A CYTOLOGICAL SURVEY OF CERTAIN AMERICAN SPECIES OF THE GENUS GRYLLUS LINNAEUS, 1758, FORMERLY ACHETA FABRICIUS, 1775, (ORTHOPTERA:GRYLLIDAE) AND THEIR HYBRIDS.

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A THESIS

Submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

October, 1960.

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ACKNOWLEDGEMENTS

The author wishes to express his thanks to the Government of the Province of Quebec, without whose financial assistance, under the Orthoptera of Quebec Grant, this research would have been impossible. I would also like to acknowledge the invaluable assistance of Professor D.K.McE. Kevan, whose ready interest and helpful criticism gave direction to the project. Professor W.F. Grant advised the author on technique and helped in the interpretation of the material, giving ungrudgingly of his time on numerous occasions. I wish to acknowledge also the assistance of Professor R.S. Bigelow for providing the breat bulk of the material examined and for his helpful criticism, both during the study and of this manuscript. Thanks are also due to Dr. R.D. Alexander for providing material.

Much of the material in this thesis has been the subject of discussion on numerous occasions, and the author would like to express his thanks to the members of the staff and graduate students of the Department of Entomology at Macdonald College for their interest and criticism.

My thanks are also due to Miss B. Robinson for typing the manuscript and to my wife for its revision and proof reading.

I. INTRODUCTION

Mayr, Linsley and Usinger (1953) have defined three levels of taxonomic study; the first or alpha level dealing with the recognition and description of new species, the second or beta level dealing with the arrangements of these described species into the hierarchical system and the third or gamma level dealing with the study of infraspecific variation and the evolution of taxa. It is probably the general rule that,in any one group, progress is made by passing through these various levels in turn as they suggest in the following quotation:

> "The three tasks of taxonomy are rarely undertaken simultaneously. Evolutionary studies cannot be pursued unless a satisfactory classification is available, and this in turn is based on the prior description of species. The taxonomy of a given group, therefore, passes through several stages. ... Actually it is quite impossible to delimit alpha, beta and gamma taxonomy sharply one from another, since they overlap and intergrade. However, the trend is unmistakeable."

If this is in fact the general trend then the present situation in the taxonomy of the field crickets of the genus Gryllus (formerly Acheta see Randell in press) must provide an interesting example of the directly opposite situation.

The work of Fulton, Alexander, Cousin and Bigelow which can only be described as gamma taxonomy, has completely reorganized the taxonomy of the group, involving generic transfers, resurrection of synonymized specific names, and the description of new species which without evidence from gamma taxonomy were completely unrecognizable. Their studies on this genus are making possible the selection of reliable anatomical characters on which a revision of the subfamily may be based.

This thesis is an account of a cytological survey to assess the value of chromosome numbers as an indication of systematic position, and to ascertain the amount and kind of chromosomal abnormality in interspecific hybrids.

II. REVIEW OF THE TAXONOMIC AND BIOLOGICAL LITERATURE (EXCLUSIVE OF CYTOLOGY)

The two generic names central in any discussion of the subfamily Gryllinae are Gryllus and Acheta. Both of these names have a long taxonomic history dating in their original use to the tenth edition of the Systema Naturae. Although Gryllus of Linnaeus is presently recognized, Acheta of Linnaeus is considered invalid since Linnaeus used it as a subgeneric name under the genus Gryllus and did not also recognize a subgenus Gryllus, (Opinion 124 of the Int. Com. Zool. Nom.).

The genus Gryllus, as defined by Linnaeus in 1758 was placed in the order Coleoptera and contained six, now invalid subgenera including all of the known Orthoptera Saltatoria, mantids and phasmids. His subgenus Acheta contained four species, all of which are now placed in separate genera, and one, Gryllus (Acheta) gryllotalpa, Linnaeus, 1758, is placed in another family (Gryllotalpidae). The remaining three species, i.e., G.(A.) domesticus, G.(A.) campestris and G. (A.) umbraculatus while now placed in separate genera are all referred to the single subfamily Gryllinae.

The very broad genera of Linnaeus were soon subjected to a narrowing of definition, Acheta of Fabricius (1775) became a genus rather than a subgenus as previously defined. This same author also described the first new world species, A.assimilis Fabricius (1775), in his Insecta Systematica. Without access to a copy of this work it is almost impossible to obtain an impression of how Fabricius interpreted the generic name Gryllus, Roberts (1941), however, cites Gryllus sibericus Fabricius as a synonym of Gryllus (Locusta) sibericus Linnaeus in his discussion of the genus Gomphocerus Thunberg, from which it is possible to suggest that Fabricius included at least a portion of the Linnaean subgenus Locusta in his definition of Gryllus while excluding crickets, which he described under the genus Acheta.

In 1810 Latreille designated Gryllus (Acheta) campestris Linnaeus, 1758, as the type of the genus Gryllus and Curtis (1830) designated G.(A.) domesticus as the type of Acheta although these designations were apparently unnocticed by later workers until Roberts (1941) drew attention to them.

Henri de Saussure's "Melanges Orthopterologiques" volume two, fascicule five (1877) is of great importance to the taxonomy of crickets, since it is the last complete revision of this group. Subsequent work is spread throughout the literature, and were it not for the scarcity of workers in this

field the taxonomy of the group would be in a worse state of confusion than it is at present. Saussure raised the equivalent of the Linnaean subgenus Acheta to the level of a family, recognizing six tribes. Of these tribes only Gryllii is important to this discussion. Within this tribe he referred G.campestris Linnaeus to the genus Liogryllus, a genus in the legion Brachytrypites, while G.(A.) domesticus and G.(A.) umbraculatus were referred to separate genera (i.e. Gryllus and Platyblemmus) in the legion Gryllites. It is interesting to note that he classified the field crickets of the New World with B.(A.) domesticus Linnaeus, separating them from G. (A.) campestris, a practice still advocated by many workers, particularly in North America. Except for changes in the two generic names and descriptions of new species in the genus to which G.(A.) domesticus is referred, the present classification of the two genera as accepted by most workers is essentially the same as that devised by de Saussure. Kirby's "Synoptic Catalogue of the Orthoptera follows a classification similar to that of Saussure although the genus Acheta is used instead of Liogryllus. The generic name of the black field crickets was finally stabilized by a decision of the International Commission, whose opinion 104 placed Gryllus on the Official List with G.(A.) campestris Linnaeus (1758) as the typespecies (see Int. Comm. Zool. Nom. 1908).

Considering for a moment the absolute size of the two genera, we see that Saussure recognized 4 species of Liogryllus (= Gryllus Linnaeus 1758) and 39 species of Gryllus (= Acheta Fabricius 1775); Kirby recognized 5 species of Acheta (= Gryllus Linnaeus 1758) and 110 species of Gryllus (= Acheta Fabricius 1775); as of 1955 the situation was as follows 5 species of Gryllus and approximately 250 of Acheta. As to new world species, Saussure recognized 12 and Kirby 35, both authors placing them in the same genus with G.(A.) domesticus Linnaeus, 1758.

Following a thorough morphological study of material from the western hemisphere Rehn and Hebard (1915) synonymized all of the previously described names, amounting to some 49 specific and subspecific epithets, under the single specific name Gryllus assimilis (Fabricius) although they recognized the possibility of subspecies within this single large species.

Of all workers on crickets Dr. Lucien Chopard has been the most prolific. His revisions of the cricket faunas of specific geographical areas have been well conceived, but the total conception of the group on a world basis is difficult, if not impossible, to assess from these fragmentary works. It is even difficult to arrange the species in some sort of order for while certain geographic areas are treated the relationship of species in the same genus but from different geographic areas is ignored. This becomes a serious difficulty

in a genus such as Acheta with some 250 recognized species. Important as this omission has been, his separation of the African species of Acheta into species groups, and his practice of providing illustrations of the male epiphallic plate have greatly simplified the identification of specimens in this genus where external morphology provides so few reliable specific characters. Until 1955, however, his classification of the crickets referable to the genera Gryllus and Acheta was essentially the same as that used by Saussure, apart from certain simplifications, the addition of certain new species, and the suppression of certain obviously synonymous specific names. By 1955 the weight of evidence provided by Cousin's hybridization of neotropical species that Chopard had referred to Acheta with Gryllus campestris and G.bimaculatus De Geer caused him to synonymize the two genera under Gryllus, the older name. It is necessary now to consider Cousin's experiments in detail, bearing in mind that Chopard, who made the determinations of Cousin's material, did not accept Rehn and Hebard's decision to synonymize all of the New World forms under G.assimilis.

In 1933, Cousin reported the hybridization of Gryllus campestris and G.bimaculatus. While this raised certain doubts in the minds of some authors as to the validity of the two species, it had no effect on the classification at the generic

level. In 1946, Cousin reported the crossing of Gryllus burmudiensis Caudell (= Acheta assimilis partim Rehn and Hebard 1915) females with Gryllus campestris males. This was the first biological indication that the placing of certain species in Acheta, especially the western hemisphere forms, was rather artificial. Further evidence was soon forthcoming. In 1954, Cousin reported the crossing of another species from the western hemisphere, A.peruviensis Saussure, with G.campestris. Later in the same year she recorded the crossing of a third species, A.argentinus Saussure also with G.campestris. Following the crossing of A.argentinus with A.capitatus Saussure and A.assimilis Fabricius reported in 1956 she recognized five New World species all of which interbreed with reduced fertility either with G. campestris itself, or with A.argentinus which interbreeds with G.campestris. These species also interbreed amongst themselves in certain combinations of males and females but are apparently infertile in others (see Plate 1, summarizing the experimental results of both Cousin and Bigelow).

Work of a similar nature has been carried out by Dr. R.S. Bigelow with the neartic species discovered by Fulton (1949, 1952) and described formally by Alexander (1957). Fulton, working in North Carolina, showed the presence of four "races" which differed from each other in song and in the characteristics of their seasonal development and ecological

niche. In 1957, Alexander demonstrated morphological differences between these "races" and associated them with previously described names where these were available, replacing Fulton's vernacular names. He also discovered a fifth species which did not occur in North Carolina. He was able, by means of the audiospectrograph, to show visually the audible differences in song originally noted by Fulton. The five "races" showed no signs of interbreeding in experiments made both by Fulton and Alexander, and this with the fact that they were sympatric made it impossible to regard them as races, i.e., subspecies, in terms of the modern subspecies concept. Alexander, therefore, raised them to distinct species, a view shared by most workers on the group.

In 1958, Bigelow recorded the presence of two temporally isolated species in Quebec, one of which has since proved to be identical to the Mountain Cricket described by Fulton (i.e., A.pennsylvanicus Burmeister sensuo Alexander 1957) the other was undescribed, being rare in collections, and having been confused with A.pennsylvanicus by Alexander (1957) because the two species are identical in song. This species was described by Alexander and Bigelow (1960) as A.veletis.

In his 1958 paper Bigelow also recorded the crossing of males of this new species with females of Fulton's Triller Cricket (i.e. A.rubens (Scudder) sensuo Alexander 1957). In a later paper (Bigelow, 1960), he recorded the

reverse cross between these two species (i.e. A.veletis female with A.rubens male), the crossing of A.rubens and A.assimilis (sensuo stricto) in both directions, and the crossing of males of A.assimilis (sensuo stricto) with a female of A.pennsylvanicus. These last two crosses are of great importance since they link the neotropical and Bermudian species studied by Cousin with the Northeastern North American species studied by Fulton, Alexander and Bigelow.

In a study of the male genitalia undertaken by the author (Randell in press), it was found that these characters paralleled the evidence previously provided by biological work. The male genitalia of material from various species and various localities in the Western Hemisphere were much more similar to G.campestris than to A.domesticus. It was, therefore, suggested that since several definite anotomical differences existed in the genitalia alone, the species described from the Western Hemisphere should be transferred to the genus Gryllus; and the genus Acheta retained for the species that resembled A.domesticus basing the generic placing on the male genitalia.

Thus the biological or gamma taxonomic work of Cousin, Fulton, Alexander and Bigelow has resulted in the resurrection of eight specific trivials, the description of two new species (not to mention the Brazilian sibling of G.assimilis recorded

by Cousin, 1956), and the transfer of these species from *Acheta* to *Gryllus*.

III. REVIEW OF THE CYTOLOGICAL LITERATURE

The cytological literature dealing with the genera Gryllus and Acheta is very sparse and much confused due to the uncertainties of their classification. In all references to only twenty papers were found nineteen of which are listed in Makino (1951). (Of these papers five were seen in the original, the others being unavailable even with Inter-Library Loan).

The information available in Makino (1951) is summarized in Table 1, with the addition of one further paper on A. domesticus.

TABLE 1

Species	2n	n	Locality	Observer	Reference
Genus : Gryllus					
campestris	29	-		Ohmachi	'29,P.I.A.(Tokyo) 5
				Ohmachi	'35,Bull.Mie 5
	29	14,15		Buchner	'09,A. Zf. 3
				Buchner	'10 A. Zf. 5
assimilis	29	14,15	Mass.	Baumgartner	'04,B.B. 8
	29	14,15	Brazil	Piza	'45,Luiz de Que. 2
bimaculatus	29			Tateishi	'32,Z.M.(Jap.)44

TABLE 1 cont'd

Species	2n	n	Locality	Observer	Reference
Genus : Acheta					
mitratatus	25	12,13	Manchuria	Honda	'26,P.I.A.(Tokyo) 2
				Honda & Iriki	'32,S.R. Tokyo B.D. 1
					'38,Ann.Zool. Jap.17.
	25	12,13	Hokkaido	Momma	'48,Oguma Comm. Vol.Cyt.Genet.
mitratus	27	-	Tokyo	Ohmachi	'27,P.I.A.(Tokyo) 3
				Ohmachi	'35,Bull.Mie, 5.
	27	13,14	Tokyo & Kyoto	Honda & Iriki	'32,S.R.(Tokyo) B.D.1
					'38,Ann.Zool. Jap.17.
	27	-	Taiwan	Tateishi	'32,Z.M.(Jap.),44
domesticus	21	10,11		Baumgartner	'04,B.B.8
	21m,22f	-		Gutherz	'07,A.M.A. 69
				Gutherz	'08,Zentr.Phys.22
				Gutherz	'09,Sitz.Ges.Nat. Fr.Berlin('09)
	21	10,11		Meek	'13,Phil.Trans.Roy. Soc.London B203
	21	-		Nath & Bhimber	'53 Res.Bull.E. Punjab Univ. No.37.
desertus	21	-		Brunelli	'09,Mem.R.Acad. Lincoi,Ser. 5a, 7.
nipponensis	19	-		Ohmachi	'29,P.I.A.(Tokyo),5
					'35,Bull.Mie,5
minor	11	-		Ohmachi	'29,P.I.A.(Tokyo),5
					'35,Bull.Mie,5.

An examination of Table 1 brings out certain facts: first that the genus Gryllus is apparently uniform in chromosome number, while Acheta contains species with widely differing numbers, i.e., 2n = 27 - 11; second that certain numbers are missing that would provide a complete series, i.e., 2n = 23, 17, 15, 13, and third that one species A.mitratus is differentiated into a northern race with 2n = 25 and a southern race with 2n = 27.

Apart from these citations of chromosome number, only one other reference to the two genera was found in the cytological literature. This was a reference by M.J.D. White in "Animal Cytology and Evolution" (1954) to the crossing experiments made by Cousin on G.campestris and G.bimaculatus.

"In the hybrids between the bed bugs Cimex lectularius and C.columbarius pairing of the autosomes is likewise complete (Darlington, 1939), so that this state of affairs may be quite usual in heteropteran species hybrids. The hybrid crickets studied by Cousin (1934, 1941) and those between the grasshoppers Trimerotrophis maritima and T.citrina obtained by Carothers (1941a, b) probably also belong in this category, since in both cases an F_2 was easily obtained."

It was in the light of the above quotation that the present study was undertaken, one of its objects being to assess the amount and kind of demonstrable abnormalities present in species hybrids obtained by Dr. R.S. Bigelow.

IV. AIMS, METHODS, AND MATERIALS

Before describing the experiments undertaken and the results obtained, it may be advisable to discuss in some detail the original aims of this study. Since from previous experience hybrid individuals might be extremely rare (see Bigelow, 1958) a technique of very high predictability was needed as only a small number of these already rare individuals could be sacrificed for cytological study. Any technique used would thus have to give the maximum number of usable slides per given amount of testicular material, and the individual crickets would have to be sacrificed when the maximum number of meiotic divisions were taking place. The preliminary experiments, then, had two aims: (a) the development of a slide making technique that gave a high yield of usable slides, and (b) the pinpointing of the exact time at which meiosis takes place, correlated if possible with some readily visible anatomical feature that would allow the use of material from the mass cultures rather than individual rearings. Although experiments on these two problems ran concurrently, and in actual practice usually involved the same material, it is felt that for discussion purposes they should be treated separately.

1. DEVELOPMENT OF THE STAINING TECHNIQUE

A variety of slide making techniques were tested, including testicular squashes and paraffin sections. Squashes of living material in acetic acid solutions of both orcein and carmine stains were uniformly unsatisfactory. Not only was the quality of the staining poor, but the number of preparations that could be made from a single testis was small and many slides were ruined during the making of permanent mounts (see appendix 1, for staining schedules). The poor quality of the cytological images obtained was not entirely the fault of the stains as preparations made from other species of Orthoptera were on the whole somewhat better than those obtained from crickets. In an attempt to improve the results obtained with stain-fixation in aceto-carmine and aceto-orcein, prefixation in several fixatives was used. The aqueous fixatives proved best in this application with Navashin's chrom-acetic-formal giving the best results (see appendix 2, for fixatives). Non-aqueous fixatives such as Newcomer's (1953) were unsuccessful in improving the quality of the preparations. Inspite of the improved quality of the slides obtained with prefixation, it was decided that sectioned material should be used - both because of the previously mentioned difficulties and because even in material which was actively dividing the relative amounts of somatic and spermatogenic material made it almost impossible to find

dividing cells in the final preparations.

Since the very soft testicular material failed to section well, despite the use of numerous different fixatives and infiltration techniques, a simplified form of double embedding (see appendix 3) developed by Peterfi (cited in Pantin, 1948) was adopted. Using this method it has proved possible to obtain good sections from every testis used.

The sections obtained were in some cases stained with Erhlich's Hematoxylin or with methyl-green and pyronin but all slides used in making counts and for the analysis of the hybrid karyotypes were stained with crystal violet, using the variation for orthopteran testicular material recommended by White (cited in Darlington and La Cour, 1947) (see appendix 1), and mounted in Euparal.

2. DETERMINATION OF THE TIME OF MEIOSIS

It has not been the practice amongst insect cytologists to carefully define the stage of development of their material. On examining large numbers of adult males of the Mountain Cricket (A.pennsylvanicus sensuo Alexander, 1957) collected in the field, it was found that meiotic divisions were either absent in the testicular follicules or at least extremely rare. Following this discovery, a careful study of the last three nymphal instars of A.domesticus was conducted. From individual

rearings of various species of crickets, it has been found that the wing buds do not become dorsally placed until the second to last nymphal stadium where they are minute and not easily seen. In the next to last instar they are still quite small, the outer, metathoracic pair reaching only a point mid-way between the anterior and posterior margins of the third abdominal tergum. While the wing buds of the next to last nymphal instar are relatively narrow, those of the last instar are broader and longer, occupying almost all of the dorsum of the first three abdominal segments, and the apical portions of the metathoracic wing buds reach a point mid-way between the anterior and posterior margins of the fourth abdominal segment. In all cases the males may be distinguished from the females by the presence of the ovipositor buds in the latter, although in the second to last instar it is sometimes necessary to examine the individual from the ventral side to determine the sex.

Cytological preparations made from nymphs in these three stages showed that mitotic divisions were common in the second to last instar, and decreased in frequency in the next to last instar, while meiotic divisions were rare in the next to last instar, common in the last instar and rare or absent in the adult. Despite the fact that there is great interspecific and intraspecific variation in the number of nymphal stadia

this pattern of wing development holds true for all species of Gryllinae pinpointing exactly the number of stadia left until adulthood, and at the same time the physiological stage of the testis.

3. MATERIAL

All of the crickets with the exception of some locally collected material of A. domesticus and G. pennsylvanicus was generously supplied by Dr. R.S. Bigelow. The origin and history of the material used in this study is outlined in Table 2.

TABLE 2

Origin and history of the material

Species	Origin	History
Genus : Gryllus		
assimilis	Field collected by Dr.R.S.Bigelow from various localities in Jamaica, April 1959.	Specimens examined belonged to the first and second laboratory reared generations
pennsylvanicus	(a) Field collected by Dr.Bigelow and the author from Bangall, New York, July 1957	Specimens examined probably belonged to the third or fourth laboratory reared generation
	(b) Field collected at Macdonald College, August 1959	Specimens examined belonged to the first laboratory reared generation

TABLE 2 cont'd

Species	Origin	History
Genus : Gryllus cont'd		
veletis	(a) Field collected by Dr. Bigelow in North Carolina, April, 1958 (b) Field collected by Dr. Bigelow in Virginia, April, 1958 (c) Field collected by Dr. Bigelow in Maryland, April, 1958	Generation of examined specimens unknown
	(d) Field collected by Dr. Bigelow at Macdonald College, April, 1959	Specimens examined belonged to the first laboratory reared generation
rubens	Field collected by Dr. Bigelow in Virginia, April, 1957	
fultoni	Supplied by Dr. R.D. Alexander, Univ., Mich., Ann Arbor	
("Texas Half-triller")	Supplied by Dr. R.D. Alexander	Specimens examined belonged to the first generation reared in this laboratory.

TABLE 2 cont'd

Species	Origin	History
Genus : Gryllus Hubrids		
Male progeny of		
(1) G.assimilis females/ G.rubens males		
(2) G.rubens females/ G.assimilis males		
(3) G.assimilis females/ male progeny of (1)		
(4) Female progeny of (1)/ G.assimilis males		
(5) Female progeny of (1)/ Male progeny of (1)		
(6) G.pennsylvanicus females/ G.assimilis males		
(7) G.fultoni females/ G.veletis males		
(8) G.assimilis females/ "Half-triller" males		
Genus : Acheta		
domesticus	(a) Hay storage room Department of Nutrition, Macdonald College	Specimens examined from both collected and first generation laboratory reared
	(b) Fluker's Cricket Farm Inc., Baton Rouge, Louisiana	Specimens examined belonged to the first reared generation
Genus : Scapsipedus		
marginatus	Field collected by Dr. Bigelow in Jamaica, April, 1959	Specimens examined belonged to the second lab-reared generation.

V. RESULTS

Using the technique outlined in the previous sections the eight species and eight hybrids were examined to determine their chromosome numbers. The results of these determinations are listed in Table 3.

As will be noted in this table all of the species of the genus Gryllus studied gave counts of 29 in mitotic metaphases in spermatogenic material. The size range amongst the various pairs was so small that it was extremely difficult to associate any of the pairs, although it was always possible to identify the X chromosome, due to its large size, and at least one pair of rather large autosomes. These three chromosomes were apparently common to the karyotypes of all the species (see Plate 2, Fig. 1). It was impossible to differentiate the karyotypes of the various species, although differences must be present since pairing is apparently suppressed in certain of the hybrids.

The hybrids of G.rubens, the "Texas Half-triller" and G.pennsylvanicus with G.assimilis provide an interesting series. The hybrids obtained between G.rubens and G.assimilis show no abnormalities in chromosome behavior in either mitosis

TABLE 3

Chromosome numbers of the species and hybrids

Species	Mitotic Metaphase	Metaphase I	Metaphase II	Remarks
Genus : *Gryllus*				
assimilis	29	15	14,15	
pennsylvanicus (a)	29	15	14,15	
(b)	29	15	14,15	
veletis (a)	29	15	14,15	
(b)	29	15	14,15	
(c)	29	15	14,15	
(d)	29	15	14,15	
rubens	29	15	14,15	
fultoni	29	15	14,15	
species (Texas-Half-triller)	29	15	14,15	
assimilis/*rubens*	29	15	14,15	
rubens/*assimilis*	29	15	14,15	
assimilis/*rubens*//*assimilis*/*rubens*	29	15	14,15	
assimilis//*assimilis*/*rubens*	29	15	14,15	
assimilis/*rubens*//*assimilis*	29	15	14,15	
pennsylvanicus/*assimilis*	29	28	variable	X plus I,II,26 I at metaphase I
assimilis/Texas Half-triller	29	20	variable	X plus 9 II, 10 I at metaphase I
fultoni/*veletis*	29	?	?	Double bridge at anaphase I
Genus : *Acheta*				
domesticus	21	11	10,11	
Genus : *Scapsipedus*				
marginatus	21	11	10,11	

or meiosis. This stability is carried over into both the backcrosses and the F_2. Hybrids obtained between G.assimilis and the "Texas Half-triller" showed suppression of pairing affecting five of the fourteen pairs of autosomes. Cells in metaphase II of meiosis were uncommon and showed a wide variety in the number of chromosomes present. Hybrids between G.pennsylvanicus and G.assimilis were even more aberrant. In these individuals all of the autosomes with the exception of one pair are in a univalent condition at metaphase I and great variation was noted in the number of bodies present in cells at metaphase II. Despite this irregularity sperms with apparently normal morphology are formed.

The situation in the hybrids between G.fultoni and G.veletis as yet defies analysis. In anaphase I of meiosis what is apparently a double bridge is formed. It has proved difficult, however, to analyse the situation as it appears in polar views of metaphase I, since the hybrid shares with G.fultoni a peculiar condition in which the various pairs of autosomes are seemingly interconnected by fine strands.

In the course of developing the technique used in the study of the other species the chromosome number of A.domesticus was determined as 2n = 21 in spermatogenic material of two populations from widely separated localities in North America.

Among material of G.assimilis collected in Jamaica specimens of an introduced species of the closely related genus"Scapsipedus"were discovered. These were identified by Dr. L. Chopard as Scapsipedus marginatus Alzelius and Brannius. Material reared from first generation lab reared adults was examined to determine the chromosome number, which proved to be 2n = 21.

VI. DISCUSSION

Apparently the only published drawing of a polar view of metaphase I in an American species of Gryllus is that published by Piza (1945). It is identical with those seen by the author in all of the species of Gryllus studied. As seen in metaphase I the karyotype consists of thirteen ball-shaped bodies, one body approximately twice as long as wide and one body somewhat longer than the preceding and showing a definite centromere (i.e., the X chromosome). This lack of morphological distinction between the various pairs of chromosomes and between the species makes it impossible to identify the pairs of chromosomes involved in abnormalities in the hybrids cytologically. Apart from the double bridge formed in the fultoni-veletis cross the other hybrids showed only a failure of synapsis of greater or lesser degree. This is quite obviously another case in which it is impossible to distinguish between Dobzhansky's (1951) chromosomal sterility and genic sterility, since without genetic analysis of the species involved, it is not obvious whether the chromosomes differ in large numbers of small structural rearrangements. White (1954) has suggested that there is little evidence for chromosomal sterility in animal hybrids and that suppression of pairing in animal hybrids is more likely due to physiological,

(i.e., genic) factors.

The presence of a double-bridge at anaphase I of meiosis in the fultoni-veletis hybrid is quite obvious and resembles nothing seen in any of the other crosses or in the pure species. As previously stated, it is difficult to suggest the cause of this phenomenon since polar views of metaphase I are confused by interconnecting strands between the bivalents. This situation also occurs at metaphase I in fultoni, but in this case the various bivalents are clearer and a count is possible. In both cases the X chromosome is not involved in the formation of the interconnected mass suggesting that the condition is not associated with sections of heterochromatin in the autosomes. Differences between the metaphase I picture in fultoni and the fultoni-veletis hybrid suggest that pairing is incomplete in the hybrid since its configuration is more complex with a greater number of darkly staining areas.

The counts made on A.domesticus and S.marginatus require no comment; in each case mitosis and meiosis are quite regular and conform to the general pattern. Some differences in the relative size of the chromosomes occur and the karyotypes are not identical although the chromosome number is the same. The differences present are best appreciated by consulting the figures at the end of the paper.

VII. CONCLUSIONS

Certain taxonomically important conclusions are bound up in these findings. It is the practice of many working systematists to synonymize species which when crossbred, either under natural or artificial conditions, produce offspring which reach maturity and in some cases even if the offspring do not reach maturity. It must be obvious from the findings in this paper and in those of many other workers that the ability to produce viable hybrid progeny, although a valuable indication of closeness of relationship does not suggest the likelihood of the two species remaining distinct. Thus although the two nymphalid butterflies Limenitis artemis Drury and L. astyanax Fabricius hybridize in a broad zone including parts of southern Canada and the North-Eastern United States, and this hybridization is apparently on some considerable age, neither species is in danger of losing its identity, at least under the conditions as they now exist. The fact that these species of Gryllus are able to maintain a broad spectrum of interspecific differences in ecological preference, developmental physiology, and behavior patterns associated with song production and with courtship and reproduction must indicate that hybridization, if it occurs in the field, does not take place with sufficient

frequency or with sufficient success to lead to the eventual merging of the various species under the present conditions. The final court of appeal must be the situation as it occurs in nature; only the fertility and Darwinian fitness of the inter se and backcross hybrids will determine the future of two interbreeding species. The ability of these crickets to speciate while retaining the ability to interbreed would perhaps, under altered environmental conditions, provide through the pooling of independently developed specializations the only hope of continuation for the stock. The cytological abnormalities evident in these hybrids between species of Gryllus are further evidence of their specific distinctness.

It would also seem logical to conclude that the chromosome number is a valuable character in the classification of crickets of the subfamily Gryllinae, since it is apparently an indication of generic placing. It is obvious, however, that anatomical evidence must also be considered since both A. domesticus and S. marginatus have the same chromosome number but differ markedly in both the morphology of the chromosomes and of the male genitalia. The chromosome number is thus a form of negative character; i.e., species with different numbers probably do not belong in the same genus. Such reasoning would only be valid in cases where it is proved applicable and the author does not mean to suggest that this criterion be applied to all groups of organisms indiscriminately.

VIII. SUMMARY

The present classification of the genus Gryllus, (Orthoptera, Gryllinae) is largely the result of gamma taxonomic work undertaken since 1949. In earlier works the members of this genus were classified in two different genera with other unrelated species. The modern classification is based on work by Cousin, Fulton, Alexander and Bigelow, whose studies on biometrics, ecology, behavior patterns, developmental physiology and hybridizations have placed it on a sound footing.

This study was undertaken to assess the value of chromosome numbers in relation to taxonomy in the subfamily Gryllinae and to ascertain the amount and kind of chromosomal abnormalities in interspecific hybrids. In all six species of Gryllus, eight inter-specific hybrids between Gryllus species and one species each from the genera Acheta and Scapsipedus were studied.

All of the species of Gryllus studied had a diploid (male) chromosome number of 29. Of the hybrids, those involving G.rubens Scudder and G.assimilis (Fabricius) were without visible abberations at any stage of gametogeesis,

while those between G.pennsylvanicus Burmeister and G.assimilis, and "Texas Half-triller" and G.assimilis showed a suppression of pairing at metaphase I of meiosis, one bivalent being present in the former and nine in the latter in each case out of a possible fourteen. Hybrids between G.fultoni and G.veletis showed what was presumably a double-bridge at anaphase I, but the true nature of the anomaly could not be ascertained due to a stickiness of the chromosomes which the hybrid shares with the female parent, G.fultoni.

Counts made on A.domesticus (Linnaeus) and S.marginatus Afzellius and Brannius, showed them to be identical in chromosome number, male diploid equals twenty-one, but the morphology of the chromosomes in the two species was entirely different.

IX. BIBLIOGRAPHY

Alexander, R.D.

1957. The taxonomy of the field crickets of the eastern United States (Orthoptera: Gryllidae: Acheta). Ann. Ent. Soc. Am. 50: 584-602.

Alexander, R.D. and R.S. Bigelow

1960. Allochronic speciation in field crickets, and a new species Acheta veletis. Evolution - In press.

Baumgartner, W.J.

1904. Some new evidence for the individuality of the chromosomes. Biol. Bull. 8: 1-28.

Bigelow, R.S.

1958. Evolution in the field cricket, Acheta assimilis Fab. Can. J. Zool. 36: 139-151.

1960. Interspecific hybrids and speciation in the genus Acheta (Orthoptera: Gryllidae). Can. J. Zool. 38: 509-524.

Chopard, L.

1955. Note sur un Grillon gynandromorphe provenant du Congo Belge. Memoires Soc.Roy. Ent. Belgique, 27: 153-157. (See note page 153).

Cousin, G.

1933. Sur l'Hybridation de deux especes de Gryllides Acheta campestris et bimaculatus. Bull. Soc. ent. Fr. 12: 189-192.

Cousin, G.

1946. Les croisements d'especes dans la famille des Gryllidae: croisement femelle Gryllus bermudiensis avec male G.campestris L.
C.R.Acad. Sci. Fr., 223: 434.

1954a. Sur la transmission de quelques caracteristiques specifiques quantitatives dans le croisement des especes Gryllus peruviensis Sauss. et Gryllus campestris L.
C.R. Acad. Sci. Fr. 239: 1429-1431.

1954b. Sur la diagnose de trois especes du genre Gryllus: Gryllus argentinus Sauss., Gryllus bimaculatus de Geer, Gryllus campestris L.
C.R. Acad. Sci. Fr. 239: 1877-1879.

1956. Biometrie et definition de morphologie quantitative des especes et de leur hybrides.
Bull. de la Soc. zool. Fr. 81: 247-289.

Darlington, C.D. and L.F. La Cour

1947. The handling of chromosomes, 180 pp.
George Allen & Unwin Ltd., London.

Dobzhansky, Th.

1951. Genetics and the origin of species, 3rd ed., 364 pp.
Columbia Univ Press, New York.

Fulton, B.B.

1949. Speciation in the field cricket, Gryllus assimilis Fab. (Abstract) - J. Elisha Mitchell sci.Soc., 65: 204-205.

1952. Speciation in the field cricket.
Evol., 6: 283-295.

Kirby, W.

1906. Synoptic catalogue of the Orthoptera, Vol. II.

Linnaeus, C.

1758. Systema naturae, tenth edition (a photographic facsimile of the first volume of the tenth edition (1758)).
Brit. Mus. (Nat. Hist.) London (1956).

Makino, S.

1951. An atlas of the chromosome numbers in animals, 2nd. ed.
Iowa State College Press, Ames, Iowa.

Mayr, E., E.G. Linsley, and R.L. Usinger

1953. Methods and principles of systematic zoology, 328 pp.
McGraw-Hill Book Co. Inc.

Nath, V. and B.S. Bhimber

1953. Spermatogenesis of Acheta domesticus Linn. with observations under the phase-contrast microscope.
Res. Bull. E. Punjab Univ., 37: 145-155.

Newcomer, E.H.

1953. A new cytological and histological fixing fluid.
Science, 118: 161.

Piza, S. de Toledo

1945. Compotamento do heterocromossomio em alguns Ortopteros do Brasil.
Ana. Escola sup. Agric. Luiz de Queiroz, 2: 173-207.

Randell, R.L.

1960. On the classification of the field crickets of the western hemisphere (Orthoptera: Gryllidae: Gryllus).
Submitted to the Can. J. Zool. (in press).

Rehn, J.A.G. and M. Hebard

1915. The genus Gryllus (Orthoptera) as found in America.
Proc. Acad. Nat. Sci. Phila., 67: 293-322.

Roberts, H.R.

1941. Nomenclature in the Orthoptera concerning genotype designations.
Trans. Am. Ent. Soc., 67: 1-34.

Saussure, H. de

1877. Melanges orthopterologiques, Vol.2, fasc. 5: 271-365.

APPENDIX 1

STAINS AND STAINING SCHEDULES

1. Carmine:- (National Analine Division Cert. NCal5)

2. Orcein:- (Dr. G. Grübler & Co., Leipzig).

Schedule:- As cited in Darlington and La Cour (1947) pp. 126, 127.

a. Small pieces of tissue fixed in acetic-alcohol or one of the fixatives listed in schedule 2 crushed with a needle in a small drop of the stain-fixative.

b. Cover slip prepared by smearing thinly with Mayer's albumen and dried quickly in the flame of an alcohol lamp.

c. Remove large debris and place cover slip in position. Heat gently in the alcohol flame, do not boil.

d. Store overnight under refrigeration, in a humid atmosphere.

e. To make permanent preparations:-

(i) Invert slide in a dish containing 10% acetic acid.

(ii) Take cover slip with adhering material through:-

1:3 acetic alcohol ..... 2 mins.

Absolute alcohol, 2 changes .. 2 mins. each Carmine
1 min. each Orcein.

(iii) Mount in Euparal

Preparation of Stain Fixatives:-

a. Aceto-carmine; as cited in Darling and La Cour (1947), p.116.

45 cc. glacial acetic acid.

55 cc. distilled water.

Heat to boiling and add 0.5 gm. of carmine. Shake well, cool and filter.

(Use soft iron, uncoated needles in disection to mordant stain.)

b. Acetic-orcein: as cited in Darling and La Cour (1947), p.116.

Dissolve by boiling 2.2 gm. orcein in 100 cc. of glacial acetic acid. Store in this form as stock solution when required dilute with distilled water to make a 1% solution of the dye in 45% acetic acid and filter.

3. Haematoxylin:- (Dr. G.Grübler & Co., Leipzig)

Schedule:- Using sectioned material prepared as outlined in Appendix 3.

a. Xylene.

b. Hydrate in graded alcohols.

c. Stain in Ehrlich's acid haematoxylin.

d. Blue in tap water.

e. Dehydrate in graded alcohols and mount in Euparal.

Preparation of Ehrlich's Acid Haematoxylin:- As outlined in Gurr, E. 1960, Encyclopedia of microscopic stains.

Haematoxylin	2 gm.
Absolute alcohol	100 cc.
Potassium alum, 2.5% aqueous	100 cc.
Glycerine	100 cc.
Glacial acetic acid	10 cc.

The haematoxylin is first dissolved in the alcohol, the other ingredients are then shaken or stirred in. After ripening for three months the solution is ready for use.

Note:- This stain gave highly uniform results and while not used in the evaluation of the material, it formed a standard against which the other stains were checked.

4. Methyl-green and pyronin (Unna Pappenheim):- (Both stains obtained from the British Drug House Ltd., London.)

Schedule: As cited in Darlington and La Cour (1947), p. 132.

a. Sectioned material prepared as in Appendix 3, hydrated as in steps a and b under haematoxylin.

b. Incubate one of a pair of slides in distilled water to which a little crystalline ribonuclease is added, at 50°C for 2-3 hours.

c. Stain both slides together in methyl-green-pyronin for 20-30 minutes.

d. Rinse in distilled water.

e. Drain and allow slide to air dry.

f. Dehydrate in a mixture of absolute alcohol 1 part, acetone 1 part and xylene 6 parts for 10 minutes.

g. Transfer to pure xylene and mount in Canada Balsam.

Preparation of Methyl-Greem-Pyronin:- As cited in Darlington and La Cour (1947), p.117.

Solution A

Phenol	0.25	gm.
Distilled water	100	cc.
Methyl green	1.0	gm.

Solution B

Phenol	0.25	gm.
Distilled water	100	cc.
Pyronin G	1	gm.

For use mix 3 parts of A with 7 parts of B.

5. Crystal violet:- (British Drug House Ltd., London)

Schedule:- As cited in Darling and La Cour (1947), p.130.

a. Sectioned material prepared as in Appendix 3, hydrated as in steps a and b under haematoxylin.

b. Stain in 0.1% crystal violet in aqueous solution for 10-60 minutes.

c. Rinse in distilled water.

d. Transfer to 80% alcohol containing 1% I_2 and 1% KI for 30-45 seconds.

e. Rinse in 95% alcohol.

f. Transfer to absolute for 4-10 seconds.

g. Differentiate under low power of microscope in clove oil for approximately 30 seconds.

h. Xylene, three changes each 10 minutes.

i. Rinse in absolute alcohol and mount in Euparal.

Note:- This method gives exceptionally good results, and for this reason was used exclusively for making counts. The times given are only approximate and should be adjusted to the material being used, there is little likelihood of

spoiling slides as destaining and restaining are quite simply accomplished without noticeable deterioration in the material.

Preparation of Crystal Violet:-

The stock solution is prepared by boiling together 1 gram of crystal violet and 100 cc. of glass-distilled water. When desired for use the stock should be diluted at the rate of one part to nine parts glass-distilled water.

APPENDIX 2

FIXATING FLUIDS

Most of the common histological and cytological fixating fluids were tried in the course of developing the final technique. Many were dropped since they offered no obvious advantages over others which were easier to prepare, or because they failed to improve the cytological images in squashes or sections. For preserved material obtained from workers not familiar with cytological work or without facilities, 3:1 acetic-alcohol either injected into the body cavity or allowed to enter through a dorsal incision has proved quite satisfactory. However to simplify the technique and insure comparability between material obtained from different species, Navashin's fluid was used in the fixation of all the material used in making the chromosome counts.

Navashin's Fluid:- As cited in White, M.J.D. (1957) Cytogenitics and systematic entomology. Ann. Rev. Ent. 2: 87.

Solution A

Glacial acetic acid ..... 1 part

1% aqueous chromic acid ..... 8 parts

Solution B

Formalin

These two solutions react with each other on contact and must be kept separate until immediately before using.

APPENDIX 3

PETERFI'S METHOD OF IMPREGNATION WITH CELLOIDIN

The testis in crickets is a semiliquid structure in the live insect, and even after fixation is delicate. It has proved quite difficult to obtain really good sections with the use of a straight paraffin embedding technique. Since the usual method of celloidin double embedding was felt to be excessively complex for what advantages it might offer, a simpler technique which offered the additional support that was needed was sought in the literature. On the advice of Professor J.E. McFarlane of the Department of Entomology, Peterfi's celloidin-paraffin was tried with outstanding success.

Peterfi's Celloidin-Paraffin Method:- As cited by Pantin, C.F.A. (1948), Notes on microscopical technique for zoologists, Cambridge Univ. Press.

a. Dehydrate in absolute alcohol.

b. Transfer to 1% celloidin in methyl benzoate for 3-5 hours.

c. Transfer to fresh celloidin-methyl benzoate, 3-5 hours.

d. Fresh celloidin-methyl benzoate for 12-24 hours, or for storage.

e. Proceed to benzene for 15 minutes.

f. Fresh benzene for 15 minutes.

g. Transfer to a saturated solution of paraffin in benzene at 30°C. for 15-30 minutes.

h. Proceed to paraffin for the completion of infiltration and embedding.

PLATE 1

PLATE 1

Map indicating the geographical relationships of species of Gryllus hybridized by Cousin and Bigelow; circles mark the approximate point of origin of the stocks used in the various crosses. Note especially the pattern in which crosses are fertile in both directions, separating the species into three geographically defined groups.

Abbreviations:-

Ar	Gryllus argentinus Saussure	
As_b	Gryllus assimilis (Fabricius)	from Brazil
As_j	Gryllus assimilis	from Jamaica
As_m	Gryllus assimilis	from Mexico
As_v	Gryllus assimilis	from Venezuela
B	Gryllus bimaculatus De Geer	
Br	Gryllus bermudiensis Caudell	
C	Gryllus campestris Linnaeus	
Cp	Gryllus capitatus Saussure	
F	Gryllus fultoni (Alexander)	
P	Gryllus peruviensis Saussure	
Pa	Gryllus pennsylvanicus Burmeister	
Rb	Gryllus rubens Scudder	
V_l	Gryllus veletis (Alexander and Bigelow)	

PLATE I

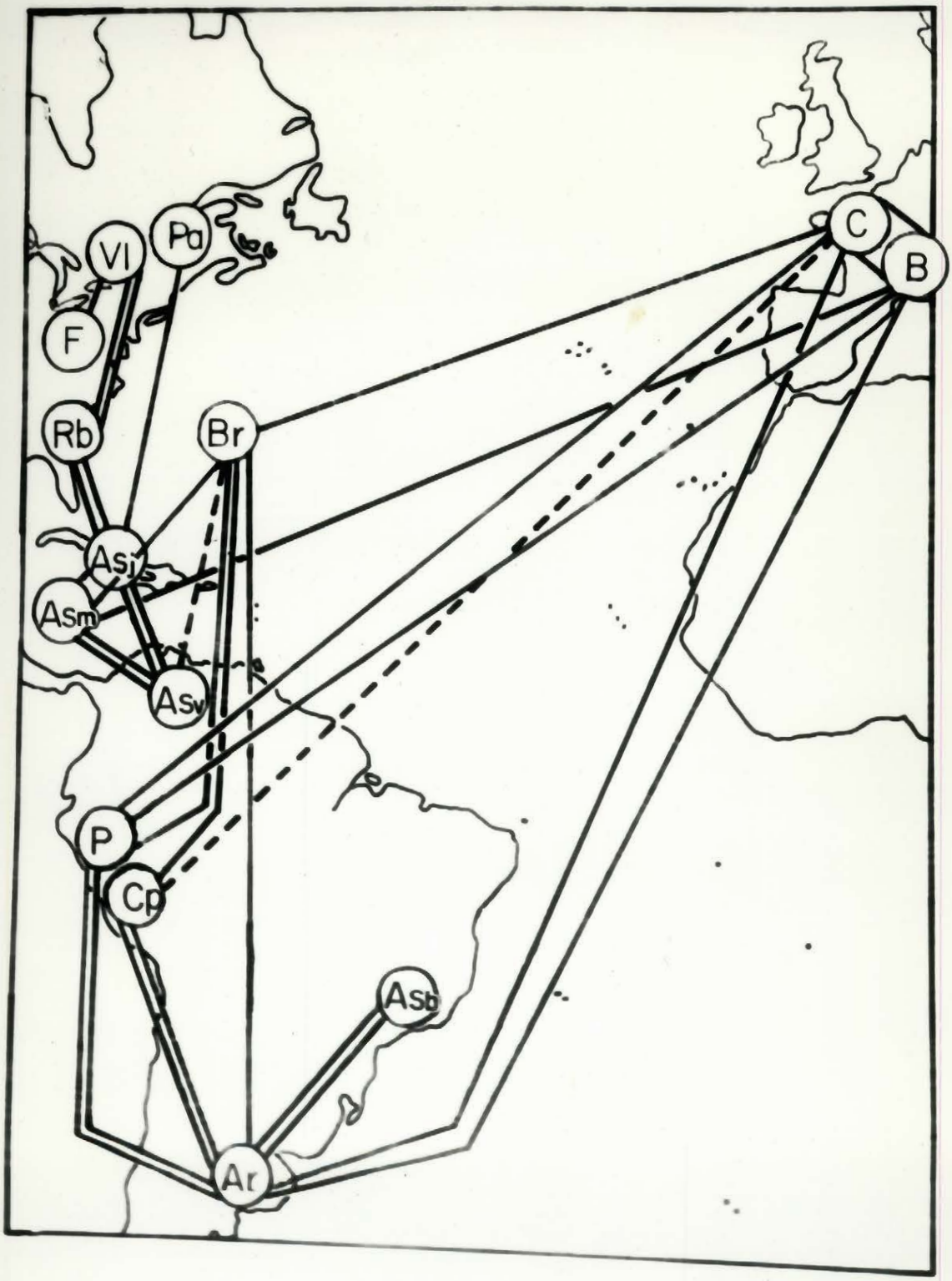


PLATE 2

PLATE 2

Fig. 1 - Polar view of metaphase I, G.assimilis.

Fig. 2 - Polar view of metaphase I, G.fultoni.

Fig. 3 - Polar view of metaphase I, hybrid between Texas Half-triller males and G.assimilis female (bivalents in outline, univalents in solid color).

Fig. 4 - Polar view of metaphase I, hubrid between G. fultoni female and G. veletis male.

Fig. 5 - Polar view of metaphase I, hybrid between G.pennsylvanicus female and G.assimilis males. ((bivalent in outline, univalents in solid color).

Fig. 6 - Side view of anaphase I, hybrid between G.fultoni female and G.veletis male. Note double bridge.

PLATE

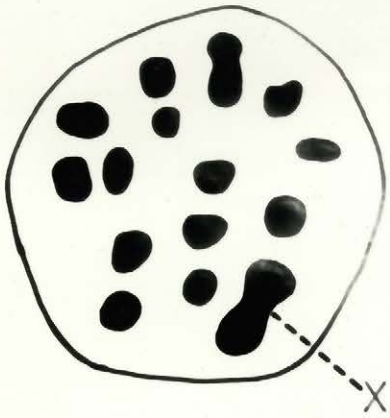


Fig. 1

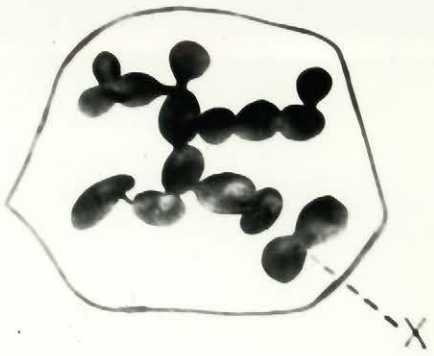


Fig. 2

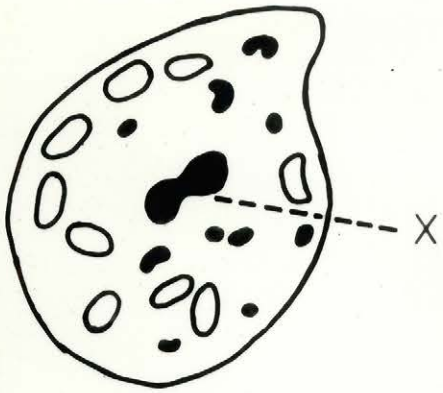


Fig. 3

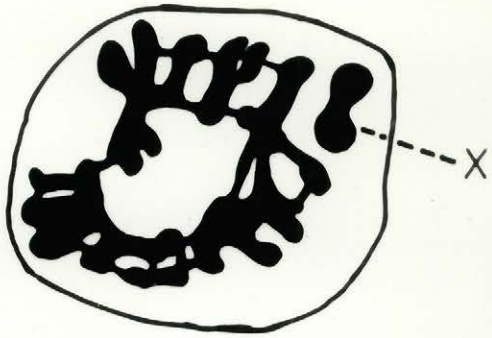
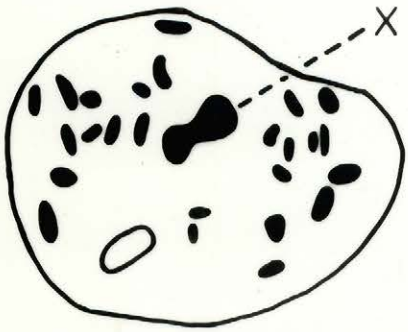


Fig. 4



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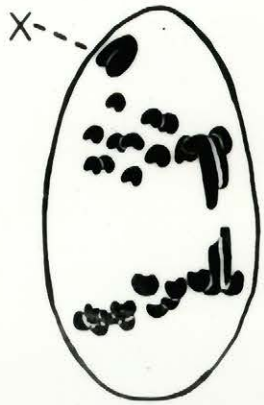

Fig. 6

PLATE 3

PLATE 3

Fig. 1 - Polar view of mitotic metaphase, A.domesticus.

Fig. 2 - Polar view of metaphase I, A.domesticus.

Fig. 3 - Polar view of metaphase I, S.marginatus.

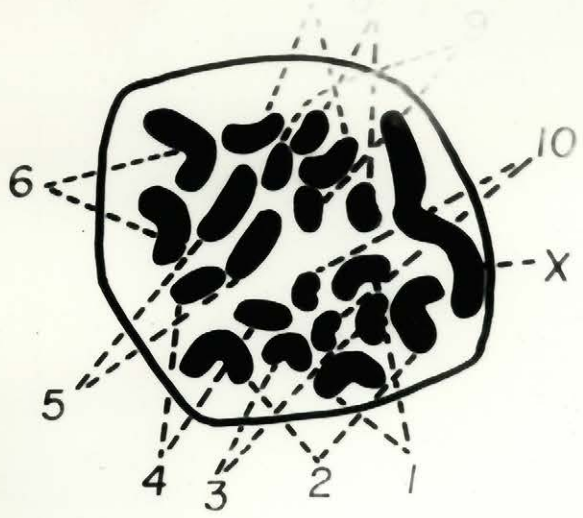

Fig. 1

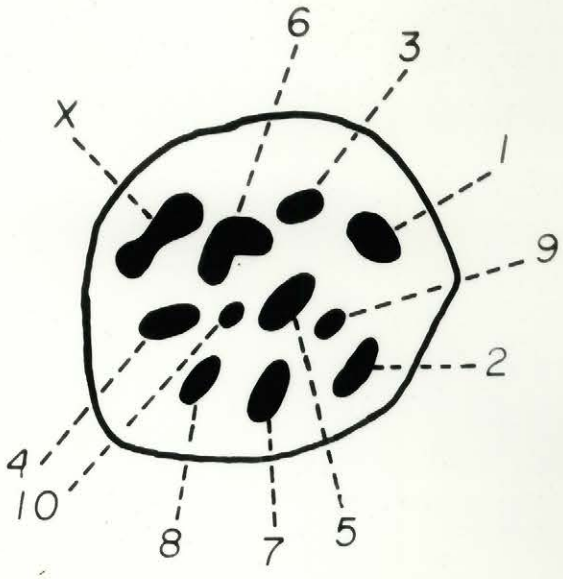

Fig. 2

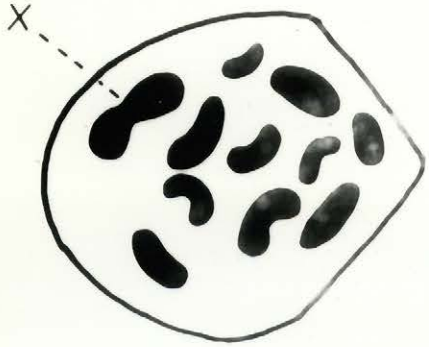

Fig. 3

PLATE 4

PLATE 4

Fig. 1

Longitudinal section of a testicular follicle in the last instar of Gryllus assimilis (Fabricius) showing cells in anaphase I.

Fig. 2

Same material as Fig. 1 at twice the magnification.

Fig. 3

Cross section of a testicular follicle in the last instar of Gryllus sp. (Texas Half-triller) showing cells in metaphase I.

Fig. 4

Cross section of a testicular follicle in a hybrid between G.assimilis female and "Texas Half-triller male, showing various stages of meiosis.