STUDIES IN INHERITANCE OF COLOR PATTERNS IN *ACRYDIUM ARENOSUM* (GROUSE LOCUST)

by

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INTRODUCTION

Distinct and easily recognizable color patterns with the added advantage of a comparatively short life cycle have made the Tettigidae (grouse locusts) a valuable group with which to carry on studies in heredity. The determination of the behavior in heredity of a small number of factors will be of value in studying other factors, not only of this group, but of animals and plants in general, and will enhance the ability to predict with considerable accuracy the possible character complexes of the offspring of matings in a wide field. It is likely that the laws of inheritance supported by the results of the study in the Tettigidae may be applied to other animals of greater economic importance but which would require more time and expense for a similar study.

The purpose of this experiment has been to make matings of which the offspring will be indicative of the linkage relations of the factors for the color pattern characteristics found in Acrydium arenosum, and to begin the construction of a chromosome map showing those relationships.

REVIEW OF LITERATURE

The most extensive inheritance studies in insects have been on the fruit-fly Drosophila by Morgan and his students.

Outstanding results have been the finding of a correlation between chromosome number and linkage groups, the promulgation of the linear hypothesis, an explanation of non-disjunction and its effects, and an advance in the knowledge of mutants and lethals.

The breeding of the grouse locust, with the purpose of the investigation of the heredity of color patterns was begun by Dr. R. K. Nabours in 1908. The diversity of patterns had been previously noted. Hancock had observed in a note of June 20, 1898 in his vivarium experiments on Tettix ornatus that "In the seven specimens...there is considerable divergence in the variety of ornamentation, showing this peculiarity extends to individuals of the same brood." (p. 168) Even the work of taxonomists has been influenced by color patterns, as was that of Harris (1841) in which he gave five names to the single species Tettix ornatus, basing the names principally on the color markings.

In the southern species Parattettix texanus twenty two very closely linked or allelomorphic factors for dominant color patterns have been found with one other certainly closely linked and two very loosely linked or on separate chromosomes. All of the color patterns with two exceptions are dominant. Parthenogenesis occurs in P. texanus; the unfertilized eggs, with rare exceptions, producing females which are usually homozygous for all the factors they carry.

Segregation and crossing over is the same in bisexually and parthenogenetically reproducing females. Polyandry has been noted, and there is no evidence of telegony. (Nabours 1917, 1927, 1929, Nabours and Foster 1929).

Apotettix eurycephalus, another southern form, has fourteen distinct color patterns in six loci. These are all actually closely linked, the extremes being on the average less than 8 per cent apart, and they are all clearly confined to the one pair of chromosomes. (Nabours 1929). Crossing over is much more rare in the males than in the females. A. eurycephalus also reproduced parthenogenetically, and segregation and crossing over are the same as in bisexually reproducing females (Nabours 1919, 1925, 1929).

Breeding stock of Tomatettix aztecus was also obtained in the south. The four dominant color patterns so far used in experimentation appear to be on one pair of chromosomes and alternative or so closely linked that no crossing over has occurred (Nabours 1929, Nabours and Snyder 1928).

Tettigidea parvipennis pennata (Bellamy 1917) has been shown to have five color pattern factors which are extremely closely linked or allelomorphic, and one pattern factor which shows about fifty per cent segregation in both males and females, and probably is on a separate pair of chromosomes. Some parthenogenesis has occurred (Bellamy 1917, Nabours 1929.)

In other Orthoptera than the grouse locusts (Tettigidae), Hancock (1916, Nabours 1928, 1929) has found that the factors for the pink and green colors of the katydid, Amblycorypha oblongifolia, make a pair of Mendelian alternatives with the pink dominant. Ingersoll(K.S.A.C. Master's Thesis, 1926) ascertained that, in the mantid Stagmomantis carolina, the nymphal colors were not influenced by the environment, and that green was recessive to the several other colors, and avellaneous body color was dominant to a dark cinnamon pink. In general, the breeding of Orthoptera, with the exception of the Tettigidae, is attended by many difficulties such as susceptibility to diseases, requirements for hibernation, and length of time required in life cycles.

INHERITANCE IN ACRYDIUM ARENOSUM

Breeding Habits

Hancock gives the locality of Acrydium arenosum as the southern United States, adding that it has been recorded also in Nebraska, Iowa, and a few other states. All individuals used in the breeding experiments described in this paper have been collected in the vicinity of Manhattan, Kansas.

They are found most numerously among the dead leaves on

the moist shaded banks of the rivers and smaller streams, and in other shaded places which are not too dry. Nymphs and adults may be collected at any time of the year, even late in the fall and early in the spring whenever the day is warm enough to bring them out of hibernation.

Only one or two generations of Acrydium arenosum have been reared each year thus far. Paratettix texanus and Apotettix eurycephalus(Nabours 1929) produce four generations in a year. The fact that one finds both nymphs and adults of A. arenosum very early in the spring before offspring of that year could have hatched, suggests the alternation of hibernating generations described by Nabours (1929); that is, adults at the beginning of the breeding season produce offspring which become adult and produce offspring which remain nymphs over the next winter. The following spring these nymphs become adult, and produce offspring which become adult and hibernate over the winter, and begin the repetition of the cycle again.

Scheme of Alternation of Hibernating Generations of Northern (U. S. A.) Grouse Locusts (from Nabours 1929)

Winter	Spring	Summer	Autumn
ADULTS produce	NYMPHS become	ADULTS produce	NYMPHS
Winter			
remain NYMPHS			
Winter	Spring	Summer	Autumn
NYMPHS produce	ADULTS produce	NYMPHS become	ADULTS
Winter			
remain ADULTS			

The eggs of Acrydium arenosum are about three millimeters long. They are deposited just below the surface of the earth in the mating jars, in compact bunches of about twenty eggs. Offspring hatch as early as seventeen days after the mating is made. The newly hatched grouse locust is a creamy white in color except that it has dark eyes. In about half an hour its pigmentation is apparent.

The males molt five times, the females six times, giving them six and seven instars, respectively. They increase in size most noticeably immediately after each molt while the chitinous exoskeleton is still very soft. The most remarkable change occurs at the last ecdysis, when the tip of the pronotum reaches, or protrudes beyond, the tip of the abdomen, and the wings take on the adult characteristics.

Method

New stock is brought in from nature each year. Those taken in the spring are collected as early as possible, assuring a majority, if not a total, of virgin females. Later in the season only males of more complex color patterns are brought in.

Pairs of grasshoppers of desirable color combinations are placed in mating jars, which are 8" x 12" glass cylinders set in bulb pots about one-third full of coarse sand or gravel and one-third of black dirt which has been well sterilized. The bottom of an inverted small flower pot protrudes at the center of the jar. Covers for the cylinders are made of 24 mesh screen wire. The food consists of finely ground filamentous and other algae grown in tanks of running water in the greenhouse, and it is placed in small amounts every other day or oftener on the inverted pot.

The offspring are carefully transferred with curved tweezers when they are four or more days old to 9" x 15" offspring jars prepared as the mating jars. The color pattern of each of the offspring is recorded when all the offspring in a jar have passed the third ecdysis. Recording individuals smaller than this contributes to the possibility of inaccuracy.

Grasshoppers not desired for further breeding are

recorded whether previous records have been made or not, and killed in eighty to ninety per cent alcohol. They are later hermetically sealed, together with a small paper tag of identification, in small vials of fresh ninety-five per cent alcohol; the vials are stored for future reference, and serve as a check on the written records.

No regulation of temperature and humidity has been made other than that an average temperature of 80° to 85° F. is maintained in the colder months when the greenhouse is heated; in the summer the temperature of the greenhouse is much influenced by that out of doors. The humidity varies greatly, even within a jar (Nabours 1925). Since the grouse locusts used are inhabitants of moist regions, the earth in the cages should never be allowed to become dry.

THE EXPERIMENTS

Breeding

In the previous years practically all of the individuals used in the first generation matings had to be secured from nature because the greenhouse stock over-wintered so poorly or failed to produce offspring if mated. In the spring of 1929, however, of 71 matings made, 49 had either the male or female or both from offspring produced in the greenhouse during June, July or August of 1928, and of these 36 were productive. Of 22 matings made in which both male and female were taken in nature in the fall of 1928 or early in

the spring of 1929 20 were productive. The average number of offspring produced by the first named type of matings is less than for the second.

The total number of matings made to May, 1929 in the breeding of Acrydium arenosum is shown in Table I.

TABLE I

TOTAL MATINGS MADE IN THE BREEDING OF ACRYDIUM ARENOSUM,

1925 TO MAY 15, 1929.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept	Oct.	Nov.	Dec.	Total Pro-ductive	Total Non Pro-ductive	Total
1925		1 0			5 1	0 1			0 1				6	3	9
1926	0 11				12 5	2 2		1 2	0 3	1 0			16	23	39
1927		2 4	1 6	5 2	3 0	7 2	9 7	1 3	0 2				28	26	54
1928		8 12	20 15	13 0	2 1	10 4	19 21		0 2				71	57	128
1929			12 6	33 8	5 3								50	17	67
Total	0 11	10 17	33 28	51 10	22 7	19 9	27 29	2 5	0 8	1 0			171	126	297

The total number of offspring recorded from the beginning of the experiments in 1925 to January 1, 1929 are as follows: 1925, 141 males, 112 females; 1926, 285 males, 330 females; 1927, 596 males, 588 females; 1928, 1607 males, 1552 females; total number of offspring recorded, 5312. The average number of offspring of each productive mating was 31.5.

Hibernation

It has been supposed that Acrydium arenosum, unlike the southern forms of Apotettix eurycephalus and Paratettix texanus (Nabours 1929) needs a period of hibernation at some time during the winter if it is to reproduce the following spring. During the winter of 1925-26 a number of individuals were placed out of doors in screen jars, in an attempt to simulate the conditions found in the natural hibernation of the species. Matings made from the few that survived were unproductive. The experiment was repeated the following winter and greater protection was given the grasshoppers but no offspring were produced in the seven matings made from the hibernated individuals. (Hartwig, Master's Thesis, 1927).

In the winter of 1927-28 about 100 individuals taken in nature in the fall were kept out of doors in offspring jars, and were covered by a thick layer of dry leaves. Of this

number one male and seven females lived to be brought into the greenhouse. All were mated, the male to one of the females, and they and four of the other females produced offspring.

In the winter of 1928-29 artificial hibernation was carried out in two ways: (1) individuals were kept in small screen cages in a refrigerator having extreme temperature ranges from 3½ to 11° C, for varying lengths of time. (2) Individuals were kept in the regular cages used in the greenhouse, but were covered with from one to five large dry leaves or a thick layer of them, and the cages were stored in a large unheated ground-floor room which has a large door open to the outside except on the coldest winter days. On the mild winter days the cages were set out of doors. In both methods the dirt in the cages was kept moist, and food was always available. Those kept in the refrigerator did not come through the treatment nearly as well as did those kept under the modified outdoor conditions; especially is this true when the fact is considered that a large per cent of them die within three days after being brought into the greenhouse again. This large death rate may be due in part to the too sudden change in temperature. Those kept in the cold room lived well, even after several weeks following the close of the period of "artificial hibernation". Factors influencing these results appear to be the water content of

the organism in relation to the decreased temperatures, (Bodine 1921), the stage of growth of the grasshopper and the resistance of the animal in that stage to low temperatures and the relative length of the period of hibernation (Bodine 1923). No definite study has been made of the significance of these factors in the hibernation of Acrydium arenosum.

The relation of the length of time and the method of hibernation to the productivity of the mating is shown in the following table which covers the period from November 30, 1927 to March 1929. Each figure denotes the number of days a certain mated individual was in hibernation. Where two numbers occur together, the first denotes the total number of hibernation days of the male, the second that of the female of the mating. The symbol* placed after a number shows that the female of the mating was taken in nature the preceding fall and hibernated during the winter; ** shows that the female of the mating was taken in nature shortly before the mating was made, and so had passed the winter out of doors under natural and uncontrolled conditions.

TABLE II. RELATION OF LENGTH OF TIME AND METHOD OF HIBERNATION TO PRODUCTIVITY OF MATING. (NOV. 30, 1927 TO MARCH, 1929)

	♂ only hibernated		♀ only		♂hib. this meth; ♀hib. other "		♀hib. this meth; ♂hib. other "		Both ♂ & ♀ hibernated	
Method of Hibernation:	Pro-ductive:	Unpro-ductive:	Pro-ductive:	Unpro-ductive:	Pro-ductive:	Unpro-ductive:	Pro-ductive:	Unpro-ductive:	Pro-ductive:	Unpro-ductive
Out of doors or in Horticulture Building	35** 35** 115 115 115 115	115	115*	115* 42	115		42* 42* 42*	42* 42* 61	111* 115* 115,22 42	115,22
In hibernation at average temperature at 8° C.	21	41* 21 21	42 42 42 21	42 42 21 21 21 21 20	41* 41* 25*	59 41 41*	177		25-42* 21 21 19	42 42 42 42,41 41,42 25 25 19,20 18 18

The Color Patterns

The elementary characters of Acrydium arenosum considered are the color patterns of the pronotum and the femora of the jumping legs. These elementary or unit characters are designated by letters of the alphabet, similar to those used for Apotettix eurycephalus and Paratettix texanus, (Mabours 1929), or by the first two or three letters of a word descriptive of the pattern, as Bl for black, Yf for yellow femora, and Fl for flecks. Twenty-five distinct patterns have so far been described, and there are undoubtedly more in nature, and possibly some in the stock in the greenhouse which have not yet been segregated or distinguished from the easily recognized patterns. The factors for the color complexes of individuals are indicated by letters, those contributed by the respective parents being separated by an oblique line, /. As far as these studies have shown, the color patterns are not in any allelomorphic series, the only allelomorph of any pattern being its normal recessive, the +.

Descriptions of the patterns included in the study follow:

(1) + is usually a dark gray, in some individuals appearing much lighter, and in some almost black.

(2) B__White. Denser chalk-white over the anterior

pronotum and lateral lobes, and at least two-thirds of the distance posteriorly in the region of the median carina, becoming more mottled near the posterior half of the lateral carina and shading into an uneven gray to the tip of the pronotum. The femora of the jumping legs are reddish brown to orange brown, and not evenly colored; the femora of the middle legs are colored by a light wash of the brown of the jumping legs; the femora of the first legs are white. The wings and pronotum are tipped with reddish brown, fading anteriorly. The posterior part of the abdomen has two streaks of white dorso-laterally. B in Acrydium arenosum is similar to B in Paratettix texanus and O in Apotettix eurycephalus.

(3) Bil—Creamy white or yellow line on either side of the pronotum, running posteriorly to the humeral angles and along the lateral carinae, fading out about half way back.

(4) Bilf—The same as Bil except that the white line begins farther forward, is a heavier line and has an extension of the Bil onto the posterior part of the superior carina of the femur of the jumping leg. This extension varies in length, but is usually very short and indistinct.

(5) Bl—Black. The entire pronotum is usually a dense black but the tip is sometimes lighter. The black extends down over the upper part of the lateral lobe, and is a characteristic of the pattern which helps to distinguish

young Bl individuals from young dark +/+ individuals. The Bl extends over the posterior two-thirds of the hind femora. The femora of the middle legs are mottled black. Bl is similar to Θ in P. texanus and A. eurycephalus (Nabours 1929).

(6) D — A heavy white spot, half way between the proximal and distal ends of the hind femur, and above the superior carina.

(7) G — A brown stripe, extending the length of the pronotum along the median carina. The basic color of the stripe is a light tawny brown, at the anterior part as wide as the distance between the anterior carinae and widening rather abruptly to the lateral carinae at the posterior end of the scapular area, and so covering the posterior half of the pronotum. With this stripe the median carina is a very dark brown to black. The outer edges of the stripe are bordered by a narrow fringe of rich chocolate brown which widens with it, leaving a central narrow lighter stripe of an equal width its entire length. The vertex and dorsal part of the eyes of individuals bearing the G pattern are a faded tan in color. G is much like the G of A. eurycephalus.

(8) Gr — Gray. A mottled light gray covers the pronotum and lateral lobes. The femora of the jumping legs are light colored, but do not bear the pattern. Posterior to the position of the middle part of the scapular area and

along the median carina there are four small indistinct black spots, spaced equidistantly. These aid in distinguishing a Gr individual from a light colored +/+ individual.

(9) H —A light brown oblong spot as in P. texanus and A. eurycephalus. At the sides of the pronotum it reaches the humeral angles; its posterior margin at the median carina is opposite the middle of the scapular area and it extends almost as far anteriorly, the anterior margin being somewhat truncate.

(10) J —A pure white spot, in the same position and shape as the H. When no other pattern obscures it, a small black spot shows near the center of the J spot, where the white is less dense. It is comparable to J in P. texanus and Y in A. eurycephalus.

(11) K —A narrow median white stripe extending the length of the pronotum. It is quite uniform in width, being from one-half to two-thirds the width of the vertex. The median carina is white except for a very short region at the posterior end of the anterior carina. The vertex and dorsal part of the eyes are whitish. K in Acrydium arenosum is almost identical to K in P. texanus and A. eurycephalus.

(12) M —One large or two or more small white spots just posterior to the humeral angle on each side of the pronotum. The size and shape of the spots are variable.

This pattern occurs in the same position as the M of A. eurycephalus.

(13) My —A deep brown (mahogany) covering the pronotum evenly in the adult, but much more strongly in the anterior portion of the pronotum in the nymph. The My color does not extend quite as far forward as the posterior end of the anterior carinae; especially in the smaller nymphs My appears only as a mahogany colored diamond-shaped pattern between the shoulders.

(14) R — A medium yellow on the pronotum and femora of the jumping legs, somewhat like the R of A. eurycephalus. The entire pronotum is covered by the R pattern, including the scapular area and the region between the humero-apical and lateral carinae. The lateral lobes are not yellow. The middle part of the femur of the jumping leg between the superior and inferior carinae, is yellow with the color spreading out at the posterior one-third to the edges of the femur. The dorsal side of the tip of the abdomen is yellowish.

(15) T —A very reddish brown, more nearly a deep red, over the pronotum, the upper part of the lateral lobes, the scapular area, the region between the lateral and humero-apical carinae, and all of the femur of the jumping leg below the superior carina including the apex of the femur. The tips of the wings and the dorso-lateral sides of the

abdomen are strongly tinged with red. This resembles the T in A. eurycephalus.

(16) Sf — One or two small white dots at the center of the region between the superior and inferior carinae of the femora of the jumping legs. It is somewhat similar to Sf in P. texanus.

(17) W —A white to brownish-yellow line, running from the distal apex of the superior and inferior carinae of the jumping leg forward along the inferior carina about three-fourths of its length.

(18) W_1—A light brownish-orange femur of the jumping leg. All of the femur, including the apex, except the anterior two-thirds of the region above the superior carina and the lower middle portion of the region below the inferior carina is of the W_1 color; a much darker shade is below the inferior carina, almost approaching a brown which shades lighter dorsal. The distal ends of the femora of the middle legs are brownish orange, slightly darker than the characteristic color of the jumping leg femora. The margin of the pronotum above the posterior half of the lateral lobe bears a brownish marking which continues as a very fine line around the humeral angle and down into and over the scapular area. The tip of the pronotum is the color of the femora. The wing tips are slightly tinged with brownish-orange.

(19) W_3—A broad yellow stipe on the femur of the

jumping leg. The yellow covers the apex and the entire posterior two-fifths of the femur, grading forward and inward so that the anterior two-thirds of the femur carries yellow only in the region between the superior and inferior carinae. The margin of the pronotum above the posterior half of the lateral lobe bears a heavy reddish yellow line. The tip of the pronotum is a clear yellow. The dorso-lateral sides of the posterior part of the abdomen are streaked with light yellow.

(20) Yf —Yellow-tipped femur of the jumping leg. This light lemon-yellow color covers the posterior fourth of the jumping leg, extending farthest forward in a small point just above the inferior carina. The dorsal side of the tip of the abdomen is light yellow, the coloring extending forward in two dorso-lateral stripes.

Six other patterns the first three of which were found in nature in the spring of 1929 are not described at length here because they have been used in such small numbers that a definite description cannot be given. (21) Fl—white flecks all over; (22) Yfext — extended Yf; (23) M — mahogany colored all over; (24) M_1 — the outer edges of M are extended a short distance posteriorly and anteriorly along the lateral carinae; (25) Ps — two black spots at the anterior end of the pronotum; (26) Myfem —reddish femora of the jumping legs and reddish shoulder markings.

Discussion of the Effects of Color Patterns on Each Other

So far as has been learned in these experiments, the D and M patterns are the only ones wholly unaffected by any other patterns with which they might occur, hence whenever they are found they are distinct and white. Bil is distinguishable and comparatively distinct when combined with any other pattern. The lines on the femora of Bilf are obscured by the brown femora associated with B, and by W_1, W_3, Yf, and the yellow femora of the R pattern. T alters the appearance of Bil so that it looks like a light colored bar on the shoulder.

It is not impossible, though it is difficult, to distinguish Sf with the brown femora of B, and with W_1; the brown spots show much more easily with W_3 and W. No combinations of Sf and T have been made in matings.

Yf blends with W_1 to such an extent that it is difficult in some individuals to discern whether or not it is present, though more often the yellow of the Yf is strong enough to be noticeably distinct from the orange-brown femora of W_1.

K is very little affected by all other patterns except G. It becomes much a part of G, and retains its individuality only in a narrow light brown stripe of uniform width through the middle of the G. In some matings the K has a very faint pinkish tinge. Whether this is caused by

some contributing genetic factor or whether it is a pattern distinct from K has not yet been determined. T makes the K slightly reddish, and B makes it very white, but since the K extends to the tip of the pronotum it is not lost when in this combination.

The characteristics of the G pattern remain unchanged when in combination with other patterns, except that the color tone may be changed. With B, G becomes considerably lighter and seems to take on some of the chalkiness of the B pattern; with Bl it becomes blackish; with My it becomes even more brown and with T it appears a reddish stripe; it is not much altered by R except that it is slightly yellowish.

H takes up only a very little of the color of Bl or R when associated with them, and even is comparatively distinct when occurring with My. Nothing is known of its appearance in combination with B or T.

J "soaks up" any color pattern near it. With B it is a distinct white; Bl lends it much black; the brown of the G stripe which crosses the J spreads unevenly over the white; My makes it brownish and almost the color of H; it becomes yellow with R and very red with T; Bil and M, however, which come in contact with it on some side remain distinct.

W is obscured to some degree by any other pattern

showing on the femur of the jumping leg. With B it is sometimes, especially in nymphs, difficult to see. R leaves it distinct but somewhat whiter, while T allows only a row of six or seven small white spots to remain visible. Yf extends forward only far enough to cover a part of it. W_1 and W_3 probably hide W in a number of nymphs. Bl leaves W practically unchanged. My makes W a distinct white line, as though the natural yellow or brownish tone of the pattern had left it to become a part of the My on the pronotum.

W_1, although occupying the same position as the femur pattern of B, is a little less uneven in coloring and slightly lighter, and because of the brownish shoulder markings it is easily distinguishable when it is in combination with B. W_1 becomes darkened by the black of Bl. The R pattern of the femur has a shape different from that of W_1 and the brownish shoulder markings are comparatively clear so that there is little difficulty in noticing W_1 with R. W_3 likewise has a pattern of different shape, resembling closely the femur of R.

The W_3 and R patterns on the femur are indistinguishable, though the presence of W_3 is shown by the reddish-brown shoulder markings. Yf and W_3 have not been made in matings.

The yellow R is perhaps the most noticeable of the

larger patterns. It hides the stripe and spot patterns less than do any of the other larger patterns. When R is with Bl, Gr, or My, each pattern covers its usual area and there is a blending of the two colors, though each retains so much of its individuality that it is readily seen when the records are made.

B lends the stripe and spot patterns some of its whiteness so they are made slightly paler. Bl makes the B a dirty white over the shoulders and the brown jumping leg femora and tip of the pronotum faintly blackish. My shows very little, if at all, on the shoulders, but makes the tip of the pronotum a deeper brown which extends forward, especially at the sides, about one-third of the length of the pronotum. T overlies B, practically covering the white on the pronotum and the brown on the jumping legs. The B stands out in sharp contrast to the red of the T on the entire lateral lobe except the very small part at the top which is also covered by T.

The dense black of Bl blends with My to such an extent that the pronotum becomes a blackish brown. The distinguishing light and darker blotches of the Gr pattern show indistinctly with Bl, the darker parts of the pattern being practically covered by the black.

Combinations not described here either have not been produced or contain new patterns not used in the data.

DISCUSSION OF THE DATA

Table III is a summary of the relations of pairs of color pattern factors so far obtained in the breeding of Acrydium arenosum. A considerable number of possible combinations are not represented here, either because they have not occurred in matings or because the sources of the color pattern complexes were not known or were doubtful. For example, in an individual from nature it would not be known from which of its parents the color patterns were derived, and hence no cross-overs could be counted in the F_1 generation. This has decreased, by more than half, the data that otherwise would be available.

TABLE III. SHOWING THE RELATIONS OF FACTORS FOR COLOR PATTERNS

Factors	Males			Females			Totals		
	Number	Cross-over	Per cent	Number	Cross-over	Per cent	Number	Cross-over	Per Cent
BBil	10	0	0.	3	0	0.	13	0	0.
BG	35	12	34.29	73	26	35.62	108	38	35.19
BK				7	3	42.86	7	3	42.86
BMy				73	44	60.27	73	44	60.27
BW				90	45	50.	90	45	50.
BilBl	98	51	52.04	133	60	45.11	231	111	48.05
BilD	20	8	40.				20	8	40.
BilG	32	4	12.5	131	58	44.27	163	62	38.04
BilK	201	48	23.88	179	101	56.42	380	149	39.21
BilM				35	17	48.57	35	17	48.57
BilMy	75	40	53.33				75	40	53.33
BilSf	20	8	40.	35	15	42.86	55	23	41.82
BilT				54	31	57.41	54	31	57.41
BilW	124	60	48.39	212	96	45.28	336	156	46.43
$BilW_1$	51	31	60.78	8	2	25.	59	33	55.93
$BilW_3$				18	11	61.11	18	11	61.11
BilYf	32	13	40.63				32	13	40.63
BilfBl	5	2	40.	40	24	60.	45	26	57.78
BilfG	33	0	0.				33	0	0.
BilfGr				38	8	21.05	38	8	21.05
BilfH	147	22	14.97				147	22	14.97
BilfM	80	38	47.5				80	38	47.5
BilfMy	33	17	51.52	143	63	45.52	176	80	45.46
BilfW	33	16	48.48	164	73	44.51	197	89	45.18
$BilfW_1$	33	20	60.60	56	23	41.07	89	43	48.31
$BilfW_3$	40	23	57.5	18	11	61.11	58	34	58.62
BlG	18	7	38.88				18	7	38.88
BlGr	45	7	15.55				45	7	15.55

TABLE III(CONTINUED)

Factors	Males			Females			Totals		
	Number	Cross-over	Per Cent	Number	Cross-over	Per Cent	Number	Cross-over	Per Cent
B1J	96	49	51.04	15	4	26.66	111	53	47.75
B1K	108	76	70.37	155	98	63.23	263	174	66.16
B1M	7	5	71.43				7	5	71.43
B1Sf	7	6	85.71				7	6	85.71
B1W	58	26	44.83	137	82	59.85	195	108	55.38
$B1W_1$	96	56	58.33	93	42	45.16	189	98	51.85
$B1W_3$	18	9	50.				18	9	50.
DK	20	10	50.	35	22	62.86	55	32	58.18
DMy	20	5	25.	35	17	48.57	55	22	40.
DSf	20	0	0.				20	0	0.
DW				37	20	54.05	37	20	54.05
DW_1				35	14	40.	35	14	40.
GJ	43	0	0.	24	10	41.67	67	10	14.93
GM	27	16	59.26	35	18	51.43	62	34	54.84
GMy	39	18	46.15	143	65	45.45	182	83	45.60
GSf	27	16	59.26	35	14	40.	62	30	48.39
GW	199	91	45.73	167	76	45.51	366	167	45.63
GW_1	33	20	60.60				33	20	60.60
GW_3	18	8	44.44				18	8	44.44
GYf	32	9	28.13				32	9	28.13
GrJ				33	22	66.66	33	22	66.66
GrW_1				6	1	16.66	6	1	16.66
HK	104	41	39.42				104	41	39.42
HM	40	29	72.5				40	29	72.5
HW_3	40	11	27.5				40.	11	27.75
JK	71	9	12.68	76	24	31.58	147	33	22.45
JMy	23	12	52.17	71	41	57.75	94	53	56.38
JW	23	16	69.57	96	41	42.71	119	57	47.89
JW_1	177	93	52.54	21	11	52.5	198	104	52.53

TABLE III(CONTINUED)

Factors	Males			Females			Totals		
	Number	Crossover	Per Cent	Number	Crossover	Per Cent	Number	Crossover	Per Cent
JW_3	54	25	46.30				54	25	46.30
KM	46	19	41.30				46	19	41.30
KMy	35	15	46.86	42	17	40.48	77	32	41.56
KT				54	30	55.55	54	30	55.55
KW	171	85	49.71	209	98	46.88	380	183	48.16
KW_1	52	32	61.54	54	34	62.96	106	66	62.26
KW_3	118	49	41.53				118	49	41.53
MSf	68	1	1.48	35	4	11.43	103	5	4.85
MW	27	26	96.29				27	26	96.29
MW_3	86	44	51.16				86	44	51.16
MySf	20	5	25.				20	5	25.
MyW	91	15	16.48	329	42	12.77	420	57	13.57
MyW_1	33	17	51.51	35	21	60.	68	38	55.88
SfW	27	26	96.29				27	26	96.29
TW	31	18	58.06				31	18	58.06
TW_1	33	18	54.54	35	19	54.57	68	37	54.41
WYf	20	11	55.				20	11	55.

Explanation of Table III

The column at the extreme left shows the pairs of factors from which the cross-over percentages were calculated. The number following each pair of factors is the total number of individuals in which non-cross-overs and cross-overs between these two factors were observed in the male parents. The number of cross-overs and the per cent of crossing-over follow. The data for the female parent and the total data for the males and females follow in the same order.

Linkage

The cross-over percentages for such factor combinations as B1lD, B1lW$_3$, B1G, B1M, B1Sf, B1W$_3$, GrW$_1$, MySf, and WYf are computed from such small numbers that their reliability is questionable and they are not used in the following considerations.

It may be noted that the per cent of crossing-over in the males and females is approximately the same.

B and B1l show no crossing-over. The number of possibilities for crossing-over between them given in Table III is very small and not conclusive, but further data secured during the summer of 1929 still show no cross-overs between these two factors. From this evidence it is very likely

that B and Bil are very closely linked or allelomorphic. B has a crossover per cent of 35 with G, and that of Bil with G is 38. The cross-over per cent of B with K is 42, that of Bil with K is 39. No data are presented in Table III for the linkage between K and G, but considering their relation to B and Bil and to other factors, they seem to be comparatively closely linked, in this order: B-Bil—GK.

H and W_3 crossed over 27 per cent, and the cross-over percentage of H and K is 39. These three factors are combined in the order of K—H—W_3 by the cross-over percentage of 41 between K and W_3. There are no data for G and H, but the 44 per cent of cross-overs between G and W_3 would again place G a little to the left of K.

My and W are two of the first factors between which linkage was apparent. There is a total number of 420 chances for crossing-over with only 57 cross-overs, giving a percentage of 13. A peculiarity of this pair of factors is that when they are linked in a parent the offspring show MyW or W, but very rarely only the My. This behavior of W suggests its position at the extreme right of the chromosome with My to the left of it. The relation of H and W_3 to My and W is not yet known. K is placed at quite a distance to the left of My and W by a cross-over percent of 41 with My and 48 with W. The position of G to the left of K is again substantiated by a cross-over percentage of 45 between G

and My, but that between G and W is also 45, and 3 per cent less than the cross-over percentage between K and W. This discrepancy is the only one opposing, with any considerable strength of numbers, the arrangement of the factors in this order: GK—H—W_3—My—W.

The cross-over numbers for B and My and for B and W are so great that they indicate independent assortment; however the numbers from which the percentages were figured are not conclusively large, and there is some possibility that there may have been inaccuracies in the records of My and W with B. Again, W has a smaller cross-over per cent, than My with factors at the opposite end of the chromosome. In a total of 75 possibilities, there was 53 per cent of crossing over between Bil and My, while out of 336 possibilities there was 46 per cent of crossing over between Bil and W. It is apparent that further study and more data are necessary to discover the cause of this difference, or to point out another arrangement of the factors. The study of couplings and repulsions may aid in the explanation. This is suggested by the fact that with Bil and My the cross-over percentage is 45 in the couplings, and 56 in the repulsions; with Bil and W it is 35 in the couplings and 51 in the repulsions (these data are not included in the table).

J seems to be closely linked to G in the males. Table III shows these two factors to have no cross-overs in the male and 41 per cent in the female. Data secured during the summer of 1929 (too late to be included in the table) show a cross-over per cent of 1.13 in the male and of 33.78 in the female; 74 males and 88 females being used. J and K have been found to have a cross-over percentage of 12 in the male and 31 in the female. Data for other factors with G and K have further indicated that they probably are closely linked. Records for 1929 (not included in Table III) have 95 females with only 5 cross-overs between K and G, a little over 5 per cent. This places J to the left of G and K in this order, J—GK. A peculiarity of the linkages among these three factors is that crossing-over is noticeably less in the male, as in A. eurycephalus and P. texanus (Mabourz 1925, 1929). The cross-over percentage between J and W_3 is 46, which is greater than that between J and K or K and W_3, and the position of J to the left of G, K and W_3 is again substantiated.

In 33 chances there was no crossing-over between Bilf and G, and only 14 per cent between Bilf and H in 147 chances in the males. This is indicative that there is a linkage between Bilf, G and H, and that Bilf and G may be close together on the chromosome, but the cross-overs between Bilf and My, W, and W_3 lessen the probability of

such a linkage.

Allowing that there is comparatively loose linkage among some of the factors, and that it approaches what appears to be independent segregation between the factors at the opposite ends of the chromosome, the following tentative chromosome diagram is presented.

BBil JG K H W_3 My W

Independent Segregation

The per cent of crossing-over is near fifty in almost half of the pairs of factors. The following showed over fifty per cent of crossing-over in one sex, and nearly fifty if not over in the other: BilBl, BilW_1, BilfBl, BilfMy, BilfW_1, BilfW_3, BlJ, BlK, BlW, BlW_1, DK, GH, GSf, JMy, JW, JW_1, KW_1, MyW_1 and WW_1. This strongly indicates either that there is very loose linkage, or more than one pair of chromosomes are involved. A large percentage of cross-overs with other factors is especially noticeable for Bl and W_1. T also has a large cross-over per cent with the few other factors with which it has been in combination, but there are no data showing the comparative percentages of crossing-over in males and females for it and any other one factor.

Harman (1915, 1920, 1925) and Robertson (1915) have

found the chromosome numbers of a number of the Tettigidae, including Paratettix texanus, Apotettix eurycephalus and Acrydium granulatum in the haploid to be six autosomal and one or two sex chromosomes, depending on the sex. The chromosome theory of inheritance holds that there may be as many linkage groups or independent factors as there are pairs of chromosomes. This has been found to be true in Drosophila (Morgan, Bridges and Sturtevant, 1925), in which over three hundred factors are considered. Further study is necessary to demonstrate whether or not more than one pair of chromosome is involved in what seems to be the separate groups of Bl, W_1, T, and B—Bil—J—G—K—H—W_3—My—W.

SUMMARY

The species of grouse locust used in these inheritance experiments was Acrydium arenosum, which is common in the vicinity of Manhattan, Kansas.

Several of the twenty-five dominant color patterns are similar in appearance to some of the patterns of the southern species Paratettix texanus and Apotettix eurycephalus, but they do not exhibit the same linkage relations.

New breeding stock is collected in nature each year since the majority of individuals kept in the greenhouse over winter do not reproduce in the following spring.

Artificial hibernation has been tried as a possible means of increasing productivity, but has been largely unsuccessful.

Each color pattern is distinct when alone or with a contrasting pattern, but the exact composition of a combination of similar patterns is sometimes difficult to determine.

The haploid chromosome number of the species in six autosomal and one or two sex chromosomes, depending on the sex.

One, or possibly two, linkage groups may be deduced from the data: B—Bil—J—G—K—H—W_3—My—W, and M—Sf—D. Bl, W_1 and T seem to be independent of the other factors.

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