SINET: Ethiop. J. Sci., 35(2):117–128, 2012
 ISSN: 0379–2897

CHROMOSOME STUDY OF SOME GRASSHOPPER SPECIES FROM DIFFERENT LOCALITIES IN CENTRAL ETHIOPIA

Samuel Berhanu [1], Kifle Dagne[1] and Emana Getu [2,*]

[1] Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia

[2] Department of Zoological Sciences, College of Natural Sciences, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia. E-mail: egetudegaga@yahoo.com

ABSTRACT: Around 200 grasshopper species have been identified in Ethiopia, hitherto. The diversity and economic importance of Ethiopian grasshoppers notwithstanding, there is only few studies done on their taxonomy, distribution and ecology. Additionally, no report on the karyology of Ethiopian grasshoppers is available prior to this study. Hence, this research is aimed at studying the chromosomes of some Ethiopian grasshopper species. The grasshopper specimens used in this study were collected from eight localities in central Ethiopia. The specimens were identified as belonging to two families (Acrididae and Tetrigidae). Chromosome preparations were made from tissues of the whole gut and the testis following colchicine pretreatment of live insects. All the insects belonging to family Acrididae showed karyotypic similarity, with all having 2n = 23 (22 autosomes + X) in males and 2n = 24 (22 autosomes + XX) in females. Morphologically all the chromosomes were telocentrics except an acrocentric X chromosome observed in some of the taxa. Despite the overall karyotypic similarity, minor variations were also observed. In the genus Paratettix (Tetrigidae), the analyzed female specimen had 2n = 20 telocentric chromosomes. In the male specimen of genus Acanthacris, some chromosome numerical instability, involving hypodiploid and hyperdiploid conditions, were observed both in the meiotic and mitotic cells of the testis. Furthermore, an extra chromosome was observed in some of these cells and this was assumed to be a B chromosome. The need for further large-scale chromosome study of Ethiopian grasshoppers is recommended.

Key words/phrases: Acridid grasshopper, central Ethiopia, chromosome, karyology, karyotypic conservatism

INTRODUCTION

Grasshoppers constitute a diversified group of insects which are positioned in the order Orthoptera together with crickets, bush-crickets, cockroaches, praying mantises and stick-insects, but, placed separately from these groups, under suborder Caelifera (Ragge, 1965; Meinzingen, 1993). There are about 11,000 species of grasshoppers known to exist around the world (New World Encyclopedia, 2008).

The pioneer work of Jago (1977), cited in Tibebu Habtewold and Landin (1992), indicated that there are at least 200 species of grasshoppers in Ethiopia. A few of them constitute high risk pests of economically important crops in different parts of the country. Tibebu Habtewold and Landin (1992) have recognized about 29 species of short-horned and long-horned grasshoppers from central Ethiopia, and these were indicated to belong to two superfamilies, three families and nine subfamilies.

In their ecological distribution, grasshoppers are found in a variety of environments but most commonly in arid environments like semi-deserts, open meadows and grasslands, as well as in disturbed areas such as crop fields and along roadsides (Tibebu Habtewold and Landin, 1992; Capinera *et al.*, 1997; Branson *et al.*, 2006).

Different cytological studies so far conducted on grasshoppers, especially on members of the family Acrididae, indicated that they show a high degree of karyotypic conservatism. They share the all-telocentric, 2n = 22 + X (male) or 22 + XX (female) karyotype of the ancestral Cryptosacci Acridoids (Roberts, 1941). However, there are

* Author to whom all correspondence should be addressed.

few exceptions to this preponderantly observed type of karyotype. For instance, *Caledia captiva* (family Acrididae), while having the chromosome number characteristic of the family Acrididae, the centromeric position varies from submedian to terminal as to the system of chromosome nomenclature set by Levan *et al.* (1964) and Shaw *et al.* (1995).

Despite the diversity and economic importance of Ethiopian grasshoppers, little is known about their taxonomy, distribution and ecology. The lack of adequate studies on these insects makes their identification difficult. In addition, no studies have been made so far regarding their karyotypes as well as other aspects of their genetics. This leaves a gap in further molecular studies of Ethiopian grasshoppers. Hence, this study is aimed at generating new information on the chromosome number and morphology (karyotype) of some of these insects based on specimens collected from different localities in central Ethiopia.

MATERIALS AND METHODS

Methods of specimen collection and collection sites

The grasshopper specimens were collected from their natural habitats such as fallow farmlands, wheat farmland, grasslands and forests using insect net and as a result of this the number of representative samples for each species is inconsistent. Hand collection was also adopted in situations where using insect net was not suitable. The specimens were collected from eight localities in central Ethiopia (Fig. 1, Table 1)

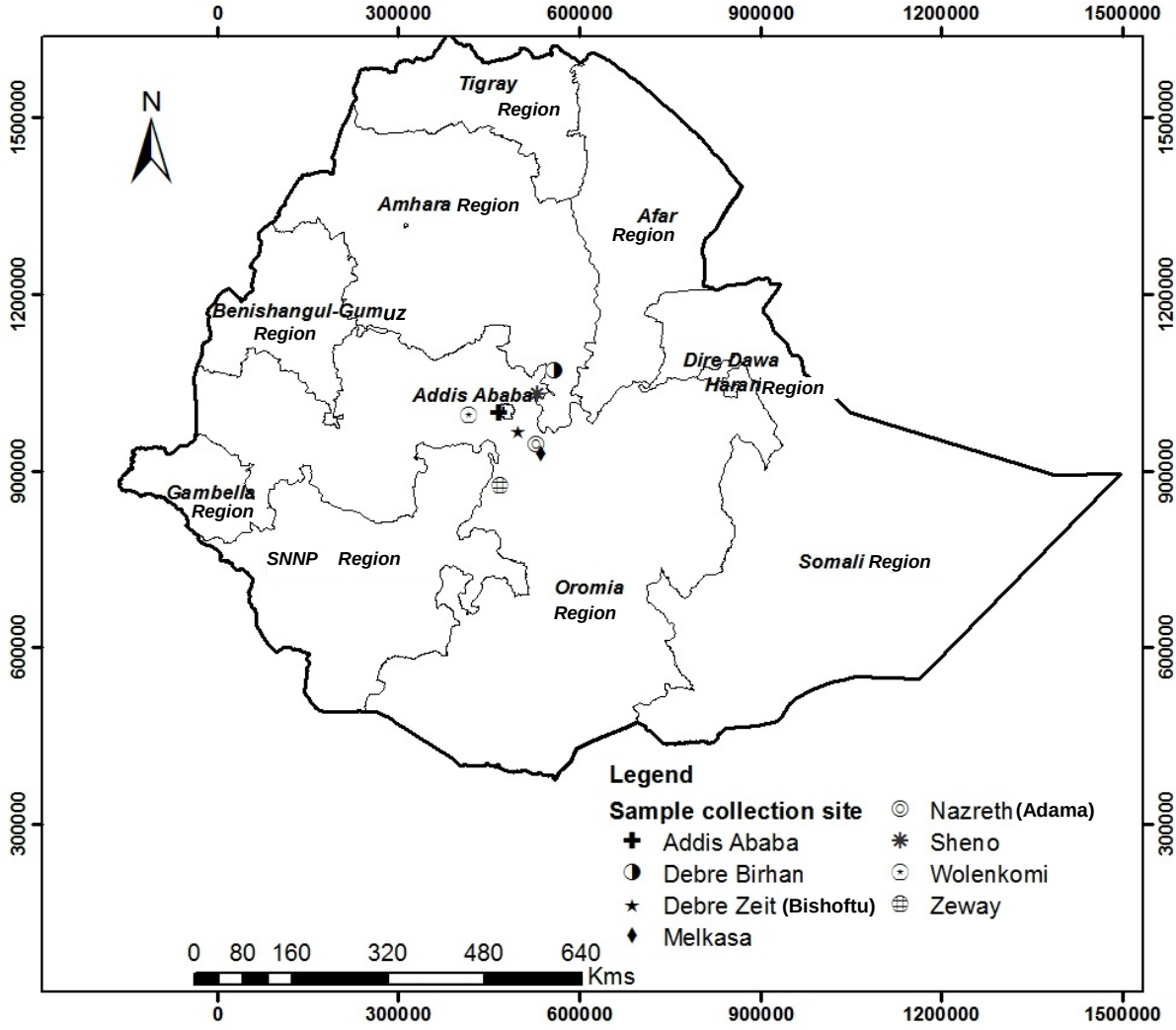

Fig. 1. Grasshopper collection sites in central Ethiopia (Source: Ethio-gis database).

Table 1. Name of localities, geographic coordinates and altitudes of collection sites and the number of male and female grasshopper specimens studied from each locality.

Locality	Coordinate (latitude, longitude)	Altitude (masl)	Number of grasshoppers chromosomally studied		
			Male	Female	Total
Addis Ababa	09°2' N, 038°42' E	2400	__	3	3
Debre Birhan	09°41' N, 039°32' E	2840	4	12	16
Debre Zeit	08°45' N, 038°59' E	1920	1	1	2
Melkasa	08°24' N, 039°20' E	1528	__	6	6
Nazreth	08°33' N, 039°16' E	1712	3	13	16
Sheno	09°19' N, 039°17' E	2842	__	1	1
Wolenkomi	09°00' N, 038°15' E	2147	6	2	8
Zeway	07°55' N, 038°43' E	1642	__	1	1
	Total		14	44	53

Taxonomic identification of the grasshopper specimens

For the taxonomic identification of the grasshopper specimen various sources from literature including books by Stretch-Lilija (1977), Rentz (1991) and Picker *et al.* (2004) and the bugguide web page (http://bugguide.net) were used.

Chromosome preparation

Using fine needle (insulin needle), the grasshopper was injected with 0.05–0.2 ml of 0.1% colchicine in the thorax and abdominal regions. After 6–8 hours following colchicine injection, the grasshopper was anesthetized and the whole gut was dissected out (Channaveerappa and Ranganath 1997) and mashed in about 1–2 ml of hypotonic solution (0.075 M KCl). Large debris of tissues and gut contents were removed by filtering the suspension through cloth gauze into a centrifuge tube and after 25–30 minutes of incubation in the hypotonic solution, the suspension was centrifuged for 5 minutes at 1000 rpm. Then, the supernatant was discarded and the pellet resuspended in 1 ml of freshly prepared fixative (3 methanol:1glacial acetic acid, v/v) and after 10–15 minutes of fixation, the suspension was centrifuged again for about 3 minutes. This particular step of removing the supernatant, addition of fixative and centrifugation, was repeated twice or thrice with the same amount of fixative. After the final centrifugation, the pellet was re-suspended in about 0.5 ml of fixative to get higher cell density.

In some cases, the testis was removed and used for meiotic chromosome slide preparation following similar procedure described for the gut tissue above.

Finally, the cell suspension was drawn with a Pasteur pipette and three to four drops were splashed on microscope glass slides inclined by about 45°. The slides were then allowed to air-dry at room temperature and stained with Giemsa stain in Sorenson's phosphate buffer solution (pH=6.8) for about 30 minutes. The slides were rinsed in distilled water air dried and mounted under a 22 mm by 50 mm cover slip in DPX mounting medium.

Cells containing complete and well spread chromosome complement were photographed with a total magnification of 1000× using a camera-fitted microscope. The photomicrographs of the chromosomes were edited using Image J software which was downloaded freely from http://rsb.info.nih.gov/ij. Two to six mitotic metaphase cells per individual were analyzed for the karyotypic description.

Since different authors use different terminologies for centromeric positions, the terminology by Levan *et al.* (1964) was used in the present study. Accordingly, a chromosome where the centromere is located at the terminal region is named as telocentric rather than acrocentric. Chromosome size was used to arrange putative homologous chromosomes into pairs to construct the karyotypes.

RESULTS

Taxonomic identification

All the specimens studied were identified as belonging to the families Acrididae and Tetrigidae. Identification to the lowest taxonomic levels was not possible for most of the specimens due to the unavailability of previous work on the taxonomy of Ethiopian grasshoppers. Because of

these difficulties, only one specimen was identified to the species level and six specimens to genus level while for two of the specimens it was not possible to go below the family level. Photographs of the representatives of the identified grasshopper specimens are shown in Figure 2 and the identification result is presented in Table 2.

Chromosome

All the taxa under the family Acrididae investigated here had the diploid chromosome number of 2n = 23 in males and 2n = 24 in females, and these consist of 22 autosomes + XO in males and 22 autosomes + XX in females. Except in rare cases, all the chromosomes are telocentric type and the autosomal fundamental number is 22. On the contrary, the single taxa under the family Tetrigidae (*Paratettix* spp.) had 2n = 20 telocentric chromosomes and the autosomal fundamental number of 18. The various minor differences observed are briefly described for each of the taxa under their respective taxonomic category as follows.

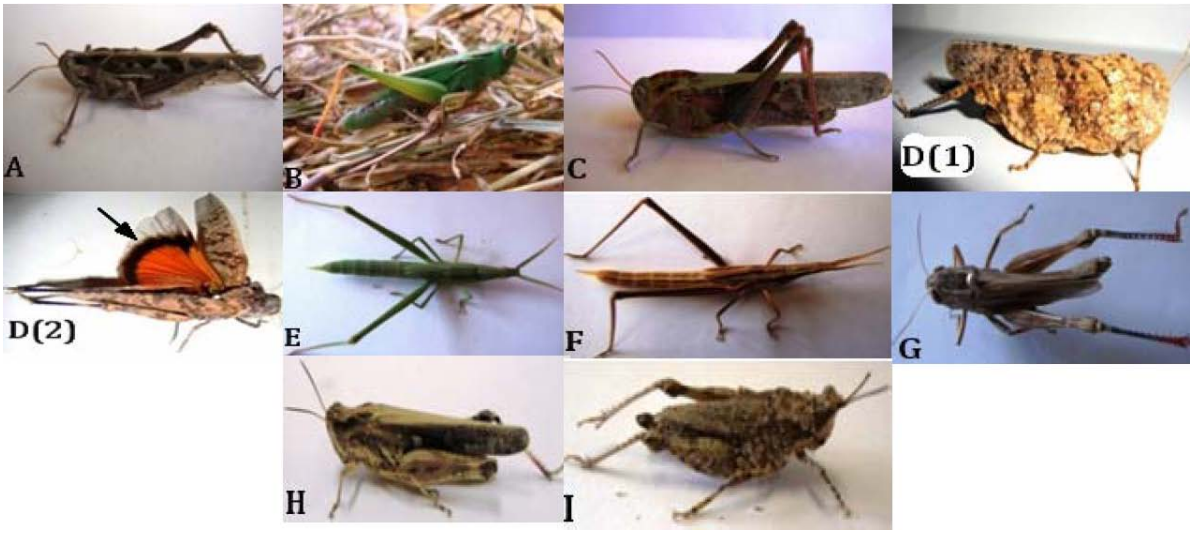

Fig. 2. Pictures of grasshopper specimens belonging to different species collected for the study from different localities of Ethiopia: (A) *Acanthacris* spp., (B) *Paracinema tricolor*, (C) *Gastrimargus* spp., (D(1) and D(2)) *Pardalophora* spp., (E) *Acrida* spp.-1, (F) *Acrida* spp.-2, (G) Acrididae MU, (H) Acrididae WDZU, (I) *Paratettix* spp.

Notes: ● In figure D (2) the hind wing with thick dark band (arrow), is the characteristic feature of the genus *Pardalophora*. (http;//bugguide.net). Specimens represented in figure E and F are distinguishable from one another by their body coloration (Picker *et al.*, 2004).

Table 2. Taxonomic identification of the grasshopper specimens used in the present study.

Taxa identified	Site of collection	Taxonomic level	Subfamily	Family
Acanthacris sp.	Wolenkomi	Genus	Cyrtancanthacridinae	Acrididae
Paracinema tricolor	Debre Birhan Addis Ababa	Species	Oedipodinae Oedipodinae	Acrididae
Gastrimargus sp.	Zeway Melkasa Debre Birhan	Genus		Acrididae
Pardalophora sp.	Nazreth	Genus	Oedipodinae	Acrididae
Acrida sp.-1	Debre Zeit Sheno Melkasa	Genus	Acridinae	Acrididae
Acrida sp.-2	Melkasa	Genus	Acridinae	Acrididae
Acrididae MU[a]	Melkasa	Family	Unidentified	Acrididae
Acrididae WDZU[a]	Wolenkomi Debre Zeit	Family	Unidentified	Acrididae
Paratettix sp.	Nazreth	Genus	Tetriginae	Tetrigidae

[a] These specimens were identified only to the family level, and designated as MU and WDZU after the names of sites where they were collected.

Taxa identified to family level only

The two types of specimens designated as Acrididae MU and Acrididae WDZU are morphologically distinct (Fig. 2 G, H). However, the two possess very similar karyotypes with a slight difference observed between the karyotypes of the two taxa. In the karyotype of Acrididae MU, the chromosome size decreases gradually from the largest to the smallest pair (Figure 3A), *i.e.*, a sharp discontinuity is not evident. On the contrary, as it is observed in Figure 3B, the chromosomes of Acrididae WDZU decrease in a gradual manner up to the 9th pair while the last three pairs (pair 10, 11 and 12) are distinctly smaller than the rest of the chromosomes.

Subfamily Acridinae

The karyotypes of female specimens of the two species of genus *Acrida* designated here as *Acrida* sp.-1 and *Acrida* sp.-2 are presented in Figure 4A and B, respectively. The two karyotypes are very similar, having 2n = 24 chromosomes and autosomal fundamental number of 22. In both cases the chromosome size decreases in a gradual manner up to the 10th pair, after which there is a large gap in size between this group and that of the last two pairs. Tiny second arm-looking chromatin is evident on the chromosomes of *Acrida* spp.-2 (Fig. 4B), particularly on chromosome pair-3.

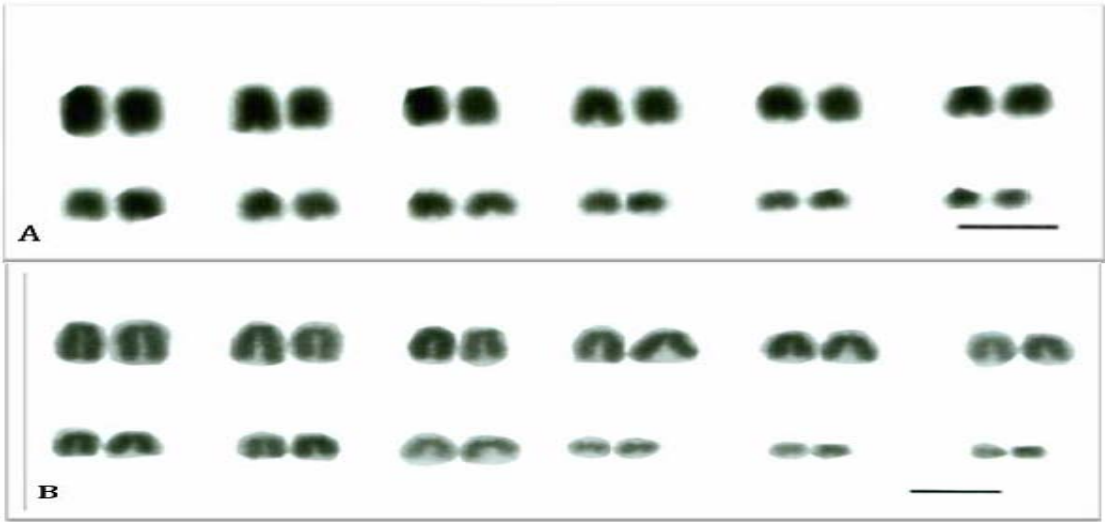

Fig. 3. Karyotypes of the two female grasshopper taxa identified only to the family level. (A) Acrididae MU, (B) Acrididae WDZU. Scale bar = 10 μm.

Fig. 4. Karyotypes of two grasshopper taxa identified to family Acrididae, subfamily Acridinae, genus *Acrida*. (A) *Acrida* spp.-1 (B) *Acrida* spp.-2. Scale bar = 10 μm.

Subfamily Cyrtancanthacridinae

Acanthacris sp.

A normal karyotype of a male specimen of this taxon is presented in Figure 5A. It consists of 2n = 23 (22 autosomes + X) chromosomes and the autosomal fundamental number is 22. All the autosomal chromosomes have small chromatin projections which apparently look like short second arm in the autosomes whereas in the X chromosome it is more distinctly recognizable second arm. The X chromosome looks like an acrocentric rather than telocentric when one considers the terminology of Levan *et al.* (1964). Size wise, there are two groups of chromosomes. The first five autosomal pairs and the X chromosome are of about equal size with only slight differences between them. The chromosomes of the second group, consisting of pairs 7 to 12, are relatively smaller than the chromosomes of the first group and vary among themselves in a gradual manner.

Chromosome numerical variability between somatic cells in the testis was observed involving diploid, hypoploid and hyperploid conditions. Figure 5B and C present a hypoploid cell (2n = 22) and a hyperploid cell (2n = 24), respectively. As the karyotypes (Fig. 5D and E) constructed for these aneuploid cells show, it seems that the same chromosome (chromosome corresponding to pair 3 or 4 of the normal karyotype) is missing in the hypoploid and exists as an extra in the hyperploid cell.

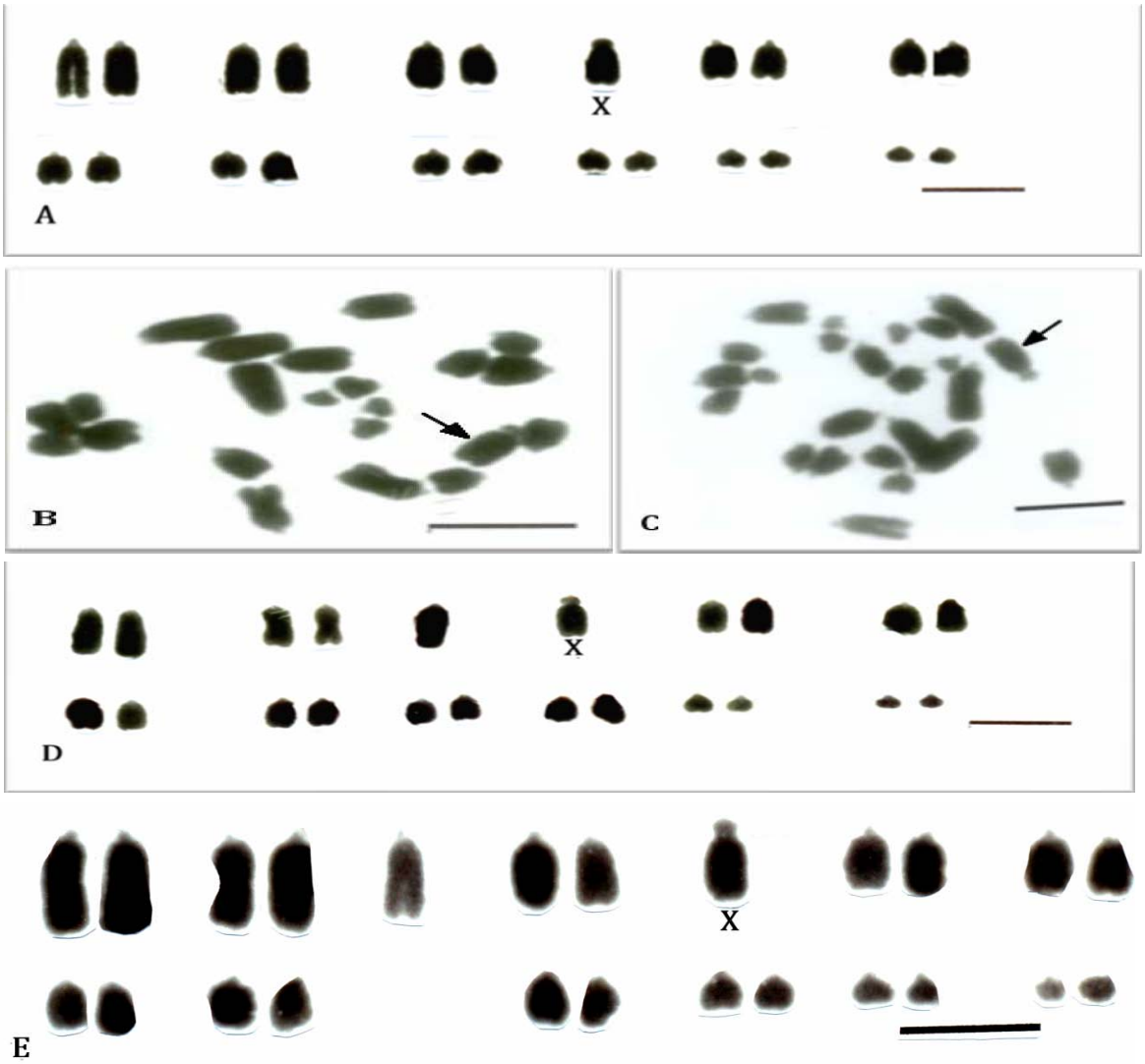

Fig. 5. Chromosome of *Acanthacris* spp. (A) male karyotype from a cell with normal chromosome number (2n = 23), (B) a cell with 2n with 2n = 22 chromosomes, (C) a cell with 2n = 24 chromosomes, (D) karyotype from cell B, (E) karyotype from cell C. X represents the X-chromosome. Scale bar = 10 µm.

Variability in chromosome number was also observed in meiotic cells of the testis of this taxa. These are illustrated in Figure 6. Both hypoploid and hyperploid cells were observed. Furthermore, an additional chromosome, looking different from the ordinary members of the complement, was documented. Like the X chromosome, the extra chromosome condenses early (Figs 6A–D) in prophase I and sometimes observed bending on itself (Fig. 6B). It is as compact as the X chromosome on most of its parts but appears slightly fussy at its ends at the stage when the bivalents of the normal autosomes take on a fussy appearance (Figs 6A and B). It is found lying independently (Figs 6A, B and C) or sometimes associated with the X chromosome either laterally (Fig. 6D) or end to end. Without considering this odd extra-chromosome, hypoploid and hyperploid cases are present. For instance, Figures 6A and B show 10II + X + the odd chromosome (2n = 22), Figure 6C shows 11II + X + the odd chromosome (2n = 24) and Fig. 6 D shows 12II + X + the odd chromosome (2n = 26).

Subfamily Oedipodinae

Gastrimargus sp.

The karyotypes of male and female individuals are presented in Figures 7A and B, respectively. The karyotype is consisting of 2n = 23 in males and 2n = 24 in females with autosomal fundamental number of 22. All the autosomal chromosomes are telocentric and the X chromosome is acrocentric, having a small second arm (Fig. 7A). This, however, is not evident in the karyotype of the female.

The X chromosome of this specimen is smaller than the X chromosome of *Acanthacris* sp. which, like the X chromosome of this specimen, also has a second arm. The size of the chromosomes varies in a gradual manner from the largest up to the 10th pair while the last two pairs of chromosomes are clearly smaller than the rest of the chromosomes, but of equal size to each other.

Figure 7C presents dyads of a male meiotic cell at anaphase I stage from the pole that includes the X chromosome (n = 11 autosome dyads + X). The interesting thing about these chromosomes is that the two sister chromatids constituting each of the dyads have opened apart making each dyad apparently look like a metacentric chromosome.

Paracinema tricolor

This was the only taxon identified to the species level in the present study. Figure 8 presents the karyotype of a female specimen consisting of 2n = 24 telocentric chromosomes. In the karyotype, the first nine pairs of the chromosomes are gradually decreasing in size whereas the last three pairs of the chromosomes are sharply smaller.

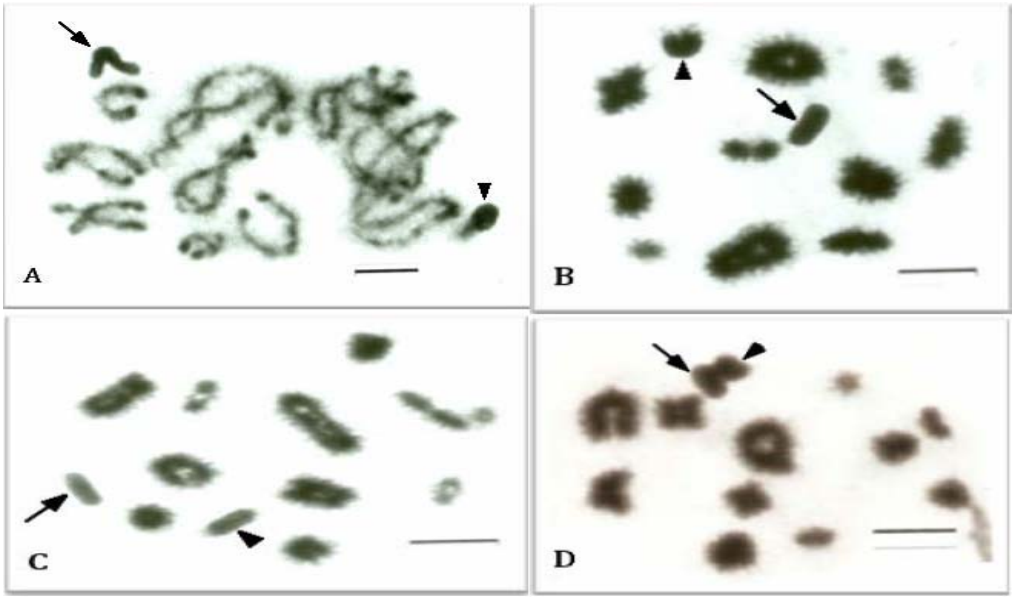

Fig. 6. Meiotic chromosomes of *Acanthacris* spp. (A) A cell in diplotene stage with 10II + X + 1E, 2n = 22, (B) A cell with 10II + X + 1E (2n = 22) (C) A cell with 11II + X + 1E (2n =24) (D) A cell containing 12II + X + 1E (2n = 26). Arrows indicate X chromosome and arrow heads indicate the extra chromosome designated here as E. Scale bar = 10 μm.

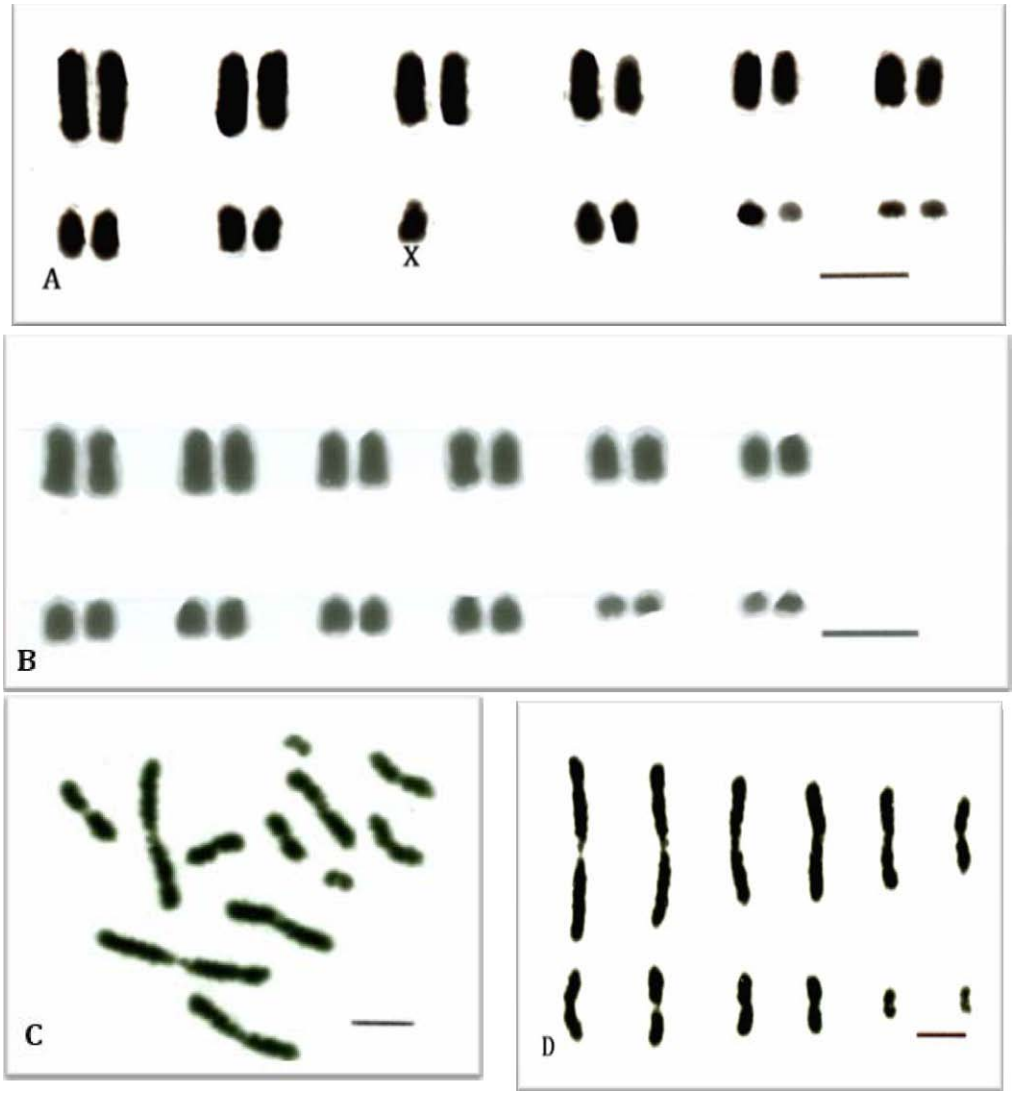

Fig. 7. Chromosomes of *Gastrimargus* species. (A) Karyotype of a male specimen, (B) Karyotype of a female specimen, (C) Meiotic chromosomes of a male anaphase I cell from the pole that includes the X-chromosome, (D) Dyads shown in Figure 7C arranged in decreasing size order in the manner of a karyotype. Scale bar = 10 μm.

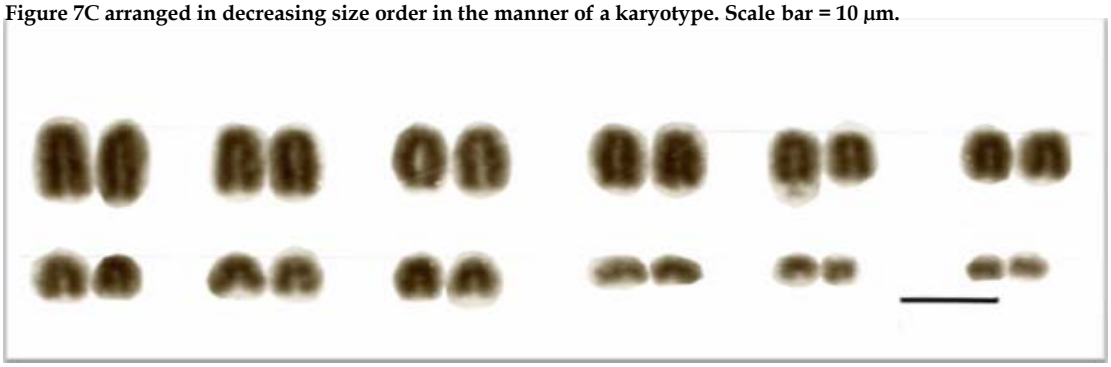

Fig. 8. Karyotype of *Paracinema tricolor* specimen. Scale bar = 10 μm.

Pardalophora sp.

Figure 9 shows the karyotype of a male insect (2n = 23). The X chromosome has a small second arm and hence, is an acrocentric chromosome. It is one of the smaller chromosomes in size. The last two pairs of chromosomes are sharply smaller than the rest of the chromosomes, which are relatively larger and vary in a gradual manner.

Family Tetrigidae

Subfamily Tetriginae

Only one taxon from this family was included in the study. This belongs to subfamily Tetriginae and genus *Paratettix*, and herein designated as *Paratettix* sp. The karyotype of a female specimen is presented in Figure 10. Unlike the other taxa considered so far, the karyotype of a female insect is consisting of 2n = 20 chromosomes (Fig. 10). The chromosomes vary in a gradual manner. Unlike the chromosomes of the other taxa considered earlier, no sharp size differences between the chromosomes (especially between the first five homologous pairs of chromosomes) were observed.

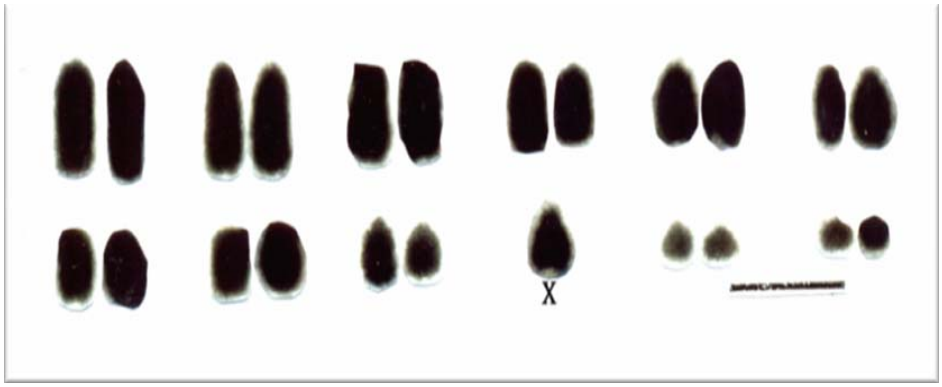

Fig. 9. Karyotype of a male *Pardalophora* spp. X indicates the X-chromosome. Scale bar = 10 μm.

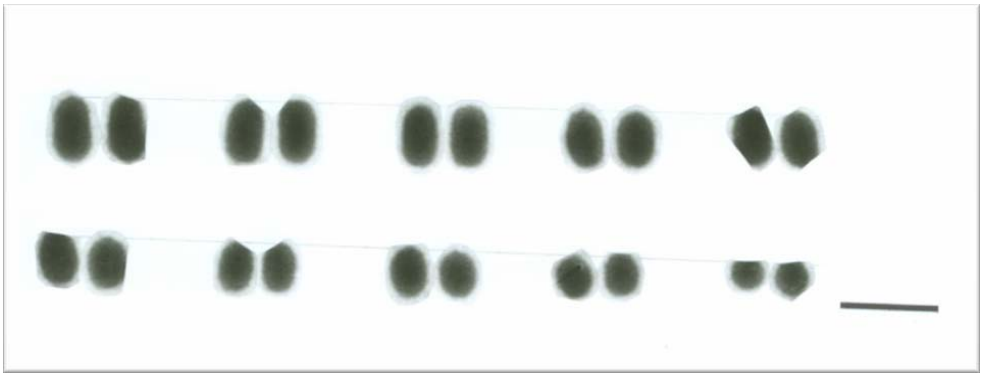

Fig. 10. Karyotype of *Paratettix* spp. of the family Tetrigidae. Scale bar = 10 μm.

DISCUSSION

In spite of the presence of a large number of grasshopper species in Ethiopia, none of them has any chromosomal descriptions to date. Also, studies on the species composition of Ethiopian grasshoppers are scanty, and to date only two research studies/publications by Stretch-Lilija (1977) and Tibebu Habtewold and Landin (1992) are available. Hence, the identification of the specimens was done based on the available materials.

According to Stebbins (1971), an asymmetrical karyotype contains chromosomes with subterminal or terminal centromeres and with different relative size. On this basis, the karyotypes in all the studied acridid grasshopper species, are asymmetrical and show slight tendency towards bimodalism.

The present result obtained for the taxa belonging to family Acrididae is in conformity with what have been described for this family elsewhere. Different studies have indicated that the species of the family Acrididae have a uniform and conserved karyotype, which predominantly are 2n = 22 autosomes + X (male)/ XX (female) telocentric chromosomes (Mesa and Fontanetti, 1983; Rocha *et al.*, 2004;

Souza and Melo, 2007). In line with this, all the species, in the present study with some exceptions, manifest karyotypic conservatism of the family in both the morphology and number of chromosomes. It was suggested by different workers that several factors are responsible for karyotypic conservatism such as climatic stability and habitat similarity (Vosa, 2005), natural selection (Vij *et al.,* 1980), and lack of heterochromatin or repetitive sequences (Jackson, 1971).

In spite of the apparent karyological conservatism in the family Acrididae, however, some cryptic chromosome structural rearrangements such as paracentric inversion, insertion, deletion, duplication or reciprocal translocation, which do not result in easily detectable morphological changes, might have occurred. Some evidence in support of this are available from C-banding comparison and molecular studies. For example, Mesa and Fontanetti (1983), Rocha *et al.* (2004), Souza and Melo (2007), have observed different species having the same chromosome number and morphology but differing in the number, location, distribution and size of interstitial heterochromatin blocks in the karyotype.

In the present study, hyper-diploid (2n = 24) and hypo-diploid (2n = 22) conditions relative to the normal 2n = 23 chromosome were observed in somatic cells of male Acanthacris spp. Similarly, the meiotic chromosome number of cells from the testis also deviated from the expected 11-bivalents + x. Similar phenomenon has been reported by Channaveerappa and Ranganath (1997) in the gonadal tissue of a different acridid grasshopper species, *Gastrimargus africanus orientalis.* They assumed this phenomenon to be due to aneuploidy, though the causes for the aneuploidy was not indicated.

In all the analyzed meiotic cells of the male of *Acanthacris* spp., an extra chromosome was observed. Although it is not possible to identify this chromosome among the somatic chromosomes, it may be responsible at least for some of the hyperploidy situations. For example, a gametic cell with 11 bivalents + X + this chromosome will have 2n = 24 chromosomes (see Fig. 6C). The nature of this extra chromosome is not clear. If it was an additional copy (trisomy) of one of the somatic chromosomes, some trivalent associations would be expected at meiosis. Since no trivalent association was observed, it is unlikely that this chromosome is a homolog of any of the somatic chromosome. Furthermore, no other chromosome was observed which behaves in the same way in meiotic cells like this particular chromosome, which would be expected if it has a homologue. This chromosome may possibly be a B-chromosome. Studies have indicated that there is high prevalence of B-chromosomes in grasshoppers, especially in the family Acrididae (Henriques-Gil and Arana, 1990; Lopez-Leon *et al.,* 1993; Bakkali *et al.,* 1999). Henriques-Gil and Arana (1990) have identified about 30 B-chromosome variants in this family. In the present study, it has been observed that this odd chromosome is sometimes associated with the X chromosome, which may imply that the two chromosomes have some homology. Probably, this chromosome might have been derived from the X chromosome or shares some homologous segment with it. Cabrero *et al.* (2003) have indicated cases in which the B-chromosomes can be derived from the X chromosome.

The result obtained for the *Gastrimargus* spp. is in concordance with the karyotype reported by Channaveerappa and Ranganath (1997) for *Gastrimargus africanus orienatlis*, although the authors observed some numerical instability in germ line cells of some individuals within the studied species of the genus *Gastrimargus*. Unlike their result, no chromosomal instability was observed in the specimens of *Gastrimargus* spp. in the present study.

Furthermore, in *Gastrimargus* spp., a special phenomenon has been observed in dyads at anaphase-I stage of meiosis. Normally, at dyad stage of meiosis the arms of sister chromatids remain apposed with only some degree of separation as the result of dissociation of cohesin complex (Gimenez-Abian *et al.,* 2004). In the present case, however, the two sister chromatids have opened apart to about 180° in such a way that individual dyads look like metacentric chromosomes. Although the exact mechanism of how this happens is not known, some workers attribute some chromosome morphological changes to the effect of higher concentration of colchicine (Rodriguez *et al.,* 2001). Whether the same factor is responsible or not for what was observed in the present study remains for future investigation.

The karyotype of the female *Paratettix* spp. was found to consist of 2n = 20 telocentric chromosomes, whereas previous studies indicated that

the family Tetrigidae is typified by the chromosome number of 2n = 13 (X0) and 14 (XX) in males and females, respectively (Harman, 1915; Henderson, 1961). In addition, Henderson (1961) and Warchalowska-Sliwa *et al.* (2005) have reported polyploidy in different species of family Tetrigidae.

Based on the male concealed genitalia, Roberts (1941) divided the superfamily Acridoidea into two groups – Chasmosacci and Cryptosacci, and according to the author, the first group comprises only family Pamphagidae and family Pyrgomorphidae, whereas the second contains all the remaining families. Those families under Chasmosacci have a karyotype of 2n = 19 (male) and 20 (female) telocentric chromosomes and are considered as primitive, while the chromosome complement in the Cryptosacci group is with 2n = 23 (male) and 24 (female) telocentric chromosomes and considered as advanced. Conversely, White (1973) considered the karyotype with 2n = 23 (X0)/ 24(XX) as a primitive karyotype, while the one with 2n = 19 (X0)/ 20 (XX) as a derivative.

In the present study, the specimens identified as *Paratettix* spp., are morphologically similar to those species under the family Tetrigidae, which is comprised by the Cryptosacci group, by having, for instance, dorsoventrally flattened body, fat hind femur and less developed fore- and hind-wings. Despite this, karyotypically, they are similar to the Chasmosacci group by having a complement of 2n = 20 telocentric chromosomes. As a result, further taxonomical and cytological studies are needed.

The high degree of karyotypic similarity observed in acridid grasshoppers implies that karyotypic data alone does not provide sufficient information about the systematics and phylogeny of acridid grasshoppers. As a result, the findings obtained from this study indicate that there is a need for integrated data from morphological, cytogenetical and molecular studies to clarify the systematics of these insects.

ACKNOWLEDGMENTS

The former Department of Biology and the Graduate Program of the Addis Ababa University are acknowledged for providing necessary labratory facilities to carry out the study and for covering the research cost. Due appreciation also goes to Prof. Jan Landin, Dr Emiru Seyoum, Agegnehu Wasse and Yohannes Lemma for their genuine provision of important literatures. Afework Desalegn is also acknowledged for his kind help in preparing the map of specimen collection sites using GIS data.

REFERENCES

1. Bakkali, M., Cabrero, J., Lopez-Leon, M.D., Perfectti, F. and Camacho, J.P.M. (1999). The B chromosome polymorphism of the grasshopper *Eyprepocnemis plorans* in North Africa. I. B variants and frequency. *Heredity* **83**:428–434.
2. Branson, D.H., Joern, A. and Sword, G.A. (2006). Sustainable management of insect herbivores in grassland ecosystems: new perspective in grasshopper control. *Bioscience* **56(9)**:743–755.
3. Cabrero, J., Bakkali, M., Bugrov, A., Warchalowska-Sliwa, E., Lopez-Leon, M.D., Perfectti, F. and Camacho, J.P.M. (2003). Multiregional origin of B chromosomes in the grasshopper. *Eyprepocnemis plorans*. *Chromosoma* **112**:207–211.
4. Capinera, J.L., Scherer, C.W. and Simkins, J.B. (1997). Habitat associations of grasshoppers at the Macarthur Agro-ecology Research Center, Lake Placid, Florida. *Fla. Entomol.* **80(2)**:253–261.
5. Channaveerappa, H. and Ranganath, H.A. (1997). Karyology of a few species of south Indian acridids. II Male germ line karyotypic instability in *Gastrimargus*. *J. Biosci.* **22(3)**:367–374.
6. Gimenez-Abian, J.F., Sumara, I., Hirota, T., Hauf, S., Gerlich, D., de la Torre, C., Ellenberg, J. and Peters, J-M. (2004). Regulation of sister chromatid cohesion between chromosome arms. *Curr. Biol.* **14**:1187–1193.
7. Harman, M.T. (1915). Spermatogenesis in Paratettix. *Biological Bulletin* **29(4)**:262–276.
8. Henderson, S.A. (1961). The chromosomes of the British Tetrigidae (Orthoptera). *Chromosoma* **12**:553–572.
9. Henriques-Gil, N. and Arana, P. (1990). Origin and substitution of B-chromosomes in the grasshopper *Eyprepocnemis plorans*. *Evolution* **44(3)**:747–753.
10. Jackson, R.C. (1971). The karyotype in systematics. *Annu. Rev. Ecol. Syst.* **2**:327–368.
11. Levan, A., Fredga, K. and Sandberg, A.A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas* **52**:201–220.
12. Lopez-Leon, M.D., Cabrero, J., Pardo, M.C., Viseras, E., Camacho, J.P.M. and Santos, J.L.

(1993). Generating high variability of B chromosomes in *Eyprepocnemis plorans* (grasshopper). *Heredity* **71**:352–362.

13. Meinzingen, W.F. (1993). A guide to migrant pest management in Africa. Food and Agricultural Organization of the United Nations (FAO), Rome.
14. Mesa, A. and Fontanetti, C.S. (1983). Karyotypes of nine Brazilian species of Acridids (Orthoptera: Acridoidea). *Brazil. J. Genetics* **6(2)**:295–305.
15. New World Encyclopedia (2008). *Caelifera*. Retrieved on June4, 2010 from http://www.newworldencyclopedia.org/entry/Caeliferaoldid=861010.
16. Picker, M., Griffiths, C. and Weaving, A. (2004). *Field Guide to Insects of South Africa.* A Division of New Holland Publishing Ltd, South Africa.
17. Ragge, D.R. (1965). *Grasshoppers, Crickets and Cockroaches of the British Isles.* Butler and Tanner Ltd, London.
18. Rentz, D.C. (1991). Orthoptera. **In:** *The Insects of Australia: A Text Book for Students and Research Workers,* pp. 369-390, (Naumann, I.D., Carne, P.B., Lawrence, J.F., Nielson, E.S., Spradbery, J.P., Taylor, R.W., Whitten, M.J. and Littlejohn, M.J., eds). 2nd ed. (Vol. 1) Melbourne University Press, Australia.
19. Roberts, H.R. (1941). A comparative study of the subfamilies of the Acrididae (Orthoptera) primarily on the basis of their phallic structures. *Proc. Acad. Nat. Sci. Phila.* **93**:201–246.
20. Rocha, M.F., Souza, M.J. and Moura, R.C. (2004). Karyotypic analysis, constitutive heterochromatin and NOR distiributon in five grasshopper species of the subfamily Leptisminae (Acrididae). *Caryologia* **57(1)**:107–116.
21. Rodriguez, E.M., Parra, M.T. and Rufas, J.S. (2001). Colchicine promotes a change in chromosome structure without loss of sister chromatid cohesion in prometaphase I-arrested bivalents. *Chromosoma* **110**:478–486.
22. Shaw, D.D., Contreras, N. and Maclean, V. (1995). The genomic and molecular organisation of centromeres in the genus *Caledia*. **In:** *Kew Chromosome Conference,* pp. 119–132, (Brandham, P.E. and Bennett, M.D., eds). Royal Botanic Garden, London.
23. Souza, M.J. and Melo, N.F. (2007). Chromosome study in *Schistocerca* (Orthoptera-Acrididae-Cyrtacanthacridinae): Karyotypes and distribution patterns of constitutive heterochromatin and nucleolus organizer regions (NORs). *Genet. Mol. Biol.* **30(1)**:54–59.
24. Stebbins, G.L. (1971). *Chromosomal Evolution in Higher Plants.* Edward Arnold Ltd., London.
25. Stretch-Lilija, C. (1977). *Short-horned grasshopper pests in Ethiopia, their identification and control.* Institute of Agricultural Research, Addis Ababa.
26. Tibebu Habtewold and Landin, J. (1992). Composition and structure of Orthopteran faunas in cereal crops in Ethiopia. *Bull. Entomol. Res.* **82**:29–39.
27. Vij, S.P., Sharma, M. and Toor, I.S. (1980). Cytological investigations into some garden ornamentals II. The genus *Aloe* L. *Cytologia* **45**:515–532.
28. Vosa, C.G. (2005). On chromosome uniformity, bimodality and evolution in the tribe Aloineae (Asphodelaceae). *Caryologia* **58(1)**: 83–85.
29. Warchalowska-Sliwa, E., Niklinska, M., Gorlich, A., Michailova, P. and Pyza, E. (2005). Heavy metal accumulation, heat shock protein expression and cytogenetics changes in *Tetrix tenuicornis* (L.) (Tetrigidae, Orthoptera) from polluted areas. *Environ. Pollut.* **133**:373–381.
30. White, M.J.D. (1973). *Animal Cytology and Evolution*. Cambridge University Press.