

LINKAGE RELATIONS OF AUTOSOMAL FACTORS IN THE FOWL¹

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INTRODUCTION

FOR the past 20 years data have been accumulated at the KANSAS AGRICULTURAL EXPERIMENT STATION on the linkage relations of factors in the domestic chicken with the objective of establishing chromosomal maps for this species. The possibility of obtaining sufficient data for locating gene markers on all the chromosomes seems rather remote, since each succeeding publication on the cytology of the chicken increases the proposed number of chromosomes. One of the earliest careful studies on the chromosomes of the fowl was by GUYER (1916), and he found the diploid number to be 15 to 19. Later SHIWAGO (1924) reported 32 chromosomes, and HANCE (1926) reported 36 chromosomes. WHITE (1932) found 66 chromosomes in the fowl and YAMASHINA, in 1944, placed the number of chromosomes at 78. Numerous other workers have reported chromosome numbers between the above mentioned extremes.

The lack of agreement among cytologists is due to the difficulty of obtaining satisfactory preparations for study and the rather large number of very small chromosomes. Whether there are 16 or 39 pairs of chromosomes in the chicken will not make too much difference to the geneticist since even the smallest proposed number makes the problem of complete mapping a complicated one. It is true, however, that the chicken is the one domestic animal which offers material that is at all promising for gene mapping. Some of the sex-linked factors in the fowl show what appears to be independent assortment among themselves. This would indicate that map distances on the sex chromosome are so great that the percentage of crossing over is large enough to obscure evidence of linkage. The earlier cytologists believed that the largest member of the chromosomal complex was the sex chromosome but more recent workers hold that several of the autosomes are larger than the sex chromosomes. If the latter interpretation is correct, we can expect additional difficulty in obtaining gene maps of the autosomes of this species because of long distances.

Fortunately, the chicken has a considerable number of non-interfering characters which are satisfactory for linkage tests. This permits the testing of several characters in one cross, thus reducing the work and facilities required. Because of mortality which may be large during growth, it is desirable to have characters which express themselves early in life, preferably in the day-old chick. Many individuals are lost because of failure to hatch. The late-stage deaths in shell may be salvaged for traits identifiable at that time, since sex may be determined then by examination of the gonads. Characters which

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TABLE 1

Results of backcrossing the heterozygote to the recessive parent for those characters studied.

TRAIT	FEMALE OFFSPRING		MALE OFFSPRING		TOTAL	
	BIRDS	BIRDS	BIRDS	BIRDS	BIRDS	BIRDS
	SHOWING TRAIT	LACKING TRAIT	SHOWING TRAIT	LACKING TRAIT	SHOWING TRAIT	LACKING TRAIT
Albinism	419	454	373	484	792	938*
Pinkeye	345	447	335	367	680	814*
Multiple spurs	390	428	332	353	722	781
Rumplessness	1624	1756	1665	1696	3289	3452*
Polydactylism	1435	1941	1365	2008	2800	3949*
Duplicate	748	790	756	814	1504	1604
Syndactylism	483	907	373	705	856	1612*
Retarded feathering	170	198	218	200	388	398
Feathered leg	1316	2106	1514	1918	2830	4024*
Naked neck	2277	2332	2355	2447	4632	4769
Crest	1846	1993	2036	2121	3862	4114*
Flightlessness	1371	1517	1463	1494	2834	3011*
Silkie	880	905	896	797	1776	1702
Ropy	189	211	202	232	391	443
Frizzling	873	972	925	1030	1798	2002*
Muff	1463	1557	1380	1477	2843	3034*
Fray	526	576	585	767	1111	1341*
Rose comb	1244	1215	1186	1314	2430	2529
Pea comb	1481	1571	1354	1653	2835	3224*
Duplex comb	860	1164	1032	1161	1892	2325*
Dominant white	1976	1823	2154	2011	4130	3834*
Recessive white	429	351	388	327	817	678*
Blue	670	709	761	769	1431	1478
White skin	1479	1429	1503	1505	2982	2934

* These are cases where the deviation from the expected 1 to 1 ratio was statistically significant.

were accompanied by low viability, or which were difficult to identify, were usually avoided in this study because of the unreliability of the resulting linkage data.

CHARACTERS UTILIZED

Albinism.—This character was reported by WARREN (1933a) as one inhibiting pigment in the eye and plumage and adversely affecting the vision of the bird. It behaves as a recessive. The character is best identified by the brilliant red eye in the day-old chick. The resulting poor vision causes low viability. Table 1 records the segregation of this character when the heterozygote is backcrossed to the recessive. The ratio of 792 albinos to 938 normals deviates significantly² from the expected one to one ratio and indicates that

² Any deviations from a one to one ratio referred to as statistically significant by the Chi-square test are at or beyond the 5 percent level. Statistical assistance was given by DR. H. C. FRYER of the Statistical Laboratory of the KANSAS AGRICULTURAL EXPERIMENT STATION.

some condition interferes with the expression of albinism. Since the red eye could be identified in a late stage embryo, some embryos dying in the later stages of incubation were included in the above totals.

Pinkeye.—Pinkeye was found by WARREN (1940b) to be inherited as a recessive to the normal eye color. In the day-old chick pinkeye was virtually indistinguishable from albinism, except that diluted pigmentation could show in the plumage unless inhibited by other factors for white. In both pinkeye and albinism, the vision of the carriers was impaired and in each, the adult iris color did not differ greatly from normal. The pinkeye factor dilutes black plumage color to a shade of blue. The total of 680 pinkeyes to 814 normals recorded in table 1 deviates from the expected equality to a statistically significant degree indicating that deviation is not due to chance. The totals include both day-old chicks and late embryonic classification.

Multiple spurs.—This trait is expressed as triple spurs instead of single spurs on each leg. Multiple spurs are a standard requirement for the Sumatra breed. The character may be identified any time in life beginning with the late embryonic development (HUTT 1941). In the day-old or younger stage, multiple spurs are identified by the absence of any spurs since the triple spurs to appear later in development then appear as oversized, flattened scales on the leg. The possibility of overlooking multiple spurs in the day-old chicks may account for the slight shortage of multiple spurs found in table 1 which records 722 multiple spurs to 781 normals. The deviation from the expected ratio is not statistically significant.

Rumplessness.—DUNN (1925) reported on the inheritance of the character, but it has been known as a heritable trait in the chicken for several centuries. The character used in this study is inherited as a dominant, although LANDAUER (1945) has reported on a similar recessive trait. The primary morphological change in the rumpless fowl is the absence of the five free caudal vertebrae and the pygostyle, and this is responsible for the absence of tail feathers. By palpation the absence of the caudal vertebrae may be detected as early as the late embryonic stage. The viability of birds carrying rumplessness is not impaired but the absence of the caudal vertebrae permits the saddle feather to hang down over the vent, thus interfering with copulation and resulting in poor fertility. The total of 3289 rumpless birds to 3452 normals deviates from the expected one to one ratio to a degree which is statistically significant.

Polydactylism.—This term is usually applied to five-toed condition as contrasted with the more common breed characteristic, four toes. The extra toe usually has one more phalanx than the No. 1 digit to which it is usually attached. In segregation there is evidence of modifying factors which influence the expression of polydactylism (PUNNETT and PEASE 1929; WARREN 1943). Polydactylism behaves as a dominant character with high variability in expression in both the heterozygote and homozygote. Even though polydactyly is dominant, an occasional heterozygote has only four toes. Polydactylous individuals seem to be fully as vigorous as the normal four-toed type. Probably due to the presence of modifying factors, there is usually a striking shortage of polydactylous segregates. The data in table 1 show 2800 polydactyls to

3949 normals, a ratio which deviates from the expected in a statistically highly significant manner.

Duplicate.—This trait was described by WARREN (1941) as a new type of *polydactylism*. It appeared as a mutation in the four-toed White Leghorn. Like polydactylism it is dominant and variable in expression, but more commonly exhibits six toes than does the former. Duplication of digits in the wing are also common in the duplicate character. The character is easily identified in the day-old chicks. In the homozygous state, duplicate sometimes causes the leg to be deformed to a degree that it cripples the possessor and impairs its viability. The penetrance of duplicate is better than that of polydactylism. The backcross data show a total of 1504 duplicates to 1604 normals, a ratio which does show a statistically significant deviation from the expected.

Syndactylism.—This trait which causes webbing between the third and fourth toes is not a breed characteristic. It has been observed commonly in the White Plymouth Rock breed, and the genetic behavior of this trait is described in a paper now in press (WARREN 1949). Syndactylism behaves as an irregular recessive with an occasional appearance of the defect in the F_1 generation and poor penetrance in the F_2 generation. There is no evidence of any influence of this character on the viability of the carriers, except that when the toes are exceptionally closely bound together walking is somewhat impeded. The large shortage of syndactyls in the backcross generation is shown in table 1 where 856 syndactyls and 1612 normals were obtained in a mating from which equality was expected.

Retarded.—This character expresses itself in the presence of the sex-linked early feathering by reducing the number of well-developed secondary flight feathers in the day-old chick from a total of 6 or 8 to a total of 2 or 3. In the 10-day-old chick it inhibits tail feather development and secondary flight feathers. The character is identifiable in day-old chicks, but not in the embryonic stage. This character was first described by WARREN (1933b). The totals of 388 retarded to 398 normals are recorded in table 1. The character behaves as a recessive and does not affect the viability of the carrier, since the ratio is virtually one to one, the deviation having no statistical significance.

Feathered legs.—Leg feathering is characteristic of the Asiatic breeds. When mated with normal (non-feathered), leg feathering behaves as a dominant. There are varying intensities of leg feathering, and it has been proposed that two factors are involved. Characters due to multiple factors are not well adapted for gene mapping, but it should be possible at least to find evidence for or against linkage. Leg feathering can be identified as early as the late embryonic stages, and the character does not affect the vigor of the individual. Most of the data reported in table 1 were obtained from matings of feather legged birds which were segregates, rather than from matings of purebreds having this feathering trait, and as a result the segregation ratios differ widely in the 47 matings supplying the data. The total (table 1) shows 2830 with feathered legs to 4024 non-feathered. This ratio differs widely from a one to one and gives support to the view that more than one factor is involved.

Naked neck.—This is a very old breed known as the "Transylvania Naked-Neck." It is characterized by the absence of all feathers on the neck. DAVEN-

PORT (1914) described it as a simple dominant. The trait is readily recognizable in late stage embryos, as well as in older individuals. Birds carrying this character have their vigor unaffected and the ratio of naked neck to normal fits closely the expected one to one ratio. Backcrosses to the recessive normal gave 4632 naked necks to 4769 normals.

Crest.—This breed trait is expressed by causing the feathers on the head to be much longer than normal and to grow more erectly. It has been shown by FISHER (1934) that homozygous crest causes the dorsal skull to be sharply domed, giving an expression which was earlier thought to be a separate character—cerebral hernia. In homozygous crest the extremely long feathers hang down over the sides of the head and impair the vision. The doming of the skull also causes pressure on the brain which probably handicaps the bird. The longer more erect down on the head makes it relatively easy to recognize crest in the day-old chick. The backcross segregates show a total of 3882 crests to 4114 normals. The shortage of crested individuals causes the proportion to deviate significantly from the expected equality.

Flightlessness.—This dominant trait was first studied by WARREN (1932). The down of the day-old chick is unaffected either in the heterozygous or homozygous state. It was observed (WARREN 1937) that the homozygote is virtually featherless at maturity, if the individual survives that long. The feathers are so defective that they break off as soon as they emerge. Homozygotes usually die at not later than early maturity age and never seem to become sexually mature. The heterozygote seems quite viable and only the large flight and tail feathers break off making the bird incapable of flight. The backcross data in table 1 gave a ratio of 2834 heterozygous flightlessness to 3011 normals. The shortage of flightless individuals is too great to be considered due to chance and may be due to poor penetrance of the character, or to some difference in viability since the trait could not be recognized until the chicks were about three weeks old.

Silkie.—This fluffy type of plumage is normal for the Silkie bantam. The fluff type of feathers found on the whole body of the silkie is ordinarily restricted to the abdomen of other breeds. The fluffing of the feather is due to the defective microscopic hooklets which, when of normal structure, hold the barbs and barbules into a firm web. This character behaves as a simple recessive with no influence on the viability. It is not recognizable until the chick is a few weeks old. The backcross data in table 1 show a total of 1776 silkies to 1702 normals. The slight deviation from a one to one ratio may be attributed to chance.

Ropy.—A heritable defective feather condition was discovered in the Kansas State College poultry flock and was called ropy. In the newly-hatched chick the down fails to fluff out and in the adult the shaft of the larger feathers is grooved. This trait behaves as a simple recessive. Birds showing this trait do not seem to have their vigor impaired. The backcross ratio in table 1 is 391 ropys to 443 normals, which does not deviate sufficiently from the expected one to one ratio to have any statistical significance. The data on the mode of inheritance have not yet been published.

Frizzling.—This trait has long been known in certain types of show ring

chickens. It causes the feathers to curl up, giving the bird a ragged appearance. LANDAUER and DUNN (1930) and HUTT (1930) each showed that the homozygote has such defective feathers that it is not provided with adequate protection for survival. Homozygotes do not survive well unless given special care. The backcross data show 1798 frizzles to 2002 normals, and the shortage of frizzles is too great to be attributed to chance.

Muff.—This peculiar trait, common to some of the continental breeds of poultry, behaves as a dominant. The factor causes the lengthening of feathers on the cheeks and chin, and is usually referred to as muffs and beard. Longer down in these regions of the head characterizes the trait in the day-old chick. Birds with the character seem fully viable. The ratio of 2843 muff to 3034 non-muffs in the backcross data shows the character not to have complete penetrance or that all muffed individuals were not recognized. Characters such as naked neck make identification of muff difficult.

Fray.—This trait is recessive to normal and causes the larger feathers of the body to have a poorly formed web, but is not so extreme as silkie. Fray is not identifiable in the day-old chick but can be seen in the first set of wing feathers after they have emerged from their enclosing sheaths. This character was found in the Ancona breed by WARREN (1938). The stocks carrying fray have generally not been vigorous, but this may have been due to the inbreeding necessary for carrying a recessive trait. In the backcross data 1111 frays to 1341 normals are reported. Again there is a statistically significant shortage of the mutant type.

Rose comb.—Rose comb varieties are known in several breeds of poultry. The low, flattened comb type is readily identifiable from the allele single comb, in the late embryonic stages. The rose comb varieties seem to be fully viable. Rose comb is completely dominant to single comb. The ratio of 2430 rose comb segregates to 2529 single comb segregates shows the character to possess good expressivity. The deviation from the expected ratio is not statistically significant.

Pea comb.—This is another varietal characteristic which is recognizable in the day-old chick and late embryos, unless the single comb to which it is crossed carries a large comb such as is characteristic of Mediterranean breeds. The large comb factor causes the heterozygous pea to vary toward the single comb type. The typical pea comb is a low type of comb with three ridges extending the length of the comb. If the single combed individual used in the cross to pea comb is a small-combed type, pea comb behaves as a complete dominant. The pea comb does not affect the vigor of the possessor. The ratio of pea comb to single comb (normal) in table 1 is 2835 pea combs to 3224 single combs. The deviation here from the expected ratio is highly significant statistically. The shortage of the pea type may result from the fact that the comb size factors affect the expression of pea comb to the extent that it may be confused with the single type, especially in immature individuals.

Duplex comb.—The duplex type of comb is a requisite in some breeds. As the name indicates, it is split single comb, the split extending for varying

distances in the direction of the length of the blade of the comb. Duplex can ordinarily be identified in the day-old chick by the use of a hand lens. Duplex is dominant to normal and has no effect on the viability of the bird. Occasionally, where the split is only slight, the character may be overlooked in the day-old chick. This may account for the statistically significant shortage of duplex in the segregates recorded in table 1. There were 1892 duplexes to 2325 normals.

Dominant white.—This character is usually found in one breed of chickens only, the White Leghorn. If the extension factor is present, it is a simple matter to identify segregates of dominant white at hatching time. No viability factors are here involved. This character shows good penetrance as is evidenced by the ratio of 4130 whites to 3834 colored in the backcross generation data found in table 1. In the case of dominant and recessive white which follows, the excess of whites over colored was statistically significant. This may be accounted for by the fact that in some instances the classification was in the day-old chick. Sometimes the Columbian pattern in combination with silver produces a chick which might at hatching be classified as white. There is also the possibility that some of the crossbred stocks used in studies of dominant white may have occasionally carried the recessive white factor and in segregation caused an excess of whites.

Recessive white.—Recessive white color is characteristic of most white varieties other than the White Leghorn. This trait does not affect the viability of the bird carrying it. The backcross data in table 1 show 817 whites to 678 birds with colored plumage, an excess of white which is statistically significant.

Blue.—Blue plumage is the color characteristic of the Blue Andalusian breed. It is often recorded as a case of blending inheritance since the bird showing the blue color is the heterozygote resulting from crossing blacks and splashed whites. The character has no effect on viability of the bird. The color can be recognized in the day-old chick which also carries the extension factor. The backcross of the blue heterozygote to colored birds gave a ratio of 1431 blues to 1478 non-blues as shown in table 1. This deviation from the expected one to one ratio is not statistically significant.

White skin.—White skin is another breed characteristic and has as its allele, yellow skin. The distinction between these two skin colors does not express itself until the chick is a few weeks old and then is most sharply defined in the legs. Skin color has no influence on the vigor of the stock. White skin is dominant to yellow. The ratio of white skin to yellow in table 1 is 2982 to 2934 and does not vary significantly from a one to one proportion.

It is to be noted that in this list of 24 characters in table 1, 12 show statistically significant deviations from the expected one to one ratio. Ten of the twelve deviations involved shortages of the mutant type. With such large numbers as are available for some of these traits, even minor factors influencing the viability of individuals showing the character may bring about deviations which have statistical significance.

LINKAGE GROUPS

The linkage groups which follow are listed in chronological order of their being reported in the literature, and are lettered for purposes of identification. All data presenting linkage tests and crossing over are from backcrossing to the double recessive. Both coupling and repulsion phases are utilized but are combined in the totals of crossover and non-crossovers, unless separation is desired for analyzing cases of questionable linkage. Usually the male was the heterozygote in the backcross, but data were used from crossing over tests in both sexes, since it has been shown (WARREN 1940a) that the percentage of crossing over is similar in both the male and female chicken. The writer previously published some data on autosomal linkage in the chicken (WARREN (1933c), WARREN and HUTT (1936)), but the totals given in the earlier publication have been revised to include recently acquired data. The data for a large number of tests for linkage are given in table 2. For each character pair combination there are given two figures, the one to the left of the dash being the parental combinations and the one to the right the new combinations. The degree to which the parental combination exceeds the new combination is taken as evidence for linkage. The letter following the two values for each test designates the worker making the test, so in the table an attempt has been made to list all published linkage test data. The spaces carrying the x indicate combinations for which no linkage data are available, but where one member of the pair belongs to a linkage group which has shown independent assortment with the other member. The spaces carrying dashes indicate character combinations which bring about interference and prevent making satisfactory tests for linkage.

Group A

This group includes the first reported case of autosomal linkage. SEREBROVSKY and PETROV (1928) found close linkage between creeper and rose comb. The later, more comprehensive data of LANDAUER (1933b) and TAYLOR (1934) showed these two factors to be very closely linked. LANDAUER reported from a total of 7408 backcross individuals a crossing over percentage of 0.39 percent and TAYLOR 0.5 percent crossing over in 2183 individuals. HUTT (1936) later reported uropygial to belong to this linkage group with 29.6 percent crossing over between it and rose comb in 348 individuals. Uropygial is a new factor affecting the development of the uropygial papilla. The present report adds no data on this linkage group since the totals are already quite adequate. The linear organization of the known genes in this chromosome and later discussed chromosome maps are shown in figure 1.

Group B

This linkage group was established by the report of SUTTLE and SIPE (1932) when they found evidence for linkage between crest and frizzling. HUTT (1933) later reported dominant white and frizzling to be linked. The combined data of WARREN and HUTT (1936) added to those of earlier workers gave a relatively

	RETARDED	ALBINO	PINKEYE	MULTIPLE SPUR	RUMPLESS	NAKED NECK	FEATHERED LEG	WHITE SKIN	ROSE COMB	PEA COMB	POLYDACTYLY	CREST	DOMINANT WHITE	FLIGHTLESS	CREEPER	SILKIE PLUMAGE	DUPLEX COMB	FRIZZLING	BLUE	MUFF	
SYNDACTYLISM				286-307K	102-127K	199-190K	130-115K	126-129K	57-47K	359-390K	621-634K	XXX	XXX	91-69K	XXX		XXX	116-115K		432-325K	
ROPY				101-90K	69-77K	63-65K	61-71K		138-154K		126-128K	99-88K	XXX	---	XXX		112-125K	54-53K		28-21K	
FRAY				XXX	272-268K	197-216K	218-205K	267-260K	158-155K	134-128K	325-337K	853-740K	972-863K	---	XXX	---	136-142K	378-398K	91-87K	131-129K	
DUPLICATE	57-83K	XXX	XXX	559-239K	54-57K	199-188K	140-154K	330-317K	205-181K	213-239K	---	637-628K	287-296K	286-283K	XXX	XXX	379-282K	266-257K	276-329K	170-192K	
RECESSIVE WHITE		---	55-49K	XXX	219-218K	146-145K	210-224D	59-54K 216-227D	163-187K 55-47F 49-45B	74-76K 34-35F	249-24K	235-207K	---	118-111K	XXX		141-130K 13-13F	80-73K	---	345-344K 17-15F	
MUFF	59-46K	62-67K	166-150K	28-37K 334-344B	364-373K	427-405K	217-203K	84-93F 340-378K 72-67B	149-175K 132-128F	605-600K 104-90F	163-144F 1108-1024K 348-367B	484-807K 388-421F 202-188B	589-588K 93-101F 129-191B	577-532K	23-20F	246-236K	587-601K 241-228F 367-344B	489-492K 45-46B	327-366K		
BLUE		---	---	XXX	234-221K	599-620K 252-173F	485-389K 29-31F	216-252K 31-42F	244-192K 246-288F	317-314K 108-93F	126-114K 162-171F	104-113K 120-119F	---	168-204K	XXX	95-107K	208-220K 47-39F	79-88F			
FRIZZLING	XXX	148-100K	16-20K	103-96K	790-717K 259-190E	167-148K 116-117E	136-110K	410-394K 68-46B	221-200K 24-24F 20-26B	XXX	367-393K 55-68B	1918-715K 225-906 68-26B	1662-356K 560-107B	69-85K	132-140E	XXX	350-328K 33-40F 128-126E 81-41B				
DUPLEX COMB	18-20K	201-228K	186-181K	477-136K 658-239B	163-179K	160-181K 102-104E	267-337K	652-538K 62-61E 138-142B	61-55F	436-371K 123-104F	579-500K 176-149F 300-217B	342-367K 225-203F 210-179B	388-415K 26-18F 240-215B	484-536K 192-183B	38-39F 145-141E	XXX					
SILKIE PLUMAGE	XXX	XXX		XXX	9-8K XXX	554-424K	164-175K	211-197K	326-373K	288-301K	228-198K	XXX	203-192K	2346-300K	XXX						
CREEPER	XXX	XXX	XXX	XXX	477-525E	XXX	XXX	18-15F XXX	295-33F 7380-28E 2175-10H	72-117F	99-91F	104-100F	96-80F	XXX							
FLIGHTLESS	177-154K	127-132K	133-120K	214-180K 120-122B	296-241K	938-807K	343-382K	449-407K	662-641K	687-482K	478-448K	405-391K	135-135K								
DOMINANT WHITE	67-88K	---	74-89K	150-188B	925-875K	842-815K	939-874K 16-16A	885-867K 24-12F 98-105B	617-594K 31-28A	640-642K 27-22F	1062-1023K 55-37F 36-23A 128-182B	3457-492K 407-75B									
CREST	238-242K	217-220K	196-210K	28-37K XXX	936-947K 246-254E	581-574K	879-846K 39-85F 23-22A	1006-987K 87-73F 73-63B	374-355K 356-282F 145-109C	293-290K 177-173F	1157-1062K 160-130F 206-182B										
POLYDACTYLY	76-88K	147-155K	251-240K	41-24K 514-172B	725-671K	893-973K 144-182F	818-824K 68-82F 17-15A	1232-1195K 39-83F 150-130B	415-432K 417-331F 27-41A	207-211K 152-160F											
PEA COMB	162-125K	149-140K	160-131K	101-106B	204-220K	1264-1267K 518-570M	762-736K	208-211K 64-65F	388-306F												
ROSE COMB	33-34K	116-102K	103-86K	228-218B	791-808K	719-721K 318-337F	667-702K 110-96F 68-63A	525-488K 62-48F													
WHITE SKIN	54-56K	114-97K	182-107K	140-138K	581-557K	767-819K 13-21F	787-823K 96-77D														
FEATHERED LEG	200-179K	268-273K	182-241K	XXX	374-408K	1101-1162K 28-25F															
NAKED NECK	56-66K	313-324K	96-103K		1328-1221K																
PINKEYE		---		252-217K	67-95K																
RUMPLESS	15-22K	319-290K																			

KEY TO AUTHORITY
DUNN & JULL ----- A
HUTT & CO-WORKERS ----- B
JULL ----- C
LAMBERT & KNOX ----- D
LANDAUER ----- E
SEREBROVSKY & PETROV -- F
SUTTLE & SIPE ----- G
TAYLOR ----- H
WARREN ----- K
HERTWIG ----- M
DEAKIN & ROBERTSON --- N

TABLE 2. Parental and new combinations of characters tested by various workers. The number to the left of the dash is the total of parental, and that to the right the total of new combinations. Instances where parental combinations exceeded the new combinations significantly indicate genetic linkages. The letter following the second number indicates the author who is listed in the key to authority. A long dash alone in the space marks combinations where one character sufficiently interferes with the expression of another to make the notation of segregation impossible. The X in the space marks character combinations where one member has already been tested for linkage with some member of the group to which the other belongs. Spaces carrying no marks are those for which no linkage data exist.

accurate location of these two genes. Since the 1936 publication, WARREN has obtained considerable additional data on this group.

The writer's total on the relationship of crest and frizzling are 1918 non-crossovers to 715 crossovers. These added to the total of 56 to 24 published by HUTT (WARREN and HUTT 1936) and the total of 316 to 90 by SUTTLE and SIPE (1932) make a grand total of 2290 non-crossovers to 829 crossovers. This gives a crossing over percentage of 26.6 for the pair of factors.

For dominant white and frizzling the total 1662 non-crossovers to 356 crossovers when added to HUTT's totals of 491 to 94 makes 2153 non-crossovers to 450 crossovers. This ratio yields a crossing over percentage of 17.3.

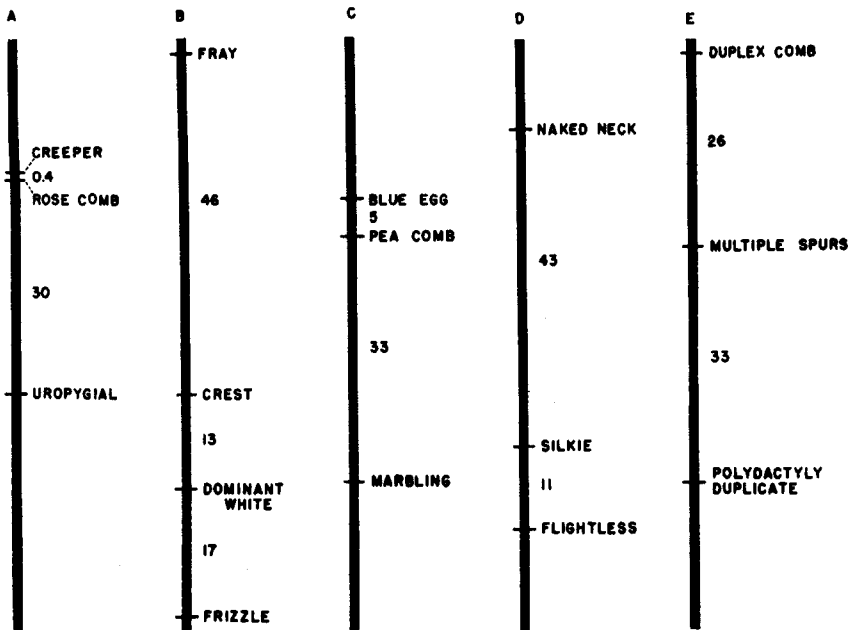


FIGURE 1.—Chromosome maps for five autosomes in the fowl.

The linkage relations of crest and dominant white have also been investigated. The total of 3457 non-crossovers and 492 crossovers have been added to the totals of HUTT, 357-57 giving 12.6 percent crossing over. It is of interest to note that the map distance between crest and frizzling is 26.6 while the sum of the distance between crest and dominant white (12.6) and dominant white and frizzling (17.3) is 29.9. Thus the difference, 3.3, would be a measure of the double crossing over percentage occurring in the 26.6 units of map distance between crest and frizzling. WARREN and HUTT (1936) published the results on a test involving these three factors at one time and set up in a manner to provide a measure of double crossing over. Out of 284 gametes, they failed to obtain any double crossovers. These results were interpreted to indicate the phenomenon of interference operates in the fowl as it does in *Drosophila*.

A factor, fray, found by WARREN (1938) has shown evidence of being linked

with both crest and dominant white. The linkage is relatively loose, but the data are extensive enough and sufficiently consistent to seem to establish the new factor as a member of group B. The total of 853 parental combinations to 740 new combinations in the test of linkage between fray and crest shows a deviation from a one to one ratio of sufficient magnitude to indicate that it is probably not due to random sampling. The crossing over percentage for the pair of factors is 46.3. In the case of dominant white and fray the totals of parental combinations and new combinations are 972 and 863. Here again the deviation from equality in the two groups is statistically significant. These totals gave a crossing over percentage of 47.0. The fact that fray shows evidence of linkage, although loose, with two members of the group, tends to strengthen the view that it also belongs in the group. In the linkage tests of fray and crest, and fray and dominant white, both coupling and repulsion phases were involved in each test, thus reducing the possibility that differential viability or penetrance might be the responsible factor rather than genetic linkage in causing deviations from the one to one ratio.

It is somewhat difficult to follow the segregation of fray and frizzling at the same time, since each affects the structure of the feather in a somewhat similar manner. A test for linkage, between these two, gave a total of 378 parental combinations to 398 new combinations, values which provide no evidence of linkage between the two. It is possible that these two factors may be located at opposite ends of the chromosome, and they have been so placed in figure 1. The slightly less crossing over between crest and fray than between dominant white and fray would be in accord with this arrangement, giving the order of fray, crest, dominant white, and frizzling.

Group C

The third linkage group was established by the report of HERTWIG (1933) that a new down pattern factor, "marbling," was linked with pea comb. HERTWIG found 32.8 percent crossing over between it and pea comb in a population of 789 individuals. Hertwig also reported loose linkage between marbling and naked neck, with 45.6 percent crossing over in 1141 individuals, a deviation which is statistically significant. The writer is inclined to place naked neck in another linkage group because of evidence that appears to be stronger than that presented by HERTWIG, and because considerable data which offer no evidence for linkage between naked neck and pea comb. A total of 2531 individuals in a backcross test for linkage between naked neck and pea comb (table 1) gave 1264 parental combinations to 1267 new combination. This is so near a one to one ratio that it cannot be considered as support for placing pea comb and naked neck in the same linkage group. HERTWIG (1933) reported that 1088 offspring from a backcross gave slightly more new combinations than parental combinations. With somewhat questionable proof in the case of marbling and naked neck and contradictory evidence for naked neck and pea, it seems that evidence favoring the placing of naked neck in this group is unconvincing. HERTWIG's theory regarding the location for the factor, naked

neck, is further weakened by evidence for linkage between naked neck and two different members of another group.

BRUCKNER and HUTT (1939) published data showing close linkage between pea comb and blue egg. A total of 35 individuals showed 5.7 percent crossing over between the two factors. They state that their evidence favors placing the characters in the order blue egg, pea comb, and marbling in the fourth chromosome group although no published data are available on the relationships of blue egg and marbling.

Group D

WARREN (1935) published preliminary data giving evidence for linkage between silkie plumage and flightless. The data to date on this pair of factors include 2646 individuals and give 11.3 percent crossing over from both coupling and repulsion phases. The data recently obtained support the placing of naked neck in the same linkage group. The linkage of naked neck with both silkie and flightless is loose, but the fact that statistically significant evidence for linkage is found in the tests for each of the relationships (naked neck and silkie, and naked neck and flightless) seems convincing. In each test both repulsion and coupling phases are involved, and in each phase the parental combinations exceeded the new combinations, thus providing proof that the evidence for loose linkage is not a result of differential viability or penetrance of the characters involved. The totals are 554 non-crossovers and 424 crossovers in the case of silkie and naked neck, with the deviation from a one to one ratio being statistically significant. For naked neck and flightless, there are 938 non-crossovers and 807 crossovers, and the χ^2 test indicates that the deviation from equality of the two groups is much too great to be due to chance. These values give 43.3 percent crossing over for naked neck and silkie, and 46.2 percent for naked neck and flightless. The slight difference in these two percentages would favor the placing of the genes in the order naked neck, silkie, flightless on the chromosome. The data of HERTWIG (1933) might be interpreted to indicate that linkage groups C and D could be combined into one, but the data in table 2 for tests between pea and silkie give 51.1 percent crossing over, which deviates in the wrong direction to favor this view. The tests for linkage of pea and flightless offer some support for combining the two groups since the total of 557 parental combinations to 482 new combinations gives a crossing over value of 46.4 with a χ^2 value equaling 5.78, 1 D. F. With marbling and naked, and naked and flightless, and flightless and pea, all showing about the same degree of loose linkage; and flightless and silkie showing close linkage, and pea and silkie with no linkage, it is difficult to place these factors in any one linear organization.

Group E

HUTT (1941) reported a fifth linkage group upon finding evidence for linkage relations between multiple spurs and duplex comb. Later HUTT and MUELLER (1943) added polydactyly to the group and reported 28 percent crossing over between duplex comb and multiple spur; 33 percent crossing over between multiple spur and polydactyly; and 42 percent crossing over between duplex

comb and polydactyly. The total data accumulated by the author on the linkage relations between duplex comb and multiple spurs are 477 non-crossovers and 136 crossovers, resulting in a crossing over percentage of 22.2 which is considerably less than the value of 27.8 percent reported by HUTT. HUTT obtained 858 non-crossovers to 239 crossovers, and if these are combined with the foregoing totals, a crossing over value of 26.2 is obtained. WARREN (1941) suggested that a new type of polydactyly, duplicate, was probably an allele of the usual type of polydactyly, and the same author (1943) later reported evidence confirming this view. For linkage relations between duplex comb and polydactyly data are available from various investigations. SEREBROVSKY and PETROV (1930) reported 176 non-crossovers to 149 crossovers. HUTT and MUELLER (1943) found 300 non-crossovers to 217 crossovers, which when added to the writer's totals of 579 non-crossovers to 500 crossovers, make a total of 1055 non-crossovers to 866 crossovers. This grand total gives a crossing over percentage of 45.1, slightly greater than the values obtained by HUTT and MUELLER. Variability in penetrance of polydactyly may affect this value, but analysis of the writer's data does not indicate the phenomenon to have much influence. The deviation from equality is highly statistically significant.

For duplicate and multiple spur, 559 non-crossovers and 239 crossovers were obtained with a crossing over percentage of 30. Duplicate and duplex comb gave 379 non-crossovers to 282 crossovers with a crossover value of 42.6. Thus the linkage relation of duplicate with multiple spurs and duplex comb of 30 and 42.6 percent, respectively, are quite similar to the values obtained for the linkage relation of polydactyly with these two factors (33 and 45.1 percent). These results together with the earlier report of WARREN (1941) that polydactyly and duplicate segregated as alleles seem to justify the conclusion that duplicate is a mutation at the same locus as polydactyly. The arrangement of the genes on this chromosome is duplex comb, multiple spurs, polydactyly and duplicate.

Independently Inherited Characters

In addition to the five linkage groups, table 2 carries several other autosomal characters which show independent assortment with members of these five groups. In table 3 are brought together those characters which have shown evidence of being independent of the five known groups and the X indicates the combinations for which tests have shown no linkage and the O combinations of two members of the same linkage group. The interrogation marks indicate tests which have a statistically significant deviation from the expected and will be discussed in the next division of this paper. The dash indicates combinations in table 3 where interference of one character with another prevents the making of a reliable test. The following characters show independent assortment with one or more members of each group: recessive white, muff, blue, white skin, feathered leg, rumplessness, syndactylism, ropy, pinkeye, albino, and retarded. This may be taken as evidence that each character has its determiner on chromosomes other than those to which the five linkage groups belong unless these characters have their genes so widely separated from known loci

TABLE 4

Tests that show no linkage among characters not belonging to any known linkage group

	RETARDED	ALBINO	PINKEYE	RUMPLESSNESS	FEATHERED LEG	WHITE SKIN	RECESSIVE WHITE	BLUE	MUFF
Retarded	0			X	X	X			X
Albino		0	—	X	X	X	—	—	X
Pinkeye		—	0	X	X	X	X	—	X
Rumplessness	X	X	X	0	X	X	X	X	X
Feathered leg	X	X	X	X	0	X	X	?	X
White skin	X	X	X	X	X	0	X	X	X
Recessive white		—	X	X	X	X	0	—	X
Blue		—	—	X	?	X	—	0	X
Muff	X	X	X	X	X	X	X	X	0
Syndactylism				X	X	X	X		X
Ropy				X	X			X	

on the chromosomes as to permit independent assortment. These eleven characters have been arranged in table 4 to show the tests that have been made among them. Rumplessness, feathered leg, and muff have been tested with each of these groups as well as with the members of the linkage groups, so the survey for linkage has been completed for the characters listed here. In some of the untested combinations, the masking action of one character upon another prevents making the test for linkage. Thus, the segregation of blue cannot be followed in the presence of albinism.

Other Possible Linkage

There are in table 2 data which indicate possible additional linkages. The Chi-square test was made in these cases and only those at or beyond the 5 percent level are considered as having a statistically significant excess of the parental combinations over the new combinations. In tables 2 and 3 the interrogation marks indicate these tests where the deviation proved to be statistically significant. Without additional supporting evidence some of these indications should not be given too much weight, since there are several other cases in table 2 where deviations in the reverse direction are beyond that which can be considered as due to chance fluctuations. In some instances it has been demonstrated that the deviation from a one to one ratio of the parental and new combinations is due to poor penetrance or viability of two of the involved factors. If both coupling and repulsion are involved, such cases usually can be detected. Results in table 1 indicate the factors in which the expressivity is not good. Even where the linkage test is in one phase, coupling or repulsion only, critical evidence may be obtained by comparing the two parental combinations one with another; or the two new combinations one with another. If the devia-

tion from a one to one ratio is due to genetic linkage, then the two new combinations should be of about equal numbers, though less than each of the parental groups. If the apparent linkage is due to poor expressivity of two of the involved factors, then one of the two new combinations should be smaller than the other.

Duplex comb shows evidence of being linked with pea. Both the data of the writer and those of SEREBROVSKY and PETROV (1928) show significant deviations from the expected one to one ratio. The fact should be noted, however, that there is some interference between pea and duplex, since duplex is not always easily recognized when combined with pea comb. The data in table 1

TABLE 5

Additional tests for linkage not included in table 2 (See table 2 for key to authority indicated by the letter following the numbers.)

	PARENTAL COMBINATIONS	NEW COMBINATIONS
Marbling—pea comb	531	258M
Marbling—naked neck	625	521M
Blue egg—pea comb	33	2B
Uropygial—recessive white	60	56B
Uropygial—rose comb	245	103B
Yellow head—dominant white	18	16N
Mottling—duplex	324	328B
Mottling—muff	312	292B
Mottling—flightless	81	114B
Mottling—crest	282	292B
Mottling—polydactyly	328	326B
Mottling—multiple spur	320	307B
Duplex—extended black	276	219F
Polydactyly—extended black	250	241F
Duplicate—syndactylism	421	148K
Duplicate—recessive white	64	52K
Recessive white—syndactylism	92	103K

show rather large shortages of both the pea and duplex types of combs. The linkage data include both the coupling and repulsion phase and in each there is an excess of the parental type. Most of the data, however, are from the repulsion phase in which the pea-duplex combination would fall in the new combination and being difficult to recognize, would cause apparent linkage. If pea comb were linked with duplex comb, it should also be expected to show linkage with polydactyly, duplicate, and multiple spurs, and there is no suggestion of linkage in any of the ratios for these factors. Considering the results as a whole, it seems that the evidence for linkage here is spurious. The evidence favoring linkage of pea comb and flightless has already been discussed.

The data on linkage relations of rumplessness and frizzling offer some support for the view that these two characters are in the same group. The data of the writer have a χ^2 value slightly below significance being 3.55, while those of

LANDAUER (1933a) show a highly significant deviation ($\chi^2 = 10.6$, 1 D. F.). Most of the deviation in the writer's data comes from an excess of the group having neither rumplessness nor frizzling, so it seems probable that the evidence favoring linkage is not reliable. Furthermore, rumplessness fails to show any evidence of linkage with either dominant white or crest, each of which is in the same linkage group as frizzling.

Rumplessness also shows a statistically significant deviation ($\chi^2 = 4.57$, 1 D. F.) when the linkage with naked neck is tested. Rumplessness shows poor expressivity and a study of the linkage data shows all the evidence for linkage coming from the coupling phase. Most of the available data were from the coupling phase but the few tests in the repulsion phase had the parental and new combination groups of virtually the same size. It would appear then that the apparent linkage of naked neck and rumplessness may be due to poor expressivity of the characters involved.

Syndactylism shows rather extreme deviations from the expected one to one ratio when tested for linkage with polydactylism and duplicate. Since the latter two belong to a multiple allelic series, this might be considered as strong evidence for placing the three characters in the same linkage group. Both syndactylism and polydactylism show poor penetrance in table 1. In the coupling phase of the test for linkage between duplicate and syndactylism the totals for the two parental groups were 271 duplicate non-syndactyls, 150 non-duplicate syndactyls, and for the two new combinations 144 non-duplicate non-syndactyls and 4 duplicate syndactyls. For the polydactylism-syndactylism coupling test the totals for the parental groups were 318 polydactyls non-syndactyls, 234 non-polydactyls-syndactyls, and for the new combinations were 303 non-polydactyls non-syndactyls and 68 polydactyls-syndactyls. These results show that much of the shortage of the new combinations is due to the exceptionally low incidence of the combinations of either polydactyly or duplicate with syndactylism. The application of the χ^2 test indicates that the shortage here is even greater than would be expected from the chance combination of two groups each showing a deficiency. To check further the evidence for linkage, stock was prepared for making the linkage test in the repulsion phase for syndactylism and polydactylism. Here again there was an obvious shortage of the syndactylous-polydactylous group, but this time the group fell in the parental groups and made a shortage of this group. The totals for the parental groups were 119 non-polydactyls non-syndactyls, and 30 polydactyls-syndactyls, and for the new combination, 139 syndactyls non-polydactyls and 124 polydactyls-non-syndactyls. This makes a total of 169 parental combinations and 263 new combinations. When these totals are added to those from the coupling phase, it gives 621 parentals to 634 new combinations, a deviation from a one to one ratio which is not statistically significant. This is a rather striking demonstration of how certain parental combinations may give spurious evidence for linkage when the characters being tested have poor penetrance.

SEREBROVSKY and PETROV (1930) reported evidence for linkage between

naked neck and blue with a parental total of 252 to 173 new combinations which is a highly statistically significant deviation from the one to one ratio. The writer's larger body of data in table 2 does not confirm the findings of Serebrovsky and Petrov since the ratio of 599 parentals to 620 new combinations is virtually one to one, the deviation being within the range to be expected from chance and in the wrong direction.

MUNRO and KOSIN (1940) reported breast ridge as a new character in the fowl, but were undecided as to whether it was a case of very close linkage with pea comb or only another expression of the pea gene.

WARREN (1945) described a case of lateral asymmetry which provided some evidence for placing body size differences, earlobe color, and skin color factors on the same chromosome. A more recent test of this theory was made by backcrossing F_1 females from the cross of the White Leghorn by the Australorp to White Leghorn males. The F_1 generation females should be heterozygous for skin color, and for factors controlling earlobe color and body size. Both body size and earlobe color are probably due to multiple factors. If factors controlling these three characters are on the same chromosome, it would be expected that the white skin segregates should have larger body size and less white earlobes when compared with the yellow-skinned segregates. From 275 segregates the evidence did not offer much support for the theory. The birds with white earlobes tended to be smaller but those of the two skin colors did not differ significantly in size. There was also no significant difference in the tendency toward earlobe color in the birds of the two skin colors.

SUMMARY

This report provides additional data on three of the five well-established autosomal linkage groups in the chicken. The character fray has been added to the crest-dominant white-frizzled group. Data are presented for including naked neck in the silkie-flightless group instead of with the blue egg-pea comb-marbling group. Duplicate, which proved to be an allele of polydactyly has been added to the duplex comb-multiple spurs-polydactyly group. Additional data were presented on the map distances on these autosomes and for independent assortment of a large number of other autosomal characters.

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