in each herd.

Table 2. Occurrence of gray on six coat areas. Percentage of animals in each of four age groups which possessed gray on any one or more of six coat areas.

Age group	Percentage of animals possessing gray on the coat area indicated					
	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
< 24 months	7.9	17.4	14.1	10.1	8.9	8.1
24-47 months	22.6	36.6	37.2	26.8	21.9	23.2
48-95 months	27.0	52.6	53.3	39.8	35.9	27.5
>95 months	35.1	64.6	63.8	62.0	57.6	31.7

Within each age group, the differences in the percentages of animals possessing gray on any one or more of six coat areas appear unlikely to have arisen by chance (Appendix Table 15). Therefore, it may be assumed that among animals within a given age range gray is more likely to appear on certain areas of the coat than on others. To obtain a clearer view of this tendency, the areas within each age group were ranked from highest to lowest in order of the percentage of animals possessing gray. This ranking may be seen in Table 3.

It is shown in Table 3 that gray occurred most frequently on the cheeks and jaws of animals in all age groups.

In each age group, a smaller percentage of animals possessed gray on the nose and on the escutcheon than on other areas.

Table 3. Occurrence of gray on six coat areas. Ranking of areas according to percentage of animals, within each age group, which possessed gray at the indicated area.

Rank	Age Group			
	<24 months	24-47 months	48-95 months	>95 months
1st	Cheeks	Jaws	Jaws	Cheeks
2nd	Jaws	Cheeks	Cheeks	Jaws
3rd	Neck	Neck	Neck	Neck
4th	Body	Escutcheon	Body	Body
5th	Escutcheon	Nose	Escutcheon	Nose
6th	Nose	Body	Nose	Escutcheon

Although this might be taken as evidence that graying on the nose and escutcheon was restricted by some unknown means, it might also simply reflect the fact that these Holsteins were more likely to be white on the nose and escutcheon than on any of the other four body areas. Since gray cannot occur on these white areas, the difference in the frequency of occurrence of gray on these two areas and on other body areas was examined more closely. First, the percentage of animals in each of the four age groups which possessed white on any one or more of the six coat areas was determined. These percentages are presented in Table 4.

Table 4. Occurrence of white on six coat areas. Percentages of animals in each of four age groups which possessed white on any one or more of six coat areas.

Age Group	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
< 24 months	37.7	2.4	12.1	1.1	3.3	30.6
24-47 months	31.1	3.2	11.0	1.2	2.9	27.6
28-95 months	31.8	2.0	9.5	0.5	2.8	30.4
> 95 months	35.4	2.2	9.2	0.7	1.8	30.3
All ages	34.4	2.5	10.9	0.9	2.9	29.9

Within each age group, the differences in the percentages of animals possessing white on any one or more of six coat areas are unlikely to have occurred by chance (Appendix Table 16). Therefore, it may be assumed that white was most likely to be found on the noses and escutcheons of these Holsteins.

In this table it may be seen that animals with white noses or white escutcheons were about 10 times as frequent as animals with white cheeks, necks, or bodies. Animals with white noses or white escutcheons were about three times as frequent as animals with white jaws. Thus, even if gray were to occur with equal frequency on any black area of the body, ratios obtained from a group of animals when the distribution of the white areas is not considered would be biased in favor of the cheeks, jaws, neck, and body. This bias may be avoided by considering

at each location only those animals which possessed or, as is in the case of gray animals, were assumed to have possessed at one time black hair coats.

Among these animals, the percentage in each of the four age groups which possessed gray on any one or more of six coat areas was determined. These percentages are presented in Table 5.

Table 5. Occurrences of gray on six black coat areas. Percentages of animals in each of four age groups which possessed gray on any one or more of six coat areas.

Age group	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
< 24 months	12.7	17.9	16.0	10.2	9.2	11.7
24-47 months	32.8	37.8	41.8	27.2	22.6	26.7
48-95 months	39.6	53.7	58.8	39.9	36.9	39.8
>95 months	54.3	66.0	70.3	62.4	58.6	45.5

Within any age group, the differences in the percentages of animals possessing gray on any one or more of six coat areas, which possessed or were assumed to have possessed at one time black hair, appear unlikely to have arisen by chance (Appendix Table 17). To obtain a clearer picture of the frequency of occurrence of animals with gray on any one or more of the six body areas, the areas within each age group were ranked from highest to lowest in order of the percentage of animals possessing gray. This ranking may be seen in the following table.

Table 6. Occurrence of gray on six coat areas. Ranking of areas according to percentage of animals, within each age group, which possessed gray at the indicated area.

Rank	Age groups			
	<24 months	24-47 months	48 - 95 months	>95 months
1st	Cheeks	Jaws	Jaws	Jaws
2nd	Jaws	Cheeks	Cheeks	Cheeks
3rd	Escutcheon	Nose	Neck	Neck
4th	Nose	Neck	Escutcheon	Body
5th	Neck	Escutcheon	Nose	Nose
6th	Body	Body	Body	Escutcheon

It is shown in Table 6 that the black coat areas of the cheeks and jaws became gray more frequently than other coat areas.

It is of interest to examine the frequency of occurrence of gray on the six coat areas of animals less than six months old. Three-hundred sixty-five calves younger than six months were examined. Thirty-four, or 9.3 per cent, were gray on one or more coat areas. These gray calves occurred in nine of the 18 herds; no gray calves, younger than six months, were observed in the other nine herds. One bull calf was observed to be gray, grade 2, on the nose and cheeks at the age of three weeks. Another calf was gray on all six coat areas at three months. The percentages of animals, younger than six months, which were gray at each of the coat areas

is presented in Table 7.

Table 7. Occurrence of gray on six coat areas of 365 calves. Percentage of animals possessing gray on any one or more of six coat areas.

Age group	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
<6 months	1.1	7.4	1.9	1.1	0.5	3.0

Among animals younger than six months, gray occurred with greatest frequency on the cheeks and with the next greatest frequency on the escutcheon. Gray in animals less than six months old occurred much less frequently than in the group of animals between six and 24 months old.

In contrast to the small percentage of animals, less than six months old, which were gray, a large percentage of animals older than 95 months were gray. Of the 271 animals more than 95 months old, 112 were gray on all six coat areas, 46 were black on all areas, and the other 113 were gray on from one to five coat areas.

Within Age Group and According to Amount of White on Head. Personal observations had suggested that Holsteins with large amounts of white on their heads were more likely to be gray than other animals of the breed. In order to test this hypothesis all animals observed to be entirely white on all of three coat areas of the head -- the cheeks,

noses, and jaws -- were considered to have large amounts of white on the head. The white-head color pattern is not popular with Holstein breeders and as a result, there is some selection against it. Among the 2,704 animals studied, only 57 had white on all three head areas. All but two of these animals were in six of the 18 herds.

Table 8. Occurrence of gray on nearly white headed animals. Comparison of ratios of gray to normal animals among white-headed and among other animals.

Age group	White headed animals		All other animals		
	Gray	Normal	Gray	Normal	Chi-square
< 24 months	8	17	140	252	0.141 n.s.
24-47 months	17	0	175	115	11.530** (P < 0.005)
48-95 months	6	2	136	38	0.042 n.s.
> 95 months	5	0	41	8	0.974 n.s.

Within the three age groups -- younger than 24 months, 47-95 months, and older than 95 months -- the differences in ratios of gray to normal animals among nearly white-headed animals and among all other animals were small enough to have occurred by chance. Within the 24-47 months age group, the differences in ratios of gray to normal animals among nearly white-headed animals and among all other animals were too large to have occurred by chance. These differences suggested

that Holsteins with large amounts of white on their heads tend to become gray at a younger age than other Holsteins.

Association of Gray on Any Two of Six Coat Areas. All possible two coat area combinations of the six coat areas were studied to determine if gray were more likely to occur jointly on both coat areas than singly on either of the two coat areas. The frequency with which graying occurred on both of two coat areas as contrasted to graying on only one of the two areas was highest for the following pairs of areas in all age groups: cheeks and jaws, neck and body, nose and cheeks, and nose and jaws. In the following pairs of coat areas, especially in the younger age groups, the frequency with which graying occurred on both areas was but a little greater than the frequency of graying on a single area: nose and escutcheon, cheeks and escutcheon, jaws and escutcheon, and jaws and neck. On the remaining pairs of coat areas, nose and neck, nose and body, cheeks and neck, cheeks and body, jaws and body, neck and escutcheon, and body and escutcheon, graying on a coat area of the head, or graying on the escutcheon occurred more frequently than graying jointly on both areas of these remaining pairs in animals younger than 48 months. In animals older than 47 months, graying jointly on both coat areas of these pairs occurred more frequently than singly on one area. The data from which these associations

Table 9. The occurrence of gray on one coat area with occurrence of gray on another coat area of animals within four age groups.

Coat area		Animals less than 24 months old									
		Cheeks		Jaws		Neck		Body		Escutcheon	
		Black	Gray	Black	Gray	Black	Gray	Black	Gray	Black	Gray
Nose	Black	583	16	573	25	592	7	587	9	524	32
	Gray	22	67	27	60	59	28	56	32	34	37
Cheeks	Black			773	44	862	0	848	12	610	34
	Gray			32	103	95	95	110	79	34	55
Jaws	Black					796	2	778	4	602	29
	Gray					76	77	88	65	37	50
Neck	Black							892	12	668	44
	Gray							26	82	19	35
Body	Black									632	59
	Gray									12	30

Coat area		Animals, 24-47 months old									
		Cheeks		Jaws		Neck		Body		Escutcheon	
		Black	Gray	Black	Gray	Black	Gray	Black	Gray	Black	Gray
Nose	Black	282	37	264	56	293	16	298	17	254	47
	Gray	33	120	33	123	80	76	89	67	58	79
Cheeks	Black			331	50	391	18	378	20	287	46
	Gray			25	204	101	147	131	113	65	102
Jaws	Black					331	12	330	10	257	37
	Gray					127	139	145	117	84	108
Neck	Black							456	28	322	78
	Gray							55	122	29	75
Body	Black									318	88
	Gray									18	72

Table 9 (concl.)

Coat area		Animals, 48-95 months old: Cheeks Black	Cheeks Gray	Jaws Black	Jaws Gray	Neck Black	Neck Gray	Body Black	Body Gray	Escutcheon Black	Escutcheon Gray
Nose	Black	204	50	187	77	241	25	223	31	216	33
	Gray	23	152	21	153	70	106	82	91	43	112
Cheeks	Black			224	53	282	13	200	23	159	44
	Gray			12	287	99	238	135	193	62	141
Jaws	Black					229	9	220	14	172	29
	Gray					125	216	158	179	97	140
Neck	Black							336	39	235	63
	Gray							64	185	43	111
Body	Black									248	68
	Gray									34	108

Coat area		Animals more than 95 months old: Cheeks Black	Cheeks Gray	Jaws Black	Jaws Gray	Neck Black	Neck Gray	Body Black	Body Gray	Escutcheon Black	Escutcheon Gray
Nose	Black	63	18	54	26	61	19	57	23	67	11
	Gray	6	89	4	91	15	80	25	69	21	53
Cheeks	Black			64	22	80	9	81	16	58	9
	Gray			7	153	21	154	43	132	30	77
Jaws	Black					67	6	57	12	56	7
	Gray					30	143	55	120	36	79
Neck	Black							80	20	69	15
	Gray							32	134	23	74
Body	Black									70	15
	Gray									23	71

were derived are presented in Table 9. The association of graying on one area with graying on another area was tested with chi-squares using the fourfold contingency method described by Snedecor (8), with the data presented in Table 9. The chi-squares are presented in Appendix Table 18.

Genetic Tests

The occurrence and expression of a supposedly hereditary trait may be modified genetically and/or environmentally. Some one of a multitude of theoretical genetic explanations may be advanced to account for the phenomena noted in the occurrence and expression of a trait. Selection of a single genetic explanation from among the many possible is most simply accomplished by examining the suitability of these explanations sequentially, commencing with the simplest and advancing to the more complex as time and the data permit. This approach was used here in studying the inheritance of graying.

All animals which showed gray of any grade on any coat area were considered to be affected. All others were considered to be normal.

Exclusion Tests for Simple Dominant or Simple Recessive. The simplest genetic explanations that may be advanced are that the trait is either a simple dominant or a simple recessive trait. If a trait is inherited as a simple recessive,

it should be present in all progeny resulting from the mating of affected with affected animals. If a trait is inherited as a simple dominant, it should be absent in all progeny resulting from the mating of normal with normal animals. The progeny obtained from such matings are tabulated in Table 10.

Table 10. Ratios of gray to normal progeny within three age groups from matings of normal with normal and affected with affected animals, with chi-square to test hypothesis that these ratios are equal.

Type of matings	Age Groups: < 24 months Gray Progeny	< 24 months Normal Progeny	24-47 months Gray Progeny	24-47 months Normal Progeny	47-95 months Gray Progeny	47-95 months Normal Progeny
Normal x normal	3	6	2	4	2	47
Affected x affected	6	2	21	8	21	71
	$X^2 = 10.77$ (1 d.f.) ($P<0.005$)		$X^2 = 1.89$ n.s. (1 d.f.)		$X^2 = 4.86$ (1 d.f.) ($P< 0.05$)	

In no instance were both parents of an animal more than 95 months old inspected; therefore, there were no available data for that age group from matings of normal with normal or affected with affected animals.

In all age groups some gray progeny resulted from the mating of normal animals. Their numbers were thought to be too large to have resulted from errors in classification or recording. The presence of these gray progeny excluded the

genetic explanation that graying is inherited as a single simple dominant trait. Because of the possibility that there might be several types of graying each specific for definite age groups, a study of the ages of the normal parents which produced gray progeny within each age group was made. Within the group between 48 and 95 months old the youngest gray animal from normal parents was 54 months old. The oldest parent of gray animals in this group was 120 months old. Therefore, graying of the hair coat of animals between the ages of 54 and 120 months is not a simple dominant trait. Likewise, graying of animals between the ages of 43 and 124 months and between the ages of 5 and 75 months is not a single simple dominant trait.

In all age groups, some normal progeny resulted from the matings of affected animals. Their numbers were thought to be too large to have resulted from errors in classification or recording. The presence of these normal progeny excluded the genetic explanation that graying is inherited as a single simple recessive trait. Because of the possibility that there might be several types of graying each specific for definite age groups, a study of the gray parents which produced these normal progeny within age groups was made. Within the age group between 48 and 95 months old, the youngest normal animal from gray parents was 50 months old. The oldest parent of normal animals within this age group was 154 months old.

Therefore, graying of the hair coat of animals between the ages of 50 and 154 months is not a single simple recessive. Likewise, graying of animals between the ages of 24 and 90 months and between the ages of one and 170 months is not a single simple recessive trait.

The differences in the ratios of gray to normal progeny resulting from the matings of normal animals and from the matings of affected animals in the age groups of less than 24 months and 48 to 95 months are too large to have occurred by chance. Therefore, it is assumed that these differences in the ratios of gray to normal progeny are due to differences in inheritance. There are too few normal matings represented in the 24-47 months age group to draw a similar conclusion from this group.

Indirect Progeny Ratio Tests for Simple Dominance. Although the observation of affected progeny from matings of normal with normal ruled out simple dominance as a mode of inheritance of several forms of graying, other tests provide additional information. The progeny ratios which were used in these additional tests are presented in Table 11.

Matings of Affected with Normal. In the matings of affected with normal, all affected animals were assumed to be heterozygous (Aa). Under this assumption and if graying were a simple dominant, normal progeny should result from not

more than 50 per cent of such matings. If the number were in excess of 50 per cent of the total progeny examined, a simple dominant could be ruled out. An excess of normal progeny from such matings was produced only in the group of animals 24 to 47 months old.

Table 11. Observed and expected ratios of gray to normal progeny within age groups from matings in which one or both animals were gray.

Age group	Mating type	Observed progeny ratio		Expected progeny ratio	
		Affected	Normal	Affected	Normal
>95 months	affected x unobserved	19	6	12.5	12.5
48-95 months	affected x normal	4	5	4.5	4.5
	affected x affected	6	2	6.0	2.0
	affected x unobserved	81	34	57.5	57.5
	Total for age group	97	41	68.0	64.0
24-47 months	affected x normal	22	42	32.0	32.0
	affected x affected	22	18	30.0	10.0
	affected x unobserved	105	53	79.0	79.0
	Total for age group	149	113	141.0	121.0
<24 months	affected x normal	26	131	78.5	78.5
	affected x affected	31	71	76.5	25.5
	affected x unobserved	86	221	153.5	153.5
	Total for age group	143	423	308.5	257.5

Matings of Affected with Affected. In the matings of affected with affected, all animals were assumed to be heterozygous (Aa). Under this assumption and if graying were a simple dominant, normal progeny should result from not more than 25 per cent of such matings. If the number were in excess

of 25 per cent of the total progeny, a simple dominant mode of inheritance of graying might be ruled out. Normal progeny significantly in excess of 25 per cent of the total were produced only in the group of animals less than 24 months old.

Matings of Affected with Unobserved. In the matings of affected with unobserved, all affected animals were assumed to be heterozygous (Aa) and all unobserved animals were assumed to be normal (aa). Under this assumption and if graying were a simple dominant, normal progeny should result from not more than 50 per cent of such matings. If the number were in excess of 50 per cent of the total, a simple dominant would be ruled out as the mode of inheritance of graying. Normal progeny significantly in excess of 50 per cent of the total were produced only in the group of animals less than 24 months old.

Test from Combined Ratios. The expected ratios of gray to normal progeny from the three previously described types of matings were added to obtain expected ratios for this test. If normal progeny significantly in excess of the expected normal progeny were produced the inheritance of graying as a simple dominant would be ruled out. The only significant excess of normal progeny occurred in animals less than 24 months old.

Indirect Progeny Ratio Tests for Simple Recessive. Matings of Normal with Affected. In the matings of normal

with affected, all normal animals were assumed to be heterozygous (Nn). Under this assumption and if graying were a simple recessive, gray should appear on not more than 50 per cent of the progeny resulting from such matings. If the number were in excess of 50 per cent of the total, a simple recessive mode of inheritance would be ruled out. There was no significant excess of affected progeny in any of the age groups.

Matings of Normal with Normal. In the matings of normal with normal, all animals were assumed to be heterozygous (Nn). Under this assumption and if graying were a simple recessive, gray should appear on not more than 25 per cent of the progeny resulting from such matings. If the number were in excess of 25 per cent of the total, a simple recessive mode of inheritance would be ruled out. The number of affected progeny was not significantly in excess of 25 per cent of the total in any age group.

Matings of Normal with Unobserved. In the matings of normal with unobserved, normal animals were all assumed to be heterozygous (Nn) and unobserved animals were all assumed to be affected (nn). Under this assumption and if graying were a simple recessive, gray should appear on not more than 50 per cent of the progeny resulting from such matings. If the number were in excess of 50 per cent of the total, a simple recessive mode of inheritance would be ruled out. In

each of the three older age groups, there was a small excess of gray progeny; however, each excess was so small that it could have occurred as a chance deviation from expectation.

Table 12. Observed and expected ratios of gray to normal progeny within age groups from the matings in which one or both animals were normal.

Age group	Mating type	Observed progeny ratio: Affected	Observed progeny ratio: Normal	Expected progeny ratio: Affected	Expected progeny ratio: Normal
>95 months	normal x unobserved	6	2	4.0	4.0
48-95 months	normal x normal	3	6	2.25	6.75
	normal x affected	4	5	4.5	4.5
	normal x unobserved	48	42	45.0	45.0
	Total for group	55	53	51.75	56.25
24-47 months	normal x normal	2	4	1.5	4.5
	normal x affected	22	42	32.0	32.0
	normal x unobserved	46	31	38.5	38.5
	Total for group	70	77	72.0	75.0
<24 months	normal x normal	2	47	12.25	36.75
	normal x affected	26	131	78.5	78.5
	normal x unobserved	52	198	126.0	126.0
	Total for group	82	376	216.75	241.25

Test from Combined Ratios. The expected ratios of affected to normal progeny from the three previously described tests were added to obtain the expected ratios for this test. If the number of observed affected progeny was significantly in excess of the expected affected progeny, a simple recessive mode of inheritance would be ruled out. The only excess of affected progeny occurred in the animals

more than 95 months old. This excess was so small that it could have occurred by chance.

The ratios from which this test and the three previously described tests were made are presented in Table 12.

Sib Method

In studying the ratios of progeny produced from heterozygous animals, some parents, due to the small number of progeny or chance, are not identified as being heterozygous because they produced only normal progeny. Failure to identify these heterozygous animals and to include their normal progeny in the combined ratios of affected to normal progeny from heterozygous parents would result in too narrow a ratio of affected to normal progeny. Weinberg, as cited by Stern (9), developed a method of correcting data to obtain the true ratios of affected to normal progeny from matings of heterozygotes. He did this by adding the ratios of affected and normal sibs of each affected progeny in each possible combination.

For several reasons this method provided very little data for study. In a total of 2,704 animals inspected, there were only 177 full sib groups, most of which consisted of two animals. Because the percentage of gray animals increased in older age groups, full sib pairs in which the animals were in different age groups were not used. Also, in some instances,

it was impossible to determine the genotype of the dams because they had no progeny from affected sires. The corrected ratios of gray to normal progeny from matings of heterozygous animals with gray animals are presented in Table 13.

Table 13. Corrected ratios of gray to normal progeny within age groups from matings of heterozygous animals with gray animals, and chi-squares to test if these ratios differed from expected 1:1 ratio.

Age group	Progeny ratios		Chi-squares
	Gray	Normal	
47-95 months	2	1	0.333 n.s.
24-47 months	0	1	1.000 n.s.
< 24 months	4	6	0.000 n.s.

If the mode of inheritance of graying were a simple recessive, a ratio of one gray to one normal animal would be produced. In all age groups the differences between the observed and expected ratios were so small, they could have occurred by chance. The results of this test did not rule out inheritance of graying as a simple recessive.

If gray were inherited as a simple recessive, matings of heterozygotes should produce progeny in the ratio of one gray to three normal animals. The corrected ratios of progeny from the matings of heterozygous animals are presented in Table 14.

Table 14. Corrected ratios of gray to normal progeny within age groups from matings of heterozygotes and chi-squares to test if these ratios differ from the expected ratio of one gray to three normal.

Age group	Progeny Ratios		Chi-squares
	Gray	Normal	
48-95 months	6	4	4.88 (1 d.f.) ($P < 0.05$)
24-47 months	0	1	0.333 n.s.
< 24 months	2	2	1.250 n.s.

The differences between the observed and expected ratios of gray to normal animals less than 48 months old were so small they could have occurred by chance. The ratios of gray to normal progeny of those ages could have resulted from the inheritance of graying as a recessive. The excess of gray progeny more than 47 months old was large enough that it was unlikely to have occurred by chance. Therefore, for animals within this age group the inheritance of graying as a recessive was ruled out.

DISCUSSION

Characteristics of Graying

The percentage of gray animals within any of the four age groups differed widely among herds. However, with few exceptions, the older age groups had higher percentages of gray animals than those groups immediately younger. These

findings support the view that at least one type of graying is associated with maturity and advancing age. However, the age of onset of graying was not restricted to either maturity or puberty as calves and yearlings were found to possess gray. It is not apparent whether this juvenile graying should be considered as a variation of the general graying phenomenon or as a separate trait.

In all age groups, gray occurred more frequently on the cheeks and jaws than on the other areas of the body. The percentages of animals with gray noses and escutcheons were lower than the percentages of animals with gray on the other coat areas. However, gray cannot occur on white coat areas and animals with white noses and escutcheons were about ten times as frequent as animals with white cheeks, necks, and bodies. Even if gray were to occur with equal frequency on any black area of the body, percentages obtained from a group of animals when the distribution of the white coat areas of the body is not considered would be biased in favor of the cheeks, jaws, neck, and body. To eliminate this bias, a study of each coat area was made from animals which possessed, or as in the case of gray animals were assumed to have possessed, black hair on that coat area. After removing the bias due to the presence of white areas on the animals, the percentage of animals with gray cheeks and jaws

remained higher than percentages of animals gray on other body areas. This suggests that these two coat areas are more prone to graying than other coat areas. The cause of this phenomenon is not known.

Studies of association of graying on one coat area with graying on another coat area indicated that for several pairs of coat areas graying was much more likely to occur jointly on both areas, than singly on one or the other of the areas. Those areas on which it was most likely to occur jointly on both areas, in all age groups, were: cheeks and jaws, neck and body, nose and cheeks, and nose and jaws. It was interesting that three of these pairs of coat areas are on the head, where graying was most likely to appear first. For some other pairs of areas--nose and neck, nose and body, cheeks and neck, cheeks and body, jaws and body, neck and escutcheon--graying was most likely to appear on one area, the head area or escutcheon in animals of the two younger age groups, then occur jointly on both coat areas of animals in the two older age groups. Graying occurred simultaneously on all three areas of the head more frequently than singly on one area or jointly on two of the three areas. It would be interesting in future studies to test the correlation of graying with the amount of white on the bodies of Holsteins.

Genetics of Graying

It was apparent from the results of critical matings, production of gray calves from matings of normal animals and the production of normal calves from the matings of affected animals, that the mode of inheritance of graying was not that of a single simple dominant or a single simple recessive.

Examination of the ages of animals in the critical matings suggested that there might be several types of graying. Thus, one type might be specific for animals of some ages in one herd. Another type might be specific for different ages in several other herds. The variation among herds in percentages of gray animals in younger age groups also suggested this possibility.

Indirect progeny ratio tests and the Weinberg sib method were used to provide additional information. The indirect progeny ratio tests, although not so discriminatory as the critical matings, made possible the use of other types of matings in the study of the data. The Weinberg sib method, useful in human genetics, had definite limitations in this study, because there are few full sib groups in dairy cattle, and because full sib groups in which the animals were in different age groups could not be used. The results of these other tests did not cause one to reject either a simple dominant or simple recessive mode of inheritance of graying.

SUMMARY AND CONCLUSIONS

The nature, occurrence, and inheritance of graying, an undesirable coat color in Holsteins, were studied. This coat color apparently results from the intermixing of black and white hairs. To the naked eye most hairs from gray coat areas appeared to be either black or white; a very few appeared to be brown. Microscopically, most hairs from gray coat areas, were indistinguishable from normal black hairs, from normal black coat areas, and normal white hairs from normal white spotted coat areas. A few hairs were of intermediate type with less pigment in the cortex than normal black hairs.

Data collected on 2,704 Holsteins furnished evidence regarding the age of onset, frequency, and site of occurrence of graying on coat areas. The data were grouped according to the age of the animal into four age groups; younger than 24 months, 24 to 47 months, 48 to 95 months, and more than 95 months old.

With few exceptions, the older age groups had higher percentages of gray than those groups immediately younger. This indicated an increase in graying as age increased. However, the percentages of young animals which were gray in some herds, as high as 49 per cent of all animals younger than two years in one herd, indicated that not all graying was due to old age.

In most animals gray appeared first on the cheeks and jaws and these coat areas had the highest frequencies of gray in all age groups. The body was usually the last coat area to become gray. The percentages of gray animals among nearly white-headed Holsteins were no higher than among all others, except in the group from 24 to 47 months old. This might indicate a tendency for these animals to become gray at a younger age.

Gray was more likely to appear jointly on the following pairs of coat areas, than singly on one or the other: jaws and cheeks, neck and body, nose and cheeks, and nose and jaws.

The inheritance of graying as a single simple dominant was excluded by gray progeny resulting from matings of normal with normal animals. Likewise, the inheritance of graying as a single simple recessive was excluded by normal progeny resulting from the matings of affected with affected progeny.

Indirect progeny ratio tests did not disprove either a simple dominant or simple recessive mode of inheritance of graying. These tests are not so discriminatory as the critical matings.

Examination of the ages of the animals in the critical matings suggested that there might be several types of gray. One type might have definite age effects in several herds. Another type might have different age effects in other herds.

Higher percentages of gray progeny from matings of

affected animals than from matings of normal animals indicated that graying was inherited to some extent. Therefore, whenever other characteristics are equal, in the selection of Holsteins for breeding purposes preference should be given to those animals which show no gray coat color. Graying of young animals is more objectionable than graying of mature animals.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Prof. F. W. Atkeson for his helpful suggestions; to Dr. George R. Barrett for his work in the collection of the data, for making it available, and for his continued interest in this study; and to Dr. Keith Huston for his counsel and encouragement throughout the preparation of this thesis.

LITERATURE CITED

1. Bogart, R., and H.L. Ibsen.
The relation of hair and skin pigmentation to colour inheritance in cattle, with some notes on guinea-pig hair pigmentation. J. Genetics. 35:31. 1937.

2. Castle, W.E.
Coat color inheritance in horses and in other mammals. Genetics. 39:35. 1954.

3. Fox, D.L.
Animal biochromes and structural colours. Cambridge University Press, New York, N.Y. 1953.

4. Gilmore, L.O.
Color and color patterns in Holstein-Friesian cattle. Color markings of your Holstein-Friesian cattle. Holstein-Friesian Association of America. 1953.

5. Holstein-Friesian Association of America.
Color markings of your Holstein-Friesian cattle. Holstein-Friesian Association of America. 1953.

6. Lambert, W.V.
Silver guinea-pigs. J. Hered. 26:279. 1935.

7. Prawochenski, R.
Correlation between the surface of white markings in the colour of Holstein-Friesian cows and their productiveness. Proceedings of the Eighth International Dairy Congress. 8:292. 1928.

8. Snedecor, G.W.
Statistical Methods. Iowa State College Press, Ames, Iowa. 5th ed., 1956.

9. Stern, C.
Principles of human genetics. W.H. Freeman & Co., San Francisco, Calif. 1949.

10. Salisbury, G.W.
The inheritance of equine coat color. J. Hered. 32:235. 1941.

11. Vitamin treatment offers little hope for gray hair. Science Newsletter. 41:204. 1942.

12. Wright, S.
On the genetics of several types of silvering in the guinea-pig. Genetics. 32:115. 1947.

APPENDIX

Table 15. Occurrence of gray on six coat areas. Numbers of animals which possessed gray on any one or more of six coat areas. Heterogeneity chi-squares to test hypothesis that the numbers are equal.

	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
Younger than 24 months old						
Number gray	87	192	155	111	98	89
Number inspected	1100	1100	1100	1100	1100	1100
	$X^2_{(5\ d.f.)} = 83.85$ ($P < 0.005$)					
24 to 47 months old						
Number gray	156	252	256	185	151	160
Number inspected	689	689	689	689	689	689
	$X^2_{(5\ d.f.)} = 81.47$ ($P < 0.005$)					
48 to 95 months old						
Number gray	174	339	343	256	231	277
Number inspected	644	644	644	644	644	644
	$X^2_{(5\ d.f.)} = 139.04$ ($P < 0.005$)					
More than 95 months old						
Number gray	95	175	173	168	156	86
Number inspected	271	271	271	271	271	271
	$X^2_{(5\ d.f.)} = 121.37$ ($P < 0.005$)					

Table 16. Occurrence of white on six coat areas. Numbers of animals which possessed only white on any one or more of six coat areas. Heterogeneity chi-squares to test hypothesis that the numbers are equal.

	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
Younger than 24 months old						
Number gray	415	26	133	12	36	337
Number inspected	1100	1100	1100	1100	1100	1100
$X^2 = 1117.4$ (5 d.f.) ($P < 0.005$)						
24 to 47 months						
Number gray	214	22	76	8	20	190
Number inspected	689	689	689	689	689	689
$X^2 = 543.70$ (5 d.f.) ($P < 0.005$)						
48 to 95 months						
Number gray	205	13	61	3	18	196
Number inspected	644	644	644	644	644	644
$X^2 = 605.89$ (5 d.f.) ($P < 0.005$)						
More than 95 months						
Number gray	96	6	25	2	5	82
Number inspected	271	271	271	271	271	271
$X^2 = 533.62$ (5 d.f.) ($P < 0.005$)						

Table 17. Occurrence of gray on six black coat areas. Numbers of animals which possessed gray on any one or more of six black coat areas. Heterogeneity chi-squares to test hypothesis that the numbers are equal.

	Nose	Cheeks	Jaws	Neck	Body	Escutcheon
Younger than 24 months old						
Number gray	87	192	155	111	98	89
Number inspected	685	1074	967	1088	1064	763
$X^2 = 73.5$ (5 d.f.) $(P < 0.005)$						
24 to 47 months old						
Number gray	156	252	252	185	151	160
Number inspected	475	667	613	681	669	599
$X^2 = 68.0$ (5 d.f.) $(P < 0.005)$						
48 to 95 months old						
Number gray	174	339	343	256	231	177
Number inspected	439	631	583	641	626	445
$X^2 = 119.43$ (5 d.f.) $(P < 0.005)$						
More than 95 months old						
Number gray	95	175	173	168	156	86
Number inspected	175	265	246	269	266	189
$X^2 = 69.36$ (5 d.f.) $(P < 0.005)$						

Table 18. Chi-squares to test hypothesis of independence of occurrence of gray on any two areas.

Areas on animal	<24 months	24-47 months	47-95 months	>95 months
Nose and cheeks	377.57	207.82	178.22	90.74
Nose and jaws	340.79	165.56	144.06	75.63
Nose and neck	446.07	121.16	128.95	62.18
Nose and body	158.79	97.74	82.66	32.82
Nose and escutcheon	133.41	79.20	142.16	47.01
Cheeks and jaws	440.87	336.76	348.24	130.22
Cheeks and neck	417.16	244.27	285.45	148.02
Cheeks and body	314.20	65.69	88.37	84.65
Cheeks and escutcheon	229.00	116.95	91.50	54.14
Jaws and neck	416.02	188.62	206.81	116.03
Jaws and body	340.26	167.82	134.82	49.02
Jaws and escutcheon	212.93	103.90	89.61	51.73
Neck and body	628.50	290.65	262.66	92.18
Neck and escutcheon	190.91	105.61	109.04	59.17
Body and escutcheon	141.18	111.94	121.14	57.61

THE INHERITANCE OF GRAYING IN HOLSTEIN-FRIESIAN CATTLE

by

JOSEPH WILLIAM MUDGE

B. S., Kansas State College of Agriculture
and Applied Science, 1942

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1957

The purposes of this study were to determine the characteristics of graying of the hair coat of Holstein-Friesian cattle and the mode of inheritance of graying.

To the naked eye, hairs from gray coat areas of registered Holsteins appeared to be either black or white. Microscopically, as whole mounts, most hairs from gray coat areas, were indistinguishable from black hairs of normal black coat areas, and white hairs from normal white spotted areas. A few hairs, intermediate in type, appeared to have less black pigment in the cortex than normal black hairs. Thus, it appeared that graying is due to an intermixture of black and white hairs.

Data from 2,704 registered Holsteins, in 17 herds and one consignment sale, in five states, were used in this study. The data were collected by George R. Barrett, who was aided in part of the collection by Keith Huston. Herds were inspected between March 28, 1950 and August 8, 1950. Because the percentages of gray animals increased as the ages of the animals increased, the animals were divided into four age groups; younger than 24 months, 24 to 47 months, 48 to 95 months, and older than 95 months. For this study the hair coat was divided into six areas: nose, cheeks, jaws, neck, body, and escutcheon.

Graying occurred most frequently on the cheeks and jaws of animals in all ages. In most animals it occurred first on

coat areas of the head. The body was usually the last coat area to become gray. On the following pairs of coat areas, gray occurred more frequently, jointly on both areas, than singly on one area or the other area: cheeks and jaws, neck and body, nose and jaws, and nose and cheeks.

The inheritance of graying as a single simple dominant was excluded because gray progeny resulted from matings of normal with normal animals. Likewise, the inheritance of graying as a single simple recessive was excluded because normal progeny resulted from the matings of affected with affected progeny.

Indirect progeny ratio tests did not cause one to reject either a simple dominant or simple recessive mode of inheritance. However, these tests are not so discriminatory as the critical matings.

Examination of the ages of the animals in the critical matings suggested that there might be several types of gray. One type might have definite age effects in several herds. Another type might have different age effects in other herds.

Higher percentages of gray progeny from matings of affected animals than from matings of normal animals indicated that graying was inherited to some extent. Therefore, whenever other characteristics are equal, in the selection of Holsteins for breeding purposes, preference should be given to those animals which show no gray coat color. Graying of young animals is more objectionable than graying of mature animals.