SPERMATOGENESIS IN HYBRIDS BETWEEN *CIRCOTETTIX VERRUCULATUS* AND *TRIMEROTROPIS SUFFUSA* (ORTHOPTERA: OEDIPODIDAE)

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INTRODUCTION

Two species of Orthoptera — *Circotettix verruculatus* and *Trimerotropis suffusa* — are especially well suited to show the cytological effects of hybridization. Differences in the shapes of the chromosomes within each species make it possible to distinguish individual chromosomes with few exceptions. Although very little work has been done on the spermatogenesis of hybrids in animals which are favorable for a detailed analysis of meiosis, a few studies have been made on the germ cells of hybrids in which the behavior of individual chromosomes or groups of chromosomes might have been followed. Dobzhansky (1934), working on hybrids between *Drosophila pseudoobscura* and *Drosophila persimilis*, could distinguish the sex-chromosomes from the autosomes and found that in some individuals the sex-chromosomes are less likely to pair than the autosomes. Klingstedt (1939) studied the cytology of one naturally occurring and two experimentally produced hybrids between *Chorthippus bicolor* and *Chorthippus biguttulus* (Acrididae). The six largest chromosomes are multiples and easily distinguished from the eleven much smaller chromosomes. Synapsis was complete and no other differential behavior was discovered. The spermatogenesis of six hybrids between *Triturus cristatus* and *Triturus marmoratus* (Urodela) was investigated by White (1946). Failure of synapsis was common and varied greatly in the spermatocytes within the individual. The number of bivalents (tetrads) in individual B varied from two to nine out of a possible twelve. No attempt was made to follow individual chromosomes but from the figures it appeared as though failure of synapsis were more frequent among the smaller chromosomes than among the larger.

The behavior of individual chromosomes in the hybrids has been studied, and it has been found that certain homologous pairs showed varying degrees of asynapsis in different individuals. This asynapsis results in a large number of unbalanced gametes in most of the individuals and may be the basis of an isolating mechanism which prevents any effective interbreeding between these species.

MATERIALS

Hybrids were made between *Circotettix verruculatus* from Mt. Desert Island, Maine, and *Trimerotropis suffusa* from Woodland Park west of Colorado Springs,

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Colorado. Reciprocal crosses were made and together they produced thirty-seven male hybribs. Three different mass matings of hybrid individuals with hybrids were made, but no offspring were derived from any of those crosses.

The hybrid individuals were reared to maturity and their testes fixed for the study of their chromosomes during spermatogenesis. All of the available spermatocytes in which the chromosome conditions could be unquestionably ascertained were counted in most of the individuals. When less than a hundred cells were studied it was because no more were available. The results have been tabulated in Table I. The numbering of the chromosomes is the same as that used by Carothers and myself in former papers (Carothers, 1917; Helwig, 1929).

OBSERVATIONS

Geographic Distribution. The geographic distribution of *Circotettix verruculatus* extends in a broad arc across North America from Newfoundland, Nova Scotia, New England, and the mountains of northern Pennsylvania to the Pacific coast in British Columbia. It is found on flat bare ledges of lichen-covered and weathered rocks.

Trimerotropis suffusa ranges from the plateau of northern Arizona and northern New Mexico to southern Alberta (Blairmore) and British Columbia (Mount Revelstoke National Park) and from the eastern slopes of the Rocky Mountains to the Pacific. Very probably it occurs in wooded areas throughout this region, going as high as ten thousand feet. It is common along roads and in open places in the forests. The northern limit of the distribution of this species in all probability overlaps the southern distributional limit of *Circotettix* (Banff, Alberta) but no records of their having been taken within the same area can be found.

Taxonomy of these Species. Unfortunately the taxonomic position of neither of these species has been accurately determined. Rehn (1921) in his revision of the genus *Circotettix* did not include *Circotettix verruculatus* because he did not consider it a member of the genus. The exact affinities of this form cannot be defined until a critical appraisal is made of some forms now included in the genus *Trimerotropis.*

Likewise, the true relationships of *Trimerotropis suffusa* cannot be assessed without a revision of the genus *Trimerotropis* as it is now constituted. This heterogeneous group consisting at present of about 43 species unquestionably contains several sections; when they are finally defined, *Trimerotropis suffusa* will not be included in the more narrowly defined genus *Trimerotropis*, since it is not in the same section as the type species, *Trimerotropis maritima.* It is not unlikely that both *Circotettix verruculatus* and *Trimerotropis suffusa* after more careful scrutiny may be placed in the same genus though they are undoubtedly not conspecific. These species are both very dark gray, often almost black, with brown or black mottlings. There is

great intraspecific variation in both forms in the definition of the color pattern which appears to be a response to environmental conditions. These two forms are not separated by any pronounced or striking morphological features but rather by differences of degree, which probably only a statistical study will define. Their habitats and behavior, however, show marked differences. *Trimerotropis suffusa* could be expected to occupy the same habitat as *Circotettix verruculatus*, but not the reverse. Except temporarily, *Circotettix verruculatus* never deserts the rocky ledges it prefers. *Trimerotropis suffusa* frequents road sides and clearings in the forests and occurs only fortuitously on rocky ledges.

On warm sunny days *Circotettix* rises into the air with a vigorous clatter, usually flying in circles, and eventually comes to rest again not far from where it arose; *Trimerotropis* does not make so loud a clatter and has no pronounced tendency to fly in circles, though both tend to fly in an undulating path. Anyone familiar with both species would never confuse them in the field.

Chromosomal Conditions in Circotettix verruculatus. This species has twenty-one chromosomes in the spermatogonia (Row 3). The discrepancy between this number and twenty-three, which is characteristic for most of the Oedipodidae, is due to the formation of multiple chromosomes. Like all *Circotettix*, this form contains some chromosomes that have terminal or telomitic fiber attachments and some with non-terminal or atelomitic attachments. The relative proportions of telomitic to atelomitic chromosomes is constant for the individual and varies from one animal to another (Helwig 1929).

The eight largest elements (pairs 9, 10, 11, 12), constituting four homologous pairs, are invariably atelomitic. The two multiples belong to this group and are indistinguishable from the other six atelomitic elements. The next two pairs (7 and 8) in the size series may be either telomitic or atelomitic, but, whatever their condition, it is constant for the individual. The members of these two pairs cannot be distinguished as to size, but, when atelomitic, chromosome 7 has a subterminal fiber insertion, while element 8 has an approximately median fiber attachment. Chromosome 8 is atelomitic in only 4.7% of all the chromosomes of this pair in the population, while 76% of all chromosomes 7 are atelomitic. Chromosome 6 is the sex-chromosome and is always atelomitic. Chromosomes 4 and 5 are invariably telomitic and indistinguishable in size. Chromosome 3 has entered into the formation of the multiple which was formed by the fusion at their proximal ends of two telomitic chromosomes that would separately have fitted in 9 and 3 of the size series. Chromosome 2 is without exception telomitic and cannot be separated from chromosome 1 as to size. Chromosome 1 may be atelomitic or telomitic, and in the population from Mt. Desert Island one member of the pair is atelomitic in twenty per cent of the individuals.

The first spermatocyte contains eleven chromosomes: nine tetrads, one octad

multiple, and the sex-chromosome which is a diad (Row 4). The four largest chromosomes (9, 10, 11, and 12) are indistinguishable. One of these four largest elements is the octad multiple. Tetrads 7 and 8 are variable in their loci of fiber attachment and may consist of two telomitic diads (homomorphic), two atelomitic diads (homomorphic) or one telomitic and one atelomitic diad (heteromorphic). These tetrads can be distinguished if one of the diads in either tetrad is atelomitic, because Tetrad 7 has a subterminal fiber insertion while that of Tetrad 8 has an approximately median fiber attachment. Chromosome 6 is always atelomitic and passes precociously to one pole of the spindle. Tetrads 4 and 5 are usually indistinguishable. Occasionally Tetrad 4 will have one or both diads "knobbed", owing to a constriction near the proximal end, but this condition is not a reliable means of separating them since the frequency of "knobbed" chromosome 4 is very small. Tetrads 1 and 2 are so similar in size that this criterion cannot be used for their

FIG. 1. Spermatogonial and first spermatocyte chromosomes of *Trimerotropis suffusa* (rows 1 and 2); *Circotettix verruculatus* (rows 3 and 4); and the hybrid (rows 5 and 6).

separation. However, in anaphase the distal ends of chromosome 2 are swollen and thereby offer a dependable means of identification. Tetrad 1 may have one or both diads atelomitic in a few individuals; when telomitic its proximal ends are never bulbous.

Chromosomal Conditions in Trimerotropis suffusa. This species has twenty-three chromosomes in its spermatogonia (Row 1). The variation in proportion of telomitic to atelomitic chromosomes is much greater in this form than in *Circotettix verruculatus*, and the relative proportions of the two are correlated with geographic distribution. The frequency of atelomitic chromosomes is much less in the southern area of its distributional range than in its northern. Nine of its chromosome pairs may have one or both of its members atelomitic in addition to the unpaired atelomitic sex-chromosome in the northern area of its distribution. However, the chromosomal conditions in its southern distributional area are more like those in *Circotettix verruculatus* from Mt. Desert Island than they are in the northern part, where the two forms probably overlap. The following description applies to individuals from southern Colorado.

In the spermatogonia (Row 1) the three largest pairs (10, 11, and 12) are invariably atelomitic throughout the distributional range of this form. Chromosomes 7, 8, and 9 may be either atelomitic or telomitic, but in the populations from southern Colorado they are telomitic in the majority of individuals. These three pairs of chromosomes are indistinguishable within the species but can be seaprated when they pair with recognizable homologues from *Circotettix*. Chromosome 6 is the sex-chromosome and is always atelomitic. Chromosomes 2, 3, 4, and 5 are always telomitic in the southern Colorado populations. Chromosome 1 may be either telomitic or atelomitic, though it is very seldom the latter.

The first spermatocyte contains eleven tetrads and one diad (Row 2). The three largest elements (10, 11, and 12) are indistinguishable. The next three tetrads (7, 8, and 9) may be heteromorphic or homomorphic, but in neither condition are they separable. Chromosome 6 is the atelomitic sex-chromosome and migrates precociously to one pole of the spindle. Tetrads 3, 4, and 5 are always telomitic and, being approximately equal in size, cannot be separated. Tetrad 2 always has both diads telomitic and has bulbous or swollen ends as in *Circotettix*. The swollen ends of this element serve to separate it from Tetrad 1 which may be either heteromorphic or homomorphic.

Cytology of the hybrids. The general histological condition of the hybrid testes differed in no conspicuous way from the normal testes of either species. The size and, consequently, the number of follicles are the same. The cysts in the hybrid contain two hundred and fifty-six primary spermatocytes, as do the cysts of *Circotettix verruculatus* and *Trimerotropis suffusa*. There are no discernible differences in the spermatogenic processes correlated with the species which provides

the male parent and that which provides the female parent. There are no abnormalities of the spindle, no supernumerary divisions, and only very occasionally polyploid cells such as are so often found in hybrids. Normally all the cells within a cyst are in the same stage of development, whereas in the hybrid this synchronism is frequently lost. The meiotic chromosomes are often long and slender in comparison with those of either parent species, though not invariably so. This condition was conspicuous in the hybrids between *Triturus cristatus* and *Triturus marmoratus* (White 1946) and in hybrids between *Chorthippus bicolor* and *Chorthippus biguttulus* (Klingstedt 1939). So far as outward appearances are concerned, normal sperm are produced, though the study of the spermatocytes shows that many of these must have an unbalanced complement of chromosomes. In some individuals there is much cellular debris mixed with the sperm indicating that many spermatids probably did not transform successfully into spermatozoa.

Chromosomal Conditions in the Hybrid. The spermatogonia have twenty-two chromosomes of which one is a multiple derived from the *Circotettix* parent (Row 5). In the first spermatocyte there are eleven chromosomes: one octad multiple, nine tetrads, and the sex-chromosome diad (Row 6). Of these chromosomes the three largest (12, 11, 10) have their constituent diads regularly atelomitic and are indistinguishable. The next in the series is the octad multiple composed of the multiple (9 and 3) from the *Circotettix* parent, which joins with the sparate elements 9 and 3 from the *Trimerotropis* parent. Number 9 from *Trimerotropis* is usually telomitic, but in six individuals it was atelomitic. With one exception, Number 8 has both diads always telomitic in the hybrid, whereas Number 7 has both diads telomitic in fourteen individuals and heteromorphic in twenty-three individuals. When both diads are telomitic Number 7 is indistinguishable from Number 8. However, since Number 7, when atelomitic and therefore separable from 8, was always the one that failed to undergo synapsis, in those individuals in which it was a question as to which of the two was failing to synapse, it was considered to be Number 7. Number 6 is the sex-chromosome diad, and since it has no homologue in the male it is not involved in these failures to synapse. As numbers 4 and 5 cannot be separated with any degree of certainty, they have been considered together. Numbers 1 and 2 are always easily separated in several ways: the fiber ends of Number 2 are usually bulbous, Number 1 may be heteromorphic, as it is in six individuals, or the diads may be conspicuously unequal in size (Fig. 2, a, c; Fig. 3, a) as they are in twelve individuals. As a result of these criteria it was always possible to distinguish chromosomes 1 and 2.

Each chromosome from one parent seems capable of uniting with a corresponding chromosome from the other parent, though this condition is not actually found in all of the spermatocytes. The frequency with which homologous chromosomes synapse varies from individual to individual. No individal was found in

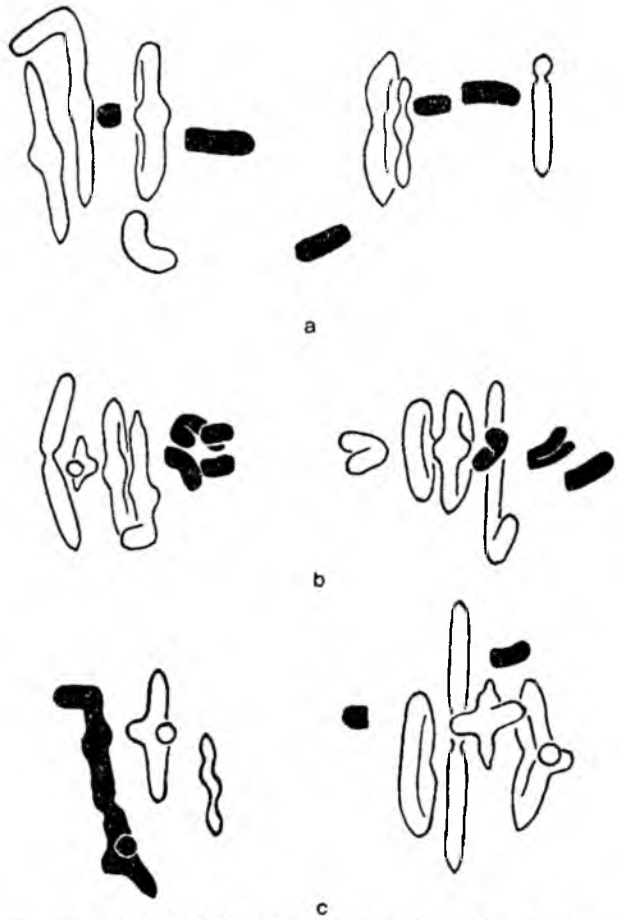

FIG. 2. Chromosomes of the hybrids. Chromosomes referred to are shown in solid black. Those in outline indicate position of center of spindle whose poles are toward the top and bottom of the plate. Not all chromosomes are shown. a. Homologues (4 and 5) have not paired. Chromosome 3 from the *Trimerotropis* parent has not synapsed with its homologue, which is part of the large J-shaped multiple. Homologues 1 have not paired. They are unequal in size in this individual. b. Chromosomes 7, (4 and 5), and 2 have not paired. Chromosome 9 from the *Trimerotropis* parent has not synapsed with its homologue, which is part of the *Circotettix*-multiple. Note the position of these unpaired chromosomes in the center of the spindle usually. c. The (9 and 3) multiple has assumed such a position on the spindle that normal segregation of homologous elements would be impossible. The normal position for the multiple is shown in Fig. 3b. Chromosomes 1 have not paired and are unequal in this individual.

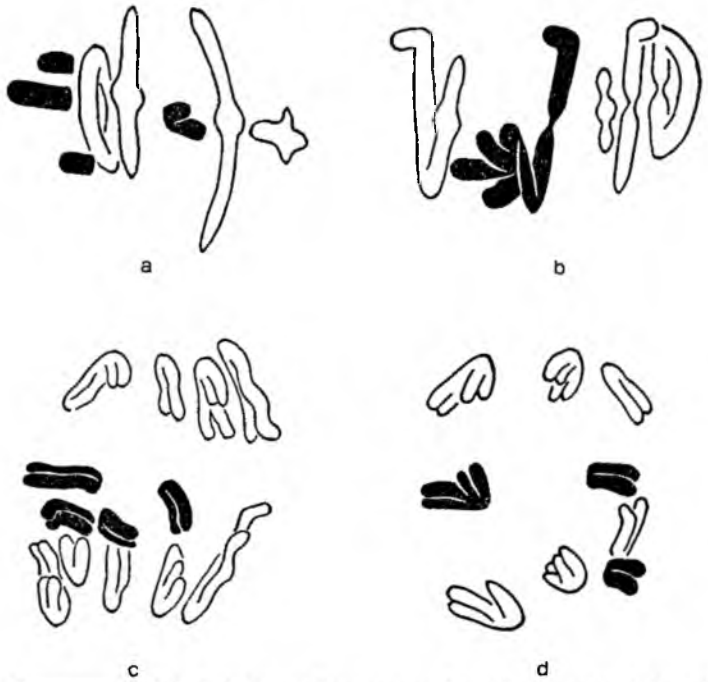

FIG. 3. Chromosomes of the hybrids. a. Chromosomes 7 and 2 are unpaired. One chromosome 7 is atelomitic and the other is telomitic. Tetrad 1 is composed of unequal elements. b. Four atelomitic chromosomes have joined to form an octad multiple as a result of a translocation. Its position on the spindle is such that homologous portions will segregate. Note the typical shape for tetrad 2. c. and d. First spermatocyte anaphases which show unpaired chromosomes lagging in the center of the spindle. In d one unpaired diad has gone undivided toward one pole.

which the incidence of synapsis for all units even approximates that found in normal individuals of either species. In every hybrid individual at least two pairs of homologues fail to synapse in some of the spermatocytes, and in most animals more than two pairs fail regularly to join. This condition holds for all the cysts within a testis, although the frequency of asynapsis may vary from one cyst to another. Very frequently more than a single pair of homologues may fail to unite within a single cell, but no record was kept of the coincidence of asynapsis in two or more pairs within the same cell in computing the percentages of asynapsis for a specific pair. A record of this coincidence was kept, however, for counting the total number of cells studied (last column in Table 1). The chromosomal conditions, as found in the thirty-seven individuals studied, are summarized in Table I. The number of spermatocytes per individual in which all the chromosomes have paired normally and hence might be expected to develop into normal sperm, ranges from 9.8% to 94.0% (Table I).

TABLE I. *Frequencies in percentage of asynapsis in the hybrids*

Individual	Complete Synapsis	Incomplete synapsis in chromosomes								Number of cells studied
		12, 11, 10	9	8	7	5, 4	3	2	1	
1	94.0					3.6	0.5	1.0	0.5	190
2	91.2					4.0	4.7		0.4	275
3	89.0					3.6	6.4	2.0		247
4	88.2					3.8	7.5		1.6	186
5	82.0				9.5	1.0	7.5			200
6	77.1					18.1	4.8			144
7	76.8					8.3	8.3	1.8	1.8	108
8	75.7	1.0			17.5		7.8			103
9	69.8	0.7				9.4	19.6		0.4	285
10	66.8				29.9	1.5	1.5	0.3	1.2	334
11	64.7				30.1		5.1		1.5	136
12	63.8				28.3	1.3	2.7	2.7	1.3	296
13	61.7					8.4	1.3	1.9	30.5	154
14	60.9				35.2	1.6	3.5		0.4	256
15	54.7				37.4	4.3	3.9	0.8		254
16	52.8		18.9		30.0	0.6				180
17	52.4				22.2		25.4			63
18	51.9	0.3	28.8		22.2	0.9	0.9	0.9		212
19	51.7				43.1	4.1	0.7	4.1		292
20	51.1				36.7	2.9	15.8			139
21	50.2		4.0		47.8					201
22*	46.0				0.4	44.8	1.7		13.7	239
23	44.4		22.9			20.1	1.4	2.8	21.5	144
24	43.8					8.9	43.1			146
25*	42.1		5.4		53.8	0.5		2.3		221
26*	37.2	13.6	1.8	1.8	1.8	9.3	40.9		1.8	161
27	35.3					13.0	36.6	1.0	31.3	306
28	35.0				22.0	10.0	27.5	1.5	3.5	200
29	34.9		2.4		34.3	13.6	26.7	1.8	9.5	169
30	34.8		8.0		50.0	4.5	2.7	1.8	8.9	112
31	27.3				47.8	2.5	3.7	9.9	32.9	161
32	25.3	0.7			38.7	7.1	37.9	2.6	20.8	269
33	25.1	1.0				55.2			5.6	287
34	24.4	1.2	1.2		43.0	14.0	26.8	5.8	12.8	86
35*	15.7	0.5			41.4	7.0	11.7	5.0	60.8	401
36	14.4	2.2				19.8	67.8	5.7	32.1	227
37	9.8	5.3			47.4	32.3	42.1			133

The following chart shows the number of individuals out of a total of 37 in which the various chromosomes failed to synapse in some of the primary spermatocytes.

Failure of pairing in the three largest pairs (12, 11, 10) occurs in only ten in-

Chromosomes	12, 11 10	9	8	7	4, 5	3	2	1
Number of individuals	10	9	4?	24	33	34	21	23

dividuals, and then the frequency of asynapsis is low. With one sure exception (individual 26, Table I) the homologues of pair 8 invariably synapse. When both diads of chromosomes 7 and 8 are each telomitic it is impossible to distinguish them, and since this condition was present in three individuals there is a possibility that in these individuals chromosomes 7 and 8 have been confused; so, at most, homologues 8 may have failed to join in at least four animals. These have been marked with an asterisk in Table I. When the smaller elements are considered, the number of individuals in which asynapsis occurs increases greatly.

In addition to the failure of synapsis of one or more pairs of homologues there are two other conditions resulting in the production of unbalanced gametes. These conditions are relatively infrequent, affecting but a small per cent of the cells, but adding to the total of the chromosomally unbalanced sperm. The more frequent condition occurs when the multiple takes its position on the spindle so that a normal segregation of its constituents is impossible (Fig. 2, c). Secondly, in sporadic cells four of the spermatogonial chromosomes may have united at synapsis to form an octad multiple (Fig. 3, b) or an unsynapsed chromosome may be joined with a tetrad to form a hexad multiple. These multiples usually assume such a position on the spindle that a normal separation of all the elements occurs; infrequently, however, they take a position on the spindle that makes normal segregation impossible. Since usually no more than two or four cells containing these multiple chromosomes are found within a cyst, the translocation which caused their formation must have occurred during the last of the spermatogonial divisions. In two hybrid individuals derived by crossing *Chorthippus bicolor* with *Chorthippus biguttulus*, Klingstedt (1939) found two cells with such multiples in one individual and seven in the other; and that is approximately their frequency in these hybrids. They sometimes occur as frequently in normal individuals of either species.

All the chromosomes except the unsynapsed ones take their normal positions on the spindle and their constituent elements segregate at anaphase. The unsynapsed chromosomes may lie almost anywhere on the spindle though usually they also take their position on the equatorial plate (Fig. 2, a and b; Fig. 3, a). When in this position, each chromatid is connected by a chromosomal fiber to opposite poles so that the long axis of the chromosome is at right angles to the long axis of the spindle. The chromatids of these elements seldom separate and the chromosome remains at the equator of the spindle after the other chromosomes have passed to the poles. (Fig. 3, c and d.) In the division of the cell these lagging diads become passively incorporated into one of the two second spermato-

cytes. Apparently they behave normally during the second meiotic division, for lagging chromosomes are rarely found.

DISCUSSION

Presence of Inversions. The various species and subspecies of *Drosophila* often differ in their gene arrangements and these differences are brought about chiefly by inversions. The presence of inversions in the heterozygotes can be detected cytologically by the appearance of bridges with accompanying fragments in some of the spermatocytes where synapsis had occurred. The Orthopteran species would appear to differ from *Drosophila* in this respect, as evidence from different levels of differentiation suggests. *Melanoplus differentialis differentialis* occurs in the eastern part of the United States, extending as far west as Minnesota and the eastern parts of Iowa, Missouri, Arkansas, and Louisiana. *Melanoplus differentialis nigricans* occurs in the western part of the United States and nearly as far south on the Mexican plateau as Mexico City. Morphologically these subspecies are separated by the much greater distal production of the aedeagal valves in *Melanoplus differentialis differentialis* as compared to *nigricans* (Roberts 1942). No other feature upon which to separate these subspecies has been found. When these are crossed, fertile hybrids are produced and an examination of their spermatocytes disclosed no failure of synapsis whatsoever and no cytological bridges nor fragments (Helwig, unpublished).

Melanoplus femur-rubrum femur-rubrum ranges over most of the United States and as far south as Central Mexico. In the south-eastern states it is replaced by *Melanoplus femur-rubrum propinquus* which extends from southern North Carolina through South Carolina, Georgia, Florida, Alabama, and as far west as Gulfport, Mississippi. These two subspecies are distinguished structurally by the length of the furcula. In *femur-rubrum* the furcula does not, or scarcely, reaches the middle of the supra-anal plate whereas in *propinquus* it reaches considerably beyond the middle of the supra-anal plate (Blatchley 1920). A cross between these two forms produces fertile hybrids and there is never any failure of pairing in the spermatocytes of the hybrid nor any cytological conditions suggesting the presence of inversions in the heterozygous condition. (Helwig, unpublished.)

In the hybrids from *Chorthippus bicolor* and *Chorthippus biguttulus* the chromosomes appear to be "sticky", and this condition results in the "abundant occurrence of bridges" which consequently makes the discovery of bridges due to structural change extremely difficult. True inversion differences were found in one cell of one individual and in three cells of a second. Since similar inversion bridges are found in both parent species, no definite conclusions can be reached as to whether or not these species differ in gene arrangements. Finally, in the hybrids between *Circotettix* and *Trimerotropis* no inversion differences are found.

The evidence indicates that sizable inversions of the gene order have not be-

come established during the phylogeny of these Orthopteran species and subspecies.

Role of Small Rearrangements. In the absence of any genetic data in these species and in the Orthoptera, in general, it is difficult to find an explanation for the asynapsis observed. Only by analogy with *Drosophila*, where both the cytology and genetics are so well known, can a possible explanation be found. That rearrangements of the chromatin have occurred during the evolution of these species there can be no doubt, as attested by the presence of atelomitic chromosomes. Chromosomes with terminal centromeres are the usual condition in the Acridoidea and undoubtedly were present in the ancestors of *Circotettix* and *Trimerotropis*.

Detailed examinations of the salivary chromosomes of several species of *Drosophila* and *Sciara* indicate that minute rearrangements of the chromatin are more numerous than large ones. (Horton, 1939; Dobzhansky and Tan 1936; Metz and Lawrence 1938; McCarthy 1945.) Horton (1939) found that a short inversion involving only two or more bands on the salivary chromosomes ordinarily prevents pairing for some distance on either side of the rearrangement. He also found asynaptic regions where the visible pattern of the bands on the homologues are similar, and he interpreted this failure of synapsis as due to an undetectable rearrangement or the inversion of a single band. McCarthy (1945) likewise found in *Sciara* short asynaptic regions in which no pattern differences could be found. He also found such regions that were apparently due to minute differences often involving a single band. An extra band in one homologue in *Sciara agraria* was sufficient to cause a small asynaptic gap between them. Carson (1944) found many small differences in *Sciara impatiens* that resulted in asynapsis.

It seems possible that the failure of pairing found in the *Trimerotropis-Circotettix* hybrids may be caused by minute intrachromosomal rearrangements of the translocation-inversion type. Since these minute rearrangements interfere with synapsis in *Drosophila* and *Sciara*, it would be expected that they might do likewise in the acridids.

In salivary chromosomes limited areas of the chromosomes may fail to pair without, however, preventing the chromosomes as wholes from undergoing synapsis (Dobzhansky and Tan 1936; Horton 1939; Cavalcanti 1948). Nevertheless, if synapsis fails in too many regions the result is almost no pairing, as in hybrids between *Drosophila azteca* and *Drosophila algonquin* (Dobzhansky 1937). Possibly so many minute rearrangements have accumulated in *Circotettix verruculatus* and *Trimerotropis suffusa* since their divergence from a common ancestor that their combined effect in the hybrids is to produce a certain amount of asynapsis. The more numerous these intra-chromosomal alterations in gene arrangements the more acute would be the competition for pairing between homologous genes. Consequently, the frequency with which synapsis fails may be directly propor-

tional to the number of different rearrangements in the homologues. For example, in individual 1, where only six per cent of the spermatocytes show asynapsis, the number of minute rearrangements whereby the homologues differ may be relatively few, whereas in individual 37, where 91.2% of the spermatocytes show failure of synapsis, the homologues involved must differ by many minute gene arrangements.

The hypothesis that some of the hybrid individuals were heterozygous for more minute rearrangements than others implies that the parental forms were highly heterogeneous cytologically. Then, if there is a close correlation between the number of rearrangements and the amount of asynapsis, the parental species should have shown a certain amount of asynapsis. Hundreds of individuals of these two species have been examined in connection with other studies (Helwig, 1929 and unpublished) and the almost total lack of any failure to pair in both species is quite striking.

Possibly, none of the homologues in either species differed by enough rearrangements to make competitive pairing sufficiently effective to prevent the synapsis of the chromosomes as wholes. But, when the accumulated rearrangements of the two species are combined in one individual the resulting greater structural heterozygosity is effective in producing some asynapsis.

Helfer (1941) found that in *Drosophila pseudoobscura* induced breakages are distributed more or less uniformly in proportion to chromosome length. Likewise, in *Circotettix* (Helwig 1933) breakage is directly proportional to the size of the chromosome, being relatively infrequent in the smaller ones. Consequently, more asynapsis resulting from transpositions would be expected in the six largest chromosomes than in the smaller ones, but actually they fail to pair much less often than the smaller elements.

These large chromosomes may have more minute rearrangements, as expected, than the smaller ones, but the proportion of the chromosomes involved in these transpositions relative to their total length is so small that even under the conditions of competitive pairing the chromosomes succeed in pairing as wholes. On the other hand, in the smaller chromosomes the transposed sections constitute a much larger proportion of the chromosomes and as a result of competitive pairing the homologues fail to synapse much more frequently or synapse so insecurely that they fall apart precociously.

The possible role of genes in causing asynapsis in these hybrids cannot be assessed, since nothing is known of the genetics of these forms and very little of the Orthoptera as a whole.

Change in Locus of Fiber Attachment. A consideration of the mode of shift in locus of fiber attachment may also give some evidence of the possible presence and extent of chromosomal rearrangements in *Circotettix* and *Trimerotropis*, for there can be no doubt that some kind of intrachromosomal transpositions have occurred

during their phylogeny. Terminal fiber attachments are the usual condition among the Acridoidea, and the two forms have unquestionably descended from ancestors that had only telomitic chromosomes. It was suggested (Helwig 1929) that the shift in locus of fiber attachment from a terminal position to a non-terminal or atelomitic one was brought about by an inversion of the proximal end of the chromosome. Since the chromosomal fiber continued to be attached to the same locus on the chromosome, such an inversion would bring about an apparent, though not a real, shift in point of fiber attachement. It is now realized that such an explanation for the change in locus of fiber attachment is too simple. In all probability, the intact proximal end could not fuse with the broken surface nor could the fractured or injured end serve as the end of the chromosome. Furthermore, if a telomere is an essential part of a chromosome, as Muller (1940) believes, it appears to be incapable of occupying an interstitial position or of being replaced by any other part of the chromosome. Both of these conditions would have to be realized if the change in the location of the centromere to a nonterminal position had been due to a simple terminal inversion.

White (1945) has suggested that the change in position of fiber attachment has come about as a result of two breaks in the chromosome and a subsequent pericentric inversion. In order to get such an inversion the centromere could not have a truly terminal locus. Some workers (White, 1935; Darlington 1936; Coleman 1943) maintain that all rod-shaped chromosomes actually have two arms, one of which may be minute. It is postulated that one break occurred in the minute arm and a second somewhere in the chromosome on the opposite side of the centromere. A pericentric inversion of the included piece would result in an apparent, though not actual, shift in point of fiber attachment. The evidence for minute arms in rod-shaped chromosomes is far from convincing. Though not concerned with the location of the centromere in her paper, Carothers (1936) shows clearly in some of her figures that the point of fiber atachment is terminal. Chromosomes in which the region of fiber attachment apparently pulls out as a small trabant from the main body of the chromosomes leave little doubt of the terminal position of their centromeres. Such conditions are relatively common in the chromosomes of the Acridoidea. Makino and Momma (1950), using experimental methods, are of the opinion that in three species of grasshoppers studied by them the centromere is terminal.

On the assumption that terminal inversions did produce the shift in the locus of the centromere, these inversions in some of the chromosomes of *Trimerotropis* and *Circotettix* would have involved as much as half of a chromosome. Then inversion loops should be present in individuals that are heterozygous for the inversion, but none have ever been found by the author nor by Coleman (1948) working with the same species. Probably also significant is the finding that terminal inver-

sions were never produced in *Circotettix* in several dozen individuals and hundreds of cells subjected to irradiation (Helwig, 1933).

Since terminal inversions require only a single break in the chromosome it would be expected that they would constitute the most numerous type of aberration in both experimental and natural populations, but such is clearly not so. One was reported in *Drosophila ananassae* (Kaufman 1936; Kikkawa 1938; Dobzhansky and Dreyfus 1943) and of this one the latter authors said, "We cannot exclude the existence of an invisible telomere persisting at the end of the chromosome despite visible variations." Pavan (1946) described an inversion which is "possibly terminal" in *Drosophila nebulosa*. Two terminal inversions were found in *Drosophila robusta* by Carson and Stalker (1947).

Carson (1944) found three terminal inversions in *Sciara impatiens* as well as other terminal differences. Of these he said, "It appears that the centromeres, and any heterochromatin that may be associated with them, have no expression in the salivary gland chromosomes. For this reason, it is unsafe to draw the conclusion that the terminal aberrations described above involve the ultimate ends of the chromosomes." Crouse (1947) found a terminal inversion in *Sciara ocellaris* and McCarthy (1945) discovered another in *Sciara prolifica*.

In spite of the possibility that terminal inversions may occur, evidence has been given to show that the shifts in the locations of fiber attachments in *Circotettix* and *Trimerotropis* are very probably not due to terminal inversions. More recently White (1948) has suggested that the shift in the position of fiber attachment is due to a transposition of the centromere "possibly with a very short region on either side". Crouse (1947) has reported what appears to be just such a centromere shift in the C-chromosome in some individuals of *Sciara ocellaris*. This shift would also demand that the centromere of the original rod-shaped chromosome was not actually terminal and, consequently, this most recent suggestion brings us no nearer to a solution of this problem.

SUMMARY

Hybrids were made between *Circotettix verruculatus* from Mt. Desert Island, Maine, and *Trimerotropis suffusa* from Woodland Park near Colorado Springs, Colorado. These two forms are probably not so distantly related as their present taxonomic designation would indicate. They are very probably species of the same genus.

Circotettix verruculatus has twenty-one spermatogonial chromosomes. The difference between this number and twenty-three, which is characteristic for most of the Oedipodidae, is due to the presence of two multiple chromosomes.

Trimerotropis suffusa has twenty-three spermatogonial chromosomes. The hybrid has twenty-two chromosomes in the spermatogonia. The first spermatocytes

have eleven chromosomes. Two of the spermatogonial chromosomes of *Trimerotropis suffusa* pair with the multiple derived from the *Circotettix* parent. The three largest tetrads are indistinguishable. Tetrads 7 and 8 are separable when at least one of the diads of chromosomes 7 or 8 is atelomitic. Chromosome 6 is the sex-chromosome. Tetrads 4 and 5 cannot be separated. Chromosome 3 is involved in the formation of the multiple and therefore distinguishable. Elements 1 and 2 can be separated by one of several criteria.

In the hybrid one or more pairs of homologous chromosomes invariably fail to synapse in some of the spermatocytes. The pair or pairs of chromosomes that fail to join are characteristic for the cells of a given individual but may vary from one animal to another. When the same pair fails to undergo synapsis in different individuals the frequency of asynapsis is not necessarily the same. Failure of synapsis involves the smallest chromosomes of the complex much oftener than the largest elements.

The presence of inversions in some species of Orthoptera is considered.

The possible role of minute rearrangements as a factor in causing asynapsis and also in effecting a shift of fiber attachment in these species is discussed.

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