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CYTOLOGY AND CYTOTAXONOMY OF ACRIDIDAE : A SUMMARY

ASHOK K. SINGH

Cytotaxonomy Research Laboratory, Zoological Survey of India,
27 Jawaharlal Nehru Road, Kolkata-700 016

CHROMOSOMES, SPECIES AND TAXONOMY

A chromosome contains a linked group of genes, each consisting of a unique number of nucleotide pairs specifying a particular gene, arranged in a precise sequence. Functionally, the genome in a species is a stable integrated system and therefore the structure of chromosomes has to be conservative in order to maintain the integrity. Speciation is a change from one stable system to another and therefore, must involve changes in the genes and their subsequent integration either at the molecular or at the structural levels or both. The taxonomists in determining the evolutionary relationships, invariably emphasize the comparison of structures, which are more conservative in evolution and have empirically determined different sets of characters in different groups of organisms for the purpose. The chromosome complement of a species is a conservative set of morphological features and structural changes in them could be a basis for the study of evolutionary relationships within and between species. In ultimate analysis, all evolutionary changes must originate in changes in the genome, but most of them are at the molecular level and therefore, not so easily accessible for studies. In the mean time cytotaxonomists have gathered a lot of useful information at the microscopic levels of changes in the chromosome structure, which are mediators of evolution.

RESEARCH WORK

Preface :

Acridid and Pyrgomorphid grasshoppers are the classic examples of karyotype stability and are thus interesting from evolutionary viewpoint.

Since the very beginning, even before the rediscovery of Mendel's work in the early part of the 20th century, grasshoppers had drawn the attention of cytologists as material for investigation.

Since then, grasshopper remained the classical animal for the learning of the grammar of chromosome biology. Their availability in most of the places and their easy maintenance in laboratory, relatively short life cycle, large size of chromosomes with low numbers in their complements made them the material of choice to animal cytologists. The ease with which meiosis can be studied and aberrations can be scored made the investigators use them as good experimental systems.

Earlier workers believed that survey of karyotypes of grasshoppers might be a useful endeavour. They had two purposes. The first was to compare closely related species or genera to discover the karyotypic variability and some trends of karyological evolution where data might also help orthopterists as an additional set of characteristics for distinguishing species and other taxononomic categories. Their second purpose was to discover material useful for cytological research, such as species with low diploid number and novel sex chromosome mechanisms.

They deferred presenting a summary of phylogenetic studies because interpretations based on gross chromosome morphology alone had led to erroneous conclusions. Related species possessed nearly identical chromosome constitutions and thus have not been able to revise taxonomy.

The situation has now changed with the advent of various banding techniques. Grasshoppers by virtue of having 'large' chromosomes are most useful, as banding minute differences that can be located and specified. In the present studies 'C' 'G' and Hoechst 33258 fluorescence banding techniques have been utilized for the study of karyotypes, meiotic patterns and species differentiation. 35 species under 32 genera and 11 sub-families have been investigated.

(A survey of literature on the cytology and cytotaxonomy of Indian Acridid and Pyrgomorphid grasshoppers has been published by the author (Singh, 2002)

MATERIAL AND METHODS

Techniques used in Chromosome Preparations :

Selected male and female grasshoppers were injected with 0.05% colchicine (0.02 to 0.04 ml) depending upon the size of the insect (colchicine is injected prior to dissection to arrest metaphase). Four to six hours after the injection, the testes and hepatic caecae were dissected out in 0.67% solution of insect saline from the males, while females were preferred to be dissected after 10–12 hours with a possibility to accumulate more metaphases in their somatic cells. The tissues thus dissected out were cleaned in the same medium. After cleaning, the tissues were transferred for a hypotonic treatment in a solution of 0.9% sodium citrate for 45–60 minutes. After that, the tissues were fixed instantly in a freshly prepared fixative of acetic acid and methanol (1 : 3) for 45–60 minutes. A slightly more hypotonic treatment and fixation were preferred for the somatic cells for better chromosomal preparation. The fixed tissues were transferred in small tubes and stored preferably at 4°C.

The slides were prepared by the air dry technique. The fixed material were squashed in 50% acetic acid on the slides cleaned in dichromate solution, and stored in the vapours of 50% acetic

acid at 4°C for over night. Next morning the slides were brought at room temperature and immersed in 1 : 3 acetic acid methanol mixture for an hour. The cover slips were removed with the help of a sharp blade in the immersed condition and dried at room temperature in a dust proof chamber. After a preliminary scanning of the unstained slides, the selected ones were stained in 2–3% solution of Giemsa [E. Merck (India) Private Ltd.] in phosphate buffer at p^H 6.8 to 6.9. Staining was checked until appropriate contrast was obtained, they were then immediately rinsed in distilled water (two changes) and rapidly air dried under a lamp. After scanning, the slides were soaked in Xylene and mounted in DPX.

a. C-banding

C-banding was carried out according to the method by Sumner (1972) with some minor modifications. The air dried slides were treated with 0.2 N Hydrochloric acid for 30–60 minutes at room temperature, rinsed in distilled water and dried. These slides were dipped in a freshly prepared 5% aqueous solution of Barium hydroxide octahydrate at 50°C for 1–10 minutes. The treatment time depends on the ageing of slides; the stored slides produce sharp bands after comparatively longer treatment. After thorough rinsing with several changes in distilled water, they were incubated for 1 hour at 60°C in 2 x SSC (0.3 M sodium chloride and 0.03 M tri-sodium citrate at p^H 7), rinsed in distilled water and dried. These slides were stained for 30 to 90 minutes in Giemsa stain; 2.5 ml of stock solution added to 50 ml of buffer (at p^H 6.8). Finally, the slides were again rinsed briefly in distilled water, blotted, allowed to be dried thoroughly under lamp, soaked in Xylene and finally mounted in DPX.

b. Fluorescence banding with Hoechst 33258

Staining of chromosomes with Hoechst 33258 has provided yet another valuable technique of chromosome banding (Hilwig and Gropp, 1972) which is popularly called Hoechst 33258 for staining heterochromatin in mouse chromosomes. A simple direct and slightly modified staining procedure given here was followed throughout. The air dried slides were first soaked in Mcllvain's buffer at p^H 5.4 for 10 minutes, then stained with freshly prepared Hoechst solution (0.05 mg/ml or 0.1 mg/ml) in the same buffer at p^H 5.4 for 15 to 20 minutes at room temperature. After staining, the slides were rinsed in the same buffer (two changes) and mounted either in buffer or in a glycerol buffer mixture. The slides were observed immediately with the help of fluorescence microscope or were stored for some days (up to a couple of weeks) in a refrigerator prior to observation. All fluorescence observations were made using Leitz ortholux photomicroscope with its transmitted light fluorescence attachments using appropriate barrier and exciter filters.

c. Photomicrography

Photomicrographs were taken with the help of Leitz ortholux microscope. Planapochromatic objectives of different magnifications were used. Filters of different combinations were used to obtain the best possible contrast. Black and white negative films (panchromatic) ranging from 50 to 120

ASA were used in fluorescence photomicrography. The negatives so obtained were printed on photosensitive Agfa bromide papers of different grades. Fine grain film and paper developer of Agfa-gevaert (A 901 and 902) and Kodak (D 76 and D 163) were used while developing negatives and positive prints. Digital cameras have also been used on several occasions for more precise results.

d. Karyotyping and construction of idiograms

The cells with good chromosome spreads were photomicrographed. The diploid number (2n) were determined by basic or most predominant number observed in the individual. The cut out of individual chromosomes which appeared similar in morphology and staining intensity were paired to construct karyotypes. All the karyotypes that could be prepared were used for the morphometric measurements. In case of C-band karyotypes the sex chromosomes were placed after the last autosomal pair. In some species very few individuals were available for study. In such cases a banding feature that was present on both the homologues of a given chromosome pair was assumed to occur throughout the species. Morphometric measurements of each chromosome were taken from several metaphase plates from either of the sex. Their mean values so obtained were used in calculating the relative length of the chromosome in percent of the total haploid length. These measurements were also used in drawing comparative idiograms.

SPECIES INVESTIGATED

(Taxonomic grouping according to Dirsh, 1961)

Name of the species	Name of the locality	Number of Specimens		
		Male	Female	Total
Order ORTHOPTERA Superfamily PAMPHAGOIDEA Family PYRGOMORPHIDAE Tribe TAGASTINI				
1. ***Tagasta indica*** Bolivar	1. Sukna forest Darjeeling (W. B.)	–	1	1
Tribe ATRACTOMORPHINI				
2. ***Atractomorpha crenulata*** (Fabricius)	1. Kunihar (H. P.) 2. Varanasi (U. P.) 3. North Bengal Univ. campus Siliguri (W. B.) 4. Jainagar 5. Matpukurdhapa 6. Golf club 7. Lake gardens 8. Narendrapur and 9. Indian Museum pond, Kolkata	65	15	80

Name of the species	Name of the locality	Number of Specimens		
		Male	Female	Total
Tribe CHROTOGONINI 3. ***Chrotogonus (Ch.) trachypterus*** (Blanchard)	1. Himachal Pradesh 2. N. B. U. campus 3. Singla and 4. Sukna forest Darjeeling 5. Matpukurdhapa, Kolkata	5	5	10
Superfamily ACRIDOIDEA Family ACRIDIDAE Subfamily HEMIACRIDINAE 4. ***Spathosternum prasiniferum*** (Walker)	1. Varanasi 2. N. B. U. campus 3. Sivok and 4. Sukna forest Darjeeling 5. Diamond harbour 6. Narendrapur and 7. Golf club Kolkata	04	18	122
Subfamily TROPIDOPOLINAE 5. ***Tristria pulvinata*** (Uvarov)	1. Golf club,	140	2	142
Subfamily OXYINAE 6. ***Gesonula punctifrons*** (Stål)	1. N. B. U. campus 2. Sivok 3. Sukna forest 4. Golf club 5. Indian Museum pond	27	8	35
7. ***Oxya fuscovittata*** (Marschall)	1. Sivok 2. Suknaforest 3. Narendrapur 4. Diamond harbour 5. Golf club	24	2	26
8. ***Oxya hyla*** Serville	1. Varanasi 2. N. B. U. campus 3. Suknaforest 4. Diamond harbour 5. Jainagar 6. Golf club	12	4	16
Oxya sp. Serville	1. Andaman Island (around Port Blair) 2. Golf club	34	2	36
9. ***Caryanda paravicina*** (Willemse)	1. Dow hill, Darjeeling	4	1	5

Name of the species	Name of the locality	Number of Specimens		
		Male	Female	Total
Subfamily COPTACRIDINAE				
10. ***Eucoptacra praemorsa*** (Stål)	1. Sukna forest	1	–	1
Subfamily CALLIPTAMINAE				
11. ***Peripolus pedarius*** (Stål)	1. Singla	7	15	22
Subfamily EYPREPOCNEMIDINAE				
12. ***Eyprepocnemis alacris*** (Serville)	1. Himachal Pradesh 2. Singla 3. Canning, Sundarbans (W. B.)	2	2	4
13. ***Eyprepocnemis rosea*** Uvarov	1. Himachal Pradesh	3	–	3
14. ***Choroedocus robustus*** (Serville)	1. Singla 2. Sukna forest	1	3	4
15. ***Tylotropidius varicornis*** (Walker)	1. Narang 2. Chail and 3. Saproon (H.P.) 4. Sukna forest	3	6	9
Subfamily CYRTACANTHACRIDINAE				
16. ***Cyrtacanthacris tatarica*** (Linnaeus)	1. Matpukurdhapa	–	1	1
17. ***Chondracris rosea*** (de Geer)	1. Singla	1	–	1
18. Patanga succincta (Johansson)	1. Chail 2. Saproon and 3. Masobra (H.P.)	2	3	5
Subfamily CATANTOPINAE				
19. ***Catantops innotabilis*** (Walker)	1. Himachal Pradesh 2. Sivoke 3. Sukna forest	8	–	8
20. ***Catantops simlae*** Dirsh	1. Masobra (H.P.)	1	1	2
21. ***Xenocatantops humilis*** (Serville)	1. Renuka lake (H.P.) 2. Singla 3. Sukna forest	16	–	16
22. ***Stenocatantops splendens*** (Thunberg)	1. Singla 2. Sukna forest	4	2	6
Subfamily GOMPHOCERINAE				
23. ***Leva cruciata*** Bolivar	1. N.B.U. carnpus	2	–	2
24. ***Chorthippus indus*** Uvarov	1. Renuka lake and 2. Saproon (H.P.)	9	–	9
25. ***Dnopherula (Aulacobothrus)*** Jago	1. Narang 2. Chail 3. Saproon and 4. Happy valley (H.P.)	15	–	15

Name of the species	Name of the locality	Number of Specimens		
		Male	Female	Total
Subfamily OEDIPODINAE				
26. ***Acrotylus inficita*** (Walker)	1. N. B. U. campus 2. Sivoke	2	7	9
27. ***Aiolopus tamulus*** (Fabricius)	1. Himachal Pradesh 2. N. B. U. campus 3. Dow hill 4. Jainagar	9	4	13
28. ***Dittopternis venusta*** (Walker)	1. Sukna forest	4	1	5
29. ***Heteropternis respondens*** (Walker)	1. N. B. U. campus 2. Singla	–	3	3
30. ***Pternoscirta cinctifemur*** (Walker)	1. Sukna forest	–	1	1
31. ***Scintharista notabilis*** Uvarov	1. Himachal Pradesh	2	–	2
32. ***Sphingonotus indus*** Saussure	1. Singla	9	1	10
33. ***Trilophidia annulata*** (Thunberg)	1. Himachal Pradesh 2. Varanasi 3. N. B. U. campus 4. Canning, Sundarbans 5. Sivok 6. Sukna forest 7. Jainagar	29	15	44
Subfamily ACRIDINAE				
34. ***Acrida exaltata*** (Walker)	1. Kunihar (H.P.) 2. Varanasi 3. N. B. U. campus 4. Singla 5. Sukna forest 6. Diamond harbour 7. Golf club	28	3	31
35. ***Ceracris nigricornis*** (Walker)	1. Dow hill 2. Singla	18	7	25
36. ***Sikkimiana darjeelingensis*** (Bolivar)	1. Singla	1	4	5
37. ***Phlaeoba antennata*** Brunner	1. Sivoke 2. Sukna forest	14	3	17
38. ***Phlaeoba infumata*** Brunner	1. Renuka lake and 2. Kunihar (H.P.) 3. N. B. U. carnpus 4. Diamond harbour 5. Jainagar 6. Golf club	61	16	77

COMPARATIVE KARYOLOGY OF THE SPECIES STUDIED

1 ***Tagasta indica*** Bolivar

1	2	3	4	5	6	7	8	9	10
15.31	14.12	12.33	11.38	9.91	9.13	8.23	7.64	6.63	5.33

The X was largest in the complement. Length of chromosomes had gradually decreased.

2 ***Atractomorpha crenulata*** (Fabricius)

17.23	13.99	12.29	10.91	9.69	9.16	8.44	7.78	6.64	3.84

The X and the last pair of chromosomes were distinct by their size. C-band was hardly perceptible in the first 2 pairs of autosomes, and in Hoechst staining also, no bright centromeric regions were noticeable. On C-band staining the centronmeric region of the smallest bivalent was prominent.

3. ***Chrotogonus trachypterus*** (Blanchard)

14.15	13.37	12.59	14.46	9.84	9.39	8.78	8.09	7.35	5.97

The X and the 1st, 2nd pairs of autosomes were large elements. Length of other chromosomes had gradually decreased.

4. ***Spathosternum prasiniferum*** (Walker)

16.21	**13.95**	11.91	10.24	9.24	8.17	7.09	6.67	6.16	4.00	3.38	2.98

Last 3 pairs were small. All the chromosomes had centromeric band. On the X it was prominent. The 7th and 9th pairs also had a band in their interstitial region. In Hoechst staining chromosomes showed brighter centromeric region. In all the bivalents random distribution of chiasmata was visible.

5 ***Tristria pulvinata*** (Uvarov)

15.92	**15.06**	13.65	11.48	9.63	8.30	7.49	6.84	5.86	3.19	2.59

Last 2 pairs were small. First 4 pairs had wider gaps than others. The 1st pair was distinct on its prominent centromeric band. The X had become marker element on its proximal band. In Hoechst staining, all the chromosomes showed brighter fluorescing centromeric region. The proximal band of the X had not fluoresced prominently. Chiasma was not restricted to any particular region in the bivalents.

6 ***Gesonula punctifrons*** (Stål)

18.18	13.60	11.40	9.81	8.30	7.61	6.89	6.41	6.13	5.53	3.59	2.50

The X was largest, and the last 2 pairs were separated as small ones. All the chromosomes had centromeric band. The 5th pair also had a proximal band. In Hoechst staining, centromeric regions were not distinct as band. Last 2 small pairs had formed chiasma in the distal region.

7 ***Oxya fuscovittata*** (Marschall)

14.71 12.55 11.21 9.85 8.91 7.71 7.13 6.87 6.48 5.93 5.18 3.62

The X was largest. The 1st, 2nd, 3rd, 4th and the last pair had bigger size difference than remaining ones. All the chromosomes had centromeric band. On the 9th pair it was hardly perceptible, whereas, proximal half region of the smallest pair was C-band positive. 6th, 7th, 8th and 10th pairs also had distal bands of different magnitude. In Hoechst staining, brighter centromeric ends were not delimited into sharp bands. All the bivalents had shown random distribution of chiasmata.

8 ***Oxya hyla*** Serville

16.05 14.17 12.55 10.53 8.89 7.97 7.36 6.84 5.84 4.45 2.92 2.41

The X was the largest. The 1st, 2nd, 3rd and 4th autosomal pairs had bigger size difference. The last 2 pairs were separated as small ones. All the chromosomes had centromeric band of equal magnitude. A proximal band was also present on the 2nd pair. All the chromosomes had brighter fluorescing centromeric band. The 7th, 8th, 9th and 10th pairs had mostly distal chiasma.

9 ***Caryanda paravicina*** (Willemse)

15.58 15.11 **11.89** 10.70 10.15 7.11 6.74 6.42 5.82 3.75 3.18 2.50

1 large 1 small and 2 medium groups were formed by 2, 3, 4 and 3 pairs of chromosomes respectively. This caryotypic feature was unique.

10 ***Peripolus pedarius*** (Stål)

15.39 13.23 11.18 10.10 **9.80** 8.28 7.39 6.81 6.40 4.81 3.61 3.0

Large size difference was noticed in the first 3 pairs. In others this difference was smaller and uneven.

11 ***Eyprepocnemis alacris*** (Serville)

12.95 11.60 **11.46** 10.25 9.78 9.17 8.29 7.28 7.15 5.46 3.57 2.90

Size difference between the pairs was not uniform. The last 2 pairs were small in size.

12 ***Eyprepocnemis rosea*** Uvarov

13.52 **13.20** 10.66 9.26 8.19 8.06 7.47 7.28 6.89 5.88 4.81 4.26

The 1st pair of autosome and the X of similar size were grouped as large elements. Size difference between other pairs was haphazard.

13 ***Choroedocus robustus*** (Serville)

14.24 13.43 10.57 **9.87** 9.78 9.03 8.55 8.00 6.24 4.79 2.77 2.54

The first 2 and the last 2 pairs were grouped as large and small ones. Among the medium ones, size difference was large between the 7th, 8th and 8th, 9th pairs.

14 ***Tylotropidius varicornis*** (Walker)

14.40 13.45 11.83 10.58 9.45 8.55 **8.17** 7.41 6.64 3.52 2.96 2.04

The last 3 pairs were considerably small in the complement. Size difference between pairs had gradually decreased.

15 ***Cyrtacanthacris tatarica*** (Linnaeus)

16.16 13.72 11.85 **10.51** 8.79 8.17 7.53 6.81 6.10 4.40 3.43 2.57

The first 3 and the last 3 pairs were grouped as large and small ones in the complement. Size difference was bigger between the pairs in the large size group. In medium and small groups this difference was small and even.

16 ***Chondracris rosea*** (deGeer)

15.98 **14.52** 13.46 11.69 9.93 8.16 6.69 6.04 5.41 3.05 2.70 2.36

The last 3 pairs of similar size were considerably small in the complement. Difference in the size of pairs was large and equal upto the 6^{th} autosomal pair. The 7^{th}, 8^{th} and 9^{th} had small size difference.

17 ***Patanga succincta*** (Johanson)

15.40 **12.90** 11.54 10.57 9.35 8.59 7.95 7.46 6.77 3.81 3.24 2.55

1 large, 7 medium and 3 small pairs of autosomes were present in the complement. Size difference between pairs of medium and small groups was similar.

18 ***Catantops innotabilis*** (Walker)

15.64 13.93 11.15 **9.04** 8.66 8.10 7.54 6.98 6.49 4.85 3.96 3.63

3 large, 5 medium and 3 small pairs were present in the complement. Large pairs had bigger size difference than others.

19 ***Catantopa simlae*** Dirsh

16.81 14.44 11.97 10.43 9.71 7.95 7.07 6.28 5.63 4.23 3.09 2.37

Grouping of chromosomes as 3 large 5 medium and 3 small sizes was however not distinct. The 1^{st}, 2^{nd} and 3^{rd} pairs had large and equal gap in their length.

20 ***Xenocatantops humilis*** (Serville)

14.26 12.23 10.90 10.27 9.25 **8.50** 8.23 7.34 6.56 5.03 4.42 3.16

Grouping of chromosomes as 2 large 6 medium and 3 small pairs of autosomes was however not distinct.

21 ***Stenocatantops splendens*** (Thunberg)

14.87 12.74 **11.51** 10.21 9.08 8.15 7.25 6.95 6.46 5.74 4.17 3.28

2 large, 7 medium and 2 small pairs of autosomes were noted in the complement. Large pairs had big gap in their length.

22 ***Chorthippus indus*** Uvarov

20.65 22.52 16.60 **8.53** 8.20 6.82 5.29 5.12 4.27

2*n* = 17(♂) consisted of 6 metacentric and 10 acrocentric autosomes and 1 acrocentric X chromosome. Smaller arms of the metacentrics were larger than the last four chromosomes. The X 4th longest in the complement, was half the size of the 3rd pair. The 1st, 2nd and 3rd largest metacentric elements were the fusion products of the 2nd and 4th, 1st and 8th and 3rd and 7th acrocentric chromosomes of the parental complement (*i.e.* 2*n* = 23 ♂).

23 ***Dnopherula*** (Aulacobothrus) sp. Jago

14.89 13.02 11.05 **10.23** 9.97 8.74 7.67 6.88 6.41 4.70 3.74 2.75

The first 2 and the last 3 pairs could be grouped as large and small ones in the complement. Large pairs had bigger size gap than the small pairs. Those in between (3rd to 8th pairs) had uneven size difference.

24 ***Acrotylus inficita*** (Walker)

15.05 12.96 11.59 **10.28** 9.74 8.50 7.93 7.30 6.20 4.67 3.48 2.35

First 3 pairs of autosomes could be separated as large ones. For other 5 and last 3 pairs medium and small size distinction was not convincing.

25 ***Aiolopus tamulus*** (Fabricius)

18.17 13.23 11.02 **10.19** 9.89 8.90 8.25 7.48 6.65 4.02 3.00 2.18

2 pairs large, 6 pairs medium and 3 pairs were small in the complement.

26 ***Heteropternis respondens*** (Walker)

15.73 13.26 11.54 10.19 8.97 7.88 7.35 6.87 6.47 5.68 3.38 2.67

Last 2 pairs were very small in the complement. Large size difference up to 6th element had however gradually reduced. In others size difference was small and equal.

27 ***Sphingonotus indus*** Saussure

15.09 13.40 11.64 9.82 **9.26** 8.16 7.36 6.84 6.52 5.89 3.31 2.78

The last 2 pairs were distinctly small. The 1st, 2nd and 3rd pairs were large elements. From 4th to 9th including X (5th longest) formed medium size group.

28 ***Trilophidia annulata*** (Thunberg)

14.88 12.88 11.57 **10.18** 9.17 8.42 7.72 7.23 6.70 5.80 3.03 2.48

3 pairs long, 6 pairs medium and 2 pairs were distinctly small in the complement. The X 4th longest had bridged the gap between long and medium.

29 ***Acrida exaltata*** (Walker)

14.54 12.79 10.93 **10.17** 9.13 8.15 7.76 7.24 6.87 6.21 3.47 2.73

The 1st and 2nd pairs were large and the last 2 pairs were very small in the complement. Others with small gap had formed medium size group. All the chromosomes had centromeric band. One small element was the supernumerary chromosome.

30 ***Sikkimiana darjeelingensis*** (Bolivar)

13.73 12.45 11.19 10.08 9.92 9.38 8.13 6.84 6.25 5.02 3.98 3.00

Grouping of chromosomes into large, medium or small was not possible. Also, the difference in the size of pairs was not uniform throughout.

31 ***Phlaeoba antennata*** Brunner

15.79 14.73 11.80 **10.28** 8.95 7.54 6.78 6.40 6.24 4.35 3.84 2.51

3 large, 5 medium and 3 small autosomes were present. The 1st and 2nd pairs had small size difference.

32 ***Phlaeoba infumata*** Brunner

14.33 12.34 11.01 **10.21** 9.15 8.34 7.74 7.13 6.36 5.62 2.97

The 1st, 2nd and 3rd pairs large, and the last pair was small in the complement. Size difference was small between medium pairs. All the chromosomes had centromeric band. On the 9th pair C-band was prominent. C-band was also present in the distal half region of the 3rd pair. Brighter fluorescing centromeric region of all the chromosomes was visible in Hoechst staining.

A SUMMARY OF KARYOLOGICAL FINDINGS

The difference in the idiograms of 32 species reveal distinct karyotype for each of them, instead of all having 23G; 24E acrocentric chromosomes.

The number of chromosomes into different size classes, or gradual seriation of the complement were the distinguishing features of the karyotypes..Position of the X in the karyotypes of different species varied from 1st to 6th and was found to be a valuable cytotaxonomic character.

The finding is encouraging since it was believed by earlier workers that Acridoidea are a group with such uniform karyotypes that their study can throw little light on taxonomic problems.

On chromosomal banding patterns these species are highly distinct from each other.

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GLOSSARY

Cytotaxonomy — The study of the natural relationships of organisms by a combination of cytology and taxonomy.

Cell — Basic structural and functional unit of all living matter.

Nucleus — The spheroidal, membrane-bounded structure present in all eukaryotic cells which contains DNA, usually in the form of chromatin.

Chromatin — The complex of nucleic acids (DNA and RNA) and proteins (histones and non-histones) comprising eukaryotic chromosomes.

Chromosome — Thread like structures in the nucleus, consisting of chromatin, and carrying genetic information arranged in a linear sequence.

Karyotype — The term karyotype is applied to a systematized array of the chromosomes prepared either by drawing, digitized imaging, or by photography, with the extension in meaning that the chromosomes of a single cell can typify the chromosomes of an individual or even a species.

Mitotic karyotypes — Chromosomes at metaphase reveal their exact morphology on which they are paired and karyotyped. The karyotype is the first character in the cytotaxonomic evaluation of a species. Sex-chromosomes and chromosomal polymorphisms are readily detected in karyotyping. Gaps, constrictions, NORs are detected on the chromosomes which serve as the marker elements in the chromosomal differentiation of species.

Idiogram — Idiogram is the diagrammatic representation of a karyotype. A comparative analysis of which forms the basis of our cytotaxonomical conclusions.

Chromosome band — A band is defined as a part of a chromosome which is clearly distinguishable from its adjacent segments by appearing darker or lighter with one of more banding techniques.

The chromosomes are visualized as consisting of a continuous series of light and dark bands, so there are no “interbands”. The banding techniques fall into two principal groups :

(i). Those resulting in bands distributed along the length of the whole chromosome, such as G-, Q-, and R-bands, including techniques that demonstrate patterns of DNA replication, and

(ii). Those that stain specific chromosome structures and hence give rise to a restricted number of bands. These include methods which reveal constitutive heterochromatin (C-bands), telomeric bands (T-bands), and nucleolus organizing regions (NORs).

C-band mitotic, meiotic and hoechst 33258 fluorescence band karyotypes— the advent of C-banding has made it possible to differentially stain constitutive heterochromatic regions at condenced stages of a division cycle, and hence, at a time when such regions are normally indistinguishable from euchromatin. Analysis of C-band karyotypes may reveal—

(1) interspecific variation, serving to distinguish related species.

(2) it may be intraspecific in which case it may be either

(a) distinguish different populations of the same species creating polytypism or else

(b) it may distinguish different individuals of the same population creating a polymorphism.

C-band meiotic karyotypes help in analysis of chiasma localisation in relation to heterochromatin distribution, and morphological alterations among bivalent types.

Hoechst-33258 fluorescence banding— in grasshoppers it has been found that at early prophase stages only the centromeres of the autosomal bivalents fluoresced brightly whereas the entire X univalent showed bright fluorescence. It has been concluded that H-fluorescence is modulated by chromosome condensation brought about by differential ratios of DNA-protein at different chromosome regions and at different divisional stages. This property helps in distinguishing chromosomes in the karyotypes.

Meiosis

In most sexually reproducing organisms, the doubling of the gametic chromosome number, which accompanies syngamy, is compensated for by a halving of the resulting zygotic chromosome number at some other point during the life cycle. These changes are brought about by a single chromosomal duplication followed by two successive nuclear divisions. The entire process is called meiosis, and it occurs during animal gametogenesis or sporogenesis in plants.

Meiotic bivalents

A pairing configuration during the first meiotic division which consists of two completely or partially homologous chromosomes. The number of bivalents per cell (meiocyte) normally corresponds to half the somatic chromosome number of diploid and genome-allopolyploid species.

Bivalent karyotypes Analysis of meiotic bivalents in their karyotypes help considerably in localization of chiasmata and the range of its variation at intra and inter individual level. Chiasma analysis further indicate the recombination potential of that sex.

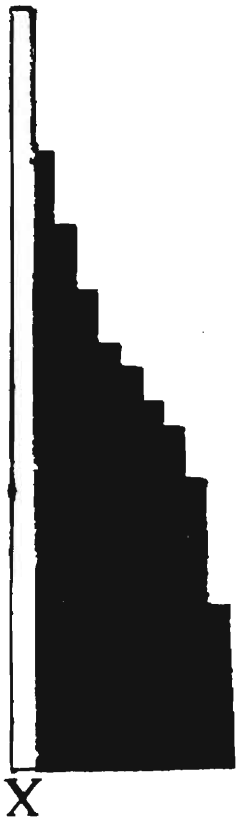
Meiotic stages Study of meiotic stages are very important in identifying the configuration of ditactic bivalents and characterizing megameric pairs. Both features act as marker elements in the cytotaxonomic identification of a species. Supernumerary chromosomes, unequal bivalents, pairing and inversions are readily distinct in meiotic stages.

PLATE I

Comparative Idiograms

Order ORTHOPTERA
Superfamily PAMPHAGOIDEA
Family PYRGOMORPHIDAE

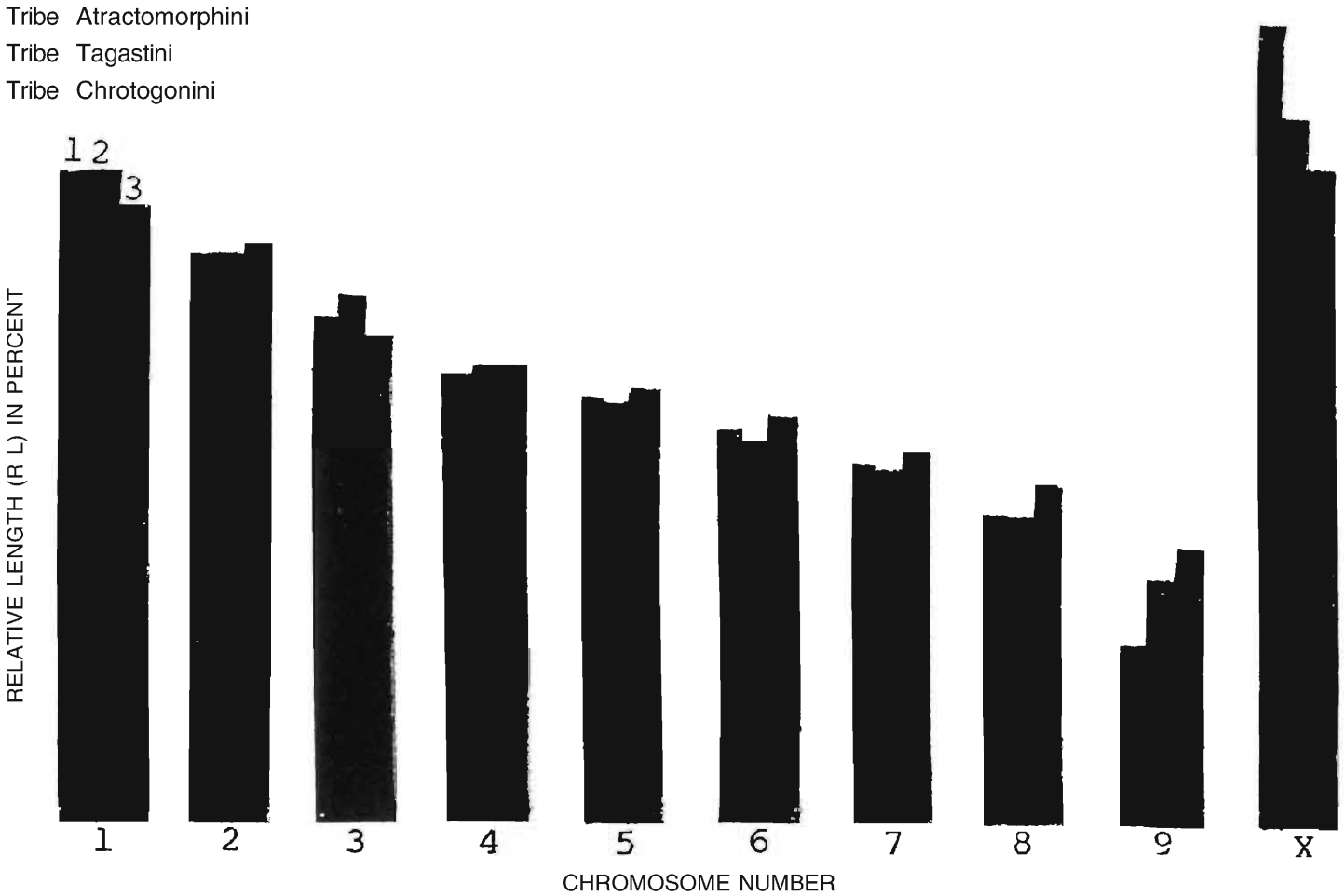
Tagasta indica
Bolivar



Atractomorpha crenulata
(Fabricius)

Chrotogonus trachypterus
(Blanchard)

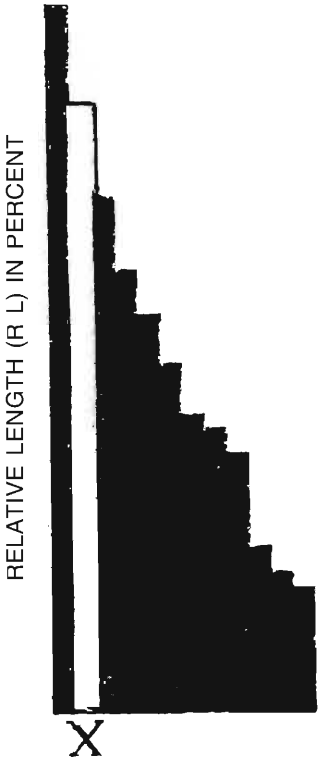
PLATE II



1. *Atractomorpha crenulata* (Fabricius); 2. *Tagasta indica* Bolivar; 3. *Chrotogonus trachypterus* (Blanchard)

PLATE III

Superfamily ACRIDOIDEA
Family ACRIDIDAE
Subfamily HEMIACRIDINAE



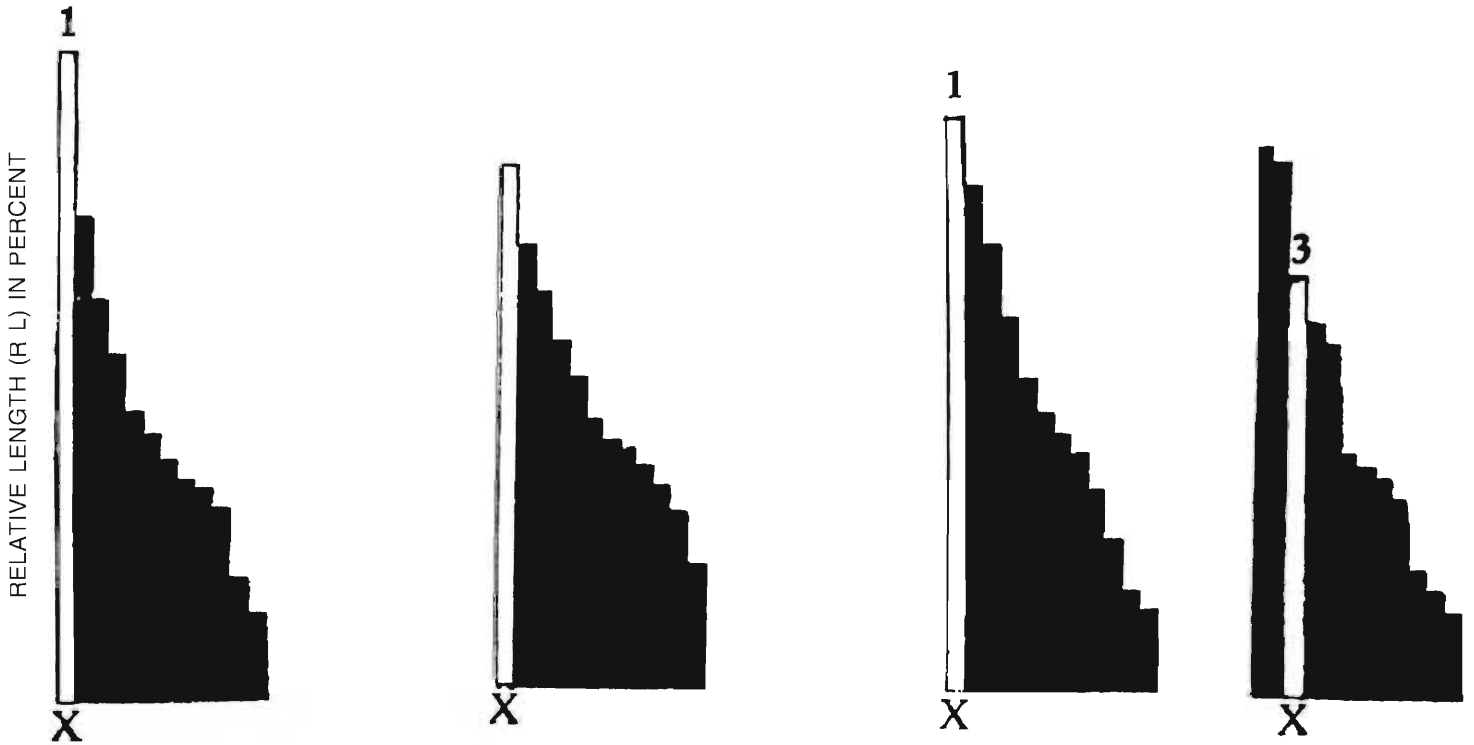
Spathosternum prasiniferum
(Walker)

Subfamily TROPIDOPOLINAE

Tristria pulvinata
(Uvarov)

PLATE IV

Subfamily OXYINAE



Gesonula punctifrons (Stål)

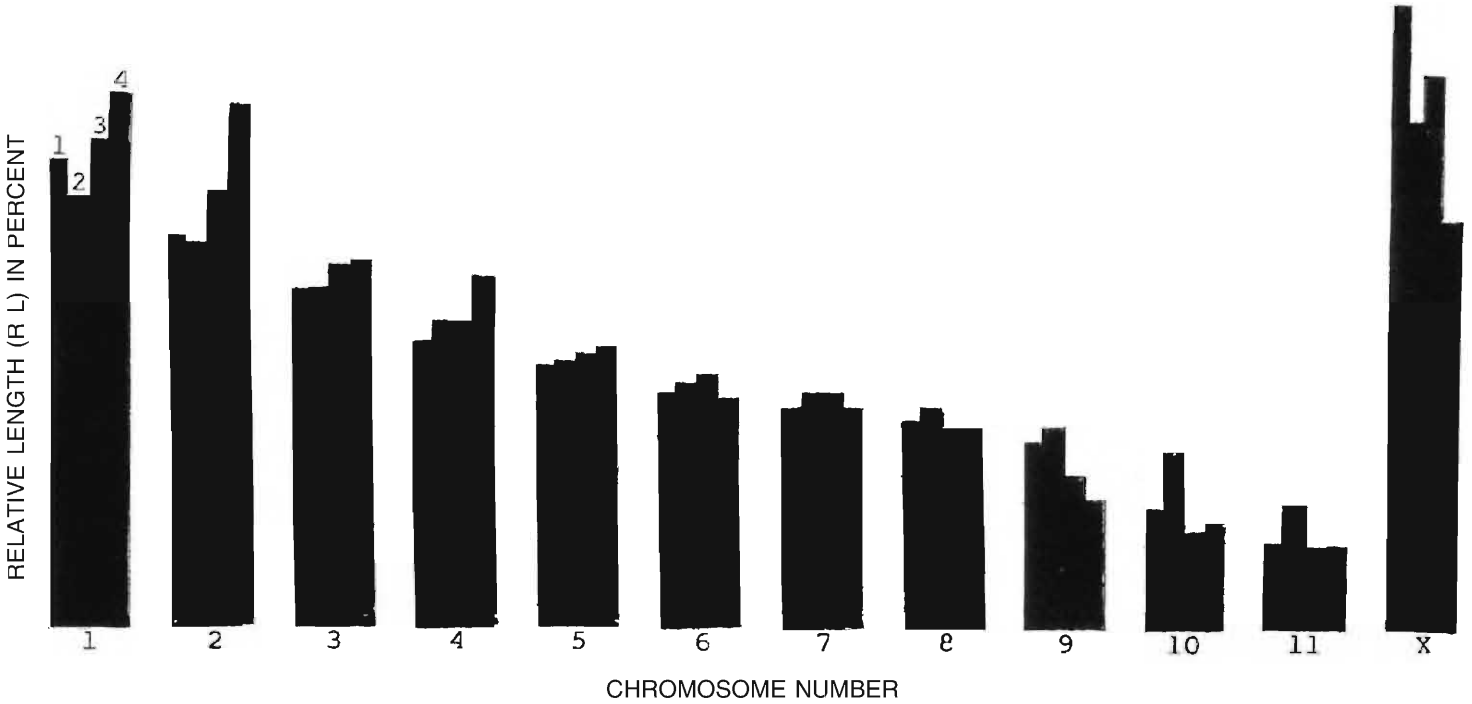
Oxya fuscovittata (Marschall)

Oxya hyla Serville

Caryanda paravicina (Willemse)

PLATE V

Subfamily OXYINAE

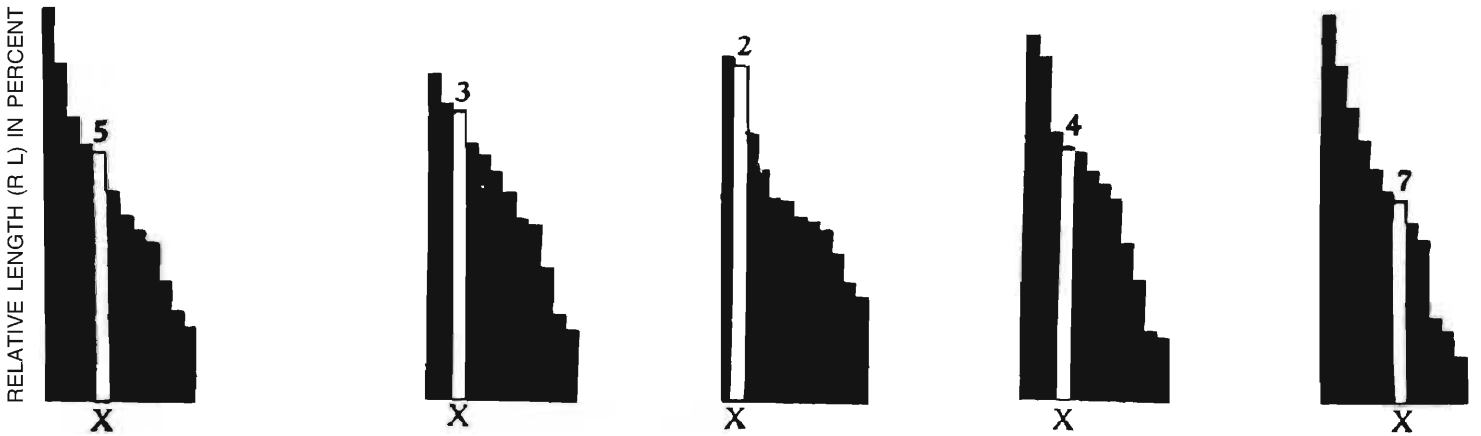


1. *Gesonula punctifrons* (Stål); 2. *Oxya fuscovittata* (Marschall);
3. *Oxya hyla hyla* Serville; 4. *Caryanda paravicina* (Willemse)

PLATE VI

Subfamily CALLIPTAMINAE

Subfamily EYPREPOCNEMIDINAE



Peripolus pedarius (Stål)

Eyprepocnemis alacris (Serville)

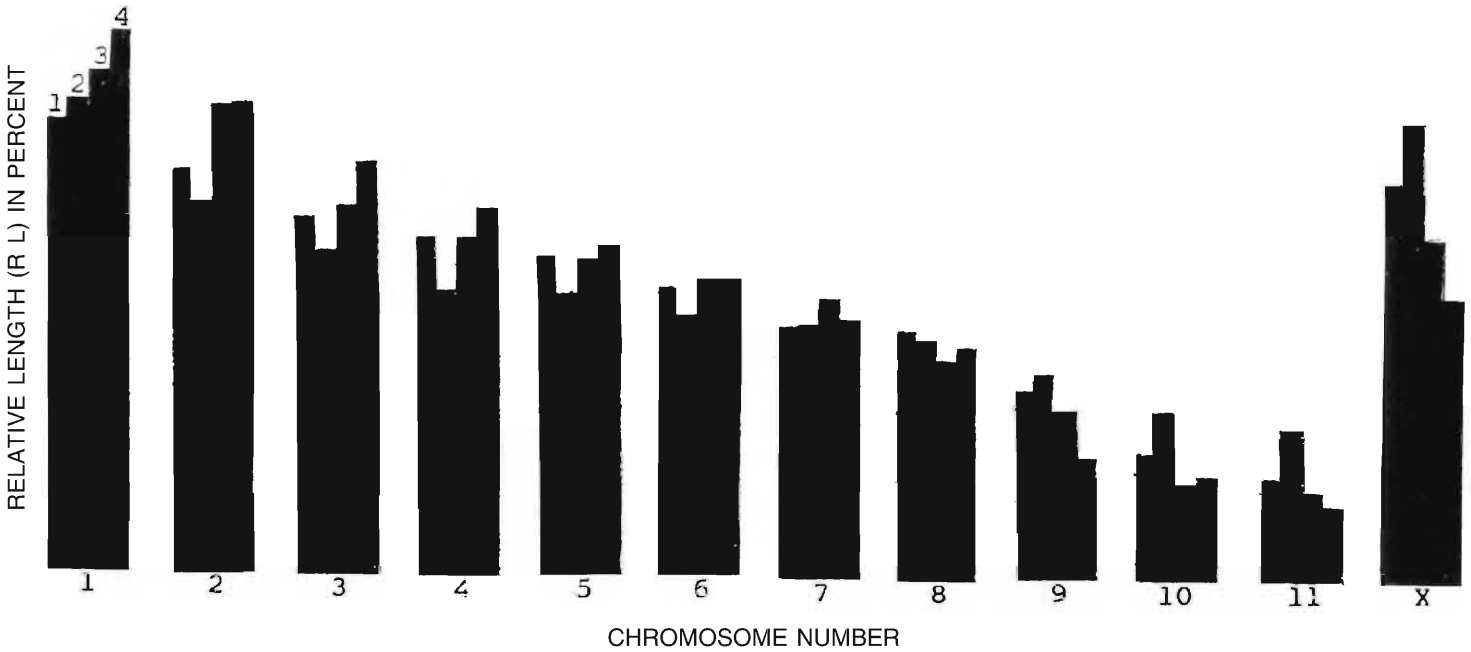
Eyprepocnemis rosea Uvarov

Chroedocus robustus (Serville)

Tylotropidius varicornis (Walker)

PLATE VII

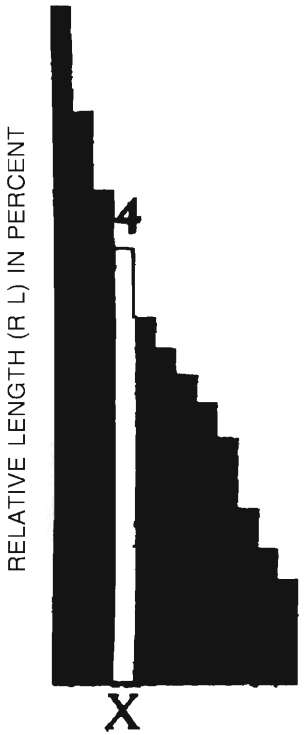
Subfamily EYPREPOCNEMIDINAE



1. *Eyprepocnemis alacris alacris* (Serville); 2. *Eyprepocnemis rosea* Uvarov
3. *Choroedocus robustus* (Serville); 4. *Tylotropidius vricornis* (Walker)

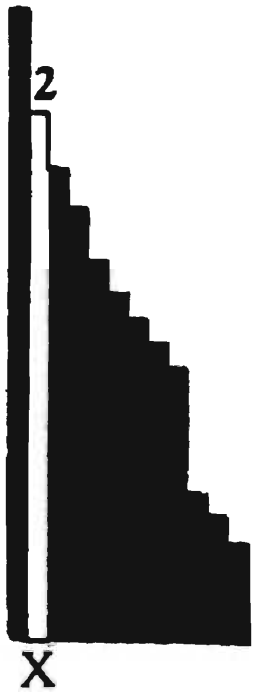
PLATE VIII

Subfamily CYRTACANTHACRIDINAE



Cyrtacanthacris tatarica
(Linnaeus)

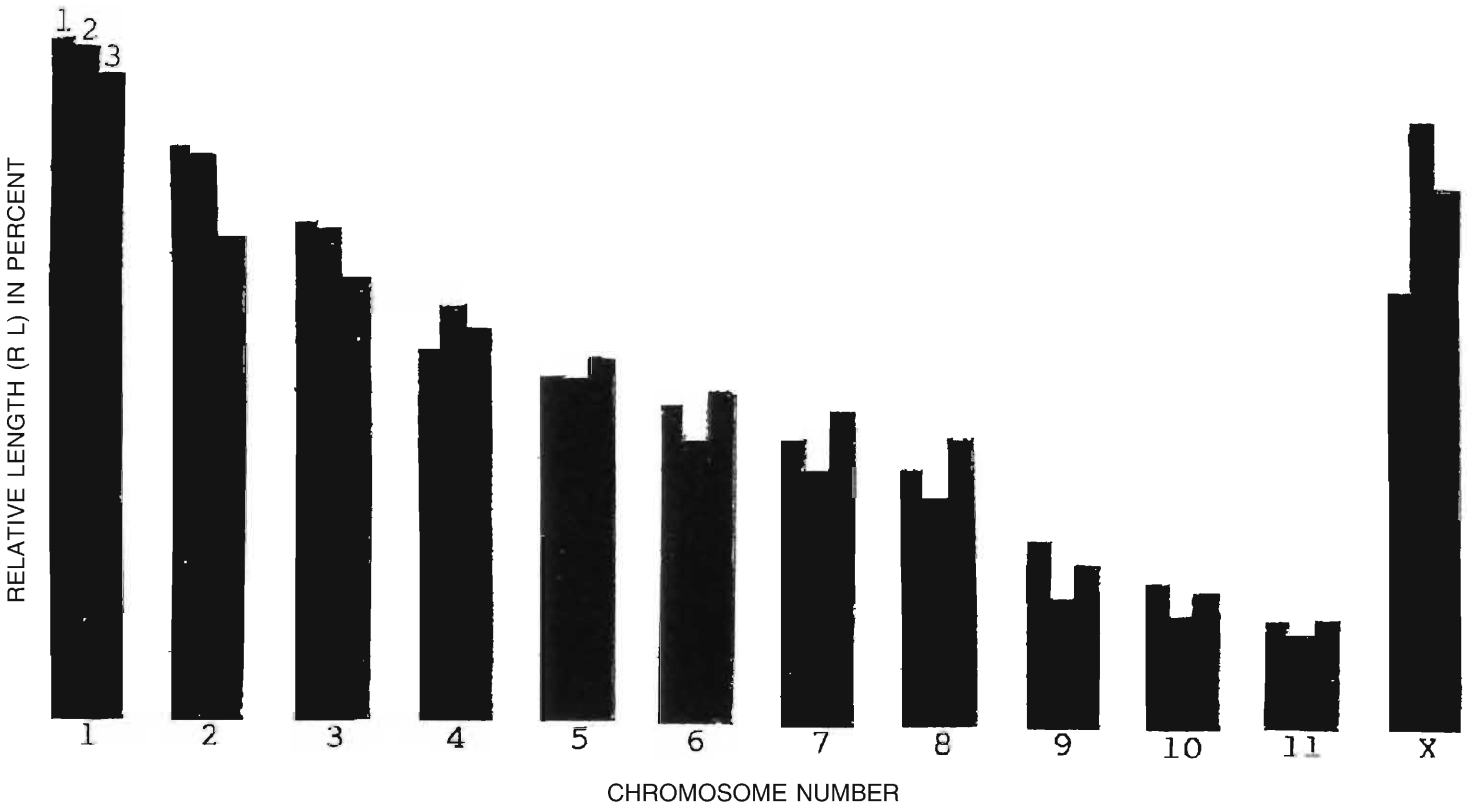
Chondracris rosea
(de Geer)



Patanga succincta
(Johansson)

PLATE IX

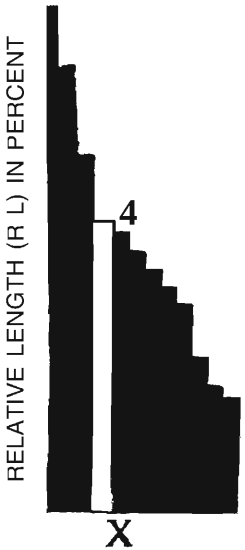
Subfamily CYRTACANTHACRIDINAE



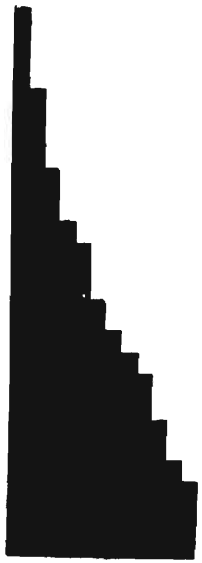
1. *Cyrtacanthacris tatarica* (Linnaeus); 2. *Chondracris rosea* (de Geer); 3. *Patanga succincta* (Johansson)

PLATE X

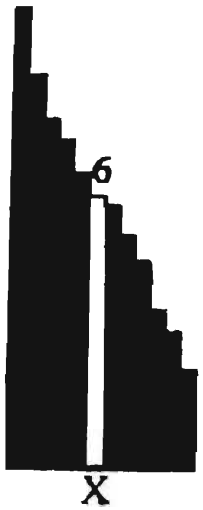
Subfamily CATANTOPINAE



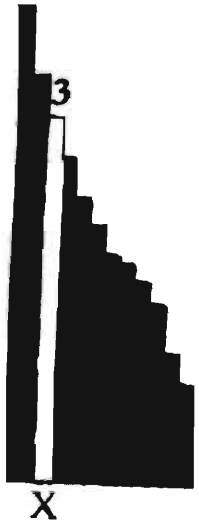
Catantops innotabilis
(Walker)



Catantops simlae
Dirsh



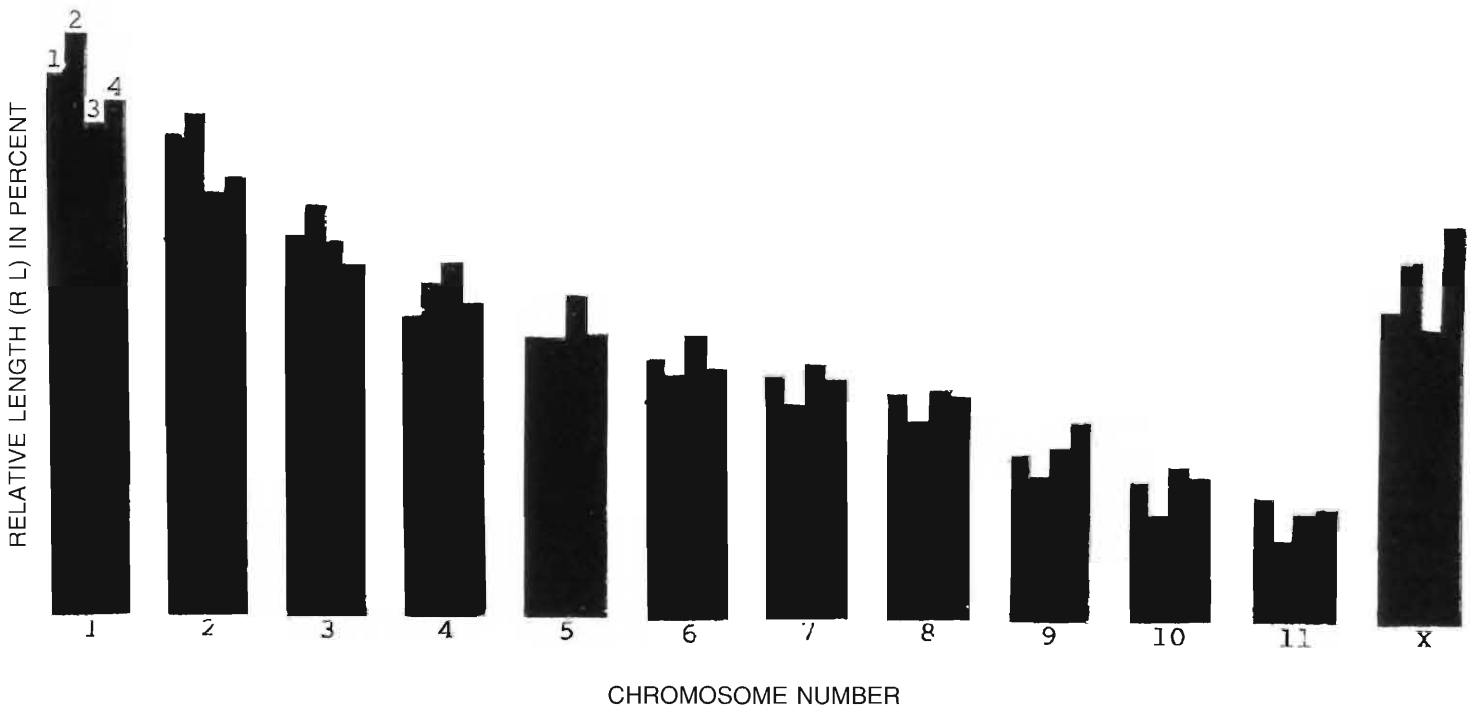
Xenocatantops humilis
(Serville)



Stenocatantops splendens
(Thunberg)

PLATE XI

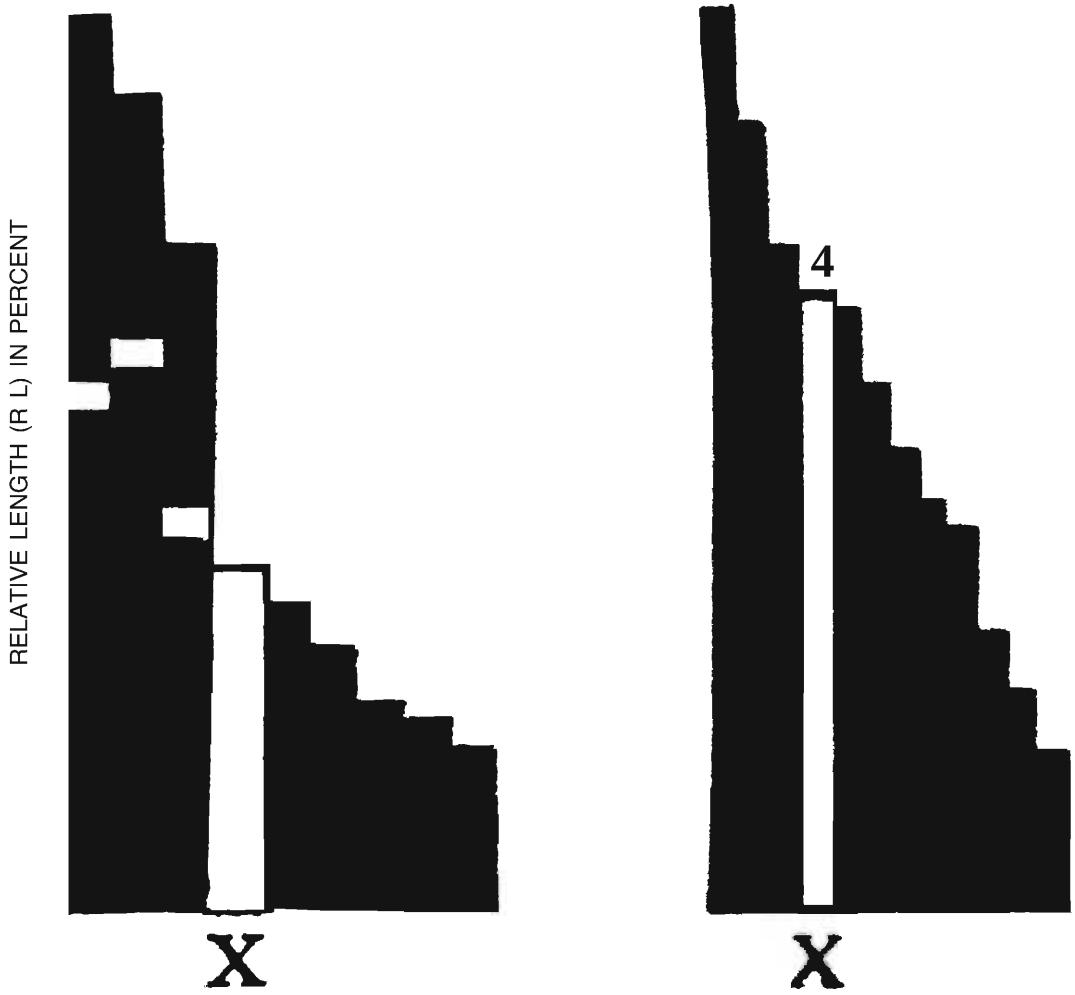
Subfamily CATANTOPINAE



1. *Catantops innotabilis* (Walker); 2. *Catantops simlae* Dirsh;
3. *Xenocatantops humilis* (Serville); 4. *Stenocatantops splendens* (Thunberg)

PLATE XII

Subfamily GOMPHOCERINAE

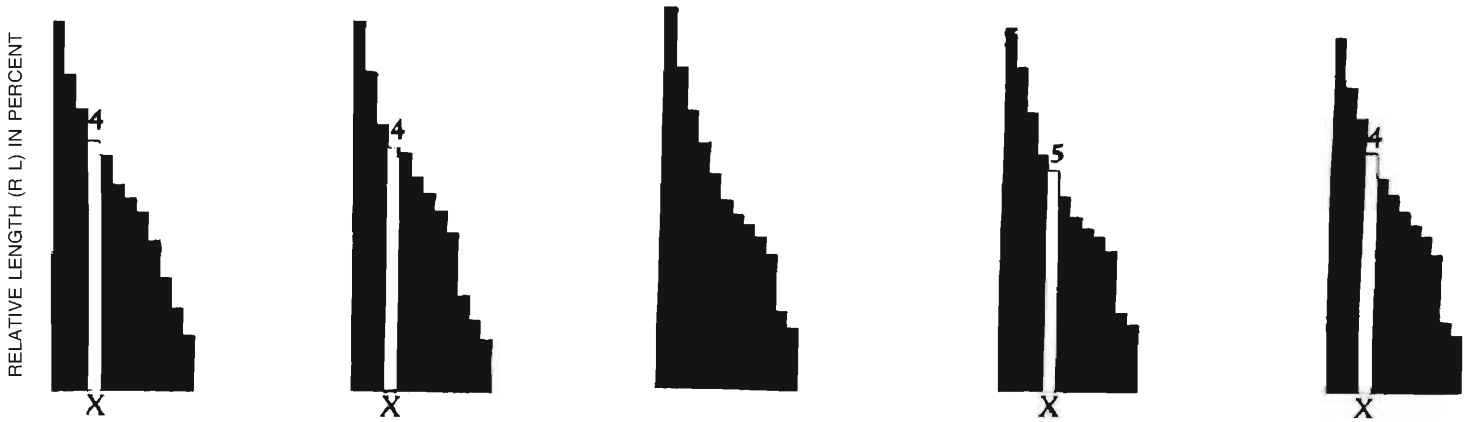


Chorthippus indus Uvarov

Aulacobothrus collinus Uvarov

PLATE XIII

Subfamily OEDIPODINAE



Acrotylus inficita (Walker)

Aiolopus tamulus (Fabricius)

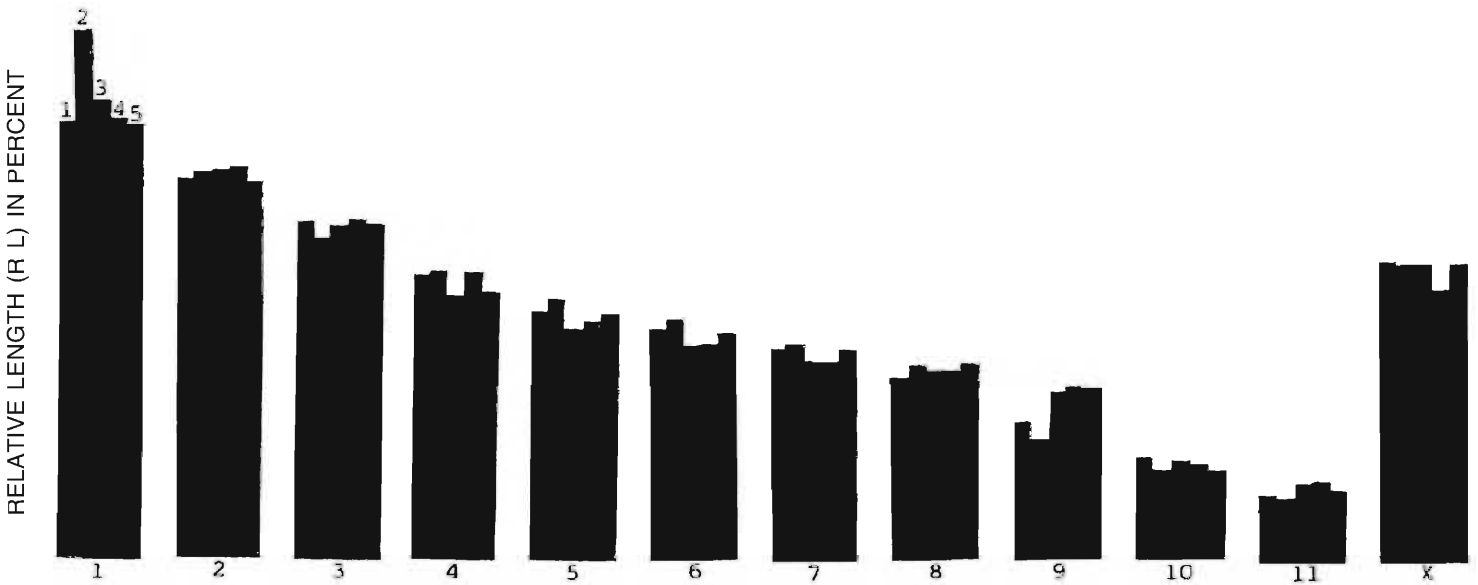
Heteropternis respondens (Walker)

Sphingonotus indus Saussure

Trilophidia annulata (Thunberg)

PLATE XIV

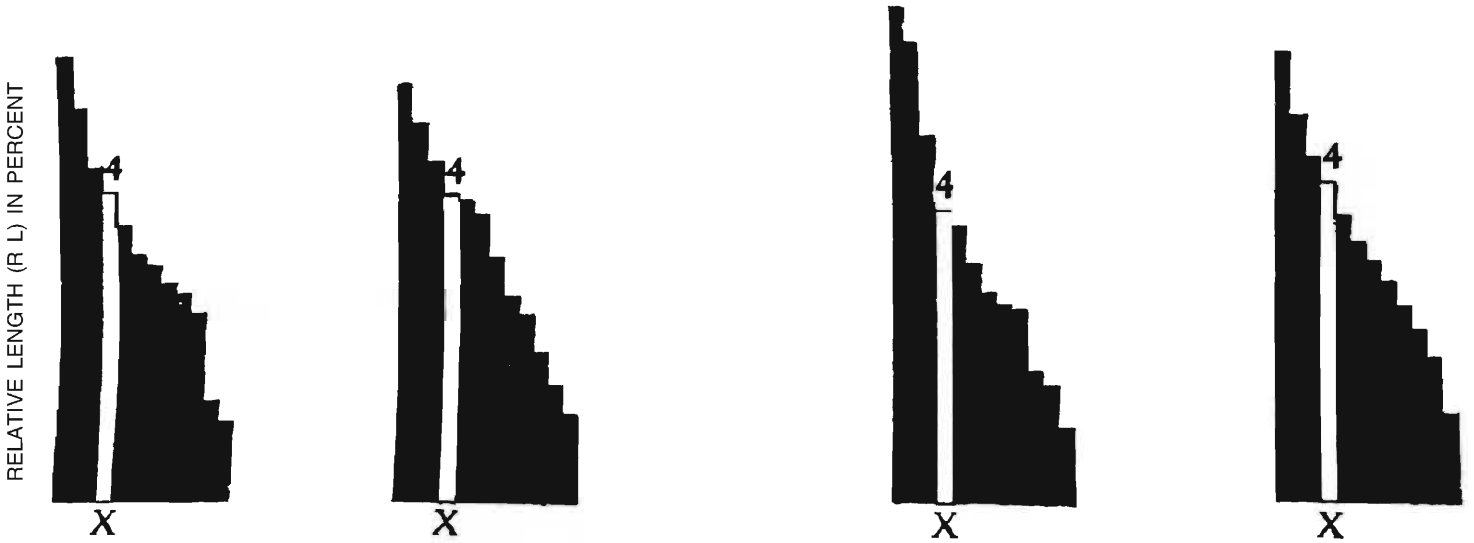
Subfamily OEDIPODINAE



1. *Acrotylus inficita* (Walker); 2. *Aiolopus tamulus* (Fabricius); 3. *Heteropternis respondens* (Walker); 4. *Sphingonotus indus* Saussure; 5. *Trilophidia annulata* (Thunberg)

PLATE XV

Subfamily ACRIDINAE



Acrida exaltata (Walker)

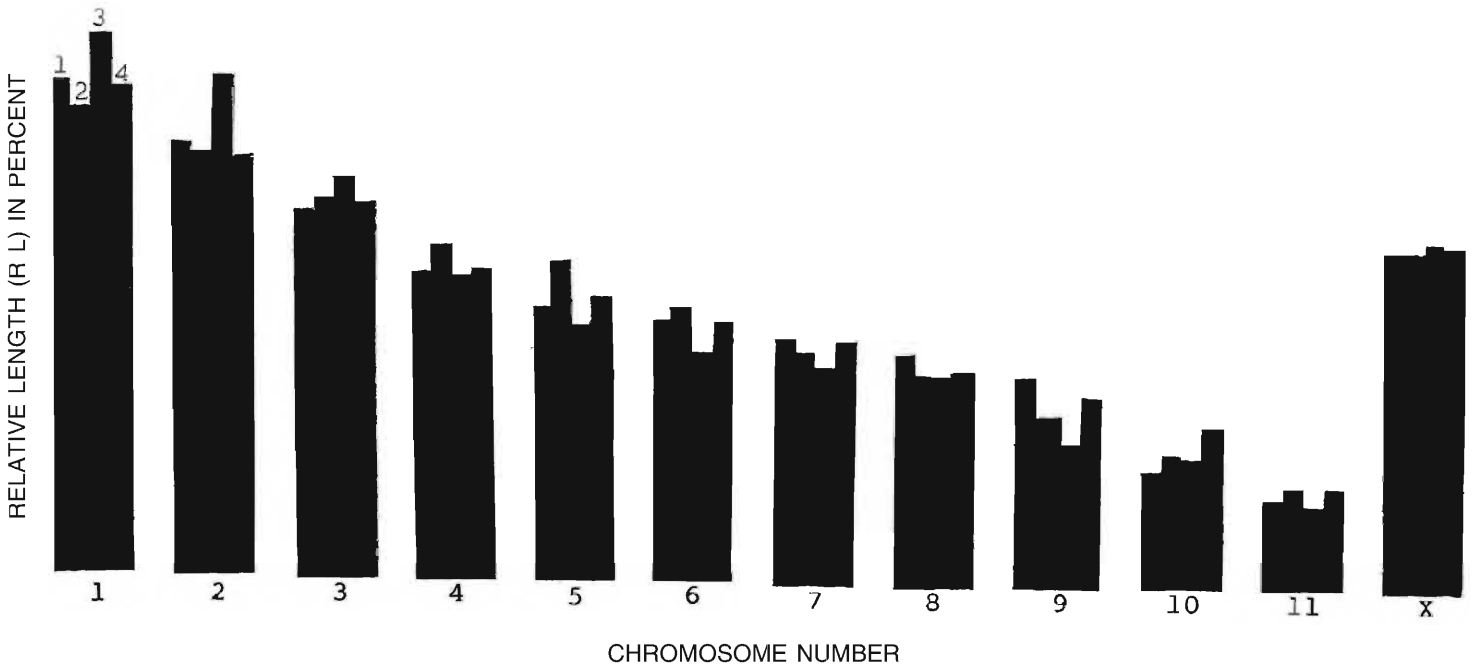
Sikkimiana darjeelingensis (Bolivar)

Phlaeoba antennata Brunner

Phlaeoba infumata Brunner

PLATE XVI

Subfamily ACRIDINAE



1. *Acrida exaltata* (Walker); 2. *Sikkimiana darjeelingensis* (Bolivar)
3. *Phlaeoba antennata* Brunner; 4. *Phlaeoba infumata* Brunner